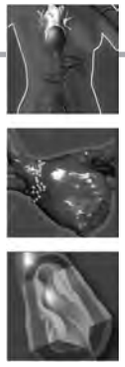

Biomarkers for diagnosis of the vulnerable atherosclerotic plaque

Atherosclerosis is a systemic disease affecting all vascular beds. The atherosclerotic plaque, as a first manifestation of the disease, involves a complex pathophysiological mechanism that is differentiated accordingly during its formation, progression and destabilization. This cascade of biomechanisms is orchestrated by the endothelium and is accompanied by the production and secretion of a variety of proteins referred to as biomarkers. The 'vulnerable' plaque is a term which describes a plaque that is destabilized and thus prone to induce atherosclerotic cardiovascular events. The determination of biomarkers related to vulnerable atherosclerotic plaques in blood serum may aid in the planning of intervention and in choosing the best medical treatment. Moreover, the utilization of biomarker panels can increase the accuracy of predicting the transformation of the atherosclerotic plaque into a vulnerable one.

KEYWORDS: atherosclerosis ■ biomarkers ■ vulnerable plaque

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Atherosclerotic cardiovascular disease is a major cause of morbidity and mortality worldwide, accounting for more than 19 million deaths per year [1]. Atherosclerotic patients present with a significant overlapping of vascular disorders implicating peripheral arterial disease (PAD), coronary heart disease and carotid arterial disease and cerebral disease. Atherosclerosis is a systemic disease affecting large and medium-sized arteries with lipid and fibrous accumulation within the intimal layer.

The genesis and progression of atherosclerotic plaques are accompanied by the release of a series of proteomic mediators of inflammation and significant chemotactic activity. These mediators carry the potential to be utilized as biomarkers, defined as measurable proteins, peptides, genes or metabolic products that represent biologic processes in an organism at a given time [2]. Biomarkers are indicators of disease states and encompass a spectrum of molecules with certain 'ideal' characteristics as proposed by Thomas *et al.* [3]. Even though atherosclerotic plaque imaging can be a much more precise predictor of destabilization, its employment – in the majority of the modalities used to acquire images – can be costly and is not as practical as the measurement of a serum biomarker. In addition, depending on the plaque anatomic location, the appropriate imaging required ranges from the simple, quick and cost effective use of ultrasound to the expensive magnetic resonance technology.

As demonstrated by many authors, the majority of plaque ruptures are clinically silent [4–6]. However, besides serving as surrogate markers of drug efficacy or as markers for patient stratification, the role of biomarkers in detecting the vulnerable (unstable, thrombogenic) plaques is a field of great interest due to its potential to also aid in the prevention of cardiovascular events.

Physiology of atherosclerotic plaque formation

The starting point for atheroma formation is endothelial dysfunction or activation [7,8]. In addition, others have suggested that the primary factor that initiates plaque formation is the response to lipoprotein retention, which in turn reduces the endothelial threshold to sheer stress [9]. At present, the most important contributors of endothelial dysfunction are hemodynamic disturbances, hypercholesterolemia and inflammation. Etiologic factors also include cigarette toxins, homocysteine and a wide spectrum of infectious agents. Chronic endothelial injury eventually results in endothelial dysfunction and increased permeability and induces LDL oxidation and accumulation in the subendothelial space of the intima [10] as well as the expression of adhesion molecules (e.g., vascular cell adhesion molecule [VCAM]-1, ICAM-1, and P selectin) and chemokines (e.g., monocyte chemoattractant peptide [MCP]-1) that participate in platelet aggregation, lymphocyte and monocyte adhesion and infiltration, thus initiating the inflammatory process [11–16]. As monocytes are

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attracted to the endothelium and migrate to the subendothelial space, they mature into macrophages and uptake oxidized LDL transforming into 'foam' cells that eventually form the lipid core of the atherosclerotic plaque after apoptosis occurs [17]. This inflammatory mediator cascade promotes a phenotype change of vascular smooth muscle cells (VSMCs) from the 'contractile' phenotype state to the active 'synthetic' state. VSMCs in the synthetic state can migrate and proliferate from the media to the intima, where they produce excessive amounts of extracellular matrix (e.g., collagen, elastin and proteoglycans) that transforms the lesion into a fibrous plaque [18]. The typical atherosclerotic plaque comprises of the lipid core and the fibrous cap, and is the most commonly classified histologically by the American Heart Association-recommended Stary classification [19].

The vulnerable atherosclerotic plaque

Vulnerable atherosclerotic plaques (high-risk or unstable plaques) are associated with an increased risk of disruption, distal embolization and vascular events. They are histological lesions with a large lipid core, a thin fibrous cap, and may contain ulceration, intraluminal thrombosis and intraplaque hemorrhage, as well as intense infiltration of macrophages and other inflammatory cells. Inflammation plays a key role in the pathogenesis of atherosclerosis, and the immune system and oxidative stress seem to be involved in the initiation, propagation and activation of such lesions in the arterial wall [19–22]. Unstable plaques are rich in inflammatory cells that destroy the fibrous cap and are responsible for endothelial denudation and therefore thrombogenicity of the plaque contents. Rupture depends on the balance between inflammatory cell activity and the VSMC-driven repair process under the influence of the hemodynamic stress exerted on it [23]. When rupture takes place, the fibrous cap appears to be eroded at the shoulder of the lesion (where the fibrous cap meets the intima of the normal segment of the vessel wall) [24].

Activated macrophages, T cells and mast cells produce a variety of molecules – inflammatory cytokines, proteases, coagulation factors, radicals and vasoactive molecules – that are expressed in the plaques and may modulate extracellular matrix remodeling, cell proliferation, cell death (apoptosis) and ultimately destabilize these lesions [19–22,25]. These molecules include VEGF, VCAM-1, ICAM-1, MCP-1, cathepsins, P selectin, endothelin-1, platelet-activating factor, NF-κB, tumor necrosis factors, interleukins and leukotactin-1. T cells in the region of the fibrous cap produce IFN-γ, a potent inhibitor of collagen synthesis, inducing apoptosis of VSMCs [25]. Furthermore, elevated expression/activity of several matrix metalloproteinases (MMPs; including -1, -3 and -9), the main physiological regulators of the extracellular matrix, seems to play an important role in plaque activation [66].

Biomarkers & vulnerable atherosclerotic plaques

Various biomarkers have been studied as candidates for monitoring the progression of atherosclerotic disease and the majority of them are implicated in different stages of the pathophysiological mechanism of plaque formation and evolution. As a consequence, the diagnostic/prognostic weight of each one of them leans either towards progression or to the direction of plaque destabilization (**Box 1**) [26]. In addition, some of the changes in circulating marker levels may be a consequence of silent plaque rupture and/or subsequent healing. Detection of vulnerable or rupture-prone lesions is of paramount importance so that necessary clinical steps can be taken to prevent the deleterious clinical sequelae associated with symptomatic plaque rupture.

Specificity and sensitivity vary for each one of these biological markers and they are also different for each vascular bed. In order to detect a vulnerable atherosclerotic plaque that is more likely to cause cardiovascular events, one should focus on studying biomarkers more associated with destabilization rather than disease progression.

■ Complement reactive protein

Complement reactive protein (CRP) is an acute phase protein, primarily synthesized by hepatocytes, and induced by IL-6 with synergistic enhancement of IL-1 or TNF [27]. A rise in CRP levels is common in both infectious and noninfectious disorders, including myocardial infarction [28]. Up until now, it is the only inflammatory marker used in clinical practice and it should be emphasized that its predictive value can be estimated only through high-precision assays. It is within these lower ranges that the hs-CRP levels seem to have predictive abilities for cardiovascular events. A hs-CRP level of >10 mg/l, for example, should be discarded and repeated in 2 weeks to allow acute inflammations to subside before retesting.

Studies have consistently reported that elevated CRP serum levels definitely have a prognostic value for cardiovascular events and

mortality [29]. CRP has been proposed to induce a prothrombotic state via induction of tissue factor expression in human monocytes [30]. It can activate or inhibit the complement system, driving the inflammation in atherosclerotic lesions [31]. CRP has also been demonstrated to decrease the expression and bioactivity of endothelial nitric oxide synthase [32] with a subsequent effect on vasodilatation. CRP downregulates both basal and VEGF-stimulated angiogenesis, whereas it promotes endothelial apoptosis in a nitrous oxide-dependent fashion [33]. CRP has also been found to synergistically enhance angiotensin II-induced proinflammatory effects, involving cellular migration and proliferation as well as lesion collagen and elastin content [34]. Finally, it induces the release of MCP-1 and endothelin-1 upregulating adhesion molecules and chemoattractant chemokines in endothelial cells and VSMCs [35].

Commercially available high-sensitivity assays for CRP are cost effective and reproducible [36]. Unlike many other inflammatory mediators, CRP is not subject to diurnal fluctuation or biological variance, and CRP concentration appears to be proportional to disease severity [37]. Unfortunately, a major limitation is that CRP is elevated in systemic inflammation [37], which may limit its use as a prognostic marker in postoperative patients.

Box 1. Proposed biomarkers for the study of initiation, progression and destabilization of the atherosclerotic plaque.

Plaque progression
- Homocysteine
- MCP-1
- ICAM-1
- P selectin
- oxLDL
- Adiponectin
- FV Leiden
- Lp-PLA_2
- PARs
- Leptin
- PAI
- LOX-1

Plaque destabilization
- CRP
- Fibrinogen
- WBC
- IL-6
- IL-18
- TNF-α
- sCD40L
- MPO
- MMPs
- OPN–OPG

CRP: Complement reactive protein; LOX: Lipo-oxygenase; Lp-PLA: Lipoprotein-associated phospholipase A; MCP: Monocyte chemoattractant protein; MMP: Matrix metalloproteinase; MPO: Myeloperoxidase; OPG: Osteoprotegerin; OPN: Osteopontin; oxLDL: Oxidized LDL; PAI: Plasminogen activator inhibitor; PAR: Protease-activated receptor; sCD40L: Soluble CD40 ligand; WBC: White blood cell.

■ Fibrinogen

Fibrinogen is a glycoprotein that circulates at a high concentration in blood and initially mediates platelet aggregation. Later in clot formation it is converted to fibrin, which in turn is organized in a matrix defining the clot shape providing strength, flexibility and stability.

More than 40 years ago, fibrinogen was demonstrated to be elevated among patients with acute thrombosis. Hyperfibrinogenemia produces a dense and tight network of fibers which demonstrates reduced fibrinolysis [38]. It increases plasma viscosity and induces VSMC proliferation. The Gothenburg Heart Study was the first prospective trial to demonstrate an association between fibrinogen levels and subsequent cardiovascular disease risk. In another study, higher levels of fibrinogen predicted subsequent acute coronary syndromes (ACS) while lower levels, despite elevated cholesterol levels, were associated with lower risks of ACS [39]. Elevated fibrinogen levels in patients with PAD are associated with increased risk of fatal cardiovascular complications [40].

However, it remains unclear whether elevated fibrinogen levels are a cause or consequence of atherosclerosis. In the Copenhagen City Heart Study the relative risk of developing a stroke was almost double in patients with higher fibrinogen levels. Nevertheless they were not associated with echolucent unstable – and therefore vulnerable – carotid plaques [41].

■ White blood cell count

The white blood cell count in peripheral blood is usually increased in inflammatory and infectious conditions and could also be affected in plaque inflammation. Higher leukocyte count is associated with a greater cardiovascular risk. In a meta-analysis of seven prospective studies comparing the top with the bottom third of the value distribution, the relative risk of coronary disease was 1.4 (95% CI: 1.3–1.5) [42], rendering leukocytes a valuable marker.

■ IL-6, IL-18 & TNF-α

Cytokines are key regulatory glycoproteins allied to inflammatory/immunological processes which modulate all aspects of vascular inflammation. Many cytokines have been implicated

in atheroma formation and complication. The Edinburgh artery [43,44], InCHIANTI [45] and MESA [46] studies have all individually established the role of IL-6 as an independent predictor of PAD in community screening, irrespective of ethnicity. IL-6 enhances cell adhesion molecule expression and enhances the production of acute phase reactants such as CRP and TNF-α by the hepatocytes. While exogenous administration of IL-18 in mice enhances atherosclerotic lesions [47], there is a converse reduction in atherosclerosis inhibiting IL-18 [48]. IL-18 has a bearing on the progression and stability of human atherosclerotic plaques [49]. TNF-α is involved in atherosclerotic progression from the initial stages of intimal thickening to the subsequent vessel occlusion. It stimulates selectin and adhesion molecule expression and MMP -1–9, -11 and -13 production in the endothelium, VSMCs and macrophages [50]. Locally, within the atheroma, it increases expression of tissue factor, a potent thrombogenic protein [51].

A limitation of IL-6 is the presence of diurnal fluctuations and large biological variance [51]. Furthermore, cytokines are present only in picogram per milliliter quantities, and the production of a standardized, sensitive and specific immunoassay is both difficult and expensive.

■ Circulating soluble CD40 ligand

Circulating soluble CD40 ligand (sCD40L), largely derived from activated platelets, activates an inflammatory reaction in vascular endothelial cells by the secretion of cytokines and chemokines. Membrane-bound CD40L and sCD40L forms interact with the CD40 receptor molecule, leading to the release of matrix MMPs and subsequent destabilization of the plaque [52]. Elevated plasma concentrations of sCD40L at baseline predict a subsequent increased risk of future cardiovascular events in apparently healthy women and in angina-stable patients.

■ Vascular calcification markers

Atherosclerotic plaque calcification enhances plaque stability and decreases the likelihood of clinical events [53]. A growing number of stimulatory and inhibitory molecules suggest that vascular calcification is an actively regulated process. Among these molecules osteopontin (OPN), an acidic phosphoprotein, and osteoprotegerin (OPG), a member of the TNF-α receptor superfamily, have recently been demonstrated to inhibit mineral deposition as well as osteoclastogenesis and they are constitutively expressed by a wide range of cell types in the vasculature [53–56].

These bone-matrix proteins, which attenuate vascular calcification, have emerged as novel markers of atherosclerotic plaque composition and cardiovascular disease prognosis. Data derived from clinical studies support the notion that increased serum levels of the aforementioned markers are positively associated with acute cardiovascular events, coronary disease severity and poor long-term cardiovascular outcomes [57–61]. Although the large prospective study by Nybo *et al.* observed no association of baseline OPG with ischemic stroke [62], recent studies have demonstrated a strong relationship of serum OPN and OPG levels with low carotid plaque echogenicity, while enhanced immunodetection of OPN and OPG in human carotid plaques indicate their contribution to plaque instability [63]. At present, only one study concerning pharmaceutical interventions with intensive lipid-lowering therapy with statins has demonstrated the attenuation of serum OPN and OPG levels and enhanced carotid plaque echogenicity, and thereafter stability, in patients with carotid stenosis [64].

■ Matrix metalloproteinases

Matrix metalloproteinases are an ever-expanding family of zinc-dependent endopeptidases with proteolytic activity toward one or more components of the extracellular matrix [65]. Growing evidence supports the strong relationship of MMPs with plaque instability and consequent cardiovascular events [66,67]. Histopathological studies and experimental models have revealed the overproduction of MMPs in the rupture-prone regions of atherosclerotic plaques [68,69]. This excessive proteolytic activity facilitates extracellular matrix cleavage and fibrous cap degradation with eventual acute plaque rupture. Concerning clinical studies, increased serum levels of MMPs or MMP genotypes (e.g., MMP-3 polymorphism) have been observed to be closely associated with atherosclerotic manifestations, such as coronary artery disease or ischemic stroke. Moreover, biomarker studies have shown greater levels of MMPs (e.g., MMP-9) in ACS, such as unstable angina and myocardial infarction compared with stable coronary artery disease.

Matrix metalloproteinases are predominantly found at several stages of atherosclerotic plaque development and their activity is tightly regulated at three levels: control of gene transcription by a wide spectrum of factors (e.g., inflammatory cytokines); secretion as latent enzymes and activation by proteases such as other MMPs; and inhibition by tissue inhibitors of MMPs (TIMPs).

Both clinical and experimental studies provide a wealth of information concerning the crucial role of MMPs and TIMPs in intimal thickening and plaque destabilization. Atherosclerotic plaque formation constitutes a net matrix deposition, in part mediated by VSMC migration and proliferation. Until now the evidence for MMP-2 activation and MMP-9 upregulation during neointima formation is abundant. At the initial stages of atherogenesis, MMPs have also been hypothesized to mediate subintimal inflammatory cell infiltration [70]. Therefore, degenerative proteases seem to additionally promote lipid-necrotic core formation in the atherosclerotic plaque [66,71,72].

In one study, MMP-9 plasma concentrations predicted stroke and cardiovascular death in patients with ≥50% carotid stenosis, though not independently [67]. These findings were further confirmed by two nonprospective studies comparing symptomatic and asymptomatic carotid arterial disease patients [73,74]. The positive predictive value of MMP-9 was significantly enhanced when combined with other members of the MMP family (MMP-7 and MMP-8 and their tissue inhibitor TIMP-1) [74] or with plaque echolucency [75]. Histological analysis of specimens obtained from patients with unstable angina has demonstrated a remarkable increase in intracellular MMP-9 levels than in stable angina. Similarly, plaques extracted from symptomatic patients within 1 month before undergoing carotid endarterectomy contain fourfold higher concentrations of MMP-9. It has been demonstrated that inhibition of TGF-β signaling alters plaque stability by altering extracellular matrix components by means of attenuating collagen deposition and increasing MMP activity due to reductions in TIMPs [76–78]. Other members of the MMP family, including excessive MMP-7, MMP-8 and MMP-12, have been observed in carotid plaques with morphological characteristics of vulnerability [75,79–81].

Finally, it has been demonstrated that cyclooxygenase/prostaglandin E (PGE) synthase-1 (COX-2/mPGES-1) are overexpressed in symptomatic plaques in association with PGE_2-dependent MMP biosynthesis and plaque rupture. This seems to be mainly achieved through PGE_2 EP4 macrophage receptor interaction inducing MMP production [82–85].

■ Myeloperoxidase

Myeloperoxidase (MPO) is a hemoprotein produced by polymorphonuclear neutrophils and macrophages and catalyzes the conversion of chloride and hydrogen peroxide to hypochlorite [86]. It is released into the extracellular fluid and general circulation during inflammatory conditions. MPO and its products are involved in the oxidation of lipids contained within LDL particles, and is thought to promote the formation of foam cells in atherosclerotic plaques [86]. Inflammatory cells producing MPO are found more frequently and in higher concentrations in the culprit lesions of patients with ACS than in patients with stable disease [87,88]. Together with MMPs, MPO degrades the collagen layer of atheroma leading to erosion or rupture of plaques and its fatal consequences [87–89]. Thus, MPO has been proposed as a marker of plaque instability even if it is not specific to cardiac diseases, as activation of neutrophils and macrophages can occur in infectious, inflammatory or infiltrative disease processes. Recently, several assays for MPO have been approved for clinical use.

Biomarkers in coronary heart disease

Despite the development of many markers associated with myocardial ischemia and injury, cardiac troponin is still the preferred marker in this category owing to its myocardial tissue specificity and related sensitivity, as well as its established usefulness for therapeutic decision-making [90–92]. However, troponin is only elevated in acute coronary syndromes and is used to assess myocardial ischemia.

The hypothesis that MPO is involved in the inflammatory process that precedes the onset of symptomatic coronary artery disease by many years was supported by findings in more than 3000 patients of the European Prospective Investigation into Cancer and Nutrition–Norfolk population study [93]. Elevated levels of MPO independently predicted future risk of coronary artery disease in apparently healthy individuals (odds ratio for the highest quartile of MPO: 1.36; 95% CI: 1.07–1.73). The potential usefulness of MPO for risk stratification was demonstrated in an analysis of 1090 patients with ACS from the CAPTURE trial [94]. At a cutoff of 350 µg/l, MPO demonstrated an adjusted hazard ratio for the 6-month incidence of death and acute myocardial infarction of 2.25 (95% CI: 1.32–3.82). The effects were particularly impressive in patients with undetectable cardiac troponin with an adjusted hazard ratio of 7.48 (95% CI: 1.98–28.29). The predictive ability of MPO was independent of the levels of cardiac troponin, CRP and sCD40L, suggesting that MPO levels reflect a different aspect of ACS.

Two additional studies of patients with ACS also demonstrated that the prognostic information from MPO was independent from that of N-terminal pro-brain natriuretic peptide [95,96]. In another cohort of 604 patients presenting with symptoms suggestive for ACS, increasing concentrations of MPO were predictive for major cardiovascular events [97]. MPO levels at baseline independently predicted the risk of acute myocardial infarction and other major adverse coronary events at 30 days, even if patients initially had undetectable cardiac troponin levels, suggesting that MPO might be helpful in the early risk stratification of patients with ACS.

Biomarkers in carotid & cerebral arterial disease

Several studies indicate a higher carotid artery stenting (CAS)-associated risk of macro- and microembolization compared with carotid endarterectomy (CEA) [98–100]. Currently, there are no studies indicating biological markers as an independent risk factor related to a higher embolic potential for CAS when compared with CEA. Biological markers have been associated with increased perioperative risk of embolization, with both CEA and CAS. In a recent study, pre-CEA levels of high senditivity CRP (hsCRP) and fibrinogen have been proposed as independent determinants of new periprocedural cerebrovascular ischemic events [101]. When comparing the group with new diffusion-weighted imaging lesions in particular, the former had significantly higher levels of fibrinogen and hs-CRP [101]. Correspondingly, pre-CAS-elevated CRP (>5 mg/l) [102] and IL-6 [103] have been demonstrated as powerful predictors of stroke, while elevated preprocedural white blood cell count independently predicted more frequent microembolic signals in transcranial Doppler [104].

A comparative study of risk-adjusted patients with elevated biological markers, randomized to CEA or CAS, would identify whether biological markers have a role in technique selection. In the absence of available data, this issue remains unresolved and is open for further investigation. The Stent Protected Angioplasty versus Carotid Endarterectomy 2 (SPACE-2) study has included substudies on biomarkers that will be available within the next 5 years [105].

Biomarkers in PAD

There is a strong clinical need for more specific biomarkers for PAD. A blood test for PAD would increase recognition of the disease and thereby improve clinical care. It is likely that a biomarker panel with high sensitivity and high specificity for PAD will be composed of biomarkers that circulate systemically but reflect the activity of local pathophysiologic processes [106–116]. Patients with PAD are at increased risk of cardiovascular morbidity and mortality. Identifying high-risk patients, especially those undergoing noncardiac vascular surgery, and aggressively managing their risk factors are the priority and the ultimate goal of a biomarker. There are multiple biomarkers that appear to stratify patients with PAD at risk of cardiovascular morbidity and mortality, but none of them are currently being used in clinical practice. With the number of biomarkers continuing to increase, other integrated strategies need to be adopted, such as the use of panels of biomarkers to improve identification of susceptible patients.

A study of a cohort of 540 high-risk individuals revealed that β2 microglobulin (β2M), cystatin C, hs-CRP and glucose were associated with PAD independently of the traditional risk factors of age, diabetes mellitus, hyperlipidemia, hypertension and tobacco use. Among the plasma markers tested, β2M and cystatin C had the highest correlation with ankle brachial index, higher than any of the conventional risk factors of age, smoking status and diabetes status. A biomarker panel score derived from β2M, cystatin C, hs-CRP and glucose had an increased association with PAD status [117], independently of the traditional risk factors. However, no association was found with the destabilization of the atherosclerotic plaque.

Conclusion

Current literature on biological markers and atherosclerotic disease is increasingly expanding, yet the subject is a complicated entity, fraught with measurement variability – as a result of either technique or effects of the disease state – as well as with complex and intertwined pathophysiology. Atherosclerosis is a systematic disease, and inflammatory markers are generated from all vascular beds including coronary circulation, cerebrovascular arterial tree and peripheral arteries. Biomarkers are substances involved in various stages of the pathophysiology of atherosclerotic plaque formation, progression and destabilization. Therefore, their diagnostic and prognostic value varies accordingly.

In order to approach the diagnosis of vulnerable plaque, a series of biological markers indicating imminent destabilization needs to be addressed. Such markers are CRP, fibrinogen, white blood cell count, cytokines, MMPs, MPO, sCD40L and vascular calcification markers (OPN and OPG). There are readily available commercial kits for determining most of these markers at costs

that should be taken into account when planning clinical or research utilization of these techniques. Finally, the special characteristics of each vascular bed should also be included in the evaluation algorithm of biomarker detection.

Future perspective

Patients with disease affecting one vascular bed are at increased risk of overall cardiovascular morbidity and mortality. There are multiple biomarkers that appear to stratify patients at risk of cardiovascular morbidity and mortality, but none are currently being used in clinical practice. In addition, they are not without limitations.

For any single biomarker (such as CRP), a certain percentage of subjects with abnormal levels will not have the disease (false positives), whereas those with disease may have normal levels (false negatives). One approach to addressing this problem is to use a panel, in which each of the biomarkers contributes independent diagnostic information. Biomarker panels and index scores are beginning to be used in medicine to refine diagnosis and to aid in prognostication. For example, such index scores incorporating novel biomarkers have been utilized to predict clinical outcomes in hepatocellular and breast malignancies [118,119]. Recently, Wang *et al.* combined multiple biomarkers from the Framingham study to predict cardiovascular outcomes and death and they found only moderate addition of predicting value on conventional biomarkers [120]. However, this study assessed biomarkers as risk factors for disease progression and incorporated all measurable biomarkers, not only those implicated in transforming the plaque into a vulnerable one. Similarly designed studies are required, focusing on the vulnerable plaque biomarker group and incorporating novel ones in search of a panel with significant predictive value.

Safe recommendations cannot be included in daily practice, as randomized controlled trials and meta-analyses are still lacking. Modern risk scores and charts for identifying patients at high risk of cardiovascular events should include biomarkers as one of their major cumulative components. Even though not all plaque ruptures are symptomatic, randomized controlled trials incorporating vascular bed-specific biomarker panel assessment are needed in order to lead us to a future of being able to detect patients with atherosclerotic plaques that are vulnerable and thus at high risk of undergoing cardiovascular events. In such a setting, decision-making about the 'how' and 'when' of interventions and medical treatment will become easier and more efficient.

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Executive summary

Introduction

- A serum biomarker should be easy and cost effective to measure.

Physiology of atherosclerotic plaque formation

- Endothelial dysfunction plays a key role in the pathophysiology of atherosclerosis.
- Various circulating proteomic mediators are involved in disease progression and can be used as biomarkers.

The vulnerable atherosclerotic plaque

- A ruptured plaque that will give rise to cardiovascular events is defined as a vulnerable one, but not all ruptures lead to symptomatology.
- The balance between vascular smooth muscle cell repair activity and inflammation determine the plaque rupture and destabilization.

Biomarkers & vulnerable atherosclerotic plaques

- Not all biomarkers are associated with plaque transformation into a vulnerable one, but they have all been used as a measure of disease progression.

Biomarkers in different vascular beds

- Biomarker value in detecting plaque vulnerability can be different in each vascular bed (coronary, peripheral and carotid/cerebral arterial vasculature).

Conclusion

- Biomarkers involved in destabilization pathophysiology are complement reactive protein, fibrinogen, white blood cell count, interleukins, soluble CD40 ligand, myeloperoxidase, metalloproteinases and vascular calcification markers (osteopontin, osteoprotegerin).
- More randomized controlled trials are needed to reach safe recommendations regarding the use of biomarkers to identify the rupture-prone atherosclerotic plaque.

Future perspective

- Serum biomarker determination and assessment in a panel may be valuable in identifying patients at high risk of cardiovascular events.

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- **The significance of the use of a panel of biomarkers.**