Journal of Cardiology (2009) **53**, 317—333

ELSEVIER

JOURNAL of CARDIOLOGY
Official Journal of the Japanese College of Cardiology
www.elsevier.com/locate/jjcc

REVIEW ARTICLE

Inflammatory biomarkers in coronary artery disease

Epaminondas Zakynthinos (MD)[a,*], Nikolitsa Pappa (MD)[b]

[a] *Critical Care Department, School of Medicine, University Hospital of Thessaly, 41110 Larissa, Greece*
[b] *Primary Health Center, Naupaktos, Greece*

Received 10 October 2008; received in revised form 24 December 2008; accepted 26 December 2008
Available online 29 January 2009

KEYWORDS
Inflammatory biomarkers;
CRP;
hs-CRP;
Cytokines;
Acute coronary syndromes

Summary Current evidence supports that inflammation is a major driving force underlying the initiation of coronary plaques, their unstable progression, and eventual disruption; patients with a more pronounced vascular inflammatory response have a poorer outcome.

Biomarkers are generally considered to be proteins or enzymes — measured in serum, plasma, or blood — that provide independent diagnostic and prognostic value by reflecting an underlying disease state. In the case of coronary artery disease (CAD), inflammatory biomarkers, have been extensively investigated; more evidence exists for C-reactive protein (CRP). Using high sensitivity (hs) assays, epidemiologic data demonstrate an association between hs-CRP and risk for future cardiovascular morbidity and mortality among those at high risk or with documented CAD. Moreover, a series of prospective studies provide consistent data documenting that mild elevation of baseline levels of hs-CRP among apparently healthy individuals is associated with higher long-term risk for cardiovascular events. Yet, the predictive value of hs-CRP is found to be independent of traditional cardiovascular risk factors.

Recent studies suggest that, besides CRP, other inflammatory biomarkers such as cytokines [interleukin (IL)-1, IL-6, IL-8, monocyte chemoattractant protein-1 (MCP-1)], soluble CD40 ligand, serum amyloid A (SAA), selectins (E-selectin, P-selectin), myeloperoxidase (MPO), matrix metalloproteinases (MMPs), cellular adhesion molecules [intercellular adhesion molecule 1 (ICAM-1), vascular adhesion molecule 1 (VCAM-1)], placental growth factor (PlGF) and A_2 phospholipases may have a potential role for the prediction of risk for developing CAD and may correlate with severity of CAD.

Finally, indications suggest that the increased risk associated with inflammation may be modified with certain preventive therapies and biomarkers may help to identify the individuals who would benefit most from these interventions.

* Corresponding author. Tel.: +32 410 681280; fax: +32 410 670838.
E-mail address: ezakynth@yahoo.com (E. Zakynthinos).

0914-5087/$ — see front matter © 2009 Japanese College of Cardiology. Published by Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jjcc.2008.12.007

Contents

Introduction

Pathophysiology of inflammation in atherosclerotic plaque

There is an extensive literature supporting the role of inflammation in coronary artery disease (CAD). Inflammatory cells, inflammatory proteins, and inflammatory responses from vascular cells play a pivotal role in the pathogenesis of various stages of atherosclerosis, including the initiation and progression of atheroma, plaque instability and rupture, and post-angioplasty and restenosis [1—3]. The vascular endothelium is subject to injury from numerous potential insults, such as hemodynamic forces [4], oxidative stress [5], and modified lipoproteins [6]. Although circulating leukocytes do not adhere to the normal vascular endothelium, injured endothelium expresses several classes of adhesion molecules that selectively bind to leukocytes [7]. Endothelial dysfunction is marked by the up-regulation of cellular adhesion molecules, such as vascular adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), and selectins, which cooperate with chemokines and mediate increased adhesion of mononuclear and neutrophil leukocytes [8]. Following adherence to the endothelium, chemoattractant cytokines, such as monocyte chemoattractant protein-1 (MCP-1), mediate transmigration of inflammatory cells into the subendothelial space [9]. In addition to MCP-1, macrophage colony-stimulating factor (M-CSF) contributes to the differentiation of monocytes into macrophages [10]. The concurrent uptake of low-density lipoproteins (LDLs) by monocyte-derived macrophages transforms them into the lipid-laden foam cells that constitute a key element of the fatty streak, the first recognizable progenitor of the advanced atherosclerotic lesion [11—13]. Mononuclear cells release cytokines, including interleukin (IL)-1 [14] and IL-6 [15]. Cytokine release from the fatty streak recruits further inflammatory cells (macrophages, mast cells, activated T cells), resulting in further uptake and oxidation of LDLs. These cytokines also stimulate smooth muscle cell proliferation and development of a collagenous fibrous cap that covers this inflammatory mixture [14]. This fibrous cap separates the pro-coagulant contents of the atheroma core from the circulating blood; collagen produced by smooth muscle cells in the lesion provides mechanical strength and stability to the fibrous cap. The synthesis and breakdown of collagen is dynamically controlled by inflammatory signals [16,17].

There are data showing that the risk for an acute coronary event has less to do with the degree of angiographic luminal stenosis than with the underlying pathology of the atherosclerotic plaque that makes it susceptible to rupture [17]. Activated T cells secrete interferon gamma (INF-γ) that decreases smooth muscle cell production of collagen. Activated macrophages secrete matrix metalloproteinases (MMPs) that proteolytically

degrade collagen, rendering the fibrous cap weak and prone to rupture [1]. Plaque rupture permits contact between the pro-coagulant lipid core and the blood, after which further inflammatory reactions lead to platelet activation, coagulation cascade, further vasomotor dysfunction, and ultimately luminal occlusion [1,18]. Adhesive interactions between vascular cells play important roles in orchestrating the inflammatory response. Recruitment of circulating leukocytes to vascular endothelium requires multistep adhesive and signaling events including selectin-mediated attachment and rolling, leukocyte activation, and integrin-mediated firm adhesion and diapedesis that result in the infiltration of inflammatory cells into the blood vessel wall [19]. During firm adhesion of leukocytes to the endothelium, members of the β2-integrin family, LFA-1 (CD11a/CD18, αLβ2), Mac-1 (CD11b/CD18, αMβ2), and p150,95 (αXβ2), as well as β1-integrins on the leukocyte surface, interact with endothelial counterligands such as ICAM-1, surface-associated fibrinogen, or VCAM-1 [20,21]. Interestingly, lipoprotein(a) [Lp(a)], which is considered a risk factor for the development of atherosclerotic disorders, seems to specifically interact with the β2-integrin Mac-1, through its apo(a) moiety Lp(a), thereby promoting the adhesion of leucocytes and their transendothelial migration. Yet, via its interaction with Mac-1, Lp(a) induces activation of the proinflammatory transcription factor NFκB, as well as the NFκB-related expression of prothrombotic tissue factor, suggesting the mechanism for the atherogenic properties of Lp(a) [22].

Percutaneous coronary intervention (PCI) produces a significant inflammatory reaction in the injured vessel wall, that may lead to the development of neointimal thickening and restenosis [23]. Balloon coronary angioplasty or stent deployment is associated with significant platelet activation, which promotes leukocyte recruitment to the injured vessel wall [24]. There is increasing experimental and clinical evidence on the molecular mechanisms that regulate the adhesion and subsequent trafficking of leukocytes to the vessel wall in the absence of the arterial endothelium such as occurs after angioplasty. Diacovo et al. [25] proposed a model of leukocyte recruitment at sites of platelet and fibrin deposition in which Mac-1 is required for polymorphonuclear leukocyte diapedesis. Additional in vitro observations indicated that platelets are capable of up-regulating and activating Mac-1 as a consequence of functional responses elicited by P-selectin on polymorphonuclears through P-selectin glycoprotein ligand-1 (PSGL-1) signaling [26]. Inoue et al. provided considerable insight into the roles of Mac-1 supporting the notion that platelets deposited at sites of arterial injury are capable of local leukocyte integrin activation in humans. They demonstrated that PCI induces activation and up-regulation of Mac-1 on the surface of neutrophils, with the maximum response at 48 h after PCI, and this is associated with restenosis [3,27]. Oxidative burst in the post-stent inflammatory process, resulting from Mac-1 dependent activation of neutrophils, probably contributes to this phenomenon of lumen loss [28].

Because of the central role of inflammation in atherogenesis, plaque stability and restenosis after PCI, several clinical studies have targeted inflammatory factors as potential markers for cardiovascular risk assessment.

The role of chronic infection in coronary artery disease

Recent data demonstrate that chronic infection may play a role in the initiation, progression, and destabilization of atherosclerotic plaques with several potential mechanisms. The effect may result from direct vessel wall colonization of the infectious agent that may damage the vessel either directly or indirectly by initiating immunologic responses. Moreover, the effect may simply be the enhancement of the pre-existing chronic inflammatory response of the body to traditional risk factors such as hyperlipidemia. Chronic infection may influence pre-existing plaque by enhancing T-cell activation or other inflammatory responses and cause destabilization of the fibrous cap [29]. The infectious agents with the most evidence to support an etiologic relationship with atherosclerosis are *Chlamydia pneumoniae* (Cp) [30—33] and cytomegalovirus [32,34,35]. A variety of other potential agents include herpes viruses [36], influenza [37,38], *Mycoplasma pneumonia* [31], and chronic infections with common bacterial agents such as periodontal disease [39,40]. Recently, Jha et al. reported that IL-6 was significantly higher in CAD patients with more than medium Cp IgA levels (>1.7 index number) than controls without CAD. The authors hypothesized that Cp IgA and elevated IL-6 may synergize to accelerate CAD [41].

Biomarkers in coronary artery disease

C-reactive protein

C-reactive protein (CRP) is the best studied of the inflammatory biomarkers in CAD. This inflammatory

biomarker has several characteristics that render it particularly attractive. It is an acute phase protein that has been shown to be a marker of systemic inflammation, elevated in response to injury, infection, and other inflammatory stimuli [42]. Hepatic production is directly related to IL-6 stimulation and, unlike other acute phase reactants, its levels remain stable over long periods in the absence of new stimuli [43]. However, CRP is not only a powerful inflammatory marker, but increasing evidence suggests that CRP may also directly participate in the inflammatory process of atherogenesis [44,45]. Ishikawa et al. suggested CRP localization in atherosclerotic plaque [44]. They also suggested that CRP plays an important role on plaque vulnerability and in the pathogenesis of unstable angina, as well as restenosis after coronary intervention [44]. Similarly, Inoue et al., demonstrated CRP is produced at the site of the culprit plaque, via the existence of CRP gradient in coronary arterial blood, sampled just distal and proximal to the culprit lesions [45]. Yet, in the same study, the transcardiac CRP gradient (coronary sinus minus peripheral blood), and activated Mac-1, increased gradually after stenting, reaching a maximum at 48 h. Further, there was a positive correlation between the transcardiac CRP gradient and activated Mac-1 at 48 h. These findings suggest CRP is produced at the site of the vulnerable plaque or the vessel wall injured by PCI and this locally released CRP may play a role in Mac-1 activation and restenosis [45].

In contrast to many other inflammatory markers, assay techniques for high sensitive (hs)-CRP are reliable, fully automated, and sensitive, providing a simple clinical tool for the careful assessment of systemic inflammation [46]. Therefore, a statement from the US Centers for Disease Control and Prevention and the American Heart Association (CDC/AHA) recommended that CRP has the assay characteristics most conducive to use in clinical practice compared with other inflammatory markers [47].

Circulating levels of CRP have been found to be related to a number of well known cardiovascular risk factors, such as obesity, smoking, blood pressure, serum triglycerides, apolipoprotein B, fasting blood glucose, heart rate, serum fibrinogen and inversely to HDL-cholesterol levels, both in children and in adults [48,49]. Healthy individuals with at least one of their parents with myocardial infarction (MI) had elevated CRP levels compared with those without heredity for myocardial infarction [50]. In a recent study, conducted on patients with various cardiovascular risk factors but no CAD (diabetic, hypertensive, smokers, and obese), in addition to healthy controls, the CRP levels, age, waist circumference, homocysteine, and triglycerides were predictors of intima-media thickness of the carotid artery assessed by Doppler, while CRP, age, and triglycerides were predictors of plaque formation [51]. Further, a small case-control study found that 60 patients with chronic stable angina had twice median CRP levels compared with controls [52].

The role of CRP for prognosis in CAD

CRP levels may be useful for short-term prognosis and long-term risk assessment after a cardiovascular event. Therefore, several studies have looked at associations between CRP levels and the risk of early death or recurrent cardiac events shortly after an acute coronary syndrome (ACS).

Morrow et al., in the study of the Thrombosis in Myocardial Infarction (TIMI) 11A trial, a randomized, dose-ranging trial of enoxaparin in unstable angina and non-Q wave myocardial infarction, found that CRP levels were higher in patients who died than in survivors (0.72 mg/L versus 1.3 mg/L). In a substudy of TIMI 11A trial, CRP was identified as an independent predictor of mortality at 14 days in ACS, including those with a negative troponin level [53]. In another large trial, the Fragmin during InStability in Coronary Artery Disease (FRISC) study, a randomized trial of low-molecular weight heparin in unstable coronary syndromes, CRP levels were independent predictors of cardiac death. Mortality was 5.7% among patients with CRP levels <2 mg/L, 7.8% among patients with CRP levels 2—10 mg/L, and 16.5% among patients with CRP levels >10 mg/L [54]. In the Global Use of Strategies to open Occluded arteries IV (GUSTO-IV) substudy, although hs-CRP elevation during the acute stage of unstable CAD was associated with an increased 30-day mortality independent of troponin levels, there was no association with an increased risk of nonfatal recurrent ischemic events [55]. Therefore, it appears that after ACS, CRP is a better predictor of death than nonfatal ischemic events.

Similarly, the association between CRP and a long-term risk assessment in patients with stable CAD or after an ACS has been efficiently investigated. In the prospective European Concerted Action on Thrombosis and Disabilities Study (ECAT), it was found that among 2121 patients with angina, each standard deviation increase in baseline hs-CRP was associated with a 45% increase in the relative risk (RR) of nonfatal myocardial infarction or sudden cardiac death over 2 years of follow-up [56]. In addition, recent data also confirm that CRP is a strong independent predictor of mortality among ACS patients who are treated with early revascularization. In a prospective study of patients who underwent early invasive therapy after non-ST

elevation ACS, CRP >10 mg/L during admission remained associated with increased risk of death over a follow-up of 20 months [57]. Yet, in 319 patients with acute MI treated with thrombolytic therapy, it was found that patients with CRP levels in the highest tertile had a lower incidence of reperfusion and a greater in-hospital mortality [58].

CRP and MI

It has been shown that CRP levels are associated with the size of the infarct [59]. In addition, it was suggested that in patients with acute MI, CRP levels correlate with the presence of plaque rupture, as assessed by intravascular ultrasound [60]. Further, an increased temperature at unstable coronary plaques, evaluated by the invasive thermogenetic catheter, has been shown to be related to CRP levels [61]. Consequently, it was suggested that CRP in the acute phase of MI could be correlated with risk assessment. However, a prospective study of 1360 patients with unstable or stable angina pectoris or acute MI found that adjusted hazard ratios for death/acute MI for CRP levels above the first tertile showed a significant risk of 1.8 with stable angina, 2.7 for unstable angina, and only 1.0 for acute MI [62]. On the contrary, in stable post-MI patients, elevated hs-CRP predicted a significantly higher risk for recurrent nonfatal MI or fatal coronary events (75% higher in the highest versus lowest quintile of hs-CRP), suggesting that CRP is not only a marker for the extent of myocardial damage [63]. Based on these data, one could suppose that CRP levels are predictive of short- and long-term cardiac events in patients with stable and unstable angina, but may not be predictive in the acute stage of MI. Therefore, the measurements should be delayed until the acute phase reaction is over and levels have returned to baseline.

Nevertheless, the results are not homogenous even in the case of stable MI. The Thrombogenic Factors and Recurrent Coronary Events (THROMBO) study was a multicenter investigation of 1045 post-MI patients that measured CRP levels 2 months after the event and again at 2 years. Although CRP levels were found to be associated with recurrent coronary events, in multivariable analyses, CRP was not proved to be an independent marker for recurrent coronary events [64].

CRP and primary prevention

Several prospective studies in apparently healthy individuals have shown that elevated hs-CRP levels are correlated with higher risk for future cardiovascular morbidity and mortality. The Multiple Risk Factor Interventional Trial (MRFIT), a nested case-control study, found that increased CRP levels predicted increased risk of cardiovascular disease in middle-aged men, but this relationship was statistically significant only for smokers [65]. The Physicians' Health Study (PHS), a prospective, nested, case-control study of men who did not have prior history of cardiovascular disease and had low rates of cigarette use, showed that those with highest baseline levels of hs-CRP had two times the risk of future stroke, three times the risk of future MI, and four times the risk to develop severe peripheral arterial disease. Cardiovascular risks were not influenced by smoking status, and were independent of lipid or other cardiovascular factors [66]. Data from European Monitoring Trends and Determinants of Cardiovascular Disease (MONICA)-Augsburg prospective study offer consistent observations regarding the prognostic capacity of hs-CRP among individuals without clinical evidence of CAD. This study followed 936 healthy, middle-aged men over 8 years and noted a 19% increase in risk for future nonfatal or fatal coronary events for each standard deviation increase in baseline hs-CRP after adjustment for multiple risk factors, including smoking status [67].

Recently, in the prospective Prevention of Renal and Vascular Endstage Disease (PREVEND) study, 8139 individuals without previous documented coronary artery disease were followed for the incidence of coronary angiography and coronary events from 1997 to 2003. hs-CRP levels were found to be associated with angiographic characteristics and clinical consequences of plaque instability during follow-up [68].

The Women's Health Study (WHS), a prospective nested case-control study of postmenopausal women showed that hs-CRP was the most powerful predictor of cardiovascular risk compared with other inflammatory markers, baseline lipid levels, and homocysteine. Women who developed cardiovascular events had higher baseline levels of CRP than control subjects; yet, those in the highest quartile of CRP had a relative risk of 4.4 of any cardiovascular event compared with those in the lowest quartile. A subgroup analysis of women with LDL levels of <130 mg/dL, a group traditionally considered to be low risk, found that those who had elevated baseline CRP were at higher risk for future events. A follow-up study of the entire cohort showed that CRP levels were stronger predictors of cardiovascular events than LDL cholesterol [69,70].

It appears that among healthy men and women, elevated CRP levels predict risk of a first cardiovascular event independently of other factors. Based on PHS [66] and WHS [69], the adjusted relative risk of a future cardiovascular event increases 26% for men and 33% for women overall for each quartile

increase in hs-CRP levels [71]. Therefore, data suggest that hs-CRP levels add to risk prediction based on lipid parameters, and may be useful for identifying patients at risk for future cardiovascular events who would have been classified as low risk.

Finally, a recent study showed that hs-CRP may be a useful marker in screening the children who are at risk of CAD in adulthood. The levels of hs-CRP were measured in 51 children (11.79 ± 3.14 years) with risk factors for CAD (hypercholesterolemia, hypertension, obesity, low HDL-cholesterol, and familial history of CAD). The results were compared with 26 children (12.98 ± 2.59 years) without any risk factors. The children with risk factors had significantly higher levels of hs-CRP compared to the control group ($P < 0.01$). The concentrations of CRP were significantly increased in children with three or more risk factors [72]. Early identification of the children with risk factors and interventions for obesity, harmful habits, and life style in childhood might decrease the incidence of coronary heart disease in adulthood.

CRP and preventive therapies

Apart from providing global risk prediction, CRP screening may also provide a method of targeting preventive interventions. In vitro and in vivo studies show that CRP is not only an indicator of inflammation, but also contributes to plaque development, plaque instability, and thrombus formation [73,74]. Therefore, the relationship between certain cardioprotective medications, such as statin and aspirin therapy, and CRP levels has been examined.

Studies have shown that statin therapy lowers CRP levels independently of lipid levels, supporting the possibility that statins have anti-inflammatory effects. Data from the Cholesterol And Recurrent Events (CARE) study, a randomized trial of pravastatin in post-MI patients, revealed that although the risk of recurrent coronary events was reduced by therapy with pravastatin among individuals with and without evidence of inflammation, the relative risk reduction was greater for those with consistent evidence of inflammation (54% versus 25%) even though the lipid profiles were similar in both groups [63]. This information suggests that statin therapy may be particularly effective among patients with elevated CRP levels. PRavastatin Or atorVastatin Evaluation and Infection Therapy (PROVE-IT) trial demonstrated that intensive statin therapy that lowered hs-CRP levels to a mean of less than 2 mg/L resulted in a reduction risk for recurrent MI or fatal coronary event, irrespective of the degree of LDL lowering [75].

In the PRavastatin Inflammation CRP Evaluation (PRINCE) study, pravastatin reduced hs-CRP levels in subjects without prior history of cardiovascular disease [76]. A further analysis of the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS) has extended the observation that statin therapy directly lowers CRP levels as primary prevention. Subjects with average levels of total cholesterol and below-average levels of HDL-cholesterol were divided into groups according to median levels of LDL cholesterol and CRP. Those with elevated LDL (above median value) were at increased risk for future cardiovascular events and benefited substantially from randomization to lovastatin, irrespective of CRP level. Those with low LDL and low CRP (below median values) were at low risk and derived little benefit from lovastatin therapy. Those with low LDL (below median value) but high CRP (above median value) were at increased risk and derived substantial benefit from lovastatin therapy [77]. Recently, the results from the Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) were reported [78]. JUPITER, was a randomized, double-blind, placebo-controlled, multicenter trial which assigned 17802 apparently healthy men and women with low LDL levels (less than 130 mg/dL) but hs-CRP of 2 mg/L or higher to receive rosuvastatin 20 mg daily or placebo, in order to assess the incidence of a first major cardiovascular event. The subjects were followed up for a median of 1.9 years (maximum, 5.0). Rosuvastatin reduced LDL cholesterol levels by 50% and hs-CRP by 37%. The rates of the primary end point were 0.77 and 1.36 per 100 person-years of follow-up in the rosuvastatin and placebo groups, respectively (hazard ratio for rosuvastatin, 0.56; 95% confidence interval [CI], 0.46—0.69; $P < 0.00001$), with corresponding rates of 0.17 and 0.37 for MI (hazard ratio, 0.46; 95% CI, 0.30—0.70; $P = 0.0002$), 0.18 and 0.34 for stroke (hazard ratio, 0.52; 95% CI, 0.34—0.79; $P = 0.002$), 0.41 and 0.77 for revascularization or unstable angina (hazard ratio, 0.53; 95% CI, 0.40—0.70; $P < 0.00001$), and 0.45 and 0.85 for the combined end point of MI, stroke, or death from cardiovascular causes (hazard ratio, 0.53; 95% CI, 0.40—0.69; $P < 0.00001$). The authors concluded that rosuvastatin significantly reduced the incidence of major cardiovascular events in apparently healthy persons without hyperlipidemia but with elevated hs-CRP levels [78]. These studies provide evidence for the non-lipid effects of statins and suggest that testing for CRP may identify many subjects, who are not eligible for statin therapy based on LDL levels, but are at high risk for future cardiovascular events and might benefit from statin therapy. Therefore, CRP levels may provide a method for targeting

Table 1 Factors affecting serum CRP levels.

Increased levels	Decreased levels
Increased blood pressure	Moderate alcohol consumption
Increased body mass index	Increased activity/endurance exercise
Metabolic syndrome/diabetes mellitus	Weight loss
Low HDL/high triglycerides	Medications; statins—fibrates—niacin
Estrogen/progestogen use	? ACE inhibitors-? Aspirin
Chronic infections (gingivitis, bronchitis)	
Chronic inflammation (rheumatoid arthritis)	

Modified from Pearson et al. [47].

statin therapy in primary and secondary prevention.

In the PHS, use of aspirin was associated with a statistically significant (55.7%) risk reduction for future MI among men who had hs-CRP levels in the highest quartile and with a nonsignificant (13.9%) reduction among those who had hs-CRP levels in the lowest quartile [66]. However, only few data are available regarding the direct effect of aspirin on CRP levels. A small randomized trial of low-dose aspirin in healthy volunteers, found that aspirin had no detectable effect on CRP [79].

Finally, the CDC/AHA statement contains recommendations for the use of CRP in the diagnosis and management of cardiovascular disease [47]. The authors recommend two separate CRP measurements, preferably 2 weeks apart. Yet, the CDC/AHA statement suggests use of hs-CRP for risk assessment in patients who are at intermediate risk for cardiovascular events (10—20% 10-year risk of coronary event) and classifies hs-CRP levels of <1.0 mg/L as low risk, 1—3 mg/L as average risk, and >3.0 mg/L as high risk. A CRP level greater than 10 mg/L indicates the presence of a significant acute phase response; usually indicates a noncardiovascular source of inflammation, and further assessment is required to determine the cause [47]. (Table 1 indicates certain patient characteristics and conditions that have been associated with increased or decreased CRP levels.) Therefore, the authors indicate the measurement of CRP for identifying patients without known CAD who may be at a higher risk than estimated by traditional risk factors. However, they suggest that further data from prospective clinical trials are needed to determine if patients with only elevated CRP levels should be treated [47].

Cytokines

Cytokines include a number of pleiotropic proteins that have been extensively implicated in the process of inflammation. Among the main cytokines are IL-1, IL-6, IL-10, tumor necrosis factor alpha (TNF-α), and MCP-1 [9,14,15]. IL-1 and IL-6 drive production of reactant proteins, including CRP. IL-6 may increase plaque instability driving expression of matrix metalloproteinases, TNF-α, and MCP-1 [80].

Elevated IL-6 levels in healthy men correlated with increased risk for future MI independently of hs-CRP [81]. Recently, a case-control study of 294 patients with clinically stable ACS (group I) and clinically stable angina pectoris (group II), showed that median IL-6 levels were significantly higher in group I than in group II ($P < 0.05$) [82]. In the Fragmin and/or early Revascularization during InStability in Coronary artery disease (FRISC-II) study, elevated IL-6 (>5 ng/L) was associated with higher 6- and 12-month mortality, independent of troponin and hs-CRP [83]. In this study patients with high IL-6 had a greater response to an invasive versus conservative strategy than patients with low IL-6 levels. These data suggest that elevated IL-6 levels may identify patients with more severe events, who would benefit from more aggressive treatment. However, the application of IL-6 as a biomarker is limited by large circadian variations and lack of confirmatory studies.

It has been shown that plasma concentrations of IL-1β are elevated in patients with hypercholesterolemia [84]. It seems that statin therapy may lower IL-1β levels in these patients [85]. However, further data from clinical studies are needed for assessment of the relationship between IL-1β and atherosclerotic clinical events.

TNF-α has been implicated in myocardial dysfunction and remodelling after acute coronary events [86]. In the CARE study, recurrent coronary events after a MI were associated with higher TNF-α levels compared with controls [87].

MCP-1, as already mentioned, is a chemokine that helps recruit monocytes into the arterial intima and activates these cells to promote atherosclerosis [9,88]. As assessed by coronary angiogram, measurements of MCP-1 in the coronary blood of patients with unstable angina demonstrated an association between MCP-1 levels and the extent of coronary atherosclerosis [89]. In the Orbofiban in Patients with Unstable coronary Syndromes (OPUS)-TIMI 16 trial, MCP-1 levels above the 75th percentile (238 pg/mL) were associated with

an increased risk of death or MI after 10 months, even after adjustment for traditional risk factors [90]. However, although MCP-1 is a promising biomarker, further research is needed to evaluate its clinical utility.

IL-10 mediates anti-atherogenic pathways. In the c7E3 AntiPlatelet Therapy in Unstable REfractory angina (CAPTURE) study, patients with elevated IL-10 levels had a decreased risk of death or nonfatal MI. Yet, those with elevated CRP and IL-10 were at lower risk than those with elevated CRP only [91]. These data suggest that IL-10 may be protective against proinflammatory mediators in ACS.

To make things more confused, in a recent study, 158 consecutive patients with angiographically identified stable CAD were enrolled. Plasma levels of the following 10 cytokines were measured: IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, TNF-α, granulocyte-macrophage colony-stimulating factor (GM-CSF), and IFN-γ. The multivariate analysis using multi-vessel disease, diabetes, and the levels of all 10 cytokines and hs-CRP showed that the only independent predictor of cardiovascular events was IL-8 (RR, 2.98; 95% CI, 1.64—7.24; $P = 0.0001$), during a 7-year follow-up period [92]. These data, in fact, suggest that the role of cytokines in the prediction of cardiovascular risk is still controversial, or, at least, we do not know yet, which one is better as a prognostic biomarker and probably a therapeutic target in CAD.

Soluble CD40 ligand

Soluble CD40 ligand (sCD40L) is a proinflammatory marker that has been shown to promote atherosclerosis and plaque instability [93]. Healthy women with high levels of sCD40L have been shown to be at increased risk for cardiovascular events [94]. In the CAPTURE trial, patients with elevated sCD40L (>5 μg/L) were at increased 6-month risk of death or nonfatal MI. Treatment of these patients with abciximab before coronary angioplasty reduced the risk. This benefit was observed in patients with and without troponin elevation, and no benefit of abciximab was observed in patients with elevated troponin values but low sCD40L values [95]. The investigators of the CAPTURE trial concluded that sCD40L may be an independent risk marker of cardiovascular events and a marker for determining benefit from therapy with glycoprotein IIb/IIIa inhibitors. Similarly, a substudy of the OPUS-TIMI 16 trial showed that patients with sCD40L levels above the median value were at an increased risk for death or recurrent MI and that sCD40L provided prognostic ability independent of troponin I and CRP [96].

Finally, a recent study of 96 patients with ST-segment elevation myocardial infarction showed that serum ratio of sCD40L/IL-10 was a better independent predictor of in-hospital adverse events than individual sCD40L and IL-10 measurements in these patients [97].

Serum amyloid A

Serum amyloid A (SAA), like CRP, is an acute phase protein. In a substudy of TIMI 11A, elevated SAA levels predicted increased risk of 14-day mortality in patients with ACS [98]. In the Women's Ischemia Syndrome Evaluation (WISE) study of women referred for coronary angiography because of suspected ischemia, elevated SAA values were correlated with angiographic severity of CAD and 3-year risk for cardiovascular events [99].

However, the results were not similar in the Thrombogenic Factors and Recurrent Coronary Events study; the measurement of SAA levels, 2 months after MI did not show significant association between SAA levels and risk of recurrent cardiovascular events over 2 years [64].

Adhesion molecules

Cell adhesion molecules (CAMs) and their counter receptors of the β2-integrin family, mainly Mac-1, play a pivotal role in the interactions between leucocytes, platelets, and vascular endothelium [8]. Adherence of circulating leukocytes to the endothelium and their transmigration into the arterial wall is an early step of atherosclerosis [8]. Soluble CAMs (sCAMs: sICAM-1, sVCAM-1 and E- and P-selectins) are shed from cell surfaces and reflect cellular activation [100]. Soluble CAMs have been studied in the early diagnosis of ACS and in risk stratification in CAD.

Hollander et al. considered that markers of platelet aggregation may detect ACS earlier than cardiac markers as plaque rupture/platelet aggregation precedes myocardial ischemia. However, although theoretically attractive, creatinine kinase MB fraction had a higher specificity for detection of acute MI, ACS, and serious cardiac events, upon emergency department arrival, than both soluble and membrane-bound P-selectin [101].

Mulvihill et al. measured CAMs in patients with ACS (unstable angina and non-Q MI) at presentation and then after 3, 6, and 12 months. CAMs levels increased within 10 h of chest pain onset and remained elevated for up to 6 months, suggesting that the inflammatory stimulus triggering expression of CAMs is long sustained, returning gradually toward control values [102].

In the WHS, elevated P-selectin levels in healthy women were associated with increased cardiovascular risk [103]. However, the role of CAMs in risk stratification in patients with CAD is not established as the results on predicting cardiovascular risk are rather confusing.

In a prospective study, among patients with stable angiographically documented CAD, CAMs (sICAM-1, sVCAM-1, and E-selectin) were measured, and follow-up information on cardiovascular events was obtained for a mean of 2.7 years. Although all CAMs were higher in patients with future death, in a model that simultaneously controlled for all inflammatory and soluble adhesion markers determined, only sVCAM-1 remained independently significant for future fatal cardiovascular events, with a 2.8-fold increase in risk. Especially sVCAM-1 added to the predictive value of classic risk factors and hs-CRP in determining the risk of death from cardiovascular causes [104].

In a prospective observational study, among patients presenting acutely with ACS (unstable angina and non-Q MI) raised concentrations of sVCAM-1 and CRP — but not sICAM-1, E-selectin, and P-selectin — were predictive of an increased major cardiovascular event within 6 months after presentation. The sensitivity of CRP >3 mg/L and sVCAM-1 >780 ng/mL for predicting future events was >90% [105]. Similarly, patients with ACS presented with increased levels of sVCAM-1 compared with patients with stable angina or healthy individuals; moreover, those with in-hospital adverse coronary events had elevated sVCAM-1 levels independently of CRP levels [106]. In patients with unstable angina undergoing coronary stenting, CRP and sICAM-1 — but not sVCAM-1 — proved useful for identifying those at higher risk of a cardiac event; yet, it was suggested that CRP may play a direct role in promoting the inflammatory component of atherosclerosis by inducing significant expression of sICAM-1 [107]. However, a prospective study of patients presenting to the emergency department with chest pain, failed to reveal any association between sICAM-1 and the risk of a serious cardiovascular event during hospital admission [108]. To make things more confused, Hillis et al. studied 126 consecutive patients presenting with clinical myocardial ischemia. In this study, only P-selectin and cardiac troponin I — but not sICAM-1, sVCAM-1, or E-selectin — were significantly higher among patients who had a serious cardiac event during the index admission or the subsequent 3 months. Moreover, both remained independently predictive in a multivariable regression equation [109].

Mac-1 expression on the surface of polymorphonuclear leucocytes is up-regulated after balloon angioplasty and coronary stenting. The maximum up-regulation of Mac-1, which is observed 48 h after PCI activation, is an early and robust predictor of late lumen loss [3,27]. Therefore, assessing the expression levels of integrin Mac-1 after coronary stenting may prove helpful in predicting risk of restenosis.

In conclusion, the utility of CAMs in predicting the outcome of individual patients seems to be limited. More prospective studies are needed for the study of secondary prevention. The counter receptor of CAMs, integrin Mac-1, may prove useful to predict restenosis after PCI.

Myeloperoxidase

Myeloperoxidase (MPO) is a heme protein produced by activated neutrophils, monocytes, and tissue macrophages, and catalyzes the modification of LDL which is a critical step in atherogenesis [110].

Several studies have suggested that there is an association between MPO levels and CAD [110]. In a prospective study of patients presenting to the emergency department with chest pain, elevated MPO levels identified patients with undetectable troponin T (TnT) levels who were at increased risk of MI during their hospital stay or after discharge [111]. According to this information, MPO may be useful for early risk stratification of patients with chest pain and non-ST elevation MI. In another well designed prospective study among patients with ACS, those with elevated MPO levels had a statistically significant increase in death or nonfatal cardiovascular events at 72 h, 30 days, and 6 months [112]; moreover, MPO levels were independent of TnT, CRP, and sCD40L levels, suggesting that MPO is an independent predictor of risk in CAD.

Finally, in the prospective European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk population study, serum MPO levels were associated with the future risk of coronary artery disease, in apparently healthy individuals [113].

Matrix metalloproteinases

Matrix metalloproteinases are endoproteases that are regulators of the extracellular matrix. They are localized at the shoulder of the plaque, have collagenase and/or gelatinase activity, and participate in vascular remodelling and plaque instability [114,115].

There are not many data for the association between MMPs and the prognosis of cardiovascular disease. Peripheral blood levels of MMP-1, MMP-2, and MMP-9 are elevated in patients with ACS [116]. Yet, in patients with either stable or unstable CAD,

those with elevated MMP-9 levels were at increased risk for future cardiovascular death [117]. A recent study enrolling 909 patients with acute MI, 466 patients with stable angina, and 1023 healthy older control subjects, showed that circulating levels of MMP-2 and MMP-9 were independently associated with the development of an acute MI rather than stable angina, as the initial clinical presentation of coronary artery disease [118]. In another study, elevations in MMP-1 at 7 and 14 days after ACS were negatively correlated with left ventricular ejection fraction [119]. However, the application of MMPs as biomarkers is limited because of the slow elevation of MMP levels after ACS and, in fact, lack of significant clinical studies, as yet.

Placental growth factor

Placental growth factor (PlGF) is a member of a family of platelet-derived proteins that function as chemoattractants for monocytes and is involved in the regulation of vascular endothelial growth [120]. PlGF appears to be stable in the circulation and may prove a strong candidate as a biomarker for plaque instability, myocardial ischemia, and prognosis for patients with ACS.

In the CAPTURE study, patients presenting to the emergency department with ACS had elevated PlGF levels compared with patients with noncardiac chest pain or stable angina. Between patients with unstable angina and NSTEMI there was no difference in PlGF concentrations. PlGF levels >27.0 ng/L were associated with increased risk of death or nonfatal MI at 72 h. Moreover, PlGF provided prognostic value independently of the levels of CRP, sCD40L, and cardiac TnT and identified a group of patients without elevated sCD40L or cardiac TnT that were at increased 30-day risk for a cardiovascular event [121].

A2 phospholipases

Lipoprotein-associated phospholipase A(2) [Lp-PLA(2)], a member of the phospholipase superfamily, seems to be a highly active enzyme in the circulation; it is also known as platelet-activating factor acetylhydrolase. Lp-PLA(2) participates in the oxidative modification of LDL by cleaving oxidized phosphatidylcholines, generating lysophosphatidylcholine and oxidized free fatty acids [122]. Recent reports indicate that Lp-PLA(2) has a distinct role in atherogenesis. Seminal findings support further the potentially damaging role that in situ release of LDL-associated oxidative products by Lp-PLA(2) may have in the formation of arterial wall lesions [123]. Local production of Lp-PLA(2) and lysophosphatidylcholine, the active product of Lp-PLA(2), in the coronary circulation were associated with early coronary atherosclerosis and endothelial dysfunction in humans [124].

Investigators measured Lp-PLA(2) in 3766 patients with stable coronary artery disease, that were followed for a median of 4.8 years for adverse cardiovascular events. In these patients, an elevated level of Lp-PLA(2) was a significant predictor of nonfatal adverse cardiovascular outcomes independent of traditional clinical risk factors and hs-CRP [125]. Similarly, in another study of 2513 patients with and 719 patients without angiographically confirmed CAD, Lp-PLA(2) predicted risk for 5-year cardiac mortality independently from established risk factors and hs-CRP concentrations [126].

A nested case-control analysis of the WHS, the ability of Lp-PLA(2) to predict the risk of cardiovascular events was not statistically significant after adjustment for traditional risk factors [127]. However, in a higher risk population of men with hyperlipidemia, in the West of Scotland Coronary Prevention Study (WOSCOPS), increased Lp-PLA(2) levels predicted risk for cardiovascular events independently of other inflammatory markers, such as CRP. A 60% statistically significant increase in risk between the highest and the lowest quintile of Lp-PLA(2) was found [128]. Yet, in the Atherosclerosis Risk in Communities (ARIC) study, Lp-PLA(2) was independently associated with CAD, in patients who had LDL levels <130 mg/dL [129]. This finding suggests that Lp-PLA(2) may have a prognostic role, similar to CRP, in identifying high-risk patients who may benefit from statin therapy and are not targeted on the basis of LDL levels. Finally, a recent study found that Lp-PLA(2) levels were associated with the extent of angiographic CAD, but they were not independently predictive after adjustment for CRP, lipid status, and other traditional risk factors [130]. In the more general setting of population studies, however, it is clear that Lp-PLA(2) is a positive risk factor for CAD and measurements of its mass may contribute to the prediction of coronary heart disease risk, especially in individuals with low LDL cholesterol levels.

Besides Lp-PLA(2), secretory type-II phospholipase A(2) [sPLA(2)-II], is also a member of the phospholipase superfamily, appearing to be an important inflammatory mediator and biomarker of cardiovascular disease [131]. An analysis of patients from the Global Registry of Acute Coronary Events (GRACE) study showed that elevated sPLA(2)-II levels were associated with increased risk of death or MI, independent of other risk factors [132].

Local activity of sPLA(2)-II in the atherosclerotic plaque seems to facilitate an inflammatory

response to induce plaque instability or rupture. In a recent study, Nijmeijer et al. studied histologically the presence of sPLA(2)-II in culprit lesions in the coronary arteries of patients with AMI or stable or unstable angina, using directed coronary atherectomy. Extracellular sPLA(2)-II was more abundantly present in atherosclerotic culprit lesions that had led to AMI, than in patients with stable or unstable angina [133]. This suggests a role for extracellular sPLA(2)-II in the development of complications of atherosclerotic lesions in coronary arteries.

In humans, the weight of evidence suggests that Lp-PLA(2) and sPLA(2)-II are positive risk factors for coronary heart disease — an observation commensurate with their position in the direct pathological sequence leading from formation of oxidized LDL in the artery wall to cellular dysfunction and formation of lesions.

Myeloid-related protein 8/14 complex

Myeloid-related protein 8/14 complex (MRP8/14), also termed calprotectin, is a heterodimer of two calcium binding proteins (S100A8 and S100A9, also referred to as MRP8 and MRP14, or calgranulin A and B) involved in calcium-dependent signaling, cell differentiation, cell cycle progression, and cytoskeleton—membrane interactions [134]. MRP8 and MRP14 are mainly expressed in monocytes and neutrophils [135]. Upon phagocyte activation, MRP8 and MRP14 form the MRP8/14 complex, which translocates to the cytoskeleton and plasma membrane, where it is secreted [136]. Therefore, complex MRP8/14 constitutes a marker of phagocyte activation, which is involved in plaque destabilization [137]. Elevated serum levels of MRP8/14 are a useful biomarker of disease activity in inflammatory disorders, such as rheumatoid arthritis and Crohn's disease [138].

Healy et al. found increased expression of platelet CD69 and MRP14 in STEMI patients at the mRNA level in patients presenting to the cardiac catheterization laboratory with STEMI than patients with stable CAD [139]. This led the investigators to quantify plasma protein levels of the most abundant form of MRP14, the MRP8/14. Plasma levels of MRP8/14 heterodimer were higher in STEMI patients (17.0 g/mL versus 8.0 g/mL, $P < 0.001$) [139]. Yet, healthy women in the validation study [140], who subsequently developed cardiovascular events during follow-up, had higher median MRP8/14 levels at baseline than women who remained free of disease (controls, $P < 0.001$). In addition, in matched-pair analysis that accounted for age and smoking status, the risk of a first cardiovascular event increased significantly with each increasing quartile of baseline concentration of MRP8/14 (P trend <0.001), such that the women in the highest versus lowest quartile had 3.8-fold elevation in risk ($P < 0.001$). Risks were independent of traditional cardiovascular risk factors and CRP.

To illustrate the potential ability of MRP8/14 to add prognostic value to lipid- or CRP-based screening, Healy et al. computed the relative risk of cardiovascular events after study participants were stratified into nine groups according to tertiles of MRP8/14 and tertiles of total cholesterol (TC):HDL or CRP. Women with low TC:HDL or CRP and low levels of MRP8/14 had the lowest RR. In contrast, women with high TC:HDL or CRP and high levels of MRP8/14 had the highest RR [139]. Importantly, even among women with low or intermediate TC:HDL or CRP levels, the risk of cardiovascular events was greater among those with high than with lower levels of MRP8/14 [139].

Altwegg et al. suggested that MRP8/14 that is expressed by monocytes and neutrophils, which are activated in plaque destabilization, might be elevated in ACS. Therefore, they compared circulating levels of MRP8/14 in patients with ACS, stable CAD, or normal coronary arteries. Systemic levels of MRP8/14 were markedly elevated [15.1 (12.1—21.8) mg/L, $P = 0.001$] in ACS when compared with stable CAD [4.6 (3.5—7.1) mg/L] or normals [4.8 (4.0—6.3) mg/L]. In addition, MRP8/14 was increased prior to necrosis markers such as myoglobin, CK-MB, and troponin, while using a cut-off level of 8 mg/L, MRP8/14 identified ACS presenting within 3 h from symptom onset [141].

In conclusion, the data suggest that MRP8/14 heterodimer can independently predict risk of future cardiovascular events and may add prognostic information to that conveyed by standard risk factors and CRP. Yet, the occurrence of elevated MRP8/14 in the systemic circulation prior to markers of myocardial necrosis is advantageous and makes it a prime candidate for the detection and management of ACS. Obviously, further studies are required to elucidate the full prognostic potential of MRP8/14 in healthy patients, as the only trial in primary prevention was conducted in apparently healthy postmenopausal women followed up in the WHS [140], as well as the diagnostic and prognostic implications in patients with chest pain.

Conclusions

Advances in understanding the pathobiology of atherosclerosis have implicated inflammation as a central contributor to the initiation and progression

of atherosclerotic vascular disease. Inflammatory biomarkers may have prognostic value for future cardiovascular risk among those at high risk or with documented cardiovascular disease. They also may be useful for identifying apparently healthy individuals, without known CAD, who may be at a higher risk than estimated by traditional risk factors. Finally, they might help to identify the subjects who are not eligible for preventive therapies based on traditional risk factors, but are at high risk for future cardiovascular events and might benefit most from these interventions.

However, until now, the data are conflicting about which biomarker is more suitable for diagnosis or prognosis of CAD. Probably, a combination of biomarkers may prove appropriate for our target.

Key points

- The role of inflammation in atherosclerotic plaque is crucial.
- High sensitivity CRP is the most extensively studied biomarker of evolving coronary atherosclerosis. Its measurement has been proposed as an adjunct to established risk factors to assess the risk for CAD.
- A cut-off level of hs-CRP of 2 (1—3) mg/L seems to discriminate high- from low-risk patients with stable or unstable coronary disease (ACS) or even apparently healthy individuals for short- and long-term prognosis of cardiovascular events. However, the measurement of hs-CRP should be delayed in the acute stage of MI until levels have returned to baseline.
- Intensive statin therapy lowers lipid and CRP levels. The significant lessening of hs-CRP value after an ACS reduces the risk for recurrent MI or fatal coronary event, irrespective of the degree of LDL lowering.
- Statins (lovastatin or rosuvastatin) reduce the incidence of major cardiovascular events even in apparently healthy persons without hyperlipidemia but with elevated hs-CRP levels. Once more, a target value of hs-CRP of less than 2 mg/L is suggested with preventive therapies.
- Cytokines (IL-1, IL-1β, IL-6, IL-8, TNF-α, and MCP-1), lipoprotein-associated phospholipase A(2) [Lp-Pla(2)], GM-CSF, sCD40L, serum amyloid A, soluble CAMs (sICAM-1, sVCAM-1, E- and P-selectins), and their counter receptors (primarily Mac-1), myeloperoxidase, matrix metalloproteinases (mainly MMP-9) and placental growth factor (PlGF) have all been implicated in the progression of plaque instability and have been considered to add prognostic information to that conveyed by standard risk factors and CRP in primary and secondary prevention of future cardiovascular events or even restenosis (Mac-1) after PCI.
- Complex MRP8/14 heterodimer, constituting a marker of phagocyte activation, seems to add prognostic value to lipid-based screening of future CAD events. Yet, using a cut-off level of 8 mg/L, MRP8/14 identifies ACS presenting within 3 h from symptom onset, prior to markers of myocardial necrosis.
- The predictive value of biomarkers is additive and beyond that of CRP suggesting the need for a ''multimarker approach'' in assessing cardiovascular risk.

References

[1] Libby P. Changing concepts of atherogenesis. J Intern Med 2000;247:349—58.
[2] Liao JK. Beyond lipid lowering: the role of statins in vascular protection. Int J Cardiol 2002;86:5—18.
[3] Inoue T, Uchida T, Yaguchi I, Sakai Y, Takayanagi K, Morooka S. Stent-induced expression and activation of the leukocyte Integrin Mac-1 is associated with neointimal thickening and restenosis. Circulation 2003;107:1757—63.
[4] Glagov S, Zarins C, Giddens DP, Ku DN. Hemodynamics and atherosclerosis: insights and perspectives gained from studies of human arteries. Arch Pathol Lab Med 1988;112:1018—31.
[5] Gong KW, Zhu GY, Wang LH, Tang CS. Effect of active oxygen species on intimal proliferation in rat aorta after arterial injury. J Vasc Res 1996;33:42—6.
[6] Steinberg D. Antioxidants and atherosclerosis: a current assessment. Circulation 1991;84:1420—5.
[7] Blake GJ, Ridker PM. Inflammatory bio-markers and cardiovascular risk prediction. J Intern Med 2002;252:283—94.
[8] Nakashima Y, Raines E, Plump A, Breslow JL, Ross R. Upregulation of VCAM-1 and ICAM-1 at atherosclerosis-prone sites on the endothelium in the ApoE-deficient mouse. Arterioscler Thromb Vasc Biol 1998;18:842—51.
[9] Gu L, Okada Y, Clinton SK, Gerard C, Sukhova GK, Libby P, Rollins BJ. Absence of monocyte chemoattractant protein-1 reduces atherosclerosis in low density lipoprotein receptor-deficient mice. Mol Cell 1998;2:275—81.
[10] Qiao JH, Tripathi J, Mishra NK, Cai Y, Tripathi S, Wang XP, Imes S, Fishbein MC, Clinton SK, Libby P, Lusis AJ, Rajavashisth TB. Role of macrophage colony-stimulating factor in atherosclerosis: studies of osteopetrotic mice. Am J Pathol 1997;150:1687—99.
[11] Stary HC. Evolution and progression of atherosclerotic lesions in coronary arteries of children and young adults. Arteriosclerosis 1989;9:119—32.
[12] Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol: modifications of low-density lipoprotein that increase its atherogenicity. N Engl J Med 1989;320:915—24.
[13] Ylä-Herttuala S, Palinski W, Rosenfeld ME, Parthasarathy S, Carew TE, Butler S, Witztum JL, Steinberg D. Evidence for the presence of oxidatively modified low density lipopro-

tein in atherosclerotic lesions of rabbit and man. J Clin Invest 1989;84:1086—95.
[14] Mantovani A, Bussolino F, Dejana E. Cytokine regulation of endothelial cell function. Fed Am Soc Exp Biol J 1992;6:2591—9.
[15] Rus HG, Vlaicu R, Niculescu F. Interleukin-6 and interleukin-8 protein and gene expression in human arterial atherosclerotic wall. Atherosclerosis 1996;127: 263—71.
[16] Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. Circulation 2002;105:1135—43.
[17] Libby P. Molecular bases of acute coronary syndromes. Circulation 1995;91:2844—50.
[18] Moreno PR, Bernardi VH, López-Cuéllar J, Murcia AM, Palacios IF, Gold HK, Mehran R, Sharma SK, Nemerson Y, Fuster V, Fallon JT. Macrophages, smooth muscle cells, and tissue factor in unstable angina: implications for cell-mediated thrombogenicity in acute coronary syndromes. Circulation 1996;94:3090—7.
[19] Simon D, Chen Z, Xu H, Li C, Dong J, McIntire L, Ballantyne C, Zhang L, Furman M, Berndt M, López J. Platelet glycoprotein Iba is a counter receptor for the leukocyte integrin Mac-1 (CD11b/CD18). J Exp Med 2000;192: 193—204.
[20] Gahmberg CG. Leukocyte adhesion CD11/CD18 integrins and intercellular adhesion molecules. Curr Opin Cell Biol 1997;9:643—50.
[21] Chavakis T, Preissner KT, Santoso S. Leukocyte transendothelial migration: JAMs add new pieces to the puzzle. Thromb Haemost 2003;89:13—7.
[22] Sotiriou S, Orlova V, Al-Fakhri N, Ihanus E, Economopoulou M, Isermann B, Bdeir K, Nawroth P, Preissner K, Gahmberg C, Koschinsky M, Chavakis T. Lipoprotein(a) in atherosclerotic plaques recruits inflammatory cells through interaction with Mac-1 integrin. Fed Am Soc Exp Biol 2006;20:5—14.
[23] De Servi S, Mazzone A, Ricevuti G, Fioravanti A, Bramucci E, Angoli L, Stefano G, Specchia G. Granulocyte activation after coronary angioplasty in humans. Circulation 1990;82:140—6.
[24] Hagberg IA, Roald HE, Lyberg T. Adhesion of leukocytes to growing arterial thrombi. Thromb Haemost 1998;80:852—8.
[25] Diacovo TG, Roth SJ, Buccola JM, Bainton DF, Springer TA. Neutrophil rolling, arrest, and transmigration across activated, surface-adherent platelets via sequential action of P-selectin and the β2 integrin CD11b/CD18. Blood 1996;88:146—57.
[26] Evangelista V, Manarini S, Sideri R, Rotondo S, Martelli N, Piccoli A, Totani L, Piccardoni P, Vestweber D, de Gaetano G, Cerletti C. Platelet/polymorphonuclear leukocyte interaction: P-selectin triggers protein-tyrosine phosphorylation-dependent CD11b/CD18 adhesion: role of PSGL-1 as a signalling molecule. Blood 1999;93:876—85.
[27] Inoue T, Sakai Y, Hoshi K, Yaguchi I, Fujito T, Morooka S. Lower expression of neutrophil adhesion molecule indicates less vessel wall injury and might explain lower restenosis rate after Cutting Balloon angioplasty. Circulation 1998;97:2511—8.
[28] Inoue T, Kato T, Hikichi Y, Hashimoto S, Hirase T, Morooka T, Imoto Y, Takeda Y, Sendo F, Node K. Stent-induced neutrophil activation is associated with an oxidative burst in the inflammatory process, leading to neointimal thickening. Thromb Haemost 2006;95:43—8.
[29] Muhlestein JB, Anderson JL. Chronic infection and coronary artery disease. Cardiol Clin 2003;21:333—62.
[30] Wald NJ, Law MR, Morris JK, Zhou X, Wong Y, Ward ME. *Chlamydia pneumoniae* infection and mortality from ischaemic heart disease: large prospective study. Br Med J 2000;321:204—7.
[31] Goyal P, Kalek SC, Chaudhry R, Chauhan S, Shah N. Association of common chronic infections with coronary artery disease in patients without any conventional risk factors. Indian J Med Res 2007;125:129—36.
[32] Liu R, Moroi M, Yamamoto M, Kubota T, Ono T, Funatsu A, Komatsu H, Tsuji T, Hara H, Hara H, Nakamura M, Hirai H, Yamaguchi T. Presence and severity of *Chlamydia pneumoniae* and cytomegalovirus infection in coronary plaques are associated with acute coronary syndromes. Int Heart J 2006;47:511—9.
[33] Wang SS, Tondella ML, Bajpai A, Mathew AG, Mehranpour P, Li W, Kacharava AG, Fields BS, Austin H, Zafari AM. Circulating *Chlamydia pneumoniae* DNA and advanced coronary artery disease. Int J Cardiol 2007;118:215—9.
[34] Eryol NK, Kiliç H, Gül A, Ozdogru I, Inanç T, Dogan A, Topsakal R, Basar E. Are the high levels of cytomegalovirus antibodies a determinant in the development of coronary artery disease? Int Heart J 2005;46:205—9.
[35] Horne BD, Muhlestein JB, Carlquist JF, Madsen TE, Bair TL, Pearson RR, Anderson JL. Cytomegalovirus: strength of antibody response and its relationship to risk of mortality among patients with angiographic coronary disease. J Am Coll Cardiol 2003;41(6, Suppl. A):367A.
[36] Rupprecht HJ, Blankenberg S, Bickel C, Rippin G, Hafner G, Prellwitz W, Schlumberger W, Meyer J. Impact of viral and bacterial infectious burden on long-term prognosis in patients with coronary artery disease. Circulation 2001;104:25—31.
[37] Madjid M, Aboshady I, Awan I, Litovsky S, Casscells SW. Influenza and cardiovascular disease: is there a causal relationship? Tex Heart Inst J 2004;31:4—13.
[38] Naghavi M, Wyde P, Litovsky S, Madjid M, Akhtar A, Naguib S, Siadaty MS, Sanati S, Casscells W. Influenza infection exerts prominent inflammatory and thrombotic effects on the atherosclerotic plaques of apolipoprotein E-deficient mice. Circulation 2003;107:762—8.
[39] Rech RL, Nurkin N, da Cruz I, Sostizzo F, Baião C, Perrone JA, Wainstein R, Pretto D, Manenti ER, Bodanese LC. Association between periodontal disease and acute coronary syndrome. Arq Bras Cardiol 2007;88:185—90.
[40] Barilli AL, Passos AD, Marin-Neto JA, Franco LJ. Periodontal disease in patients with ischemic coronary atherosclerosis at a University Hospital. Arq Bras Cardiol 2006;87:695—700.
[41] Jha HC, Srivastava P, Sarkar R, Prasad J, Mittal A. *Chlamydia pneumoniae* IgA and elevated level of IL-6 may synergize to accelerate coronary artery disease. J Cardiol 2008;52:140—5.
[42] Deodhar SD. C-reactive protein: the best laboratory indicator available for monitoring disease activity. Cleve Clin J Med 1989;56:126—30.
[43] Macy E, Hayes T, Tracy R. Variability in the measurement of C-reactive protein in healthy subjects: implications for reference interval and epidemiologic applications. Clin Chem 1997;43:52—8.
[44] Ishikawa T, Hatakeyama K, Imamura T, Date H, Shibata Y, Hikichi Y, Asada Y. Involvement of C-reactive protein obtained by directional coronary atherectomy in plaque instability and developing restenosis in patients with stable or unstable angina pectoris. Am J Cardiol 2003;91:287—92.
[45] Inoue T, Kato T, Uchida T, Sakuma M, Nakajima A, Shibazaki M, Imoto Y, Saito M, Hashimoto S, Hikichi Y, Node K. Local

release of C-reactive protein from vulnerable plaque or coronary arterial wall injured by stenting. J Am Coll Cardiol 2005;46:239—45.
[46] Wilkins J, Gallimore R, Moore E. Rapid automated high sensitivity enzyme immunoassay of C-reactive protein. Clin Chem 1998;44:1358—61.
[47] Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon 3rd RO, Criqui M, Fadl YY, Fortmann SP, Hong Y, Myers GL, Rifai N, Smith Jr SC, Taubert K, Tracy RP, Vinicor F, Centers for Disease Control and Prevention; American Heart Association. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement of healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation 2003;107:499—511.
[48] Cook DG, Mendall MA, Whincup PH, Carey IM, Ballam L, Morris JE, Miller GJ, Strachan DP. C-reactive protein concentration in children: relationship to adiposity and other cardiovascular risk factors. Atherosclerosis 2000;149:139—50.
[49] Mendall MA, Patel P, Ballam L, Strachan D, Northfield TC. C reactive protein and its relation to cardiovascular risk factors: a population based cross sectional study. Br Med J 1996;312:1061—5.
[50] Margaglione M, Cappucci G, Colaizzo D, Vecchione G, Grandone E, Di Minno G. C-reactive protein in offspring is associated with the occurrence of myocardial infarction in first-degree relatives. Arterioscler Thromb Vasc Biol 2000;20:198—203.
[51] El-Gendi SS, Bakeet MY, El-Hamed EA, Ibrahim FK, Ahmed R. The value of lipoprotein (a), homocysteine, and Doppler of carotid and femoral arteries in assessment of atherosclerosis in asymptomatic cardiovascular risk patients. J Cardiol 2008;52:202—11.
[52] Ikonomidis I, Andreotti F, Economou E, Stefanadis C, Toutouzas P, Nihoyannopoulos P. Increased proinflammatory cytokines in patients with chronic stable angina and their reduction by aspirin. Circulation 1999;100:793—8.
[53] Morrow DA, Rifai N, Antman EM, Weiner DL, McCabe CH, Cannon CP, Braunwald E. C-reactive protein is a potent predictor of mortality independently of and in combination with troponin T in acute coronary syndromes: a TIMI 11A substudy. J Am Coll Cardiol 1998;31:1460—5.
[54] Lindahl B, Toss H, Siegbahn A, Venge P, Wallentin L. Markers of myocardial damage and inflammation in relation to long-term mortality in unstable coronary artery disease. N Engl J Med 2000;343:1139—47.
[55] James SK, Armstong P, Barnathan E, Califf R, Lindahl B, Siegbahn A, Simoons ML, Topol EJ, Venge P, Wallentin L. Troponin and C-reactive protein have different relations to subsequent mortality and myocardial infarction after acute coronary syndrome: a GUSTO-IV substudy. J Am Coll Cardiol 2003;41:916—24.
[56] Haverkate F, Thompson SG, Pyke SD, Gallimore JR, Pepys MB. Production of C-reactive protein and risk of coronary events in stable and unstable angina. European Concerted Action on Thrombosis and Disabilities Angina Pectoris Study Group. Lancet 1997;349:462—6.
[57] Mueller C, Buettner HJ, Hodgson JM, Marsch S, Perruchoud AP, Roskamm H, Neumann FJ. Inflammation and long-term mortality after non-ST elevation acute coronary syndrome treated with a very early invasive strategy in 1042 consecutive patients. Circulation 2002;105:1412—5.
[58] Zairis M, Manousakis S, Stefanidis A, Papadaki OA, Andrikopoulos GK, Olympios CD, Hadjissavas JJ, Argyrakis SK, Foussas SG. C-reactive protein levels on admission are associated with response to thrombolysis and prognosis after ST-segment elevation acute myocardial infarction. Am Heart J 2002;144:782—9.
[59] de Beer FC, Hind CR, Fox KM, Allan RM, Maseri A, Pepys MB. Measurement of C-reactive protein concentration in myocardial ischemia and infarction. Br Heart J 1982;47:239—43.
[60] Sano T, Tanaka A, Namba M, Nishibori Y, Nishida Y, Kawarabayashi T, Fukuda D, Shimada K, Yoshikawa J. C-reactive protein and lesion morphology in patients with acute myocardial infarction. Circulation 2003;108:282—5.
[61] Stefanadis C, Diamantopoulos L, Dernellis J, Economou E, Tsiamis E, Toutouzas K, Vlachopoulos C, Toutouzas P. Heat production of atherosclerotic plaques and inflammation assessed by the acute phase proteins in acute coronary syndromes. J Mol Cell Cardiol 2000;32:43—52.
[62] Zebrack JS, Anderson JL, Maycock CA, Horne BD, Bair TL, Muhlestein JB, The Intermountain Heart Collaborative (IHC) Study Group. Usefulness of high-sensitivity C-reactive protein in predicting long-term risk of death or acute myocardial infarction in patients with unstable or stable angina pectoris or acute myocardial infarction. Am J Cardiol 2002;89:145—9.
[63] Ridker P, Rifai N, Pfeffer M, Sacks FM, Moye LA, Goldman S, Flaker GC, Braunwald E. Inflammation, pravastatin, and the risk of coronary events after myocardial infarction in patients with average cholesterol levels. Circulation 1998;98:839—44.
[64] Harb TS, Zareba W, Moss AJ, Ridker PM, Marder VJ, Rifai N, Miller Watelet LF, Arora R, Brown MW, Case RB, Dwyer Jr EM, Gillespie JA, Goldstein RE, Greenberg H, Hochman J, et al. Association of C-reactive protein and serum amyloid A with recurrent coronary events in stable patients after healing of acute myocardial infarction. Am J Cardiol 2002;89:216—21.
[65] Kuller LH, Tracy RP, Shaten J, Meilahn EN. Relation of C-reactive protein and coronary heart disease in the MRFIT nested case-control study. Am J Epidemiol 1996;144:537—47.
[66] Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin and the risk of cardiovascular disease in apparently healthy men. N Engl J Med 1997;336:973—9.
[67] Koenig W, Sund M, Fröhlich M, Fischer HG, Löwel H, Döring A, Hutchinson WL, Pepys MB. C-reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle aged men. Circulation 1999;99:237—42.
[68] Geluk CA, Post WJ, Hillege HL, Tio RA, Tijssen JG, van Dijk RB, Dijk WA, Bakker SJ, de Jong PE, van Gilst WH, Zijlstra F. C-reactive protein and angiographic characteristics of stable and unstable coronary artery disease: data from the prospective PREVEND cohort. Atherosclerosis 2008;196:372—82.
[69] Ridker P, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med 2000;342:836—43.
[70] Ridker P, Rifai N, Rose L, Buring JE, Cook NR. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. N Engl J Med 2002;347:1557—65.
[71] Ridker PM. High sensitivity C-reactive protein: potential adjunct for global risk assessment in the primary prevention of cardiovascular disease. Circulation 2001;103:1813—8.
[72] Guran O, Akalin F, Ayabakan C, Dereli FY, Haklar G. High-sensitivity C-reactive protein in children at risk

for coronary artery disease. Acta Paediatr 2007;96: 1214—9.
[73] Pasceri V, Willerson J, Yeh E. Direct pro-inflammatory effect of C-reactive protein on human endothelial cells. Circulation 2000;102:2165—8.
[74] Deveraj S, Xu D, Jialal I. C-reactive protein increases plasminogen activator inhibitor-1 expression and activity in human aortic endothelial cells: implications for the metabolic syndrome and atherothrombosis. Circulation 2003;107:398—404.
[75] Ridker PM, Cannon CP, Morrow D, Rifai N, Rose LM, McCabe CH, Pfeffer MA, Braunwald E. C-reactive protein levels and outcomes after statin therapy. N Engl J Med 2005;352:20—8.
[76] Albert MA, Danielson E, Rifai N, Ridker PM. Effect of statin therapy on C-reactive protein levels: the pravastatin inflammation/CRP evaluation (PRINCE): a randomized trial and cohort study. J Am Med Assoc 2001;286:64—70.
[77] Ridker PM, Rifai N, Clearfield M, Downs JR, Weis SE, Miles JS, Gotto Jr AM. Measurement of C-reactive protein for the targeting of statin therapy in the primary prevention of acute coronary events. N Engl J Med 2001;344:1959—65.
[78] Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto Jr AM, Kastelein JJ, Koenig W, Libby P, Lorenzatti AJ, MacFadyen JG, Nordestgaard BG, Shepherd J, Willerson JT, Glynn RJ, JUPITER Study Group. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. N Engl J Med 2008;359:2195—207.
[79] Feldman M, Jialal I, Devaraj S, Cryer B. Effects of low-dose aspirin on serum C-reactive protein and thromboxane B2 concentrations: a placebo-controlled study using a highly sensitive C-reactive protein assay. J Am Coll Cardiol 2001;37:2036—41.
[80] Schieffer B, Schieffer E, Hilfiker-Kleiner D, Hilfiker A, Kovanen PT, Kaartinen M, Nussberger J, Harringer W, Drexler H. Expression of angiotensin II and interleukin 6 in human coronary atherosclerotic plaques: potential implications for inflammation and plaque stability. Circulation 2000;101:1372—8.
[81] Ridker P, Rifai N, Stampfer MJ, Hennekens CH. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. Circulation 2000;101:1767—72.
[82] Ozdemir O, Gundogdu F, Karakelleoglu S, Sevimli S, Pirim I, Acikel M, Arslan S, Serdar S. Comparison of serum levels of inflammatory markers and allelic variant of interleukin-6 in patients with acute coronary syndrome and stable angina pectoris. Coron Artery Dis 2008;19:15—9.
[83] Lindmark E, Diderholm E, Wallentin L, Siegbahn A. Relationship between interleukin-6 and mortality in patients with unstable coronary artery disease. J Am Med Assoc 2001;286:2107—13.
[84] Ferroni P, Basili S, Vieri M, Martini F, Labbadia G, Bellomo A, Gazzaniga PP, Cordova C, Alessandri C. Soluble P-selectin and proinflammatory cytokines in patients with polygenic type IIa hypercholesterolemia. Haemostasis 1999;29:277—85.
[85] Ferroni P, Martini F, Cardarello CM, Gazzaniga PP, Davi G, Basili S. Enhanced interleukin-1beta in hypercholesterolemia: effects of simvastatin and low-dose aspirin. Circulation 2003;108:1673—5.
[86] Nian M, Lee P, Khaper N, Liu P. Inflammatory cytokines and postmyocardial remodeling. Circ Res 2004;94:1543—53.
[87] Ridker P, Rifai N, Pfeffer M, Sacks F, Lepage S, Braunwald E. Elevation of tumor necrosis factor-alpha and increased risk of coronary events after myocardial infarction. Circulation 2000;101:2149—53.
[88] Charo IF, Taubman MB. Chemokines in the pathogenesis of vascular disease. Circ Res 2004;95:858—66.
[89] Serrano-Martinez M, Palacios M, Lezaun R. Monocyte chemoattractant protein-1 concentration in coronary sinus blood and severity of coronary disease. Circulation 2003;108:e75.
[90] de Lemos J, Morrow D, Sabatine M, Murphy SA, Gibson CM, Antman EM, McCabe CH, Cannon CP, Braunwald E. Association between plasma levels of monocyte chemoattractant protein-1 and long-term clinical outcomes in patients with acute coronary syndromes. Circulation 2003;107:690—5.
[91] Heeschen C, Dimmeler S, Hamm C, Fichtlscherer S, Boersma E, Simoons ML, Zeiher AM, CAPTURE Study Investigators. Serum level of the anti-inflammatory cytokine interleukin-10 is an important prognostic determinant in patients with acute coronary syndromes. Circulation 2003;107:2109—14.
[92] Inoue T, Komoda H, Nonaka M, Kameda M, Uchida T, Node K. Interleukin-8 as an independent predictor of long-term clinical outcome in patients with coronary artery disease. Int J Cardiol 2008;124:319—25.
[93] Schonbeck U, Libby P. CD40 signaling and plaque instability. Circ Res 2001;89:1092—103.
[94] Schönbeck U, Varo N, Libby P, Buring J, Ridker PM. Soluble CD40L and cardiovascular risk in women. Circulation 2001;103:2266—8.
[95] Heeschen C, Dimmeler S, Hamm C, van den Brand MJ, Boersma E, Zeiher AM, Simoons ML, CAPTURE Study Investigators. Soluble CD40 ligand in acute coronary syndromes. N Engl J Med 2003;348:1104—11.
[96] Varo N, de Lemos JA, Libby P, Morrow DA, Murphy SA, Nuzzo R, Gibson CM, Cannon CP, Braunwald E, Schönbeck U. Soluble CD40L: risk prediction after acute coronary syndromes. Circulation 2003;108:1049—52.
[97] Dominguez-Rodriguez A, Abreu-Gonzalez P, Garcia-Gonzalez MJ, Kaski JC. Soluble CD40 ligand:interleukin-10 ratio predicts in-hospital adverse events in patients with ST-segment elevation myocardial infarction. Thromb Res 2007;121:293—9.
[98] Morrow D, Rifai N, Antman E, Weiner DL, McCabe CH, Cannon CP, Braunwald E. Serum amyloid A predicts early mortality in acute coronary syndromes: a TIMI 11A substudy. J Am Coll Cardiol 2000;35:358—62.
[99] Johnson BD, Kip KE, Marroquin OC, Ridker PM, Kelsey SF, Shaw LJ, Pepine CJ, Sharaf B, Bairey Merz CN, Sopko G, Olson MB, Reis SE. Serum amyloid A as a predictor of coronary artery disease and cardiovascular outcome in women. Circulation 2004;109:726—32.
[100] Blankenberg S, Barbaux S, Tiret L. Adhesion molecules and atherosclerosis. Atherosclerosis 2003;170:191—203.
[101] Hollander JE, Muttreja MR, Dalesandro MR, Shofer FS. Risk stratification of emergency department patients with acute coronary syndromes using P-selectin. J Am Coll Cardiol 1999;34:95—105.
[102] Mulvihill N, Foley J, Murphy R, Crean P, Walsh M. Evidence of prolonged inflammation in unstable angina and non-Q wave myocardial infarction. J Am Coll Cardiol 2000;36:1210—6.
[103] Ridker P, Buring JE, Rifai N. Soluble P-selectin and the risk of future cardiovascular events. Circulation 2001;103:491—5.
[104] Blankenberg S, Rupprecht H, Bickel C, Peetz D, Hafner G, Tiret L, Meyer J. Circulating cell adhesion molecules and death in patients with coronary artery disease. Circulation 2001;104:1336—42.
[105] Mulvihill N, Foley J, Murphy R, Curtin R, Crean PA, Walsh M. Risk stratification in unstable angina and non-

Q wave myocardial infarction using soluble cell adhesion molecules. Heart 2001;85:623—7.
[106] Rallidis L, Gika H, Zolindaki M, Xydas TA, Paravolidakis KE, Velissaridou AH. Usefulness of elevated levels of soluble vascular cell adhesion molecule-1 in predicting in-hospital prognosis in patients with unstable angina pectoris. Am J Cardiol 2003;92:1195—7.
[107] Doo Y, Han S, Park W, Kim SM, Choi SH, Cho GY, Hong KS, Han KR, Lee NH, Oh DJ, Ryu KH, Rhim CY, Lee KH, Lee Y. Associations between C-reactive protein and circulating cell adhesion molecules in patients with unstable angina undergoing intervention and their clinical implication. Clin Cardiol 2005;28:47—51.
[108] Hillis G, Terregino C, Taggart P, Killian A, Zhao N, Kaplan J, Dalsey WC, Mangione A. Soluble intracellular adhesion molecule-1 as a predictor of early adverse events in patients with chest pain compatible with myocardial ischemia. Ann Emerg Med 2001;38:223—8.
[109] Hillis G, Terregino C, Taggart P, Killian A, Zhao N, Dalsey WC, Mangione A. Elevated soluble P-selectin levels are associated with an increased risk of early adverse events in patients with presumed myocardial ischemia. Am Heart J 2002;143:235—41.
[110] Zhang R, Brennan ML, Fu X, Aviles RJ, Pearce GL, Penn MS, Topol EJ, Sprecher DL, Hazen SL. Association between myeloperoxidase levels and risk of coronary artery disease. J Am Med Assoc 2001;286:2136—42.
[111] Brennan ML, Penn MS, Van Lente F, Nambi V, Shishehbor MH, Aviles RJ, Goormastic M, Pepoy ML, McErlean ES, Topol EJ, Nissen SE, Hazen SL. Prognostic value of myeloperoxydase in patients with chest pain. N Engl J Med 2003;349:1595—604.
[112] Baldus S, Heeschen C, Meinertz T, Zeiher AM, Eiserich JP, Münzel T, Simoons ML, Hamm CW, CAPTURE Investigators. Myeloperoxidase serum levels predict risk in patients with acute coronary syndromes. Circulation 2003;108: 1440—5.
[113] Meuwese MC, Stroes ES, Hazen SL, van Miert JN, Kuivenhoven JA, Schaub RG, Wareham NJ, Luben R, Kastelein JJ, Khaw KT, Boekholdt SM. Serum myeloperoxidase levels are associated with the future risk of coronary artery disease in apparently healthy individuals: the EPIC-Norfolk Prospective Population Study. J Am Coll Cardiol 2007;50:159—65.
[114] Galis Z, Sukhova G, Lark M, Libby P. Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. J Clin Invest 1994;94:2493—503.
[115] Creemers E, Cleutjens J, Smits J, Daemen MJ. Matrix metalloproteinase inhibition after myocardial infarction: a new approach to prevent heart failure? Circ Res 2001;89:201—10.
[116] Eckart R, Uyehara C, Shry E, Furgerson JL, Krasuski RA. Matrix metalloproteinases in patients with myocardial infarction and percutaneous revascularization. J Interv Cardiol 2004;17:27—31.
[117] Blankenberg S, Rupprecht HJ, Poirier O, Bickel C, Smieja M, Hafner G, Meyer J, Cambien F, Tiret L. Plasma concentrations and genetic variation of matrix metalloproteinase 9 and prognosis of patients with cardiovascular disease. Circulation 2003;107:1579—85.
[118] Hlatky MA, Ashley E, Quertermous T, Boothroyd DB, Ridker P, Southwick A, Myers RM, Iribarren C, Fortmann SP. Matrix metalloproteinase circulating levels, genetic polymorphisms, and susceptibility to acute myocardial infarction among patients with coronary artery disease. Am Heart J 2007;154:1043—51.
[119] Soejima H, Ogawa H, Sakamoto T, Miyamoto S, Kajiwara I, Kojima S, Hokamaki J, Sugiyama S, Yoshimura M, Suefuji H, Miyao Y, Fujimoto K, Miyagi H, Kishikawa H. Increased serum matrix metalloproteinase-1 concentration predicts advanced left ventricular remodelling in patients with acute myocardial infarction. Circ J 2003;67:301—4.
[120] Autiero M, Luttun A, Tjwa M, Carmeliet P. Placental growth factor and its receptor, vascular endothelial growth factor receptor-1: novel targets for stimulation of ischemic tissue revascularization and inhibition of angiogenic and inflammatory disorders. J Thromb Haemost 2003;1:1356—70.
[121] Heeschen C, Dimmeler S, Fichtlscherer S, Hamm CW, Berger J, Simoons ML, Zeiher AM, CAPTURE Investigators. Prognostic value of placental growth factor in patients with acute chest pain. J Am Med Assoc 2004;291:435—41.
[122] Caslake MJ, Packard CJ, Suckling KE, Holmes SD, Chamberlain P, Macphee CH. Lipoprotein-associated phospholipase A2, platelet activating factor acetylhydrolase: a potential new risk factor for coronary artery disease. Atherosclerosis 2000;50:413—9.
[123] Caslake MJ, Packard CJ. Lipoprotein-associated phospholipase A2 (platelet-activating factor acetylhydrolase) and cardiovascular disease. Curr Opin Lipidiol 2003;14:347—52.
[124] Lavi S, McConnell JP, Rihal CS, Prasad A, Mathew V, Lerman LO, Lerman A. Local production of lipoprotein-associated phospholipase A2 and lysophosphatidylcholine in the coronary circulation: association with early coronary atherosclerosis and endothelial dysfunction in humans. Circulation 2007;115:2715—21.
[125] Sabatine MS, Morrow DA, O'Donoghue M, Jablonksi KA, Rice MM, Solomon S, Rosenberg Y, Domanski MJ, Hsia J, PEACE Investigators. Prognostic utility of lipoprotein-associated phospholipase A2 for cardiovascular outcomes in patients with stable coronary artery disease. Arterioscler Thromb Vasc Biol 2007;27:2463—9.
[126] Winkler K, Hoffmann MM, Winkelmann BR, Friedrich I, Schäfer G, Seelhorst U, Wellnitz B, Wieland H, Boehm BO, März W. Lipoprotein-associated phospholipase A2 predicts 5-year cardiac mortality independently of established risk factors and adds prognostic information in patients with low and medium high-sensitivity C-reactive protein (the Ludwigshafen risk and cardiovascular health study). Clin Chem 2007;53:1440—7.
[127] Blake GJ, Dada N, Fox JC, Manson JE, Ridker PM. A prospective evaluation of lipoprotein-associated phospholipase A2 levels and the risk of future cardiovascular events in women. J Am Coll Cardiol 2001;38:1302—6.
[128] Packard CJ, O'Reilly DS, Caslake MJ, McMahon AD, Ford I, Cooney J, Macphee CH, Suckling KE, Krishna M, Wilkinson FE, Rumley A, Lowe GD. Lipoprotein-associated phospholipase A2, as an independent predictor of coronary heart disease. N Engl J Med 2000;343:1148—55.
[129] Ballantyne CM, Hoogeveen RC, Bang H, Coresh J, Folsom AR, Heiss G, Sharrett AR. Lipoprotein-associated phospholipase A2, high sensitivity C-reactive protein, and risk for incident coronary heart disease in middle aged men and women in the Atherosclerosis Risk in Communities (ARIC) study. Circulation 2004;109:837—42.
[130] Brilakis ES, McConnell JP, Lennon RJ, Elesber AA, Meyer JG, Berger PB. Association of lipoprotein associated phospholipase A2 levels with coronary artery disease risk factors, angiographic coronary artery disease, and major adverse events at follow-up. Eur Heart J 2005;26:137—44.
[131] Niessen H, Krijnen P, Visser CA, Meijer CJ, Erik Hack C. Type II secretory phospholipase A2 in cardiovascular dis-

ease: a mediator in atherosclerosis and ischemic damage to cardiomyocytes? Cardiovasc Res 2003;60:68—77.
[132] Mallat Z, Steg PG, Benessiano J, Tanguy ML, Fox KA, Collet JP, Dabbous OH, Henry P, Carruthers KF, Dauphin A, Arguelles CS, Masliah J, Hugel B, Montalescot G, Freyssinet JM, et al. Circulating secretory phospholipase A2 activity predicts recurrent events in patients with severe acute coronary syndromes. J Am Coll Cardiol 2005;46:1249—57.
[133] Nijmeijer R, Meuwissen M, Krijnen PA, van der Wal A, Piek JJ, Visser CA, Hack CE, Niessen HW. Secretory type II phospholipase A2 in culprit coronary lesions is associated with myocardial infarction. Eur J Clin Invest 2008;38:205—10.
[134] Schafer BW, Heizmann CW. The S100 family of EF-hand calcium-binding proteins: functions and pathology. Trends Biochem Sci 1996;21:134—40.
[135] Hessian PA, Edgeworth J, Hogg N. MRP-8 and MRP-14, two abundant Ca(2+)-binding proteins of neutrophils and monocytes. J Leukoc Biol 1993;53:197—204.
[136] Stroncek DF, Shankar RA, Skubitz KM. The subcellular distribution of myeloid-related protein 8 (MRP8) and MRP14 in human neutrophils. J Transl Med 2005;3:36.
[137] Murao S. Two calcium-binding proteins, MRP8 and MRP14: a protein complex associated with neutrophil and monocyte activation. Acta Histochem Cytochem 1994;27:107—16.
[138] Liao H, Wu J, Kuhn E, Chin W, Chang B, Jones MD, O'Neil S, Clauser KR, Karl J, Hasler F, Roubenoff R, Zolg W, Guild BC. Use of mass spectrometry to identify protein biomarkers of disease severity in the synovial fluid and serum of patients with rheumatoid arthritis. Arthritis Rheum 2004;50:3792—803.
[139] Healy AM, Pickard MD, Pradhan AD, Wang Y, Chen Z, Croce K, Sakuma M, Shi C, Zago AC, Garasic J, Damokosh AI, Dowie TL, Poisson L, Lillie J, Libby P, et al. Platelet expression profiling and clinical validation of myeloid-related protein-14 as a novel determinant of cardiovascular events. Circulation 2006;113:2278—84.
[140] Ridker PM, Cook NR, Lee IM, Gordon D, Gaziano JM, Manson JE, Hennekens CH, Buring JE. A randomized trial of low-dose aspirin in the primary prevention of cardiovascular disease in women. N Engl J Med 2005;352:1293—304.
[141] Altwegg L, Neidhart M, Hersberger M, Muller S, Eberli F, Corti R, Roffi M, Sutsch G, Gay S, von Eckardstein A, Wischnewsky M, Luscher T, Maie W. Myeloid-related protein 8/14 complex is released by monocytes and granulocytes at the site of coronary occlusion: a novel, early, and sensitive marker of acute coronary syndromes. Eur Heart J 2007;28:941—8.