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EM - REVIEW

Inflammatory biomarkers and coronary heart disease: from bench to bedside and back

Gina Biasillo · Milena Leo · Roberta Della Bona · Luigi Marzio Biasucci

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Abstract Inflammation plays a pivotal role in all stages of atherosclerosis from endothelial dysfunction and plaque formation to plaque destabilization and disruption. Inflammatory biomarkers, originally studied to better understand the pathophysiology of atherosclerosis, have generated increasing interest among clinicians, because of their utility in the challenging problems of diagnosis and risk assessment of patients with suspected or proved coronary heart disease. Moreover, in fascinating perspective, they could be used as therapeutic target, counteracting initiation, progression, and development of complications of atherosclerosis. In this review, we will provide an overview of the more promising inflammatory biomarkers, focusing on their utility and limitations in the clinical setting.

Keywords Inflammation · Biomarkers · Atherosclerosis · Coronary heart disease

Introduction

Inflammatory biomarkers, widely used in other pathological conditions, have been originally studied in cardiology to better understand the pathophysiology of Coronary heart disease (CHD). Subsequently they have generated increasing interest, also among clinicians, because of the important clinical implications of their use. Inflammatory biomarkers, in fact, have assumed an established role in the challenging problems of diagnosis and risk assessment of patients with suspected or proved CHD [1].

It is well established that the inflammatory process plays a pivotal role in all stages of atherosclerosis, from endothelial dysfunction and plaque formation to plaque destabilization and disruption with superimposed thrombosis: activated inflammatory cells as neutrophils, lymphocytes, monocytes and resident macrophages, pro-inflammatory cytokines, and molecules are principal players in this scenario.

Biomarkers of inflammation, therefore, may provide unique information to the clinician either on risk stratification or on the identification of plaque vulnerability in CHD patients. Moreover, because of the key pathogenic role of inflammation in the setting of atherosclerosis, in a fascinating perspective, inflammatory biomarkers might be used as therapeutic targets, counteracting the initiation, progression, and development of complications of CHD through inhibition of inflammatory pathways.

Currently studied inflammatory biomarkers include acute-phase reactants (CRP, SAA), cytokines (IL-6, IL-18, IL-10, MCP-1, TNFα), cellular adhesion molecules (sICAMs, sVCAMs, sSelectins), markers of plaque destabilization and rupture (MPO, MMPs, PIGF, PAPP-A, sCD40L) and markers of lymphocytes and monocytes activation (Fig. 1). In particular, patients with Acute Coronary Syndrome (ACS) have an increased frequency of CD4+ T lymphocytes with defective cell surface expression of CD28, a costimulatory molecule involved in determining the outcome of antigen recognition by T cells. CD4+ CD28null T cells infiltrate unstable coronary plaques, undergo clonal expansion, release large amounts of interferon (IFN)-gamma and activate monocytes and macrophages. Moreover, expansion of these unusual T cells is strongly associated with the recurrence of acute

G. Biasillo · M. Leo · R. Della Bona · L. M. Biasucci (✉)
Institute of Cardiology, Catholic University of Sacred Heart-Rome, L.go A. Gemelli, 8, 00168 Rome, Italy
e-mail: lmbiasucci@virgilio.it

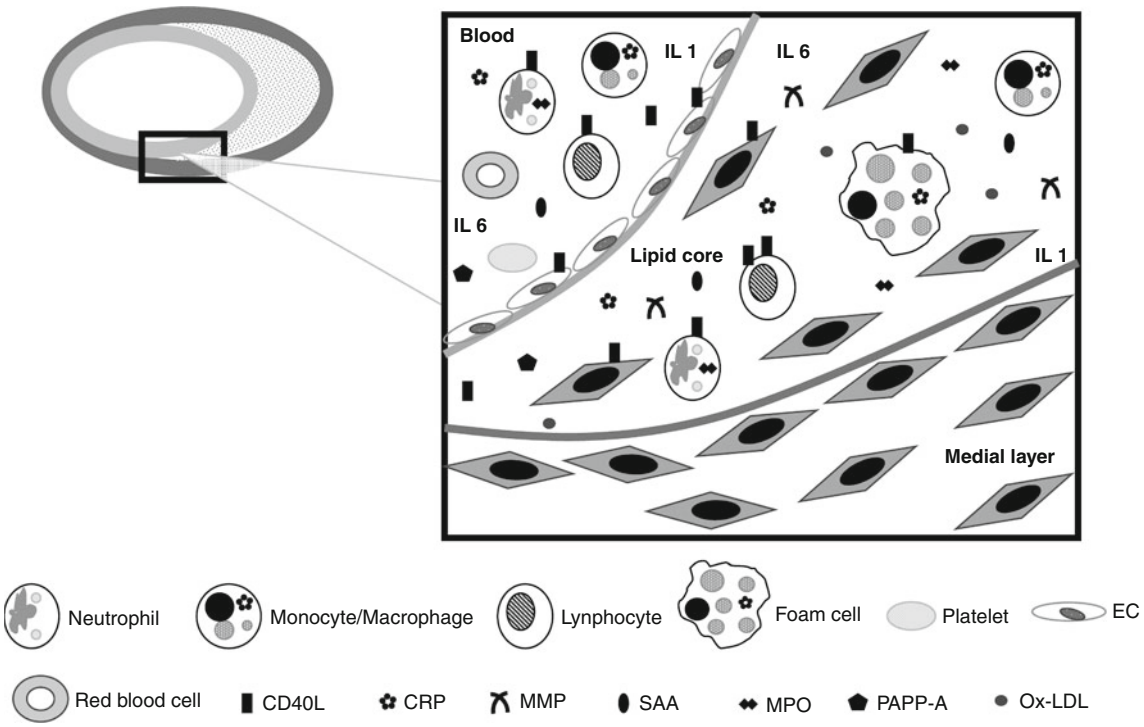

Fig. 1 Inflammatory biomarkers and vulnerable plaque

coronary events, thus conceivably playing a key pathogenetic role in plaque instability [2].

Other markers, as Fibrogen, von Willebrandt Factor, and PAI-1 are usually considered as typical pro-thrombotic markers, but, actually, they depend upon inflammatory stimulation, and may therefore be considered as inflammatory markers as well.

In this paper, we will review the available literature on the inflammatory biomarkers that are more promising from the clinical point of view, providing a critical overview on their utility and limitations.

Acute phase reactants

C-reactive protein (CRP)

CRP is an old biomarker, still the gold standard to detect and to monitor infective diseases, with a half-life of 19 h, and a well-established range of increase after an inflammatory stimulus up to 100-fold and more from baseline levels. CRP is the prototypic acute phase response protein; a member of the pentraxin family, is mainly produced by hepatocytes, and in a small amount by monocytes/macrophages and possibly by smooth muscle cells, in response to pro-inflammatory cytokines, such as IL-1 and IL-6. The biological roles of CRP are still partially unknown, the definitely proven effects being only the formation of complex with complement and the opsonisation of cell debris.

The interest of CRP in CHD derives from its sensitivity for the inflammatory process, but also from its long half-life, and robustness and reliability of its methods of assessment. Following any tissue injury, CRP levels begin to rise within 4–6 h, and continue to increase exponentially, doubling every 8–9 h, peaking within 24–48 h, and returning to normal once tissue integrity is restored. Since the first studies in the 1990s by Liuzzo e Biasucci [3], several authors have investigated the role of this biomarker in CHD. The introduction of Troponin I and T allowed researchers to dissect the role of inflammation from myocardial damage, recognizing inflammation as a primary component of the syndrome. This was described by Liuzzo [3] in ACS patients with negative TnI, and later by Ridker in the first study showing the association of CRP with the risk of future cardiovascular events in a healthy population. In the latter study, obviously, no tissue damage could have produced CRP elevation [4]. To date, CRP is the inflammatory marker most extensively assessed in prognostic studies, both in primary and in secondary prevention, and the only one recommended in guidelines. More than 20 different prospective studies have reported a significant and independent association between increased concentrations of CRP and future cardiovascular events in apparently healthy subjects. Increasing quartiles of CRP are associated with an

increasing risk of future CHD at up to 10-years follow-up in apparently healthy subjects, in elderly subjects. CRP may add important information to the Framingham risk score and to the family history in the population at intermediate risk, thus allowing an improved reclassification of these subjects in either high or low risk.

Zethelius [5] has shown that the simultaneous addition of several biomarkers of cardiovascular and renal abnormalities, such as TnI, NT-proBNP, Cystatin C, and CRP, in a population of elderly men with or without prevalent cardiovascular disease, substantially improves the risk stratification for death from cardiovascular causes over a 10-year follow-up period beyond that of a model that is based only on established risk factors.

Furthermore, in the JUPITER trial, the statin-mediated reduction of the CRP levels in subjects with normal LDL-cholesterol levels (<130 mg/dl), not candidates for statin therapy on the basis of NCEP III guidelines, is associated with a lower incidence of cardiovascular events at 2 years follow-up [6]. Finally, a recent metaanalysis has shown a clear association between CRP concentration and the risk of CHD, stroke and death from vascular and non-vascular diseases in individuals without a history of vascular disease. Although this association is attenuated by adjustment for conventional risk factors and other markers of inflammation, data emerging from the analysis support the idea that some processes related to persistent inflammation are linked to vascular disease [7]. So far, current evidence supports a key role of CRP as predictor of future cardiovascular events, particularly of death, in apparently healthy subjects.

In patients with Non ST segment-Elevation-ACS (NSTE-ACS), large studies have confirmed an independent strong value of CRP in predicting the recurrence of cardiac events, such as death, myocardial infarction and need for coronary revascularization procedures, especially in the mid to long term. In the short term, available data are contradictory. After the study by Liuzzo [3], showing a strong prognostic value of CRP for in-hospital death, Acute Myocardial Infarction (AMI), recurrent ischemia, and urgent revascularization in a selected population of patients with Unstable Angina (UA) and negative TnT, larger studies [8] have confirmed the association between CRP and short term mortality. Other studies, however, such as CAPTURE trial [9], fail to show a significantly increased risk of in-hospital events among patients with UA or Non ST segment-Elevation Myocardial Infarction (NSTEMI) and elevated CRP levels.

Stronger data are available about the prognostic value of CRP in the mid to long term, demonstrating that CRP levels predict recurrence of cardiac events, especially death, for up to 5 years, either in medically and in surgically or invasively treated patients; in these patients, pre-procedural CRP levels correlate with the incidence of restenosis after stent implantation [10].

To date, CRP is the only inflammatory biomarker currently recommended by NACB guidelines for risk assessment of patients with ACS (class IIa, level of evidence A) [11]. According to Centre of Disease Control/American Heart Association expert panel, held in 2004, CRP levels of 10 mg/L should be used as the cut-off in the acute phase of ACS, and a cut-off of 3 mg/L should be used in the follow-up [12].

In patients with ST segment-Elevation Myocardial Infarction (STEMI), no large study has prospectively assessed the value of CRP for both short- and long-term risk stratification; however, most of the available data suggest an association between CRP levels and recurrence of cardiac events. Several studies involving fewer than 200 patients with STEMI find an association between CRP concentrations and short-term (but not long-term) mortality at extremely high cut-off concentrations of hsCRP (e.g. >200 mg/L). A study of 1044 patients with STEMI finds an increased long-term risk of death with concentrations of hsCRP > 13.5 mg/L [13]. In OPUS-TIMI, 16 patients who presented with a STEMI and an increased concentration of hsCRP (>10 mg/L) had an increase in the long-term risk of death [14]. Growing evidence suggests a fascinating role of CRP as a therapeutic guide, both in patients with ACS and in healthy subjects, and, intriguingly, as direct therapeutic target. In patients with ACS, treatments associated with reduced mortality, such as Aspirin, Clopidogrel, gp IIb/IIIa inhibitors, ACE inhibitors, sartans and especially statins, are also associated with lowering of the CRP, and are most effective in patients with high CRP levels [15]. Different trials have shown a marked improvement in clinical outcome only when both LDL and CRP levels are lowered [16]. So far, it has been clearly shown that the presence of elevated CRP levels (>3 mg/L) at admission or their persistence after optimal revascularization demands an aggressive medical treatment, and the therapeutic goal of very low CRP levels seems very important and likely to be pursued. In addition, in apparently healthy subjects, data from the JUPITER trial [6] suggest that CRP screening might be an effective method to identify subjects who are more or less likely to benefit from statin therapy for cardiovascular risk reduction, regardless of cholesterol levels.

Of note, elevated CRP levels may be predictive of early relapses of atrial fibrillation after electrical cardioversion [17]; moreover, they may be used to guide implantation of cardioverter defibrillators, because of their correlation with the occurrence of ventricular tachydysrhythmia.

Intriguingly, the knowledge of pro-atherogenic effects of CRP and the results of the first experimental studies showing a beneficial effect of CRP inhibition, suggest that

CRP might be a valid therapeutic target. Pepys et al. [18], in particular, recently report that a small molecule, called 1,6 *bis*(phosphocholine) hexane, that binds and inhibits human CRP, reduces the size of MI in a rat model. Whether this molecule might turn out to be useful also in the prevention of ACS is currently unknown.

There are at least four potential targets for CRP inhibition: transcriptional inhibition of hepatic CRP synthesis, antisense therapy, blockade of CRP-mediated complement activation, and blockade of CRP receptors. The development of a specific anti-CRP therapy would also help to clarify the challenging relationship between CRP and CHD.

So far, work on CRP inhibitors is still in progress: further studies are needed to evaluate the efficacy and the feasibility of anti-CRP drugs: the potential for parallel pathways and redundancy of inflammatory contributors might limit their effect on CRP tissue damage; moreover, any adverse consequences of inhibiting CRP must be identified in humans, given the possibility that other inflammatory or infectious conditions can coexist in patients with acute myocardial infarction.

Serum amyloid A (SAA)

SAA is another acute-phase reactant recently tested as a marker of risk in CHD and directly implicated in atherosclerotic disease because of its pro-inflammatory and prothrombotic activity. SAA induces expression of IL-1, IL-18 and TNF in neutrophils, IFN-gamma in lymphocytes, Tissue Factor (TF) in monocytes; moreover, it promotes monocytes chemotaxis and adhesion and activates matrix-metalloproteinases (MMPs). Of interest, some authors observe SAA deposits in atherosclerotic lesions. As well as for CRP, SAA circulating levels seem to be associated with recurrent instability [3, 19] rather than myocardial necrosis. During AMI, SAA tends to increase within 24 h, peaking within 3 days, and the magnitude of the SAA response seems to be greater than CRP. Johnson et al. [20] find that SAA levels, but not CRP levels, are related to the severity of coronary artery disease in women who undergo coronary angiography for suspected myocardial ischemia; Kosuge et al. [21] observe a correlation between elevated SAA levels and adverse prognosis in NSTE-ACS patients: this association is independent of the raise in the CRP levels: moreover, elevated CRP levels with normal SAA are not associated with an adverse prognosis. A TIMI 11A substudy, conducted by Morrow et al. [22], finds that elevated levels of SAA, like CRP levels, are predictive of short-term mortality in a similar population of patients.

In contrast, SAA is not found to be associated with long-term risk in the ECAT study [23], and is only marginally associated with long-term risk in a study by Biasucci et al. [19], different from CRP. This lack of association could be explained in light of a different regulation of SAA and CRP production by cytokines and of a greater variability in SAA levels compared with CRP. Moreover, measurement of SAA in stable post-infarction patients in the THROMBO Study shows no significant association between SAA and risk of recurrent cardiovascular events over 2 years [24]: in this study, however, neither SAA or CRP are found to be independent markers of recurrent instability.

In conclusion, the role of SAA remains controversial, and because it does not provide incremental prognostic value over CRP in ACS patients, its routine use is not recommended.

Cytokines

Cytokines are proteins secreted by a wide variety of cells, such as leukocytes, platelets, endothelial cells (ECs), and smooth muscle cells (SMCs), important for regulating fundamental biological processes such as inflammatory and immune responses, but also involved in a wide range of diseases including CHD. Among all cytokines, IL-6 and IL-1 have been advocated as inflammatory biomarkers.

IL-6 is a multifunctional cytokine, secreted by many cell types, such as macrophages, ECs, SMCs, and T cells in response to a variety of stimuli through the production of IL-1, interferon-γ, and TNF. IL-6 promotes hepatic production of acute-phase reactants, including CRP, and differentiation of myeloid cells. Interestingly, IL-6 has been found in human atherosclerotic plaques, particularly at the shoulder region, where it can stimulate SMCs proliferation, increase plaque instability by driving expression of MMPs and TNF-α, and facilitate thrombogenicity by the release and activation of platelets. Several prospective studies have consistently shown that baseline levels of IL-6 are powerful predictor of cardiovascular events in apparently healthy subjects [25]. Furthermore, IL-6 levels are higher in patients with UA compared with those with stable angina, and if measured at 48 h after presentation for ACS, correlate with an adverse in-hospital prognosis, similar to acute inflammatory and septic diseases. In the FRISC-II study, elevated IL-6 (>5 ng/L) levels are associated with a higher 6- and 12-month mortality, and are additive to and independent of cardiac TnT status and of CRP levels. In the same study, elevated IL-6 levels, but not CRP, also identify a subgroup of patients who derive the greatest reduction in mortality from an early invasive strategy [26].

IL-1 is a pro-inflammatory cytokine, existing in two forms, IL-1α and IL-1β. Increased levels of IL-1β mRNA have been detected in human arteriosclerotic plaques, suggesting an involvement of this cytokine in the local inflammatory cascade and in the proliferation or differentiation of monocyte-derived cells and increased vascular permeability. IL-1Ra, another member of IL-1 gene family,

natural inhibitor of IL-1, whose production increases under the same conditions that stimulate IL-1α and IL-1β, is often measured as an indicator of IL-1 production because, different from IL-1α and IL-1β that are only marginally secreted, it is released into the systemic circulation. After the first study by Biasucci et al. [27], showing a strict association between an increase of IL-1Ra levels at 48 h after admission and complicated in-hospital course in UA patients, other small studies confirm their prognostic role in Stable Angina (SA) patients and ACS patients. The rise in IL-1Ra levels may precede the release of markers of necrosis, and, therefore, may also be an important early marker for the diagnosis of AMI in the Emergency Department (ED).

So far, cytokines such as IL-1 and IL-6 are promising biomarkers of CHD, but pre-analytical and analytical concerns (large circadian variations, a relatively short half-life, high-cost assays) and especially the lack of confirmatory and of large volume studies limit their inclusion in clinical routine.

However, better understanding of the role played by cytokines in atherosclerosis has stimulated the search for novel therapeutic strategies targeting the cytokine network: biologic agents inhibiting their pro-inflammatory activities such as cytokine receptor antagonists, some of which are natural endogenous inhibitors, anticytokine monoclonal antibodies, inhibitors of cytokines intracellular pathways as caspases or JAK/STAT pathway. In particular, Rizzello et al. [28] show that blockade of TNF-alpha inhibitor may modulate inflammation: the percentage of CD4+ CD28null cells is significantly reduced after incubation with infliximab, an anti TNF-alpha monoclonal antibody.

Although some of these therapeutic strategies are successfully used in rheumatological diseases, given the lifelong evolution of the atherosclerotic disease, the benefit of such approaches in CHD is likely to be lost after the withdrawal of treatment, implying a need for an indefinite drug administration, with the risks of chronic adverse side effects, including immunosuppression. Many studies are in progress to evaluate the feasibility and efficacy of these treatments in human atherosclerotic disease.

Myeloperoxidase (MPO)

MPO is a cationic protein, found predominantly in azurophilic granules of neutrophils and in some subtypes of monocytes/macrophages. Secreted after leukocyte activation, MPO induces the formation of potent oxidants, not only contributing to innate host defenses, but also promoting tissue injury. MPO, therefore, is implicated in several inflammatory conditions, including atherosclerosis. Both MPO and its reactive oxidants species have been detected in atherosclerotic lesions. In particular, among MPO oxidative derivatives, oxidized-LDL and HDL are involved in all stages of atherosclerosis. Moreover, MPO reduces Nitric Oxide (NO)-synthetase activity and catalytically consumes NO, contributing to endothelial dysfunction. Inactivation of protease inhibitors and activation of latent forms of elastases and MMPs contribute to degradation and rupture of the fibrous layer of the atherosclerotic plaque. MPO can also contribute to adverse ventricular remodeling through leukocyte migration to the perinecrotic zone, and reperfusion exposing the ischemic territory to further inflammatory and oxidative stresses.

Multiple lines of evidence recently have proposed MPO as a useful marker in CHD. In the EPIC-Norfolk Prospective Population Study, Mauwese et al. first demonstrate that elevated levels of MPO in apparently healthy individuals are associated with the risk of future development of CHD. However, although association between MPO and CHD is independent of other risk factors, there is not a significant increase in the Area Under Curve by adding MPO to the Framingham risk score [29]. In addition, Zhang et al. [30] observe that among patients undergoing cardiac catheterization, individuals with higher MPO values are more likely to have abnormal coronary angiograms. Intriguingly, in ACS, MPO seems to be associated with the presence of instability. Studies by Biasucci and Buffon [31] suggest that MPO is more than a marker of oxidative damage, and is independent of myocardial necrosis. In fact, MPO levels are related to the activation and degranulation of neutrophils, a critical event in coronary inflammation, preceding myocardial injury.

Recently, several studies evaluated the role of MPO as prognostic or diagnostic marker in ACS. Baldus et al. [32] observe an increased risk for subsequent cardiac events in the short and medium term among CAPTURE patients. The observation that MPO levels do not correlate with myocardial injury reinforces the hypothesis that MPO release is a prerequisite rather than a consequence of myocardial injury, while the lack of correlation between MPO and CRP levels suggests that activation of neutrophils precedes the release of other inflammatory markers. These findings are confirmed by Brennan [33], who evaluated MPO in patients with chest pain: its levels independently predict an early risk of AMI and major adverse cardiac events, and, in contrast to other biomarkers, MPO levels identifies patients at high risk even in absence of myocardial necrosis. Other studies show MPO utility in long-term risk stratification, up to 5 years [34]. In addition to this evidence evaluating the prognostic role of MPO, a small number of studies aim at the assessment of the effects of drugs on MPO levels. Zhou et al. [35] observe a significant reduction of the MPO and CRP levels in ACS patients after the administration of Atorvastatin in respect to individuals randomized for

treatment without cholesterol-lowering drugs. Baldus et al. [36], instead, evaluated the effect of Heparin administration, and observe that an increase of MPO serum levels, induced by Heparin, correlates with an improvement of endothelial function. Because of its pro-atherogenic properties, there is a great interest in development of therapeutic interventions to inhibit the MPO pathway. The main difficulty, at present, is related to the impairment in the role of this enzyme in host immune defense, but only individuals with a complete MPO deficiency have a significantly increased risk to develop infections, so it could be possible to target only extracellular MPO in order to maintain the leukocyte ability to kill microbes.

Some limitations to the utilization of MPO are related to analytic and pre-analytic factors: samples should be collected only in EDTA-containing tubes and centrifuged within 2 h. Moreover, administration of Heparin, determining separation of MPO from endothelial cells, as mentioned above, may increase MPO serum levels, thus affecting its measurement.

Matrix-metalloproteinases (MMPs)

MMPs are a family of genetically related proteins, sharing a common Zn^{2+}-based catalytic mechanism. MMP activity is increased by the transcription of MMPs pro-form genes and activation of proenzymes by proteolytic cascades, while inactivation is largely by binding to endogenous tissue inhibitors of MMPs (TIMPs). MMPs are widely expressed in monocytes/macrophages, endothelial cells and SMCs, fibroblasts, and neoplastic cells. Collectively MMPs have the ability to completely degrade collagen and most other components of extracellular matrix. They are involved in morphogenesis and wound healing, but also in vascular and cardiac remodelling, including atherogenesis and plaque destabilization, because of deregulated activation of these enzymes. MMPs are highly expressed in macrophage rich areas of the atherosclerotic plaque; they may modify soluble and cell surface proteins, including cytokines and chemokines, thus inducing death of macrophages and SMCs, and promote weakening of the fibrous cap, eventually increasing plaque instability.

Several studies demonstrate raised levels of MMPs in atherosclerotic diseases, such as peripheral artery disease [37] and ACS; in particular, MMP-1, MMP-2 and MMP-9 are significantly increased in patients with ACS compared to healthy controls, or in patients with more advanced CHD, although the time course of elevation is quite controversial. In the study by Kalela et al. [38], MMP-9 levels are also found to correlate with the extent of Coronary Artery Disease (CAD) in a cohort of 61 ACS patients. Few data exist on the association between MMPs levels in ACS and cardiovascular outcomes. In the AtheroGene study [39], enrolling 1227 patients with angiographically confirmed CAD but not definite ACS, increased concentrations of MMP-9 are associated with an increased risk of future cardiovascular death. Interestingly, in this study, as in others, higher concentrations of TIMPs are also predictive of future cardiovascular events. Of note, MMP-9 or MMP-1 levels seem to correlate with myocardial damage extent, thus being helpful in evaluating the ventricular remodelling that occurs after AMI, and in predicting a worse left ventricular ejection fraction after ACS. Further studies are needed to prove their real clinical usefulness as biomarkers of risk.

Because all MMPs play an important pathogenic role in plaque destabilization and in ventricular remodelling after AMI, they also represent an active area of investigation as therapeutic target. Besides natural TIMPs, drugs as barimastat, batimastat, ag3340, bay-219566 are at an advanced stage of experimentation in cancer disease in randomized studies versus placebo [40]. In patients with ACS, the need for an indefinite drug administration, with the risks of chronic adverse side-effects and the fear of losing the potential positive effects deriving from plaque remodelling by MMPs, limit, to date, the usefulness of these drugs.

Pregnancy-associated plasma protein A (PAPP-A)

PAPP-A is a protease, mainly produced by the placental syncytiotrophoblast during pregnancy, but also by other tissues and cells (fibroblasts, osteoblasts, vascular SMCs). PAPP-A produced during pregnancy forms a complex with an endogenous inhibitor, proMBP. The non-pregnancy related form is not bound to proMBP. "Free" PAPP-A has a metalloproteolytic activity, leading to cleavage of IGF-BP4, and eventually to the increase of IGF-1. Several studies demonstrate that IGF-1 promotes atherogenesis through migration and proliferation of smooth muscle cells, chemotaxis, and release of pro-inflammatory cytokines. On the other hand, a few authors show anti-inflammatory and anti-oxidant effects of IGF-1, suggesting that activation of the PAPP-A/IGF-1 system may promote an anabolic and reparative process. Whether PAPP-A is mainly involved in development and growth or in healing of atherosclerotic lesions is not completely clear; increasing evidence, however, suggest a correlation between early activation of the PAPP-A/IGF-1 system and cardiovascular diseases. Bayes-Genis et al. [41] first demonstrate an expression of PAPP-A within ruptured and eroded plaques, but not in stable ones.

PAPP-A has been evaluated in patients with chronic SA, in whom it is a strong predictor of the occurrence of adverse events in the long-term [42]. Increased PAPP-A

levels are also observed in asymptomatic patients with hyperlipidaemia. An interesting correlation between PAPP-A and the lesion status of the carotid artery is found in a similar population. Taken together, these observations suggest that PAPP-A may reflect an inflammatory state involving remodeling of subendothelial extracellular matrix, being a very early marker of the degree of atherosclerotic process.

The role of PAPP-A in ACS still remains controversial. Since the first study conducted by Bayes-Genis et al. [41] demonstrating an increase in its levels in NSTE-ACS patients compared with stable ones, other authors have evaluated the role of PAPP-A both in selected ACS patients and in heterogeneous populations of patients presenting to the ED with chest pain [43]. PAPP-A is useful in the early diagnosis and risk assessment of ACS, even among TnT negative patients. The lack of correlation between PAPP-A and TnT suggests that PAPP-A is not produced and released in response to necrosis, thus reinforcing the hypothesis of its involvement in plaque destabilization. In light of these findings, it is possible that this molecule is synthesized by activated cells and released into the extracellular matrix, thus contributing to fibrous cap weakening, and eventually to rupture. Therefore, PAPP-A might identify patients in the phase of instability preceding the rupture of a plaque, when it is most beneficial to receive more intensive treatment. In contrast to previous observations, some authors find no difference in PAPP-A levels between AMI and no-AMI patients [44]. Moreover, a study evaluating PAPP-A levels in patients presenting to the ED with chest pain reveals that it has only a modest predictive value and does not perform better than TnT, being similarly sensitive, but less specific [43]. There is no correlation between PAPP-A and TnT, as reported in previous studies. The reason for the differences observed in the studies may lie in different study populations and in the pre-analytical and analytical factors affecting PAPP-A measurement. As reported above, two forms of PAPP-A exist, one complexed with proMBP, and one defined free PAPP-A. The latter form is more specific for ACS, but assays applied in the majority of the studies detect total PAPP-A (both complexed and uncomplexed form). Although a direct measurement of PAPP-A would be the ideal method, a specific assay is not yet available. Two separate double monoclonal assays, one for total PAPP-A and one for PAPP-A/proMBP complex, with the difference representing free-PAPP-A can be used.

In conclusion, PAPP-A may represent a marker of plaque instability, providing information on top of other markers. Further studies, however, could better address whether PAPP-A causes plaque instability, or myocardial ischemia is a trigger for release of PAPP-A, which eventually contributes to healing and repair of the lesions.

Soluble CD40 Ligand (sCD40L)

CD40 belongs to the TNF receptor superfamily, and is expressed by B lymphocytes, ECs, SMCs and macrophages; CD40L is expressed on ECs, SMCs, T lymphocytes and platelets. CD40L is cryptic in unstimulated platelets, and is expressed on their surface within seconds of activation, and cleaved to generate a soluble fragment, sCD40L, released into the circulation. Both CD40 and CD40L are expressed in human atheroma, and their interaction may mediate several proatherogenic processes: adhesion of immunocompetent cells, expression and release of chemokines, TH1-mediated response, migration and proliferation of SMCs, loss of extracellular matrix through the activation of MMPs, determination of a procoagulant status through expression of TF, and formation of neovessels via expression of VEGF by activated ECs or release of IL-8 [45]. Micro-particles expressing CD40L, isolated from human atheroma, stimulate endothelial cell proliferation and promote angiogenesis. sCD40L has pro-inflammatory and pro-thrombotic activity, and can mediate several events within the vascular wall. However, "soluble CD40L" is a pool of free soluble, microparticle-bound and microvescicle-associated forms, the proportion of which is very highly variable.

Markedly elevated plasma sCD40L predicts an increased risk of CV events among apparently healthy women. The lack of association between sCD40L and atherosclerotic risk factors or measures of subclinical atherosclerosis may suggest that sCD40L performs better as a marker of plaque instability than a marker of plaque burden, and that a high level of plasma sCD40L reflects aspects of risk distinct from those provided by traditional risk factors. A few studies evaluate the role of sCD40L in ACS. Aukrust et al. [46] first observe significantly higher sCD40L levels in ACS patients with respect to controls and SA patients, thus indicating a significantly increased risk of CV events, even among TnT negative patients. Evaluation of this biomarker among a population presenting to the ED with chest pain, confirms that sCD40L identifies patients at higher risk of adverse events [47]. These findings suggest that sCD40L may constitute a reliable marker of inflammatory and thrombotic processes leading to plaque instability; moreover, sCD40L may provide information beyond those provided by Troponin. Nevertheless, other authors recently find only a weak trend toward increased risk in ACS patients with elevated levels of sCD40L treated with gpIIb/IIIa antagonists [48]: despite the possible influence of antiplatelet therapy on sCD40L levels. These results raise some caution regarding the strength of sCD40L as a biomarker for clinical application.

Intriguingly, in stable patients who undergo angiography, sCD40L levels do not predict CHD, and are not

associated with an increased risk of cardiovascular events. Moreover, higher sCD40L levels are associated with a decreased risk of CHD [49]. This apparently paradoxal finding can be explained considering sCD40L as an indicator of platelet activation and thrombotic activity, upregulated in ACS, but not in stable CAD rather than being a marker of chronic vascular inflammation.

Finally, preanalytical and analytical factors can interfere with measurements of sCD40L: it is very important to define standard techniques of measurement, in order to better interpret results of the studies and to clarify the clinical relevance of this biomarker.

Many studies have shown that several therapeutic agents may modulate sCD40L levels. Statins have been shown to reduce sCD40L levels in hyperlipidemic patients, without correlation with the degree of cholesterol lowering. Interesting data emerge from the MIRACL study: although administration of Atorvastatin markedly reduces the risk of recurrent events associated with higher sCD40L in ACS patients, no significant difference is observed in sCD40L levels over the treatment period [50]. This apparently paradoxical observation can be explained taking into account that the statins target many functions that are downstream from CD40 ligation, such as expression of adhesion molecules or TF, release of cytokines, or activation on MMPs. As for antiplatelet agents, Aspirin does not seem to decrease sCD40L [46], whereas both Clopidogrel and gpIIb/IIIa inhibitors [47] diminish sCD40L levels. Because of the relevance of the CD40/CD40L interaction in the pathophysiology of atherothrombosis, inhibition of this system stimulates an increasing interest in sCD40L as a direct therapeutic tool. In vivo studies demonstrate an improvement in the outcomes of animals with experimental chronic inflammatory diseases treated with anti-CD40L antibodies. Although these findings seem to shed new light on the management of atherosclerosis, attention should be paid in their interpretation, as for clinical implications. Long-term interruption of the CD40 pathway may trigger severe immunodeficiencies, exposing individuals to infectious diseases. Distinct pathways involving different atheroma-associated cell types may be more specific therapeutic targets, but at present, no studies have investigated this fascinating hypothesis.

Conflict of interest None.

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