REVIEW ARTICLE

Inflammation, Atherosclerosis, and Cardiovascular Disease Risk: PAPP-A, Lp-PLA2, and Cystatin C. New Insights or Redundant Information?

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It is well-known that inflammation plays a role in atherogenesis, atherosclerotic plaque progression, and acute coronary syndrome. Inflammatory cells, and cytokines and other biomolecules are implicated in these processes, and have, therefore, been investigated as potential markers of atherosclerotic plaque progression and cardiovascular disease risk. The best characterized and most widely studied is C-reactive protein. However, its role in the clinical setting is still debated. Emerging novel biomarkers that may provide information complementary to that derived from C-reactive protein include pregnancy-associated plasma protein A, lipoprotein-associated phospholipase A2, and cystatin C. This article focuses on the potential value of these three new markers in patients with coronary heart disease, and their use as markers of disease risk in apparently healthy individuals.

Key words: *Inflammation. Atherosclerosis. C-reactive protein. Pregnancy-associated plasma protein A. Lipoprotein-associated phospholipase A2. Cystatin C.*

Inflamación, aterosclerosis y riesgo cardiovascular: PAPP-A, Lp-PLA2 y cistatina C. ¿Nuevas aportaciones o información redundante?

La inflamación tiene un papel establecido, tanto en la iniciación como en la progresión del proceso aterosclerótico. Factores de transcripción nuclear, macrófagos y linfocitos participan y modulan los mecanismos inflamatorios asociados con la rotura o la erosión de la placa que culmina en muchos casos con el síndrome coronario agudo (SCA). Estas biomoléculas constituyen objetivos de medición para tratar de identificar y monitorizar el proceso inflamatorio. La lista de biomarcadores estudiados en la enfermedad cardiovascular se ha expandido rápidamente en los últimos tiempos. En este artículo se analizan las características y el valor potencial de 3 nuevos marcadores de actividad aterosclerótica, la proteína plasmática A asociada al embarazo (PAPP-A), la fosfolipasa A2 asociada a lipoproteína (Lp-PLA2) y la cistatina C, que podrían tener utilidad como complemento de la proteína C reactiva (PCR) en el ámbito de la enfermedad coronaria, tanto en la prevención primaria en sujetos aparentemente sanos como en el pronóstico de los sujetos con eventos coronarios agudos.

Palabras clave: *Inflamación. Aterosclerosis. Proteína C reactiva. Proteína plasmática A. Fosfolipasa A2 asociada a lipoproteína. Cistatina C.*

INTRODUCTION

Our knowledge of the pathophysiology of atherosclerosis and the development of acute coronary syndrome (ACS) has progressed over the last few decades thanks to the large number of studies on the proliferation of smooth muscle cells, growth factors, and the biology of the vascular bed. The prominent role played by inflammation in the pathogenesis of atherosclerosis has become apparent over the last decade. The role of inflammation at the start of atherosclerotic processes and during their progression and in the complications present in the plaques has been well established through many clinical and experimental studies.[1,2] Knowledge of these processes has helped provide an understanding of the beneficial effect of certain therapeutic interventions, such as treatment with lipid-lowering drugs, angiotensin converting enzyme (ACE) inhibitors, antiplatelet agents, etc. On the other hand, investigation of different pathways and identification of what triggers this inflammatory process may unveil new therapeutic targets.

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The action of cytokines and other biomolecules and cells characteristic of inflammation is implicated in all stages of atherosclerosis, and so such molecules and cells have been considered as potential markers to identify and monitor the different stages of the disease. In recent years, a number of studies have correlated different biomarkers with cardiovascular disease,[3] leading to a rapid increase in the number of biomarkers available.[4] These biomarkers are useful in that they can identify a population at risk of an acute ischemic event and detect the presence of so-called vulnerable plaques. Biomarkers must have certain characteristics to be a potential predictor of incident or prevalent coronary disease. Measurements have to be reproducible in multiple independent samples, the method for determination should be standardized and variability controlled, and the sensitivity and specificity should be good. In addition, the biomarker should be independent of other markers and established risk markers, improve on the predictions of risk with established risk factors, be associated with cardiovascular events in population studies and clinical trials, and the cost of the assays has to be acceptable.[5]

The third Adult Treatment Panel (ATP III)[6] established that biomarkers constitute emerging risk factors and so could be used to adjust an estimate of overall risk, as there is a large proportion of patients with intermediate risk for whom there are no clearly defined strategies for stratification.

Many biomarkers of activity have been studied in different clinical contexts (Table 1). This article presents the evidence available on 3 new biomarkers of activity which are currently under study within the broad field of investigation of biomarkers of coronary disease. As we shall show below, the biomarkers analyzed in this review reflect the different pathogenic pathways of atherosclerosis or complement the determination of C-reactive protein (CRP) in predicting coronary events, in both apparently healthy subjects and in those with coronary artery disease.

INFLAMMATION, C-REACTIVE PROTEIN, AND CARDIOVASCULAR RISK

C-reactive protein, determined by highly sensitive techniques (hs-CRP), is the most widely studied marker of inflammation in the field of atherosclerosis. It currently appears to be the most promising biological marker, although there is still controversy regarding its use in clinical practice. Increased concentrations of CRP have been associated with a range of factors, such as hypertension, body mass index (BMI), smoking, metabolic syndrome, diabetes mellitus, obesity, hormone replacement therapy, and chronic infections and inflammation. Physical activity, weight loss, and treatment with statins, niacin, or fibrates are associated with a decrease in hs-CRP.[7]

The potential usefulness of CRP in clinical practice lies in its high predictive value for coronary artery disease in the apparently healthy population.[8-11] In a meta-analysis that included 22 prospective studies, the relative risk (RR) of the population with the most elevated CRP concentrations was 1.58 (95% confidence interval [CI], 1.48-1.68), although there was some variation in the factors that were fitted in the different studies comprising the meta-analysis.[12]

C-reactive protein has added information to that provided by classical risk factors for predicting cardiovascular disease. In the Women's Health Study,[13] a concentration above 3 mg/L showed almost the same prognostic value for event-free survival in the univariate analysis as the presence of metabolic syndrome, and the concentrations of CRP and the total cholesterol/high-density lipoprotein cholesterol (HDL-C) were the only independent predictors of cardiovascular events after adjustment for traditional risk factors. Likewise, CRP proved to be a risk factor in women with concentrations of low-density lipoprotein cholesterol (LDL-C) below 130 mg/dL.[10] In the Atherosclerotic Risk in Communities (ARIC) Study,[14] the relative risk (RR) of coronary artery

TABLE 1. Biomarkers of Activity Studied for Coronary Artery Disease*

Biomarkers of Atherosclerotic Platelet Activity	
Cytokines	IL1β, IL6, IL8, IL10, IL18, TNF-α, sCD40 ligand, myeloperoxidase
Adhesion molecules	sICAM-1, sVCAM-1, p selectin
Acute phase reactants	Fibrinogen, AAS, CRP
White blood cells	
Erythrocyte sedimentation rate	
Neopterin	
Heat shock proteins	
Adiponectin	
Pregnancy associated plasma protein A	
Lipoprotein associated phospholipase A2	
Placental growth factor	
Cystatin C	

*IL indicates interleukin; CRP, C-reactive protein; AAS, angiotensin-aldosterone system; sICAM, soluble intercellular adhesion molecule; sVCAM, soluble vascular adhesion molecule; TNF-α tumor necrosis factor α.

disease after adjusting for risk factors was 1.72 (95% CI, 1.24-2.39) for CRP concentrations above 3.0 mg/L. In the MONICA study, the hazard rate ratio was 2.21 (95% CI, 1.41-3.27) after adjusting for factors of the Framingham scale.[9] Similar results were obtained for women included in the Nurses' Health Study and for men included in the Health Professionals Follow Up Study,[15] and the meta-analysis published recently by Danesh et al[12] also presented similar findings. These data suggest that CRP is an additional measure for estimating risk of coronary artery disease. However, the RR associated with a 3.0 mg/L cutoff point compared to one of less than 1 mg/L is probably smaller than suggested by the current guidelines for clinical practice (approximate RR of 1.5 vs 2.0).[7]

In patients with stable coronary artery disease and those with ACS, CRP has been shown to predict recurrent events and mortality independently even of cardiac troponin levels and after adjustment for other prognostic factors.[16-20] However, the optimum cutoff point for CRP concentration in this context has not yet been determined, and there is no evidence to suggest that this marker can help identify patients with ACS who would benefit from a particular treatment.[7]

C-reactive protein has also been considered as a therapeutic target, and it has been shown that statins lower CRP concentrations through mechanisms other than their effects on lipid concentrations. This anti-inflammatory response was observed in studies with pravastatin, atorvastatin, lovastatin, cerivastatin, and simvastatin, suggesting that a class effect is in operation.[21] Moreover, the extent of benefit derived from statins in clinical trials is greater than that expected based solely on the decrease in LDL-C, and patients who receive statins seem to have a better prognosis even when they have similar concentrations of LDL-C.[22] In the Cholesterol and Recurrent Events (CARE) trial,[19] which studied secondary prevention with pravastatin, most benefit was obtained in patients with the highest CRP values, and the use of pravastatin decreased CRP concentrations regardless of LDL-C concentrations.[23] The AFCAPS/TexCAPS[24] and Physicians Health Study[8] both reported similar findings. In the AFCAPS/TexCAPS,[24] patients with LDL-C below the median concentration (149.1 mg/dL) who showed a reduction in risk with lovastatin therapy were those with high hs-CRP, and this reduction was almost identical to that obtained in patients with hyperlipidemia. However, the usefulness of hs-CRP as a therapeutic goal and as a parameter for monitoring the response to drugs such as statins, particularly in primary prevention, has not been fully established and it is currently not recommended to measure CRP for this end (class III, level of evidence C).[7] In short, measurement of hs-CRP is useful at present to complement stratification of cardiovascular risk in a healthy population with moderate cardiovascular risk (10%-20% in 10 years) (class IIa, level of evidence B). The cutoff points established are less than 1 mg/L (low RR), 1-3 mg/L (intermediate RR), and greater than 3 mg/L (high RR). Indiscriminate use to assess cardiovascular risk is not recommended (class III, level of evidence C).[7] Thus, it should be confirmed whether decreased CRP concentrations are associated with a reduction of risk of coronary artery disease[25] and the benefits of categorizing the risk of coronary artery disease with CRP remains to be determined.

PREGNANCY ASSOCIATED PLASMA PROTEIN A

The pregnancy associated plasma protein A (PAPP-A) is a zinc-binding enzyme belonging to the metalloproteinase superfamily,[26-28] with a high molecular weight. It was first identified as a circulating protein in the serum of women in advanced stages of gestation.[29] Measurement of PAPP-A is useful for screening the fetus for Down syndrome in the first 3 months of pregnancy, as decreased circulating concentrations of this protein are associated with abnormal placental function.[30] In addition to placental tissue, PAPP-A is present in a wide variety of reproductive tissues and organs, such as the testicles and endometrium, and nonreproductive tissues, such as the kidney and colon,[31] but at much lower concentrations than those found during gestation. Pregnancy associated plasma protein A is also secreted by osteoblasts, cells of the granular layer of the ovary, and vascular smooth muscle cells.[32]

The circulating form of the protein comprises a heterotetrameric complex formed of 2 subunits of 200 kDa and 250 kDa, bound by covalent bonds to 2 molecules of 50 kDa and 90 kDa that belong to the proform of eosinophil major basic protein, an endogenous inhibitor of the proteolytic activity of PAPP-A.[33] A highly sensitive immunoassay is required to detect the protein in normal clinical situations because the concentrations of PAPP-A are 100 times less in the normal population than in gestating women.[31]

The protein is a specific protease whose substrate is insulin growth factor (IGF), a factor similar to insulin, and one of the IGF binding proteins, IGFBP-4. When IGF is released from its binding to this protein, PAPP-A appears as a growth modulator in local proliferative responses to IGF, such that it influences the role played by IGF in the pathogenesis of atherosclerosis.[34] These actions would give it an important role in the progression of atherosclerosis and the development of restenosis after coronary interventions.

Pregnancy associated plasma protein A was first considered as a biological marker of unstable atherosclerotic plaques after a study by Bayes-Genis et al,[35] who investigated culprit unstable coronary plaques and stable plaques from 8 patients who had died suddenly of cardiac causes. These authors found high levels of PAPP-A in the cells and the extracellular matrix of the plaques that showed rupture or erosion compared to stable plaques.[35] Given then that this is a marker of

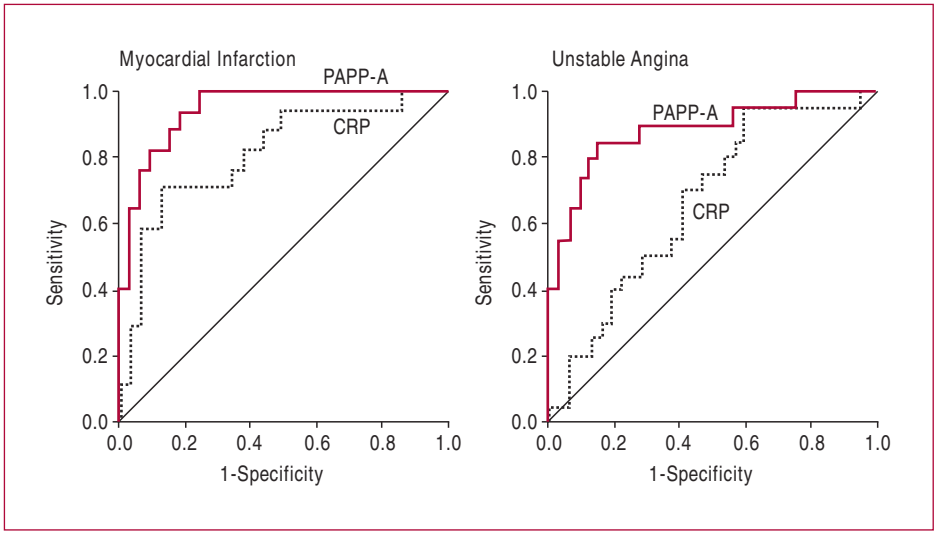

Figure 1. Receiver operating characteristics (ROC) curve for PAPP-A and C-reactive protein (CRP) concentrations in 17 patients with acute myocardial infarction (AMI) and 20 patients with unstable angina. The mean (SD) area under the curve for PAPP-A was 0.94±0.03 for patients with AMI and 0.88±0.05 for those with unstable angina. For CRP, the mean area under the curve was 0.81±0.07 and 0.67±0.08, respectively. The areas under the curve for both markers in the group of patients with AMI and in the group with unstable angina differed significantly from the areas under the ROC curve of the corresponding control group. Adapted with permission of Bayés-Genis et al.[35]

plaque instability, its usefulness in the control and stratification of patients who visit the emergency room with chest pain has also been assessed. Several studies have shown that circulating concentrations of PAPP-A are higher in patients with ACS than in those with stable coronary artery disease and control subjects.[35] In the study by Bayés-Genis et al, circulating PAPP-A concentrations above 10 mU/L allowed identification of patients with ACS with a sensitivity of 89.2% and a specificity of 81.3% (Figure 1). Likewise, PAPP-A concentrations were correlated with free IGF-I and CRP, but not with markers of myocardial damage (creatine kinase MB isoenzyme [CK-MB] and troponin I [TnI]). This finding differs from that obtained in the study by Khosravi et al,[31] who reported a correlation between concentrations of PAPP-A and troponin. These authors also found significantly higher concentrations of PAPP-A in patients with ACS than in those who were suffering from chronic coronary artery disease (P<.001) and in control subjects (P<.001). In these patients with ACS, the pattern of release of PAPP-A is very variable—significant elevations have been reported as long as 30 hours after the index event.[36] The kinetics of PAPP-A release and the corresponding protocols for obtaining optimum samples have yet to be fully established. Contrary to the findings of these studies, a recent study by Domínguez-Rodríguez et al[37] found no differences between the PAPP-A concentrations of 80 patients with ST-elevation ACS compared to control subjects. The authors concluded that PAPP-A is not a valid early marker of acute myocardial infarction (AMI). (This same study also did not find any correlation between PAPP-A and markers of myocardial necrosis.) The samples were taken a mean±SD of 6.3±2.8 hours after the onset of symptoms.

To investigate the prognostic value of determining PAPP-A in patients with coronary artery disease, Laterza et al[38] studied patients with clinical signs and symptoms of ACS (n=346 patients, of whom 33 suffered adverse events [3 deaths, 14 AMI, and 23 revascularization procedures]). On analysis of the receiver operating characteristics (ROC) curves, cardiac troponin T (TnT) was found to be a better predictor of events after 30 days than PAPP-A. For a cutoff point of 0.22 mU/L, PAPP-A had a significantly worse specificity than cardiac TnT, thus according to this study, PAPP-A was a modest predictor of adverse coronary events 30 days after the index event.[38]

In another study with 200 consecutive patients with suspected ACS, patients with undetectable concentrations of TnT and PAPP-A concentrations greater than 2.9 mU/L were at a significantly higher risk of cardiovascular death, a first episode of nonfatal AMI, or need for revascularization after 6 months of follow-up. The predictive value of PAPP-A remained after adjusting for age, sex, smoking habit, hypertension, prior AMI (RR=4.6; 95% CI, 1.8-11.8; P=.002).[39]

Heeschen et al[40] also showed in a study published recently that determination of PAPP-A provides additional prognostic information in patients with ACS. In their study, which included 547 patients with ACS, the authors found that patients with PAPP-A concentrations in the fourth and fifth quintiles, that is with PAPP-A above 12.6 mU/L, had a higher incidence of death or nonfatal AMI, with an odds ratio of 2.74 (95% CI, 1.44-5.22; P=.002) after 72 hours, 2.84 (95% CI, 1.55-5.22; P=.001) after 30 days, and 2.44 (95% CI, 1.43-4.15; P=.001) after 6 months (Figure 2). This predictive value of the PAPP-A concentrations was maintained in patients who did not present increased TnT. An interaction between PAPP-A and interleukin (IL) 10 was shown, such that the predictive value of the composite endpoint of death and nonfatal AMI was limited to patients with circulating IL10 concentrations below 3.5 ng/mL. The authors therefore concluded that the balance between proinflammatory and anti-

inflammatory cytokines determined the course of the disease in these patients, who in turn, had a higher rate of revascularization procedures. In this study, PAPP-A was also weakly correlated with other biological makers, such as hs-CRP and CD40L, although no correlation was found with TnT.

The possible value of PAPP-A as a marker of complex coronary lesions in coronary angiography has also been investigated in patients with stable coronary disease by Cosin-Sales et al.[41] In their study, which included 396 patients, the authors reported evidence that the patients with complex coronary lesions according to coronary angiography had significantly higher circulating concentrations of PAPP-A (5.89 [1.64] mU/L) compared to patients free of such lesions (5.07 [1.39] mU/L; *P*<.001). In the same study, the authors investigated the hypothesis that the PAPP-A/pro-MBP ratio could be an indicator of proteolytic activity of PAPP-A and that the ratio could be used as a marker of vulnerable atherosclerotic plaques in patients with chronic stable angina. The proform of eosinophil major basic protein is the endogenous inhibitor of this proteolytic activity of PAPP-A. Cosin-Sales et al[41] reported that patients with complex coronary lesions had a significantly higher PAPP-A/pro-MBP ratio (3.13 [1.17] mU/L vs 2.66 [0.82] mU/L; *P*<.001). In the multivariate analysis, the PAPP-A/pro-MBP ratio was an independent predictor of the number of complex lesions, as was male sex and extent of coronary artery disease. Elevated concentrations of PAPP-A were also associated with the presence of atherosclerotic carotid lesions, which were hyperechoic or isoechoic (type V or greater according to the American Heart Association classification)[42] in ultrasonography of the carotid arteries of asymptomatic subjects with hyperlipidemia and at a high cardiovascular risk. The patients with such lesions had significantly higher plasma levels than those with hypoechoic lesions (*P*<.05) and those with normal lipid levels (*P*<.05).[43] In these patients, determination of PAPP-A was related to CRP levels.

The possible relationship of PAPP-A with other cardiovascular risk factors, such as hypercholesterolemia, have been analyzed but with contradictory results.[43,44] Stulc et al[44] studied 27 patients with untreated hypercholesterolemia and no clinical manifestations of atherosclerosis. The authors reported significantly higher concentrations of PAPP-A in patients than in control subjects (*P*<.018), indicating a potential role of PAPP-A as a marker of preclinical atherosclerosis, although more studies would be needed to confirm the value of PAPP-A as such a marker, as well as its value as a marker of plaque instability. However, in a study of 64 hyperlipidemic subjects performed by Beaudeux et al,[43] no differences were found between subjects with hyperlipidemia and control subjects. Similarly, no correlation was reported between PAPP-A and cholesterol concentrations (or between PAPP-A and CRP, high density lipoproteins [HDL], and triglycerides) and PAPP-A concentrations

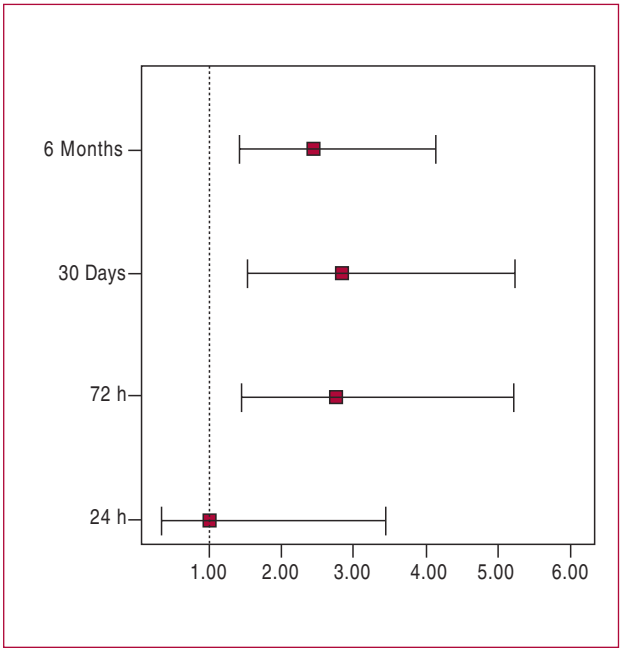

Figure 2. Relationship between plasma concentrations of PAPP-A >12.6 mU/L and cardiovascular events: odds ratio, 95% confidence interval. Taken from Heeschen C et al.[40]

remained unchanged after 10 weeks of treatment with 20 mg of atorvastatin, even though total cholesterol, LDL-C, and CRP decreased sharply. The fact that statin treatment did not affect PAPP-A levels, unlike other inflammatory markers, may be partly explained by the role of PAPP-A in the proliferative responses of the plaques rather than plaque inflammation.[44] In short, the available evidence seems to suggest that measuring plasma concentrations of PAPP-A could play a role as a marker of unstable atherosclerotic plaques and have prognostic value in patients with ACS. Such measurements could also add information to that provided by markers of myocardial damage, particularly in patients where such markers are not elevated.

LIPOPROTEIN ASSOCIATED PHOSPHOLIPASE A2

Lipoprotein associated phospholipase A2 (Lp-PLA2), also known as platelet-activating factor acetylhydrolase, is a 50 kDA, Ca^{2+} independent enzyme associated with LDL-C, and in particular with small dense LDL-C particles, which have a long half life, are very proatherogenic, and readily undergo oxidative modification.[45] The enzyme is a subtype of a growing family of A2 phospholipases and is secreted mainly by macrophages/monocytes, mast cells, and T lymphocytes. Two thirds of plasma Lp-PLA2 in circulation is bound to LDL molecules, whereas the remainder is distributed between HDL-C and very low density lipoproteins.[46] The enzyme has proinflammatory properties, as it hydrolyzes

TABLE 2. Clinical and Epidemiological Studies to Investigate the Relationship Between Baseline Concentrations of Lp-PLA2 and the Risk of Future Coronary Events in the Apparently Healthy Population*

Study and Literature Reference	Study Population	Follow-Up, Years	Primary Endpoint	Risk, 95% CI
WOSCOPS[50]	Middle-aged men; 560 cases and 1160 controls	5 years	Cardiovascular death, nonfatal AMI, or revascularization	RR=1.18 (1.05-1.33)†
ARIC[14]	608 men and women aged 45-64 years, and 740 controls	6 years	Cardiovascular death, nonfatal AMI, or revascularization	HR=1.15 (0.81-1.63)‡
MONICA[51]	934 men aged 45-64 years with moderately high cholesterol	14 years	Cardiovascular death, nonfatal AMI	HR=1.21 (1.01-1.45)†
Women's Health Study[52]	Women; 123 cases and 123 controls	3 years	Cardiovascular death, nonfatal AMI, or stroke	RR=1.17 (0.45-3.05)‡
Rotterdam[53]	308 cases and a cohort of 1820 men and women ≥55 years	7 years	Coronary events$	HR=1.97 (1.03-3.79)‡

*HR indicates hazard ratio; AMI, acute myocardial infarction; CI, confidence interval; RR, relative risk.
†In the WOSCOPS and MONICA studies, risk calculated for an increase of 1 SD above baseline values.
‡In the Women's Health Study and the Rotterdam study, the risk expressed was the comparison of the population in the upper quartile with respect to the lower quartile, and in the ARIC study Lp-PLA2 concentrations in the upper tercile were compared with the lower tercile.
$The Rotterdam study also independently assessed the relationship between Lp-PLA2 and stroke, and in this study the activity instead of mass of Lp-PLA2 was measured. The risk expressed was that associated with the risk of coronary events.

oxidized phospholipids to lysophosphatidylcholine and free oxidized fatty acids, and thus is the enzyme responsible for most of the increased lysophosphatidylcholine content of oxidized LDL particles (oxLDL).[47] The atherogenic potential of oxLDL has been attributed to this high lysophosphatidylcholine content. Both lysophosphatidylcholine and free oxidized fatty acids are biologically active and can act as monocyte chemoattractants, as reflected by the predominantly proinflammatory activity of Lp-PLA2 in atherosclerosis. In contrast, Lp-PLA2 could also have anti-inflammatory properties, as it also participates in the hydrolysis of platelet-activating factor and other phospholipids.[48] Nevertheless, growing evidence suggests that Lp-PLA2 plays an important role in the development of atherosclerosis and its clinical consequences. Expression of Lp-PLA2 is upregulated in macrophages of the fibrous plaques vulnerable to rupture.[49] The West of Scotland Coronary Prevention Study (WOSCOPS),[50] which studied the use of pravastatin in primary prevention in 6595 men free of coronary disease, provided the first evidence of the association of Lp-PLA2 and the risk of future cardiovascular events (Table 2 and Figure 3). A case-control analysis was done of middle-aged men in the WOSCOPS, with a follow-up of 5 years (560 cases and 1160 controls). The baseline measurement of Lp-PLA2 turned out to be an independent predictor of future cardiovascular events. Increased baseline concentrations of Lp-PLA2 were associated with an 18% increase in risk, after adjusting for other risk factors and other inflammatory markers, and subjects with Lp-PLA2 concentrations in the upper quintile were at almost twice the risk as those with concentrations in the lower quintile. An increase of 1 SD corresponded to an increase in risk of 1.18 (95% CI, 1.05-1.33; $P<.005$). The predictive value of the other inflammatory markers assessed (CRP, white blood cells) was attenuated in the multivariate analysis and statistical significance was only found for the upper quintile. Measurement of the plasma concentrations of Lp-PLA2 remained significantly associated with the risk of coronary events for all quintiles, and was the only inflammatory marker whose measurement was not affected by smoking.

Similar findings were reported in the monitoring of trends and determinants in cardiovascular disease (MONICA) study. The study population included 934 apparently healthy men aged 45 years to 64 years with moderately high cholesterol concentrations, who were monitored from 1984 to 1998. Baseline values of Lp-PLA2 were associated with a risk of future coronary events after adjustment for possible confounding factors—an association that was independent of the CRP concentration (hazard ratio [HR]=1.21; 95% CI, 1.01-1.45).[51] An increase of 1 SD in the concentration of Lp-PLA2 corresponded to an increase in risk of 21%. The findings of this study point to the additional value of measuring Lp-PLA2 and CRP for predicting the risk of coronary disease, as the combination of Lp-PLA2 greater than 290.8 μg/L and hs-CRP greater than 3 mg/L was significantly associated with a higher risk than that of each marker on its own, with a hazard ratio of 1.93 (95% CI, 1.09-3.40) after overall adjustment. The Atherosclerotic Risk in Communities (ARIC) Study[14] was designed to evaluate the presence of atherosclerosis in a 6-year period in 12 819 apparently healthy men and women. Measurement of plasma concentrations of Lp-PLA2 complemented determination of CRP in identifying subjects with a risk of future coronary events

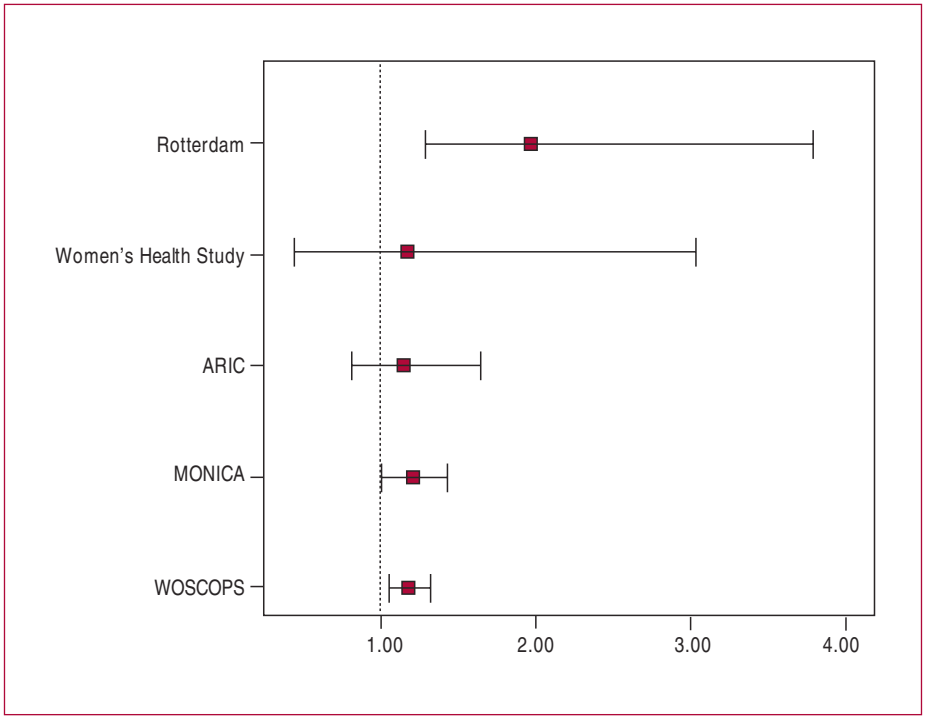

Figure 3. Clinical and epidemiological studies to investigate the relationship between baseline levels of Lp-PLA2 and the risk of future coronary events in the apparently healthy population. In the WOSCOPS and MONICA study, risk is calculated for an increase of 1 SD above the baseline values. In the Women's Health Study and the Rotterdam study, the risk is expressed as the comparison of the population with Lp-PLA2 concentrations in the upper quartile with respect to the lower one, and in the ARIC study, the comparison of Lp-PLA2 concentrations in the upper and lower tercile is presented.

and low LDL-C. In this study, a cohort of 608 patients aged between 45 years and 46 years was studied for 6 years to 8 years, along with 740 control subjects. Once again, Lp-PLA2 concentrations were greater in patients than in controls, but after multivariate adjustment for traditional risk factors, the association of Lp-PLA2 with the risk of coronary events was attenuated and no longer statistically significant. The authors did find, however, a relationship between LDL-C and plasma concentrations of Lp-PLA2—patients with LDL-C less than 130 mg/dL and increased concentrations of Lp-PLA2 (>422 µg/L) were at a significantly higher risk and remained so after adjustment for other risk factors and for hs-CRP (HR=2.08; 95% CI, 1.20-3.62). This study included women and men, blacks, and a higher percentage of patients with diabetes mellitus (DM) than in the MONICA study. The study population also had a wide range of LDL-C concentrations, as would be expected in the North American population. The annual rate of events was 0.9%. The WOSCOPS included middle-aged men with hypercholesterolemia (range, 174-232 mg/dL) and a high prevalence of other risk factors as well as a high rate of events (1.6% annually in the placebo group). In the Women's Health Study,[52] which included women at a low cardiovascular risk, measurement of plasma Lp-PLA2 was not an independent predictor of events after adjustment for traditional risk factors and for hs-CRP concentrations, even though significantly higher concentrations of Lp-PLA2 were reported in the population with cardiovascular events. These differences with respect to previous studies were attributed to sex, as the Women's Health Study only included women with a low incidence of events (0.2% annually), and fewer Afro-American subjects and fewer patients with diabetes than the other studies discussed. Only 123 cases (49 of which were stroke) and 123 controls were included in the analysis of Lp-PLA2. Another factor which may have affected the result is hormone replacement therapy (HRT), as women receiving such therapy had significantly lower concentrations of Lp-PLA2, although there were no differences between women with HRT among cases and controls and the results remained unchanged on adjusting for HRT.

The Rotterdam study[53] included 308 cases of coronary events and a cohort of 1820 men and women aged 55 years or more (mean age, 70 years) were analyzed, with a median follow-up of 7 years. In this study, activity of Lp-PLA2 was measured, unlike the other studies which determined the mass of Lp-PLA2. This activity was associated with risk of coronary events, such that after adjustment for cardiovascular risk factors and hs-CRP, the hazard ratio for subjects with Lp-PLA2 activity in the upper quartile in comparison with those with activity in the lower quartile was 1.97 (95% CI, 1.04-1.39; $P<.01$). The Lp-PLA2 activity was also an independent predictor of coronary events in individuals with non-HDL-C below the median concentration. This was the first prospective study to show that Lp-PLA2 activity is an independent predictor of ischemic stroke and its association is incremental, such that the hazard ratio for the upper quartile compared to the lower quartile was 1.97 (95% CI, 1.03-3.79; $P<.03$). Given that cholesterol is not a strong indicator of stroke,[54] the association between Lp-PLA2 activity and risk of stroke suggests

that, although Lp-PLA2 circulates bound to LDL, it can be associated with a different risk. The weight of evidence points to a role for inflammation in the pathogenesis of ischemic stroke,[55] and statins have decreased the incidence of stroke even in patients without hypercholesterolemia.[56] The anti-inflammatory effect of statins could be the key mechanism by which they reduce the incidence of stroke.

With regard to the correlation between Lp-PLA2 and other risk factors, 4 studies have reported that Lp-PLA2 was correlated with total cholesterol or LDL-C, although discrepancies were found for HDL-C. In the MONICA study and WOSCOPS, a weak correlation was observed with HDL-C, whereas in the WHS, the ARIC study, and the Rotterdam study, an inverse correlation was found. Taken together, a weak correlation with CRP was found, associated more strongly with traditional risk factors. This difference in associations could mean that Lp-PLA2 and CRP act via different pathophysiological mechanisms in the atherosclerotic process.

In patients with angiographically documented coronary artery disease, as in the study by Caslake et al,[46] concentrations of Lp-PLA2 were also higher than reported for controls. The authors of this study also showed that this increase was independent of LDL-C levels and other risk factors. The potential use of Lp-PLA2 as a marker of subclinical cardiovascular risk was investigated by Iribarren et al,[57] who observed a significant association between Lp-PLA2 and the presence of coronary calcifications. In the study, both the mass and activity of Lp-PLA2 were measured in young adults, and it was found that both values were greater in cases (266 cases) than in controls (266 controls). Nevertheless, after adjusting for a range of covariates, the only persistent significant association was mass of Lp-PLA2 and the risk of coronary calcification, with an OR of 1.28 (95% CI, 1.03-1.60) for every SD. The OR associated with the upper tercile of the mass of Lp-PLA2 (2.2) was similar to the OR for diabetes (2.6), hypertension (2.2), or smoking (2.2) according to the multivariate analysis. In this same study, a correlation with LDL-C and an inverse correlation with HDL-C were reported for both mass and activity of Lp-PLA2, and no correlation was found with CRP concentrations, once again indicating that these markers represent different metabolic pathways. Likewise, significant differences were found in Lp-PLA2 concentrations between men and women and among ethnic groups, such that white men had higher concentrations of Lp-PLA2 (mass and activity) and black women had lower concentrations.[57]

Brilakis et al[58] measured plasma Lp-PLA2 in 504 consecutive patients who underwent coronary angiography for clinical reasons. Increased Lp-PLA2 at baseline was associated with a higher risk of cardiovascular events after adjusting for other risk factors and for CRP (hazard ratio per SD, 1.30; $P<.010$). However, Lp-PLA2 was not an independent predictor of the extent of the coronary artery disease, contrary to the findings of Caslake et al,[46] although fewer patients were included in this latter study. The study by Brilakis et al[58] also included women (38%) and the extent and severity of the coronary artery disease were documented rather than just whether it was present. Levels of Lp-PLA2 were correlated with male sex, total cholesterol, LDL-C and HDL-C (negatively), fibrinogen, and creatinine. No correlation was found with CRP.

In short, these findings suggest that Lp-PLA2 is a predictor of coronary events in apparently healthy middle-aged subjects regardless of their cholesterol concentrations. Measurement of Lp-PLA2 could find an application as a new risk marker complementary to CRP. Furthermore, statins and fibrates have been shown to lower plasma levels of Lp-PLA2[59-61] and other drugs are currently under development to lower plasma concentrations of Lp-PLA2. These drugs may become one of the therapeutic options in the treatment of atherosclerosis in the future.[62]

CYSTATIN C

Cystatin C is a cysteine protease inhibitor that participates in protein catabolism. It is synthesized by all nucleated cells at a constant rate of production, filtered by the renal glomeruli,[63] and almost completely reabsorbed and catabolized in the proximal tubule cells. Cystatin C participates in the immune system by inhibiting chemiotaxis of polynuclear cells.[64]

Recently, the determination of renal function, either by estimating creatinine clearance with the Cockroft-Gault equation or by measuring plasma creatinine, has been shown to be of prognostic value in the population of patients with suspected or confirmed ACS.[65,66] However, estimation of the glomerular filtration rate from creatinine concentrations is not very reliable because creatinine is affected by factors such as age, sex, muscle mass, physical activity, and diet,[67] and it does not bear a linear relationship with the glomerular filtration rate. On the other hand, creatinine clearance (Crcl) also overestimates true glomerular filtration because creatinine is additionally secreted by the tubules.[68] Measurement of cystatin C has been shown to be a better endogenous marker of the glomerular filtration rate than creatinine.[69] Cystatin C values seem to be sensitive to small changes in the filtration rate and so could be an ideal marker for the process.[70] The value of cystatin C for predicting future cardiovascular events in patients with coronary artery disease was studied by Koenig et al[71] in a cohort of 1033 patients diagnosed with coronary artery disease in the 3 months prior to inclusion. The mean age of the cohort was 59 years and mean follow-up lasted 33.5 months. The concentrations of cystatin C were in the upper quintile in patients with renal failure compared to those with mild renal failure or normal renal function. In accordance with the incidence of

cardiovascular events (cardiovascular death, nonfatal AMI, stroke, or transient ischemic attack), no significant differences were reported between patients with different extents of renal dysfunction as assessed by plasma creatinine (incidence of events of 5.4% in patients with creatinine >106 µmol/L vs 7% in patients with creatinine <106 µmol/L; *P*=.63) or by Crcl (incidence of events of 7% in patients with Crcl <60 mL/min, 9% in patients with Crcl of 60-90 mL/min, and 6.3% in patients with Crcl >90 mL/min; *P*=.1). However, for cystatin C, there were significant differences in the probability of presenting with a cardiovascular event according to the quintile of cystatin C to which the patient belonged (14% for the upper quintile, 7.7%, 4.3%, 3.9%, and 5% for the other quintiles in descending order; *P*<.0001). In the multivariate analysis, after adjustment for age, sex, traditional risk factors, and other factors such as body mass index (BMI), history of diabetes mellitus, treatment with ACE inhibitors, HDL-C, and CRP, cystatin C was an independent predictor of risk of cardiovascular events with a hazard ratio for the upper quintile of 2.27 (95% CI, 1.05-4.91). The hazard ratio was even higher after subsequent adjustment for Crcl. In this study, cystatin C was strongly correlated not just with creatinine and Crcl but also with severity of coronary artery disease, increased age, history of diabetes, and positively with concomitant treatment with diuretics or ACE inhibitors, but negatively with beta-blockers. Although it has been mentioned that cystatin C might not be influenced by inflammation, Koenig et al[71] have found a correlation with hs-CRP. Knight et al[72] assessed factors that determined cystatin C in a study in which they also observed an association with CRP. Apart from CRP, age, sex, active smoking habit, increased weight, and greater height were also independently associated with cystatin C.

Measurement of cystatin C as a prognostic factor in patients with ACS has been assessed in a study that included 726 patients with suspected or confirmed ACS monitored for a median of 40 months for mortality and 6 months for AMI.[73] The risk of death increased with increasing baseline concentrations of cystatin C. In the Cox regression model, cystatin C was independently associated with mortality, such that patients with concentrations in the upper quartile (≥1.25 mg/L), corresponding to a glomerular filtration rate of ≤58 mL/min, had a RR for death compared to those with concentrations in the lower quartile of less than 4.28 (95% CI, 1.64-11.2; *P*<.003). However, after adjustment for other variables, cystatin C was no longer an independent predictor of new AMI. These findings remained unaltered when patients were stratified according to the final diagnosis (non-ST-elevation ACS [NSTE-ACS], other cardiac causes, and noncardiac or unknown causes of chest pain). In comparison with other markers of renal function, cystatin C has a greater capacity to differentiate between those who survive and those who do not. On classification of the patients into quartiles, it was a better marker at discriminating between high- and low-risk patients, mortality being 12 times higher in patients in the upper quartile compared to those in the lower quartile. Cystatin C was weakly correlated with CRP and TnT, and moderately correlated with N-terminal prohormone brain natriuretic peptide (NT-proBNP) and creatinine concentrations.

These findings could suggest cystatin C might be more than just a marker of renal insufficiency. Nevertheless, more studies are required to elucidate the true role it plays in cardiovascular disease and to determine whether patients with mild or moderate renal dysfunction and an acute coronary event should be treated differently to those patients with ACS and normal renal function.

COMMENT

The evidence available on PAPP-A, Lp-PLA2, and cystatin C is promising and these biomarkers of activity may become useful as a complement to CRP in predicting cardiovascular risk, both in the healthy population and in the population suffering from coronary artery disease. However, the findings are still preliminary and require further evidence and more studies to fully determine the true role of these markers and their application in clinical and/or therapeutic practice and to standardize the measurements and corresponding protocols to determine the plasma concentrations of these markers, as recommended by Apple et al[74] on behalf of the Committee for Standardization of Markers of Cardiac Damage of the International Federation of Clinical Chemistry. Although measurement of LDL-C remains one of the cornerstones of the guidelines for clinical practice in the primary prevention of coronary artery disease, new markers are required to identify clinical presentation of the disease and the population at risk, estimate the overall risk, and indicate treatment for prevention of the disease in the population at risk because more than half the coronary events currently occur in subjects who are not hyperlipidemic.[75] This fact reflects the influence of other risk factors. It is even possible that different biomarkers arise that provide specific information applicable in certain clinical situations, and so a strategy of determination of multiple markers should be assessed, as well as other pathophysiological pathways of atherosclerotic disease. The Systemic Inflammation Evaluation in patients with non-ST segment elevation Acute coronary syndrome (SIESTA) study[76] is a multicenter, observational, prospective study that aims to answer some of the questions about biomarkers in patients with NSTE-ACS admitted to hospital. The study aims to define the prognostic value of different biomarkers of inflammation and endothelial activation, to compare this prognostic value with other established clinical, electrocardiographic, and biochemical indicators of risk, and to address the prognostic importance of

persistently high values compared to transient elevation. Future investigation into biomarkers should help to identify other pathophysiological pathways of atherosclerosis and determine exactly when one or several biomarkers would be useful in clinical and therapeutic practice. Perhaps the main effort should not so much be directed at determining the relevance of inflammation in the pathophysiology of atherosclerosis and ACS but rather at identifying relevant biomarkers that can be easily and reliably measured, that provide clinically relevant information, and whose value is unquestioned because of doubts about the statistically validity of certain findings.[77]

REFERENCES

1. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. Circulation. 2002;105:1135-43.
2. Ross R. Atherosclerosis: an inflammatory disease. N Engl J Med. 1999;340:115-26.
3. Panteghini M. Role and importance of biochemical markers in clinical cardiology. Eur Heart J. 2004;25:1187-96.
4. Marian AJ, Nambi V. Biomarkers of cardiac disease. Expert Rev Mol Diagn. 2004;4:805-20.
5. Mosca L. C-reactive protein: to screen or not to screen? N Engl J Med. 2002;347:1615-7.
6. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA. 2001;285:2486-97.
7. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO, III, Criqui M, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation. 2003;107:499-511.
8. 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.
9. Koenig W, Sund M, Frohlich M, Fischer HG, Lowel H, Doring A, et al. C-Reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992. Circulation. 1999;99:237-42.
10. Ridker PM, 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.
11. Ridker PM, Buring JE, Shih J, Matias M, Hennekens CH. Prospective study of C-reactive protein and the risk of future cardiovascular events among apparently healthy women. Circulation. 1998;98:731-3.
12. Danesh J, Wheeler JG, Hirschfield GM, Eda S, Eiriksdottir G, Rumley A, et al. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. N Engl J Med. 2004;350:1387-97.
13. Ridker PM, 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.
14. Ballantyne CM, Hoogeveen RC, Bang H, Coresh J, Folsom AR, Heiss G, et al. 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.
15. Pai JK, Pischon T, Ma J, Manson JE, Hankinson SE, Joshipura K, et al. Inflammatory markers and the risk of coronary heart disease in men and women. N Engl J Med. 2004;351:2599-610.
16. Morrow DA, Rifai N, Antman EM, Weiner DL, McCabe CH, Cannon CP, et al. 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. Thrombolysis in Myocardial Infarction. J Am Coll Cardiol. 1998;31:1460-5.
17. Heeschen C, Hamm CW, Bruemmer J, Simoons ML. Predictive value of C-reactive protein and troponin T in patients with unstable angina: a comparative analysis. CAPTURE Investigators. Chimeric c7E3 AntiPlatelet Therapy in Unstable angina REfractory to standard treatment trial. J Am Coll Cardiol. 2000;35:1535-42.
18. Mueller C, Buettner HJ, Hodgson JM, Marsch S, Perruchoud AP, Roskamm H, et al. 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.
19. Ridker PM, Rifai N, Pfeffer MA, Sacks FM, Moye LA, Goldman S, et al. Inflammation, pravastatin, and the risk of coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events (CARE) Investigators. Circulation. 1998;98:839-44.
20. Zebrack JS, Anderson JL, Maycock CA, Horne BD, Bair TL, Muhlestein JB. 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.
21. Ridker PM. Connecting the role of C-reactive protein and statins in cardiovascular disease. Clin Cardiol. 2003;26 Suppl 3:III39-44.
22. MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 20,536 high-risk individuals: a randomised placebo-controlled trial. Lancet. 2002;360:7-22.
23. Ridker PM, Rifai N, Pfeffer MA, Sacks F, Braunwald E. Long-term effects of pravastatin on plasma concentration of C-reactive protein. The Cholesterol and Recurrent Events (CARE) Investigators. Circulation. 1999;100:230-5.
24. Ridker PM, Rifai N, Clearfield M, Downs JR, Weis SE, Miles JS, et al. 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.
25. Ridker PM. Rosuvastatin in the primary prevention of cardiovascular disease among patients with low levels of low-density lipoprotein cholesterol and elevated high-sensitivity C-reactive protein: rationale and design of the JUPITER trial. Circulation. 2003;108:2292-7.
26. Oxvig C, Sand O, Kristensen T, Kristensen L, Sottrup-Jensen L. Isolation and characterization of circulating complex between human pregnancy-associated plasma protein-A and proform of eosinophil major basic protein. Biochim Biophys Acta. 1994;1201:415-23.
27. Oxvig C, Sand O, Kristensen T, Gleich GJ, Sottrup-Jensen L. Circulating human pregnancy-associated plasma protein-A is disulfide-bridged to the proform of eosinophil major basic protein. J Biol Chem. 1993;268:12243-6.
28. Lawrence JB, Oxvig C, Overgaard MT, Sottrup-Jensen L, Gleich GJ, Hays LG, et al. The insulin-like growth factor (IGF)-dependent IGF binding protein-4 protease secreted by human fibroblasts is pregnancy-associated plasma protein-A. Proc Natl Acad Sci USA. 1999;96:3149-53.
29. Lin TM, Galbert SP, Kiefer D, Spellacy WN, Gall S. Characterization of four human pregnancy-associated plasma proteins. Am J Obstet Gynecol. 1974;118:223-36.
30. Brambati B, Tului L, Bonacchi I, Shrimanker K, Suzuki Y, Grudzinskas JG. Serum PAPP-A and free beta-hCG are first-trimester screening markers for Down syndrome. Prenat Diagn. 1994;14:1043-7.

31. Khosravi J, Diamandi A, Krishna RG, Bodani U, Mistry J, Khaja N. Pregnancy associated plasma protein-A: ultrasensitive immunoassay and determination in coronary heart disease. Clin Biochem. 2002;35:531-8.
32. Bayes-Genis A, Schwartz RS, Lewis DA, Overgaard MT, Christiansen M, Oxvig C, et al. Insulin-like growth factor binding protein-4 protease produced by smooth muscle cells increases in the coronary artery after angioplasty. Arterioscler Thromb Vasc Biol. 2001;21:335-41.
33. Overgaard MT, Haaning J, Boldt HB, Olsen IM, Laursen LS, Christiansen M, et al. Expression of recombinant human pregnancy-associated plasma protein-A and identification of the proform of eosinophil major basic protein as its physiological inhibitor. J Biol Chem. 2000;275:31128-33.
34. Bayes-Genis A, Conover CA, Schwartz RS. The insulin-like growth factor axis: A review of atherosclerosis and restenosis. Circ Res. 2000;86:125-30.
35. Bayes-Genis A, Conover CA, Overgaard MT, Bailey KR, Christiansen M, Holmes DR Jr, et al. Pregnancy-associated plasma protein A as a marker of acute coronary syndromes. N Engl J Med. 2001;345:1022-9.
36. Qin QP, Laitinen P, Majamaa-Voltti K, Eriksson S, Kumpula EK, Pettersson K. Release patterns of pregnancy associated plasma protein A (PAPP-A) in patients with acute coronary syndromes. Scand Cardiovasc J. 2002;36:358-61.
37. Domínguez-Rodríguez A, Abreu-González P, García-González M, Ferrer J, Vargas M. Circulating pregnancy-associated plasma protein A is not an early marker of acute myocardial infarction. Clin Biochem. 2005;38:180-2.
38. Laterza OF, Cameron SJ, Chappell D, Sokoll LJ, Green GB. Evaluation of pregnancy-associated plasma protein A as a prognostic indicator in acute coronary syndrome patients. Clin Chim Acta. 2004;348:163-9.
39. Lund J, Qin QP, Ilva T, Pettersson K, Voipio-Pulkki LM, Porela P, et al. Circulating pregnancy-associated plasma protein a predicts outcome in patients with acute coronary syndrome but no troponin I elevation. Circulation. 2003;108:1924-6.
40. Heeschen C, Dimmeler S, Hamm CW, Fichtlscherer S, Simoons ML, Zeiher AM. Pregnancy-associated plasma protein-A levels in patients with acute coronary syndromes: comparison with markers of systemic inflammation, platelet activation, and myocardial necrosis. J Am Coll Cardiol. 2005;45:229-37.
41. Cosin-Sales J, Christiansen M, Kaminski P, Oxvig C, Overgaard MT, Cole D, et al. Pregnancy-associated plasma protein A and its endogenous inhibitor, the proform of eosinophil major basic protein (proMBP), are related to complex stenosis morphology in patients with stable angina pectoris. Circulation. 2004;109:1724-8.
42. Stary HC. Natural history and histological classification of atherosclerotic lesions: an update. Arterioscler Thromb Vasc Biol. 2000;20:1177-8.
43. Beaudeux JL, Burc L, Imbert-Bismut F, Giral P, Bernard M, Bruckert E, et al. Serum plasma pregnancy-associated protein A: a potential marker of echogenic carotid atherosclerotic plaques in asymptomatic hyperlipidemic subjects at high cardiovascular risk. Arterioscler Thromb Vasc Biol. 2003;23:e7-10.
44. Stulc T, Malbohan I, Malik J, Fialova L, Soukupova J, Ceska R. Increased levels of pregnancy-associated plasma protein-A in patients with hypercholesterolemia: the effect of atorvastatin treatment. Am Heart J. 2003;146:E21.
45. Chapman MJ, Guerin M, Bruckert E. Atherogenic, dense low-density lipoproteins. Pathophysiology and new therapeutic approaches. Eur Heart J. 1998;19 Suppl A:A24-30.
46. Caslake MJ, Packard CJ, Suckling KE, Holmes SD, Chamberlain P, Macphee CH. Lipoprotein-associated phospholipase A(2), platelet-activating factor acetylhydrolase: a potential new risk factor for coronary artery disease. Atherosclerosis. 2000; 150:413-9.
47. Macphee CH, Moores KE, Boyd HF, Dhanak D, Ife RJ, Leach CA, et al. Lipoprotein-associated phospholipase A2, platelet-activating factor acetylhydrolase, generates two bioactive products during the oxidation of low-density lipoprotein: use of a novel inhibitor. Biochem J. 1999;338:479-87.
48. Tjoelker LW, Wilder C, Eberhardt C, Stafforini DM, Dietsch G, Schimpf B, et al. Anti-inflammatory properties of a platelet-activating factor acetylhydrolase. Nature. 1995;374:549-53.
49. Sudhir K. Lipoprotein-associated phospholipase A2, a novel inflammatory biomarker and independent risk predictor for cardiovascular disease. J Clin Endocrinol Metab. 2005;90:3100-5.
50. Packard CJ, O'Reilly DS, Caslake MJ, McMahon AD, Ford I, Cooney J, et al. Lipoprotein-associated phospholipase A2 as an independent predictor of coronary heart disease. West of Scotland Coronary Prevention Study Group. N Engl J Med. 2000;343: 1148-55.
51. Koenig W, Khuseyinova N, Lowel H, Trischler G, Meisinger C. Lipoprotein-associated phospholipase A2 adds to risk prediction of incident coronary events by C-reactive protein in apparently healthy middle-aged men from the general population: results from the 14-year follow-up of a large cohort from southern Germany. Circulation. 2004;110:1903-8.
52. Blake GJ, Dada N, Fox JC, Manson JE, Ridker PM. A prospective evaluation of lipoprotein-associated phospholipase A(2) levels and the risk of future cardiovascular events in women. J Am Coll Cardiol. 2001;38:1302-6.
53. Oei HH, Van dM, I, Hofman A, Koudstaal PJ, Stijnen T, Breteler MM, et al. Lipoprotein-associated phospholipase A2 activity is associated with risk of coronary heart disease and ischemic stroke: the Rotterdam Study. Circulation. 2005;111:570-5.
54. Bots ML, Elwood PC, Nikitin Y, Salonen JT, Freire DC, Inzitari D, et al. Total and HDL cholesterol and risk of stroke. EUROSTROKE: a collaborative study among research centres in Europe. J Epidemiol Community Health. 2002;56 Suppl 1:i19-i24.
55. Lindsberg PJ, Grau AJ. Inflammation and infections as risk factors for ischemic stroke. Stroke. 2003;34:2518-32.
56. Collins R, Armitage J, Parish S, Sleight P, Peto R. Effects of cholesterol-lowering with simvastatin on stroke and other major vascular events in 20536 people with cerebrovascular disease or other high-risk conditions. Lancet. 2004;363:757-67.
57. Iribarren C, Gross MD, Darbinian JA, Jacobs DR Jr, Sidney S, Loria CM. Association of lipoprotein-associated phospholipase A2 mass and activity with calcified coronary plaque in young adults: the CARDIA study. Arterioscler Thromb Vasc Biol. 2005;25:216-21.
58. 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.
59. Tsimihodimos V, Karabina SA, Tambaki AP, Bairaktari E, Goudevenos JA, Chapman MJ, et al. Atorvastatin preferentially reduces LDL-associated platelet-activating factor acetylhydrolase activity in dyslipidemias of type IIA and type IIB. Arterioscler Thromb Vasc Biol. 2002;22:306-11.
60. Tsimihodimos V, Kakafika A, Tambaki AP, Bairaktari E, Chapman MJ, Elisaf M, et al. Fenofibrate induces HDL-associated PAF-AH but attenuates enzyme activity associated with apoB-containing lipoproteins. J Lipid Res. 2003;44:927-34.
61. Caslake MJ, Packard CJ. Lipoprotein-associated phospholipase A2 (platelet-activating factor acetylhydrolase) and cardiovascular disease. Curr Opin Lipidol. 2003;14:347-52.
62. Blackie JA, Bloomer JC, Brown MJ, Cheng HY, Elliott RL, Hammond B, et al. The discovery of SB-435495. A potent, orally active inhibitor of lipoprotein-associated phospholipase A(2) for evaluation in man. Bioorg Med Chem Lett. 2002;12:2603-6.
63. Laterza OF, Price CP, Scott MG. Cystatin C: an improved estimator of glomerular filtration rate? Clin Chem. 2002;48:699-707.
64. Leung-Tack J, Tavera C, Martínez J, Colle A. Neutrophil chemotactic activity is modulated by human cystatin C, an inhibitor of cysteine proteases. Inflammation. 1990;14:247-58.
65. Al Suwaidi J, Reddan DN, Williams K, Pieper KS, Harrington RA, Califf RM, et al. Prognostic implications of abnormalities in

renal function in patients with acute coronary syndromes. Circulation. 2002;106:974-80.
66. Januzzi JL, Cannon CP, DiBattiste PM, Murphy S, Weintraub W, Braunwald E. Effects of renal insufficiency on early invasive management in patients with acute coronary syndromes (The TACTICS-TIMI 18 Trial). Am J Cardiol. 2002;90:1246-9.
67. Hsu CY, Chertow GM, Curhan GC. Methodological issues in studying the epidemiology of mild to moderate chronic renal insufficiency. Kidney Int. 2002;61:1567-76.
68. Shemesh O, Golbetz H, Kriss JP, Myers BD. Limitations of creatinine as a filtration marker in glomerulopathic patients. Kidney Int. 1985;28:830-8.
69. Larsson A, Malm J, Grubb A, Hansson LO. Calculation of glomerular filtration rate expressed in mL/min from plasma cystatin C values in mg/L. Scand J Clin Lab Invest. 2004;64:25-30.
70. Swan SK. The search continues: an ideal marker of GFR. Clin Chem. 1997;43:913-4.
71. Koenig W, Twardella D, Brenner H, Rothenbacher D. Plasma concentrations of cystatin C in patients with coronary heart disease and risk for secondary cardiovascular events: more than simply a marker of glomerular filtration rate. Clin Chem. 2005; 51:321-7.
72. Knight EL, Verhave JC, Spiegelman D, Hillege HL, de Zeeuw D, Curhan GC, et al. Factors influencing serum cystatin C levels other than renal function and the impact on renal function measurement. Kidney Int. 2004;65:1416-21.
73. Jernberg T, Lindahl B, James S, Larsson A, Hansson LO, Wallentin L. Cystatin C: a novel predictor of outcome in suspected or confirmed non-ST-elevation acute coronary syndrome. Circulation. 2004;110:2342-8.
74. Apple FS, Wu AH, Mair J, Ravkilde J, Panteghini M, Tate J, et al. Future biomarkers for detection of ischemia and risk stratification in acute coronary syndrome. Clin Chem. 2005;51:810-24.
75. Ridker PM, Brown NJ, Vaughan DE, Harrison DG, Mehta JL. Established and emerging plasma biomarkers in the prediction of first atherothrombotic events. Circulation. 2004;109 Suppl 1:IV6-19.
76. Kaski JC, Cruz-Fernández JM, Fernández-Berges D, García-Moll X, Martin JL, Mostaza J, et al. Marcadores de inflamación y estratificación de riesgo en pacientes con síndrome coronario agudo: diseño del estudio SIESTA (Systemic Inflammation Evaluation in patients with non-ST segment elevation Acute coronary syndromes). Rev Esp Cardiol 2003;56:389-95.
77. García-Moll X. Marcadores de inflamación y de antiinflamación en el síndrome coronario agudo: ¿listos para usarlos en la práctica clínica? Rev Esp Cardiol 2005;58:615-7.