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REVIEW

Markers of Atherosclerosis: Part 1 – Serological Markers

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Atherosclerosis is a major contributor to morbidity and mortality worldwide. With therapeutic consequences in mind, several risk scores are being used to differentiate individuals with low, intermediate or high cardiovascular (CV) event risk. The most appropriate management of intermediate risk individuals is still not known, therefore, novel biomarkers are being sought to help re-stratify them as low or high risk. This narrative review is presented in two parts. Here, in Part 1, we summarise current knowledge on serum (serological) biomarkers of atherosclerosis. Among novel biomarkers, high sensitivity C-reactive protein (hsCRP) has emerged as the most promising in chronic situations, others need further clinical studies.

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However, it seems that a combination of serum biomarkers offers more to risk stratification than either biomarker alone. In Part 2, we address genetic and imaging markers of atherosclerosis, as well as other developments relevant to risk prediction.

Keywords Biomarkers • Atherosclerosis • High sensitivity C-reactive protein

Introduction

Atherogenesis is the net effect of endothelial dysfunction, prothrombotic state and vascular inflammation with intertwined pathologic pathways resulting in plaque formation [1]. The first signs of atherosclerosis can be noticed in infants with regression in childhood, reappearance in puberty and progression over time [2].

Of the 56 million global deaths in 2012, 17.5 million (31%) were due to cardiovascular diseases (CVD). Atherosclerosis is the leading cause of morbidity and mortality in most developed countries and it is estimated that, by 2020, it is going to be the leading cause of death worldwide as prevalence in developing countries is expected to increase [3,4].

Nowadays, morbidity and mortality in developed countries are already decreasing through rigorous standard risk factors awareness, control and timely primary and secondary prevention. Identification of individuals who would benefit from primary prevention therapy in clinical practice is done through several algorithms, such as the American College of Cardiology/American Heart Association 13 (ACC/AHA13) score [5], Framingham Risk Score (FRS), the Systematic Coronary Risk Evaluation (SCORE) model, and the Reynolds Risk Score. All mentioned algorithms estimate the 10-year cardiovascular risk integrating standard risk factors and classify an individual's risk in percentage and some in categories (low, intermediate or high risk). However, around 50% of people having coronary artery disease (CAD) are classified as low or intermediate risk [6]. For that reason, novel risk factors are continuously being sought, some are already well researched, others less so. Novel risk factors are searched in pathogenesis pathways of atherosclerosis, either as serum or genetic biomarkers. In this two-part review, we will briefly review current knowledge on these biomarkers as well as knowledge on ultrasonographic markers. In Part 1, we discuss serum biomarkers.

Methods

For this narrative review, we searched the PubMed database using the terms ''atherosclerosis'', ''markers'', ''biomarkers'', ''coronary artery disease'', ''serum'', ''inflammatory'', ''oxidative stress'', ''endothelial dysfunction'', ''novel'', ''genetic'', ''genomic-wide association studies'', ''GWAS'', ''epigenetics'', ''DNA methylation'', ''histone chemical modifications'', ''micro RNA'', ''miRNA'', ''ultrasonographic'', ''carotid intima-media thickness'', ''proteomics'', ''metabolomics'' in different combinations connected with Boolean operators AND and OR. We selected articles by abstract and reviewed in three different sections: serum biomarkers (Part 1), and genetic biomarkers and imaging biomarkers (Part 2).

Inflammatory Biomarkers

High Sensitivity C-reactive Protein (hsCRP)

Inflammation is suggested to be a fundamental feature of atherosclerosis. C-reactive protein (CRP) is an acute-phase reactant synthesised in the liver in response to an acute inflammatory process. However, in atherosclerosis, CRP is secreted in minor quantities, therefore high sensitivity assays are needed to correctly quantify its concentration. CRP is secreted in response to interleukin-6 (IL-6) by foam cells in vascular plaques. It is generally involved in the opsonisation of bacteria and the activation of a complementary system, but in vascular tissue it induces a prothrombotic state through local endothelial activation and its damage [7].

High sensitivity C-reactive protein is, to date, the most promising biomarker of preclinical atherosclerosis. Most recent data from the Multi-Ethnic Study of Atherosclerosis (MESA) study confirmed significant linkage of hsCRP protein (along with other inflammatory biomarker GlycA) with the inflammatory processes in the surrounding of vessel wall induced by acute and chronic cigarette smoking [8]. The association of hsCRP with atherosclerosis and usefulness of patient screening was first described in the Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) trial [9]. Since then, its prediction value has been shown in more than 20 trials. Levels of hsCRP are not influenced by conventional risk factors and higher values (more than 2 or 3 mg/L) are independently associated with higher risk for cardiovascular (CV) events and mortality [10,11]. Moreover, if hsCRP is combined with the FRS it helps in reclassification of patients due to improvement in coronary risk prediction [12]. It is already used as an adjunct in the ACC/AHA13 score. Cardiovascular risk estimation is limited when acute inflammatory process, infection or tissue injury is present, but in chronic conditions, it is a consistent variable. It has also been suggested that repeated hsCRP measurements can be used to some extent to monitor statin therapy effect [13]. Its efficacy in routine clinical practice is yet to be determined.

Serum Amyloid A

Serum amyloid A (SAA) is, like CRP, an acute phase reactant secreted from the liver in response to inflammation. It is also expressed in cells forming atherosclerotic lesions. When secreted in the serum, it replaces apolipoprotein AI from the high density lipoprotein (HDL) molecule thus attenuating its anti-atherogenic, anti-oxidative, anti-inflammatory,

anti-apoptotic and anti-thrombotic function [14]. Some studies showed that elevated plasma SAA levels are associated with CAD [15,16], its severity [16], and may indicate early mortality [17].

Cytokines

Cytokines are high molecular weight peptides that are involved in inter-cell signalling.

Interleukins (IL) are a class of the cytokines that act specifically as mediators between leukocytes. The majority of interleukins are synthesised by helper T lymphocytes, along with monocytes, macrophages and endothelial cells. They are the main actors in the development of inflammatory cells as well as initiators of inflammatory processes [18,19]. Conversely, their involvement is also implicated in vascular inflammation. Among the numerous interleukins, IL-6, IL-18 and monocyte chemoattractant protein 1 (MCP-1) have been more extensively studied for their relationship with preclinical atherosclerosis. IL-6 concentrations were shown to be elevated in individuals who developed ischaemic heart disease, stroke, and heart failure [11,20–22], and were independently associated with peripheral artery disease [23,24]. IL-18 was researched in a couple of studies, some of them yielding a positive association with atherosclerosis and even declaring it as an independent predictor of coronary events [25], some studies found polymorphisms influencing its serum concentrations but with no association with plaque presence or risk of future congenital heart disease (CHD) [22,26,27]. In patients with acute coronary syndrome (ACS), higher MCP-1 levels have been related to an increased risk of death and recurrent ischaemic events. Combined with other biomarkers (hsCRP and B-type natriuretic peptide [BNP]) it improved prognostic value for death, stroke, myocardial infarction (MI), and heart failure after ACS [28].

Tumour necrosis factor α (TNFα) is another cytokine involved in systemic inflammation and is a member of a group of cytokines that stimulate the acute phase reaction. It is involved in several stages of atherogenesis. It was shown to be significantly and independently associated with the severity of CAD [29] and also predicted CVD events and mortality [11] in several studies. TNFα predicts short-term cardiovascular events in contrast to CRP, which provides prognostic information for long-term outcomes, since it seems to be involved in earlier pathogenic processes than CRP [28]

Circulating Adhesion Molecules

One of important factors in vascular inflammation is the synthesis, expression and function of a large family of adhesion molecules that aid in the binding and recruiting of circulating leukocytes, and allowing their migration into subendothelial space. Local inflammation is the key trigger in promoting expression of adhesion molecules on the intimal side of endothelial cells. To a minor extent, these molecules can also be found in plasma in circulating forms. Vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), E-selectin and P-selectin have been described to have a role in atherogenesis [30]. The Atherosclerosis Risk in Communities (ARIC) study [31] found higher levels of ICAM-1 and E-selectin in individuals with carotid atherosclerosis and higher incidence of CAD. All of the mentioned molecules were found to be significantly associated with severity of CAD in another two studies [29,30] and were shown to be indicators of the presence of CAD [30]. ICAM-1 was associated with the risk of developing myocardial infarction (MI). On the other hand, the MESA study [32] did not find an association of ICAM-1 levels with coronary artery calcium [33]. All mentioned studies clearly show that, to some extent, levels of circulating adhesion molecules are connected with atherogenesis; nevertheless more studies are warranted.

Pentraxin 3

Related to CRP, there is another member of the pentraxin family that shows some potential, pentraxin 3 (PTX3). It is also an acute phase reactant that is, in contrast to CRP, believed to be secreted predominantly locally from inflammatory spots—eg. atherosclerotic plaques—from endothelial, smooth muscle cells (SMCs), as well as macrophages and neutrophils [19]. It can be detected early in inflammatory disease, which was shown by a couple of studies investigating the correlation of its coronary sinus levels and stable CAD [34]. In contrast, its serum concentrations were not related to stable angina pectoris but were significantly higher in unstable angina [35] and were higher in individuals with a higher degree of CAD [36] and in those with vulnerable plaques [37]. Its clinical usefulness is yet to be determined.

Fibrinogen

Fibrinogen is a protein produced by the liver and has many functions. On the one hand, it serves as a fibrin source, and contributes to platelet aggregation through Gp IIb/IIIa binding and is a major determinant of plasma viscosity. On the other hand, it also serves as an acute phase reactant. Its levels and changed structure were shown to be related to premature atherosclerosis in young CAD patients [38] and were connected with its severity [39]. A later, relatively large study found no association with CAD or type 2 diabetes mellitus (T2DM) [40]. Inconclusive results were also reported regarding mortality.

Apolipoprotein-Associated Phospholipase A2

Apolipoprotein-associated phospholipase A2 (Lp-PLA2) is another promising biomarker. It is secreted from inflammatory cells and has a high affinity for lipoproteins. In atherogenesis, it lyses oxidised LDL (oxLDL) to two oxidative molecules aggravating oxidative damage and is therefore directly related to vascular inflammation. Its concentrations are higher in vulnerable plaques and it is thought to be a marker of plaque instability [7,18]. Many studies have investigated its association with CHD but varying results have been reported. Most studies agree on a positive association between its activity [9,41] and CHD but different results regarding its serum concentrations [8] have been obtained. Differences are thought to be due to non-standardised assays. Most recently, Garg et al. [42] analysed the association

of Lp-PLA2 with incident peripheral arterial disease in the MESA study consisting of 4622 participants. During median follow-up of 9.3 years, 158 incident peripheral arterial diseases were assessed. Investigators did not find a significant interaction according to ethnicity for Lp-PLA2 activity and for Lp-PLA2 mass. Similarly, no evidence of an association between Lp-PLA2 and incident peripheral arterial disease was observed [42]. On the other hand, Ge et al. demonstrated that Lp-PLA2 levels in human plasma were positively associated with the severity of CHD in the MESA study (662 males and 249 females from 11 ethnicities). Moreover, the study showed an apparent positive interaction between Lp-PLA2 and classical risk factors in predicting coronary heart disease [43].

Soluble CD40 Ligand

Another proposed marker of plaque instability and progression of atherosclerosis is soluble CD40 ligand (sCD40L). It is released with platelet activation and binds to the CD40 receptor found on B cells, monocytes, macrophages, endothelial and smooth muscle cells in atheroma. CD40 activation stimulates endothelial and smooth muscle cells to express VCAM-1, ICAM-1 and E-selectin thus promoting leukocyte adhesion, plaque instability and atherogenesis [44–46]. sCD40L levels have been proven to be significantly higher in patients with MI and unstable angina [47], but not in patients with stable CAD. It has also been linked with severity of CAD in ACS patients [45] and its prognosis [48]. On the other hand, there were contradictory findings regarding its association with CAD incidence in previously healthy subjects [44]. Nonetheless, findings are indicative that sCD40L is a marker of plaque instability, since more thrombocytes are activated in ACS than in stable CAD.

Biomarkers Of Oxidative Stress

Matrix Metalloproteinases (MMP)

Matrix metalloproteinases (MMPs) are enzymes that degrade extracellular matrix, regulating its composition. In regard to atherosclerosis, they are mainly activated in plaque shoulder regions by myeloperoxidase (MPO) thus promoting plaque vulnerability by fibrous cap thinning and its consequential rupture [19,49]. Increased levels of MMP-2 and MMP-9 are thought to be useful markers of plaque vulnerability [49]. In addition, MMP-9 was identified to be predictor of CV mortality [29,50], and an 8-year follow-up study found plasma levels of MMP-9 to be independently associated with first-time CHD events [51]; however, serum levels in other studies were inconsistent, presumably due to different assays used. Among others, MMP-3 and MMP-10 were also reported to be useful to identify subclinical atherosclerosis [52,53].

Myeloperoxidase

Myeloperoxidase (MPO) is an enzyme secreted by monocytes, macrophages and neutrophils in response to inflammatory stimuli. It produces a strong oxidant–hypochlorous acid (HOCl), and has been shown to induce low density lipoprotein (LDL) oxidation, formation of foam cells and oxidation of other molecules contributing to local oxidative stress especially in rupture prone plaques [1,7,18,19]. MPO has been widely investigated and comprehensive reviews done [54,55]. Reviews showed that MPO levels may be independently related to early detection of otherwise subclinical CAD, its severity, diagnosis of MI, prediction of adverse events and monitoring the response to therapy. Also, some conflicting results were reported due to different non-standardised assays used and due to MPO being nonspecific (increased levels in other inflammatory processes, although it is generally less involved in systemic inflammation than CRP). More studies are warranted to establish its role in clinical practice.

Oxidised LDL

Oxidised LDL (OxLDL) is thought to be one of the main initiators of atherosclerosis. It has a ready uptake by local macrophages, transforming them into foam cells. Measuring oxLDL could, therefore, serve as an indicator of atherosclerosis and its severity. Problems arise when direct measurements of its levels are attempted, since the oxLDL molecule is versatile in its nature and is quickly cleared from circulation by Kupffer cells. Currently, its quantification can be done using autoantibodies or murine monoclonal antibodies. Some studies are using oxLDL after-products (e.g. MDA-LDL) as indicators of its levels. Until now, disappointing and inconsistent results have been reported regarding its connection with atherosclerosis. However, this may be due to questionable sensitivity and specificity of its assays. When appropriate assays are developed, oxLDL could probably serve as a great atherosclerosis biomarker [7,19,56].

Lipid Hydroperoxides

Lipid hydroperoxides (LOOH) are the major primary products of LDL oxidation. OxLDL contains a number of free radical-induced LOOH. The aldehyde products of lipid hydroperoxide breakdown are responsible for the modification of the LDL apoproteins, which are characterised by the altered receptor affinity. Consequently, they are scavenged by macrophages with the development of foam cells and the initiation of the atherosclerotic lesion [57]. Moreover, LOOH contribute to inflammation and endothelial dysfunction, as the main features of atherogenesis process. Circulating LOOH have been found in the positive correlation with the risk of myocardial ischaemia and other CVDs [58]. Walter et al. [59] evaluated serum levels of LOOH and consequently the data were correlated with clinical events over a 3-year period in 634 patients with angiographic evidence of CAD. The authors summarised that increased levels of LOOH have been shown as significant predictive markers of nonfatal vascular events and procedures in patients with stable CAD, which were independent of traditional risk factors and inflammatory markers.

Isoprostanes

Isoprostanes (F2-IsoP) are eicosanoids that are formed directly from arachidonic acid by non-enzymatic reaction with reactive oxidative species (ROS). They have many biological functions, they act as vasoconstrictors, and stimulate

SMC proliferation and platelet aggregation. It is thought that F2-IsoP levels are associated with nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity [56]. Its levels are readily measured and are believed to be a marker of oxidative stress. A systematic review [60] investigating F2-IsoP association with CHD in 22 studies indicated a positive association in 20 studies. All studies investigating outcomes found a positive association with CHD mortality. A positive association has been established for CAD, and stroke; somehow, a weaker link was established with peripheral arterial disease (PAD) [60].

NADPH Oxidase

NADPH oxidase is one of the chief sources of superoxide (O_2^-) in vascular cells, monocytes and neutrophils. The P22phox subunit is its main catalytic subunit and has been widely investigated regarding its polymorphisms and CAD [1]. Another catalytic subunit, NOX2, is essential for NADPH oxidase activation [56]. It was shown that patients with CVD risk factors had higher levels of NOX2 and flow-mediated dilatation was inhibited in PAD patients with higher NOX2. Also statins were shown to reduce NOX2 levels, confirming their antioxidant effect [56]. For NOX2 levels to be a regarded as a biomarker of atherosclerosis, large prospective studies are needed.

Advanced Glycation End-Products

Advanced glycation end-products (AGEs) are various molecules derived from enzyme-free glycation in excess of free glucose. Mostly, they have been studied in conjunction with diabetes mellitus, where they have been found to play a crucial role in the development of its vascular complications. One of the main proposed mechanisms of their action is through induction of ROS formation, increasing oxidative stress [1]. On the other hand, AGEs were also related to connective tissue diseases, neurological conditions, and end-stage renal disease. They seem to be higher in patients that develop CVD, their levels are also greater with PAD and carotid stenosis. It is not yet clear if they are a CVD risk factor or just a measure of its severity [56].

Asymmetric Dimethylarginine

Asymmetric dimethylarginine (ADMA) is a principal endogenous inhibitor of nitric oxide (NO) synthase and regulator of NO formation. Consequently, it greatly contributes to NO/ROS balance. In the last couple of years, many studies investigated its connection with CHD. A recent meta-analysis [61] reported significantly increased levels in individuals with CAD, MI, and unstable and stable angina pectoris; in addition, an association with CAD severity was documented [62]. Biomarkers of oxidative stress related to atherosclerosis are summarised in Figure 1.

Biomarkers Of Haemostasis

The haemostatic system plays a pivotal role in developing atherosclerotic complications such as plaque rupture leading to, for example, occlusion of coronary artery-MI. Various haemostatic molecules have been reported to be independently associated with preclinical or manifest CAD.

Fibrinogen was discussed previously, in ''Inflammatory biomarkers'' above.

D-Dimer

D-dimer is a fibrin degradation by-product and is mostly used in the clinical evaluation of venous thromboembolism. It is considered as a global marker of the turnover of cross-linked fibrin and of activation of the haemostatic system [63]. It was reported to be strongly and independently associated with the presence of CAD in individuals with stable angina pectoris [63]. It has also been shown to be associated with the extent of CAD in ACS patients, in hospital-complications [64] and major clinical events [65]. The recent, large epidemiological 18-year ARIC study reported D-dimer to be an independent risk marker of ischaemic stroke, especially cardioembolic, however it was only weakly associated with CAD incidence [66]. Due to the lack of specificity of D-dimer, its future clinical usefulness as an atherosclerosis risk marker is questionable, if used in this regard all other fibrinolytic states have to be excluded beforehand.

Von Willebrand Factor

von Willebrand factor (vWF) plays a crucial role in platelet adhesion and aggregation. It is mainly produced and released by endothelial cells in response to versatile stimuli including inflammation [67]. Many studies investigated its correlation with atherosclerosis. A more-or-less weak link has been reported regarding its biomarker capabilities that diminished after adjustment for common CAD risk factors [68]. Nonetheless, as can be deduced from its function, it was reported to be significantly associated with mortality, reinfarction, re-hospitalisation and was predictive of the degree of myocardial necrosis [67]. Studies are predominantly conclusive that VWF is not a prognostic marker for the presence of CAD and for MI prognosis but rather directly pathogenically involved as a causative agent [67].

Plasminogen Activator Inhibitor-1 and Tissue Plasminogen Activator

Plasminogen activator inhibitor-1 (PAI-1) is an enzyme that functions as the principal inhibitor of tissue plasminogen activator (t-PA) and urokinase, the activators of plasminogen (PLG) and therefore fibrinolysis. T-PA lyses PLG to plasmin, which degrades extracellular matrix by itself or in conjunction with MMPs [69]. Excess PAI-1 and lower t-PA would therefore increase the risk of thrombosis. Epidemiological studies showed increased levels of PAI-1 in individuals with CHD, its levels were also correlated with MI and prognosis [69]. Higher t-PA activity was reported in individuals with CAD [70] and in first degree relatives [71]. Decreased levels of t-PA may reflect instability of atherosclerotic plaques and could, therefore, serve as predictor of adverse events [72].

Other Biomarkers

Lipoprotein(a)

Lipoprotein(a) (Lp(a)) is a complex molecule consisting of one LDL particle covalently bonded with apolipoprotein(a) (apo(a)). Apo(a) is PLG-like except for its fibrinolytic activity.

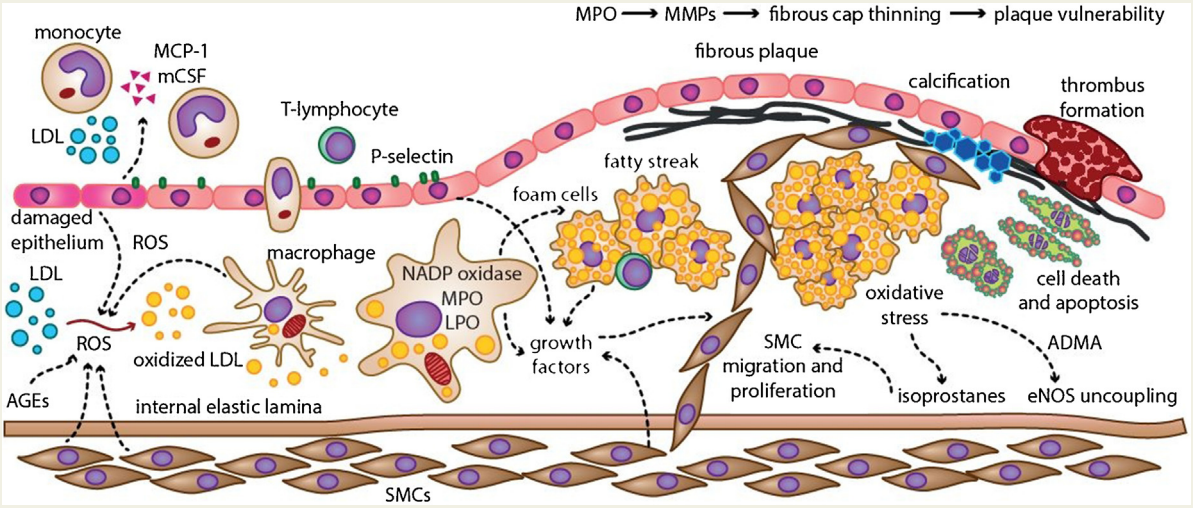

Figure 1 Oxidative stress in the pathogenesis of atherosclerosis.
All components of the vascular wall-endothelial cells, SMCs, and macrophages produce ROS, which oxidise LDL in the subendothelial space at the sites of endothelial damage produce ROS. Enzymatic sources of ROS include MPO, LPO, NADPH oxidase and the mitochondrial electron chain transport. These events initiate the changes (formation of foam cells, SMCs migration, proliferation and calcification) that culminate in the constitution of a fibrous plaque. Rupture of fibrous plaque leads to thrombus formation and occlusion of the vessel.
Abbreviations: ADMA, asymmetric dimethylarginine; AGEs, advanced glycation end-products; eNOS, endothelial nitric oxide synthase; LDL, low-density lipoproteins; LPO, lipid peroxide; mCSF, macrophage colony-stimulating factor; MCP-1, monocyte chemoattractant protein-1, MMPs, metalloproteinases; MPO, myeloperoxidase; NADP, nicotinamide adenine dinucleotide phosphate; ROS, reactive oxygen species; SMCs, smooth muscle cells.

Lp(a) is synthethised in liver and its concentrations vary from 0.1 mg/dL to 200 mg/dL (more than 1000-fold) and are predominantly dependent on different polymorphisms in the LPA gene [73]. Several theories regarding is physiological and pathophysiological role exist. Since apo(a) shares a high degree of homology with PLG, Lp(a) interferes with process of thrombosis/fibrinolysis and provides a link between the cholesterol transport system and fibrinolysis. It has been reported to be a competitive antagonist of PLG activation by t-PA and other PLG activators, it was found to inhibit fibrin and fibrinogen binding and also enhancement of PAI-1 activity [73]. Since its concentrations vary greatly among individuals, it is interesting that its concentrations do not seem to be correlated with thromboembolisms [74]. Another theory suggests that Lp(a) binding to fibrin helps to deliver cholesterol to healing tissues and, as a side effect, also to inflamed plaque sites, where it contributes to cholesterol deposition and inhibition of fibrinolysis. Another proposed mechanism is its action as monocyte chemo-attractant and stimulator of IL-8 expression in macrophages [73]. In contrast to thromboembolism, its association with CHD has been more firmly established [74,75]. Nordestgaard et al., in the European Heart Journal [76], even recommend screening for elevated Lp(a) in individuals at intermediate or high CVD/CHD risk and use of niacin in individuals with Lp(a) levels of more than 50 mg/dL with the aim of global CVD/CHD risk reduction. Niacin has only been reported to lower CHD risk, progression and prognosis before statin therapy became the standard of care [77], but, when used in conjunction with statin therapy, it did not show clinical benefit despite effective reduction of LDL levels [78]. It is still unknown what therapy would effectively lower Lp(a) and, possibly, CHD risk.

ApoB and Small LDL-P

Steffen et al. assessed whether elevated lipoprotein or apolipoprotein measures are linked with carotid atherosclerosis and plaque progression independently of CV risk factors. The authors included standard lipids in a subcohort of 2,228 MESA participants. Results showed that ApoB and small LDL-P provide atherosclerosis risk information that is not revealed by typical CV risk factors [79].

Endothelin-1

Endothelin-1 (ET-1) is a potent vasoconstrictor derived from endothelial cells, SMCs and macrophages in response to endothelial dysfunction. In addition, it exerts pro-inflammatory actions, mitogenic and proliferative effects, stimulation of free radical formation and platelet activation [80]. It was reported to be elevated in individuals with CAD, ACS and connected with its severity. It was also reported to be lower after statin therapy [81–83]. Therefore, it may serve not only as a risk marker but also as a therapeutic target.

B-Type Natriuretic Peptide

The natriuretic peptides (NP)—B-type natriuretic peptide (BNP) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) —are cardiac-derived hormones with favourable cardiometabolic actions. Low NP levels are associated with

increased risks of hypertension and diabetes mellitus, conditions with variable prevalence by race and ethnicity [84]. BNP is protein produced and secreted predominantly in ventricular myocardium in response to volume and pressure overload. NT-proBNP is its precursor. Its connection with the diagnosis and prognosis of heart failure is well established. Studies investigating its levels in individuals without heart failure reported higher risk of death and cardiovascular events [85]. A large meta-analysis of 40 studies reported a strong association with CVD risk, but only modest improvements in risk discrimination, slightly better than hs-CRP [86]. NT-proBNP is supposed to be a marker of a response to myocardial strain and possibly fibrosis. Liu et al. analysed the linkage between cardiac magnetic resonance (CMR) measures of fibrosis and the serum levels of NT-proBNP in the MESA study enrolling a total of 1,334 participants [87]. Regression analyses (univariate and multivariable) adjusting for demographics, cardiovascular risk factors, and left ventricular mass were used. Results showed increased serum NT-proBNP levels which were associated with subclinical fibrosis in a community-based setting. Another most recent study assessed whether NT-proBNP predicts PAD risk [88]. Results demonstrated significant association between serum NT-proBNP levels and incident PAD, mainly its severe form, critical limb ischaemia. In addition, NT-proBNP and cardiac tropinin C levels were independently linked with incident PAD [88].

GlycA

Serum glycoprotein N-acetyl glucosamine residues have recently been linked with the incident CVD [89,90]. The identification of circulating N-acetyl glycan groups is evaluated using the plasma glycan biosignature (GlycA). Lawler et al. [91] assessed the longitudinal association of circulating GlycA and mortality for CVD among initially-healthy individuals. Analysis of correlations and mortality linkages adjusted for hsCRP, fibrinogen, and ICAM-1 showed that GlycA reflects summative risk associated with the multiple signalling related to systemic inflammation. Another clinical study found an interaction between GlycA and smaller HDL subclasses. Increasing GlycA levels attenuated the inverse association of smaller HDL subclasses with CVD mortality [90].

Collagen Peptides

Disturbances in the synthesis and breakdown of the extracellular matrix of arterial walls have emerged as a key characteristic of atherogenesis. An important marker of these processes is collagen peptide turnover. Procollagen type III N-terminal propeptide (PIIINP) and collagen type I carboxy-terminal telopeptide (ICTP) specifically reflect the collagen synthesis and degradation in the cell. The PIIINP is a marker with the most prognostic value. Its higher levels predict an adverse outcome after a myocardial infarction and chronic heart failure and predict abdominal aortic aneurysm expansion and risk of rupture. The PICP predicts adverse outcomes following myocardial infarction and in chronic heart failure [92]. The predictive role of these two biomarkers was evaluated in the MESA study (a total of 3,068 healthy participants). Both biomarkers predicted chronic inflammatory-related severe hospitalisation and total death. Authors of this study suggested that both collagen markers may help differentiate healthy from unhealthy ageing [93].

Lipoprotein Particles

The lipoprotein plasma particles LDL and HDL that can occur independently of any change in the cholesterol content of these lipoproteins are considered important biomarkers for major coronary HD events and coronary HD death [94]. In this regard, the discordance of LDL- and HDL-cholesterol particles versus cholesterol concentrations seems to be a predictor of CVD [95]. In the MESA study, the investigators examined the changes in the plasma levels of LDL and HDL levels (with cholesterol percentiles), LDL-C, LDL-P, HDL-C, and HDL-P in relation to incident CHD and CVD events in subjects with diabetes mellitus and metabolic syndrome without DM. The authors concluded that LDL discordance and higher LDL-P in metabolic syndrome patients and higher LDL-C with lower HDL-P in diabetic patients predicated coronary HD and CVD [95].

Cardiac Troponins

Cardiac troponin (cTn) assays are used in the diagnosis of MI. In the last several years, high sensitivity assays became available which detected troponin I (Tn I) or T (TnT) levels in a healthy population. Several large prospective studies identified hsTnT and hsTnI to be good predictors of coronary HD, heart failure and mortality in the general population and, in addition, it was concluded that hsTn measurements could significantly contribute to risk stratification [96–98]. The MESA study evaluated both cTnT and NT-proBNP levels in 5,592 White, Black, Hispanic, and Chinese individuals. troponin T (TnT) and NT-proBNP have been found as predictors of incident CHD, independent of established risk factors and ethnicity, within a multi-ethnic population without known CVD. Moreover, the authors concluded that changes in NT-proBNP levels may provide additional prognostic information [99].

Cholesterol Efflux Dapacity

Cellular cholesterol efflux toward acceptors in the extracellular compartment represents a mechanism by which macrophages control the cholesterol homeostasis to prevent accumulation of intracellular cholesterol. The efflux of cholesterol from human macrophages occurs toward lipid-poor or lipid-free apolipoprotein AI (apo AI) which is the major protein component of mature HDL particles [100]. Cellular cholesterol efflux is a key step in reverse cholesterol transport in organisms. It has been documented that cholesterol efflux capacity (CEC) is inversely linked (independently of HDL cholesterol levels) with carotid intima-media thickness, the presence of angiographic coronary artery disease, and the incidence of cardiovascular disease [101]. Most recently, Tejera-Segura et al. [102] showed that CEC is related to subclinical atherosclerosis in patients with rheumatoid arthritis. It seems that serum CEC is not linked to traditional

risk factors in very old, cardiovascular event-free individuals, on the other hand, it has been found with apparently elevated values in comparison with healthy and younger individuals [103]. However, the role of CEC as a biomarker of CVD initiation must be resolved in future clinical studies.

Microalbuminuria

Microalbuminuria is a well-established risk factor, independently of traditional risk factors, of all-cause and cardiovascular mortality and CVD events in patients with diabetes or hypertension and in the general population [104]. The pathophysiological mechanism of this observation is based on the hypothesis that microalbuminuria can reflect subclinical vascular damage in the kidneys and also systemic endothelial dysfunction. Although the linkage between microalbuminuria and CVD is not fully understood, atherosclerosis and microalbuminuria appear to have certain pathogenic mechanisms in common [105]. Recently, Park et al. showed that the occurrence of microalbuminuria may imply subclinical CAD, even in an asymptomatic population [104]. A slight increase of albuminuria appears to be a significant marker of carotid intima-media thickness (CIMT), independently of traditional cardiovascular risk factors [106]. Moreover, the authors of the same study pointed to the importance of intensive measures for the early detection of atherosclerosis when the microalbuminuria is found in elderly subjects, although with normal kidney function.

Miscellaneous

Placental growth factor is a key molecule in angiogenesis, expressed in atherosclerotic plaques and is reported to be a marker of plaque instability, ischaemia and thrombosis [46].

Pregnancy associated plasma protein-A (PAPP-A) is a metalloproteinase that activates insulin like growth factor-1 (IGF-1) a potent activator of SMCs and inflammation and was associated with vulnerable plaques and may be a predictor of cardiovascular disease and mortality [107].

Growth differentiation factor 15 (GDF-15), a member of transforming growth factors with a role in regulating inflammatory and apoptotic pathways in injured tissues, has been reported to lower cardiovascular and all-cause mortality [108], in combination with other biomarkers it was reported to significantly improve risk discrimination and seems to be a good predictor of unstable angina pectoris [109].

Ischaemia modified albumin is already a well established marker of ischaemia [110]. It is derived from albumin in instances of ischaemia, where OH- (consequence of oxidative stress) alters albumin so it cannot bind to transition metal ions. It is regarded as an end product of oxidative stress. It is elevated well before cardiac troponins.

Microparticles (MP) belong to a family of extracellular vesicles released from apoptotic or activated cells. Its levels have been reported to be increased in individuals with cardiovascular risk factors, atherosclerosis and other cardiovascular disorders [6].

In recent years, metabolomics has been increasingly used in different studies also regarding atherosclerosis biomarkers, as explained in Part 2 of this article. A recent study reported higher serum phenylalanine and monounsaturated fatty acid levels to be associated with increased cardiovascular risk, while higher omega-6 fatty acids and docosahexaenoic acid levels were associated with lower risk even after adjustment for other risk factors [111].

A number of other serum biomarkers were and are also being investigated with different potential. Association with atherosclerosis was also described for Heat Shock Protein-27, sTWEAK, osteopontin, cathepsins, cystatin C and many others.

Conclusion

Atherosclerosis is a major contributor to morbidity and mortality, worldwide. With therapeutic consequences in mind, several risk scores are being used to differentiate individuals with low, intermediate or high CV event risk. The most appropriate management of intermediate risk individuals is still not known, therefore novel biomarkers are being sought to help re-stratify them as low or high risk. Proposed biomarkers are intertwined in inflammation, oxidation, haemostatic and other processes involved in atherosclerosis. Among novel serum biomarkers, hsCRP has emerged as most promising in chronic situations; others need further clinical studies either because of inconsistency among used assays (such as with Lp-PLA2, MMP, MPO, and oxLDL), contradictory results (e.g., SAA, and sCD40L) or because of small studies with not enough power. Either way, it seems that a combination of serum biomarkers offers more to risk stratification than either biomarker alone.

Conflict of Interest

Authors declare no conflict of interest.

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