Chapter 1
Inflammation Biomarkers and Cardiometabolic Risk

Flávio Reis and Filipe Palavra

Abstract The ominous presence of inflammation in all phases of atherosclerosis has prompted the evaluation of emergent biomarkers of inflammation as tools to help in identifying patients at high risk for future cardiovascular events, to improve diagnostic and prognostic abilities, and to monitor disease activity and efficacy of therapy. Acute-phase reactants, pro- and anti-inflammatory cytokines, cell adhesion molecules, chemokines, and other mediators involved in the pathogenesis of atherosclerosis have been shown to have predictive value to determine future cardiovascular events and/or death, but until now, none, with the exception of hsCRP, has demonstrated additive value to the Framingham Risk Score.

Keywords Inflammation • Atherosclerosis • Cardiometabolic risk • Biomarkers

Inflammation in Health and Disease: Overview

Inflammation, derived from the Latin word *inflammare*, means "to set on fire." Inflammation is a part of the host defense system that counteracts insults incurred by internal or external stimuli, and the typical clinical signs include redness, heat, swelling, pain, and loss of function. Inflammation is not injurious in its essence and is necessary for the removal of challenges faced by the organism and consequent homeostasis restoration. In fact, the inflammatory responsiveness should be viewed as part of the physiological mechanisms operating to respond to stress experienced by cells, tissues, and organs.

Inflammation can be classified as acute or chronic. Chronic and acute inflammatory processes were traditionally thought to be motivated by different causes, through the activities of different cells and mediators, resulting in different outcomes. Nevertheless, a more modern vision indicates that processes are connected

F. Reis, Ph.D. (✉) • F. Palavra, M.D.
Laboratory of Pharmacology and Experimental Therapeutics, Institute for Biomedical Imaging and Life Sciences (IBILI) and CNC.IBILI Research Unit, Faculty of Medicine, Sub-Unit 1 (Pólo III), University of Coimbra, Coimbra 3000-548, Portugal
e-mail: freis@fmed.uc.pt

F. Palavra et al. (eds.), *Biomarkers of Cardiometabolic Risk, Inflammation and Disease*, DOI 10.1007/978-3-319-16018-4_1

in order to give organisms the ability to deal with distinct insults in a robust and flexible manner, thus regulating key homeostatic functions [1].

Acute inflammation is an immediate response of the body and is required to remove injurious pathogens. Facing infection, tissue damage, or acute inflammation, the host undergoes a series of biochemical and physiological changes known as acute-phase response. This process involves a cascade of events and is mediated by several distinct cells and molecules that locate pathogens or damaged tissue, recruit other cells and molecules, and then eliminate harmful agents, finally restoring body equilibrium. In brief, in a normal inflammatory response, tissue injury induces the release into the surrounding area of pro-inflammatory mediators, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, histamine, leukotrienes, and prostaglandins, by mast cells and resident macrophages, resulting in vasodilation and leaky blood vessels. Complement plasma proteins are released and call for phagocytic cells (i.e., monocytes and neutrophils) to the area to remove necrotic tissue, invading bacteria, and debris. The final step of inflammation is resolution and occurs due to neutrophil-evoked conversion of prostaglandins and leukotrienes in lipoxins, thus initiating the termination sequence, as well as due to production and release of anti-inflammatory factors, such as transforming growth factor (TGF)-β and IL-10, by activated macrophages. In addition, neutrophils and reparative fibroblasts infiltrate the area, releasing matrix metalloproteinases (MMPs) for tissue remodeling and producing extracellular matrix (ECM) and collagen. Macrophages finally leave the site through lymph vessels. When these steps are firmly followed, acute inflammation resolves without tissue damage [2].

When inflammation persists for a longer time, the type of cells at the site of damage changes, leading to chronic inflammation, which is a delayed response. Chronic inflammation involves persistent acute inflammation due to a deregulated resolution phase, which could result from incapacity to remove the inflammatory stimulus, an incessant procession of leukocytes which are responsible for the production of pro-inflammatory cytokines and reactive oxygen species (ROS) that persistently damage and remodel tissue, as well as due to a condition that maintains leukocytes at the site of inflammation [2].

Chronic inflammatory diseases (which are known to initiate due to persistent or deregulated inflammation) define an extremely important part of human pathology, and several examples can be cited as follows: asthma, systemic lupus erythematosus, rheumatoid arthritis, prostatitis, ulcerative colitis, Crohn's disease, wound healing, reperfusion injury, sarcoidosis, transplant rejection, vasculitis, chronic obstructive pulmonary disease, psoriasis, sepsis, cancer, Alzheimer's disease, as well as atherosclerosis, diabetes, and obesity [2].

Numerous molecules and factors are implicated in the regulation of inflammation at the molecular level, including cytokines (such as IL-l, IL-2, IL-6, IL-12, and TNF-α), chemokines (i.e., monocyte chemoattractant protein 1, IL-8), pro-inflammatory transcription factors (NF-κB, STAT3) and enzymes (COX-2, 5-LOX, 12-LOX, MMPs), prostate-specific antigen (PSA), C-reactive protein, adhesion molecules (intercellular adhesion molecule-1 [ICAM-l], vascular cell adhesion

molecule-1 [VCAM-1], endothelial leukocyte adhesion molecule [ELAM-1]), vascular endothelial growth factor (VEGF), and TWIST. During the last year, several of these mediators and factors have been studied as putative disease/risk markers in distinct inflammatory conditions, including in cardiovascular and metabolic disorders.

Inflammation and Cardiometabolic Risk

Regardless of the availability of successful treatment strategies for dyslipidemia and hypertension, cardiovascular diseases (CVD) account for one-third of all deaths worldwide, and prevalence still increases [3]. CVD comprise a class of diseases that involve heart and systemic blood vessels. In coronary heart disease, cerebrovascular disease, or peripheral arterial disease, impaired blood vessel function leads to an inadequate blood supply of organs. Several factors influence the risk of developing CVD, including lifestyle habits (such as unhealthy diet, physical inactivity, smoking, obesity, diabetes, high blood pressure, and dyslipidemia) as well as genetic, epigenetic, and environmental factors.

Atherosclerosis is the most common pathological process that leads to CVD, including myocardial infarction (MI), heart failure, stroke, and claudication. A central event is the development of atherosclerotic plaques in the inner lining of arteries, which is characterized by necrotic cores, calcification, and accumulation of modified lipids and foam cells, but also other cell types such as smooth muscle cells, vascular dendritic cells, T cells, and endothelial cells are involved in lesion formation [4]. The multifactorial background makes it difficult to unravel initial pathological events, which are suggested to occur in a variety of cell types in a very early phase of disease, when symptoms are subclinical. While in the past atherosclerosis was viewed primarily as a passive process of cholesterol accumulation, recent evidence indicates that it is a highly active process involving components of the vascular, immune, metabolic, and endocrine systems [4].

Even though the conventional risk factors (age, male sex, hypercholesterolemia, hypertension, and smoking) in the Framingham Risk Score (FRS) account for most of the risk of coronary heart disease (CHD), about one-third of individuals with none or only one risk factor indeed develop CHD, and up to 40 % of subjects with cholesterol levels below the population average die from CHD [5, 6]. In addition, many cardiovascular (CV) events occur in patients treated with statin therapy [7]. Regardless the important role of cholesterol in atherosclerosis, many individuals who experience acute MI present total and/or LDL cholesterol below thresholds. Statin therapy alone appears to be insufficient in decreasing the high level of residual risk of further CV events, which remains at 50–75 % of that of control groups [7]. In addition, absolute CV risk remains high: about one in six patients treated with a statin in monotherapy experiences further events over a 5-year period [7], and one in five patients with a history of acute coronary syndrome who is treated with a

statin dies within 30 months [8]. This convergence of clinical findings highlights the need for improving our ability to predict CV risk.

A major shift in the paradigm of our understanding of the pathogenesis of atherosclerosis has been seen in the last decade. The biology of the atherosclerotic plaque, rather than the degree of stenosis, is now recognized as a pivotal feature in determining plaque stability. Inflammatory mechanisms play a crucial role in all phases of atherosclerosis, from initial recruitment of circulating leucocytes to the arterial wall to eventual rupture of the unstable plaque. An abundant presence of inflammatory cells, including monocyte-derived macrophages and T lymphocytes, was found at the site of rupture or superficial erosion, which is preceded by endothelial cell dysfunction with production of adhesion molecules that interact with inflammatory cells [9, 10]. Macrophages secrete various cytokines, chemokines, growth factors, and disintegrins that cause activation and proliferation of smooth muscle cells (SMCs) and lesion progression; finally, weakening of vulnerable plaque occurs by matrix degradation of its fibrous cap [11]. Several factors are found in the atherosclerotic plaque maintaining and amplifying the inflammatory mechanisms in the atherosclerotic region, including adipocytokines, angiotensin II (ANG II), heat shock proteins (HSPs), immune complexes, ROS, and pro-inflammatory cytokines.

Advances in vascular biology have established the interaction of the innate immune system with atherosclerosis; in fact, inflammation is pivotal to the initiation and progression of atherothrombosis and to triggering CVD [12–14]. Inflammation in cells involved in atherosclerosis is elicited by many other risk factors associated with atherosclerosis, including cigarette smoking, insulin resistance/diabetes, and arterial hypertension [15]. Thus, the inflammatory pathways involved in both innate and adaptive immune responses appear to transduce many of the traditional risk factors for atherosclerosis.

The ominous presence of inflammation in atherosclerosis has prompted the evaluation of emergent biomarkers of inflammation as tools to help in identifying patients at high risk for future CV events.

Epidemiologic, experimental, preclinical, and clinical studies have led to the identification of key non-modifiable and modifiable risk factors for CVD, which were able to allow discrimination of risk between individuals and serve as basis for scores of risk calculators, being the most widely used the FRS. However, the burden of obesity and diabetes has been changing the way we classically look for CV risk factors. Organizations around the world have defined the metabolic syndrome (MetS) as a cluster of metabolic abnormalities, with insulin resistance and adiposity as central features [16, 17]. Diagnostic criteria for MetS have been defined by the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III): central obesity, dyslipidemia (high triglycerides, low high-density lipoprotein [HDL] cholesterol), hypertension, and impaired fasting glucose. The presence of three of these features is considered sufficient to diagnose the syndrome [17].

Inflammation has also been identified as one relevant feature of the metabolic abnormalities found among individuals with MetS [18], which have an increased burden of CVD [19]. Besides the effect on CV morbidity and mortality, the components of MetS have been associated with diabetes. In fact, meta-analyses have clearly shown that MetS increases relative risk of CVD outcomes by about 1.5–2.0 times and of type 2 diabetes by three to five times [20, 21].

Regardless of the fact that the diagnosis of MetS is useful to alert physicians and patients about the risk associated with a sedentary lifestyle and wrong nutritional habits that cause abdominal obesity and its related constellation of metabolic abnormalities, its presence cannot appropriately predict absolute CVD risk [22]. Thus, the risk related to the contribution of MetS to global CVD risk was incorporated in the algorithms through the concept of cardiometabolic risk [22]. In a simple way, the cardiometabolic risk can be defined as the absolute CVD risk determined by traditional risk factors to which we add the additional risk associated with the features of the MetS, thus linking inflammation, obesity (excess visceral/ectopic fat), and insulin resistance to CVD (Fig. 1.1) [18].

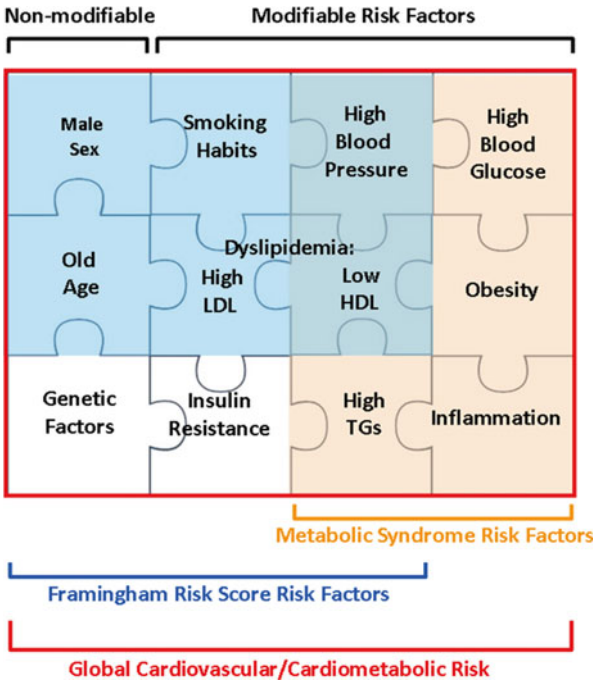

Fig. 1.1 Global cardiovascular/cardiometabolic risk factors (RFs), including the established Framingham Risk Score RFs and the emergent metabolic syndrome RFs related with obesity and inflammation

Established vs. Emergent Biomarkers: Experimental and Clinical Data

Biomarkers and/or risk factors can be either biochemical, physiological, anatomical, or physical, and they can be classified into three broad categories, genetic (including tissue or cellular) biomarkers, imaging biomarkers, and circulating biomarkers, and further into traditional and nontraditional (novel or emergent) risk factors or biomarkers.

The distinction between risk factors and biomarkers for a disease is subtle. The traditional view considers inflammatory biomarkers as risk markers rather than risk factors. A risk factor has a biological role in disease, while a biomarker that is also a risk factor is able to measure a step in pathological processes. A risk marker is statistically associated with the disease, but causality is not an obligatory characteristic of the marker: it is an indirect measure of disease processes and may be a response to other risk factors indeed involved in the pathogenesis, as was previously revised [23, 24].

During the last decades, advances in biomarker research and discovery have allowed remarkable progress in diagnosis and management of several diseases. The US Food and Drug Administration (FDA) defined biomarker as a substance that can be objectively measured as an indicator of normal biologic or pathogenic processes or of pharmacologic responses to therapeutic intervention [25]. In fact, biomarkers provide a more direct measure of a disease pathway and are ubiquitous tools that can aid in understanding disease mechanisms as well as in predicting, diagnosing, and monitoring disease processes.

CVD are caused by a number of potentially modifiable etiologies and risk factors and have been one of the major areas of biomarker research and application, especially because CHD remains the leading cause of morbidity and mortality in the developed world. Biomarkers are crucial for the quantitation of this risk. The concept of global risk assessment was introduced by the Framingham Heart Study more than 50 years ago; however, atherosclerosis is now clearly recognized as having an inflammatory signature, recommending upgrade of risk prediction tools. Understanding the inflammatory cascade in the development of atherosclerosis allows the consideration of a number of inflammatory markers as potentially useful predictors of CVD. Although there is no debate that the inflammatory process is essential to the atherosclerotic lesion, the question raised is whether the inflammatory response characterized by elevated circulating levels of biomarkers is an epiphenomenon or has a causal role. In other words, independently of cholesterol and regulators of blood pressure, could inflammatory biomarkers further report on different aspects of the pathogenic mechanisms underlying the disease?

As stated by Rao et al. [26], for the routine clinical use of a biomarker, several requirements must be accomplished: “1) the ability to control the standardization of the assay and variability of the measurement, 2) consistency in epidemiologic findings from prospective studies with clearly defined endpoints, 3) evidence that the marker adds to risk prediction over and above that already achievable through the use of established risk factors, 4) availability of population norms to guide

interpretation of results, 5) generalizability to various population groups, 6) cost-effectiveness—the incremental cost of the test should be justified by a reduction in other costs and the indirect costs of a positive test should not be limiting" [7].

The list of putative biomarkers of inflammation has considerably grown during the last years, accompanying the extensive research on this area of knowledge. In 2003, a Centers for Disease Control and Prevention (CDC)/American Heart Association (AHA) scientific statement on markers of inflammation and CVD considered several inflammatory markers as potentially useful predictors of CVD (adhesion molecules; cytokines; acute-phase reactants, including C-reactive protein [CRP], serum amyloid A protein [SAA], and fibrinogen; white blood cell [WBC] count; and erythrocyte sedimentation rate [ESR]). However, the list of putative biomarkers of inflammation under evaluation during the last years is larger (Table 1.1).

Several other molecules have been associated with inflammation and CVD, but their primary nature is not inflammatory, including oxidative and carbonyl stress compounds; advanced lipoxidation end products (namely, malondialdehyde);

Table 1.1 List of putative biomarkers of inflammation associated with cardiovascular disease

Acute-phase reactants
High-sensitivity C-reactive protein (hsCRP)
Fibrinogen
Serum amyloid A (SAA)
Pro-inflammatory cytokines
Interleukin-1 (IL-1), IL-6, IL-18
Tumor necrosis factor alpha (TNF-α)
Interferon gamma (IFN-γ)
CD40/CD40 ligand
Anti-inflammatory cytokines
Interleukin (IL)-4, IL-10
Transforming growth factor (TGF-β)
Adiponectin
Other adipocytokines (leptin, resistin, visfatin)
Cell adhesion molecules
E-selectin, P-selectin
Intercellular cell adhesion molecule-1 (ICAM-1)
Vascular cell adhesion molecule-1 (VCAM-1)
Platelet endothelial cell adhesion molecule-1 (PECAM-1)
Chemokines
Interleukin-8 (IL-8)
Migration inhibitory factor (MIF)
Monocyte chemoattractant-1 (MCP-1)
Other molecules/mediators of inflammation
Matrix metalloproteinases (MMPs)
Myeloperoxidase (MPO)
Myeloid-related protein (MRP) 8/14
White blood cell (WBC) count
Erythrocyte sedimentation rate (ESR)

advanced glycosylation end products (AGEs), such as plasma F2α-isoprostanes, advanced oxidation protein products, pentosidine, and carboxymethyl lysine; hemostatic and endothelial injury/dysfunction factors, including plasminogen activator inhibitor type 1, von Willebrand factor, and asymmetric dimethylarginine; homocysteine; lipid-associated markers, i.e., oxidized LDL and antibody to oxidized LDL, small dense LDL particles, lipoprotein (a), lipoprotein-associated phospholipase A2 (Lp-PLA2); heat shock protein (HSPs); and RANTES (regulated on activation, normal T cell expressed and secreted), among others.

Regarding clinical utility of putative inflammatory biomarkers of CV risk, it is important to consider some questions: does the biomarker add any information to that available from existing and well-established risk factors? Is it a suitable analyte? Is it stable in terms of diet influences and variations intra- and inter-day? Preferably, the biomarker should provide additional and independent information on cardiovascular risk; it should be easy to measure using standardized commercial assays with low variability and reasonably priced and not requiring specialized plasma collection or assay techniques. CRP has emerged as a robust, yet controversial clinical marker, since some of the previous requirements are accomplished.

This chapter starts exploring the most relevant data available and controversies and doubts concerning the putative use of CRP as a clinical biomarker of CV risk. Other molecule candidates to act as inflammatory biomarkers will be also debated.

High-Sensitivity C-Reactive Protein

High-sensitivity C-reactive protein (hsCRP) was first discovered in 1930, but its link to CHD was reported more than 60 years later. hsCRP is an acute-phase reactant mainly produced in hepatocytes in response to several cytokines, including IL-6 released from activated leukocytes in response to infection or trauma and from vascular SMCs during atherosclerosis lesion evolution. The role of CRP in atherosclerotic plaque formation is complex, acting in many cells involved in the process (Fig. 1.2) [27]. Although previous reports suggested a role of CRP as a surrogate of the underlying inflammatory process of atherothrombosis, accumulating evidence from in vitro and in vivo studies in clinical and experimental models robustly indicate a role of CRP as a proatherogenic factor [27–30].

In brief, CRP induces endothelial cell activation and dysfunction by several distinct activities, including directly binding with highly atherogenic oxidized LDL-C (oxLDL); increasing adhesion molecules (VCAM-1, ICAM-1, E-selectin, MCP-1); decreasing eNOS, prostacyclin, and tPA; impairing endothelial progenitor cell (EPC) number and function; and increasing pro-inflammatory cytokines and other important mediators of endothelial lesion (such as PAI-1, IL-8, CD40/CD40L, MMP-1, and ET-1) underlying atherosclerosis; in addition, it facilitates monocyte adhesion and transmigration into the vessel wall, a critical early step in the atherosclerotic process, and promotes other important modifications on monocytes (increasing tissue factor, superoxide, and myeloperoxidase contents, decreasing

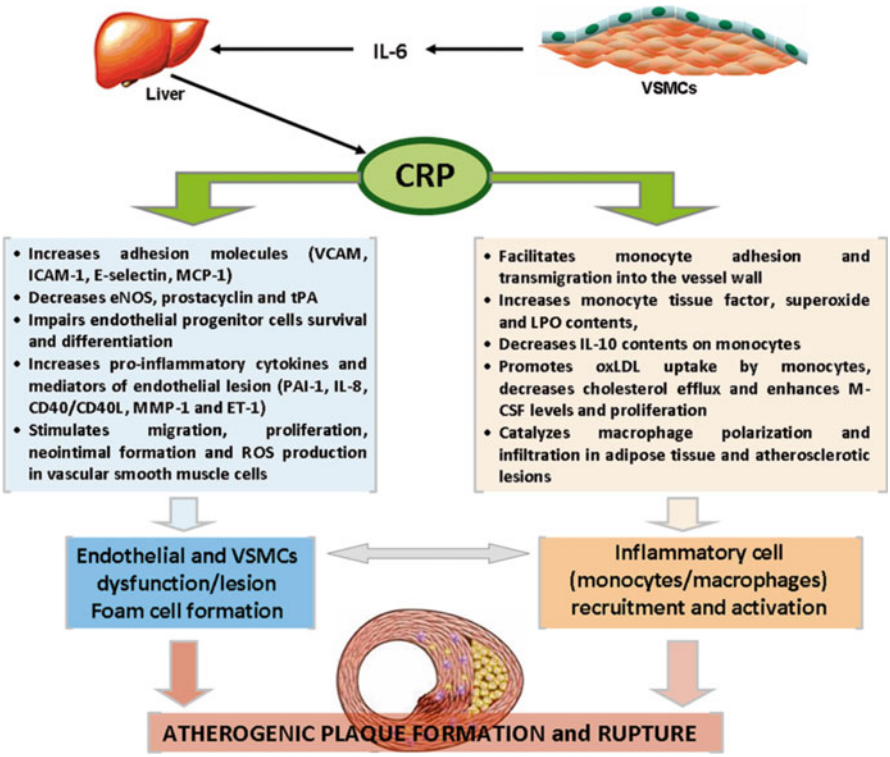

Fig. 1.2 Potential mechanisms of C-reactive protein (CRP) involvement in the pathogenesis of atherosclerotic plaque formation and rupture. While it remains uncertain whether CRP is directly involved in the pathogenesis of atherosclerosis or it is just a surrogate marker (an epiphenomenon) of other processes, several lines of evidence have been suggesting that CRP is localized within atherosclerotic lesions and exerts pro-inflammatory and proatherogenic effects

IL-10 amounts, promoting oxLDL uptake, decreasing cholesterol efflux, and enhancing macrophage colony-stimulating factor [M-CSF] levels and proliferation); CRP also catalyzes macrophage polarization, which is a pro-inflammatory trigger in plaque deposition, leading to macrophage infiltration of both adipose tissue and atherosclerotic lesions [27–30].

Several lines of evidence have indicated that inflammation plays a central role in all stages of the atherothrombotic process. In clinical terms, translation of the atherosclerosis inflammatory hypothesis to practice has been based on observational evidence linking inflammatory biomarkers to the risk of future vascular events, namely, using hsCRP [31–33]. In fact, large-scale prospective studies demonstrate that CRP strongly and independently predicts adverse CV events, including MI, ischemic stroke, and sudden cardiac death [14, 33, 34]. The inclusion of CRP to classical cholesterol screening improves CV risk prediction independently of LDL-C, suggesting that increased CRP may identify asymptomatic individuals at high risk for future events, despite average cholesterol concentrations [33]. Furthermore,

CRP concentration monitoring adds relevant prognostic information on CV risk at all LDL-C concentrations, but also at all levels of the FRS [33, 34]. As shown in the meta-analysis of Kaptoge et al., the magnitude of CV risk associated with a one-standard-deviation increase in hsCRP levels is at least as large as that associated with a one-standard-deviation increase in either total cholesterol or blood pressure [35]. Additionally, increased plasma CRP concentrations correlate with the components of MetS, such as central obesity, increased plasma triglyceride concentrations, low plasma concentrations of HDL-C, hypertension, and increased concentrations of blood glucose [33], and CRP contributes to risk prediction of MetS patients [36]. This evidence led to the development of the Reynolds Risk Score, which adds CRP to the FRS and improves global CV risk prediction in women by reclassifying >50 % of women considered at intermediate risk into higher- or lower-risk categories [37].

CRP was classified as an independent marker of CV risk by an expert panel assembled by the CDC and the AHA, as a way to improve risk stratification in populations' primary prevention [38]. The panel recommended that global risk prediction in asymptomatic individuals deemed at intermediate risk for CVD by classical risk factors should include CRP measurement and the cutoff points of <1 mg/L for low-risk and >3 mg/L for high-risk individuals.

Further than being used as an adjunctive tool in risk prediction and reclassification, there is interest in using hsCRP levels to select patients for statin initiation and to tailor intensity of therapy. Statins reduce hsCRP in an LDL-independent manner, and the benefits are superior in patients with inflammation [39]; the lower the hsCRP levels, the lower the risk [40, 41]. These evidences raised the question of whether patients that do not meet criteria for statin prescription (given the low/average LDL-C concentrations) would benefit from that medication if they had hsCRP > 2 mg/L, indicative of an enhanced inflammatory response, thus suggesting that statins could have a dual influence: reduction of LDL-C levels and direct anti-inflammatory effects. These questions/hypotheses were the basis for the JUPITER trial (Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin) [42], which enrolled 17,802 men and women with no evidence of CV disease and average or low LDL-C contents, for testing the putative benefit of rosuvastatin (20 mg po daily) treatment. JUPITER trial showed a major reduction in CV events (54 % in MI and 51 % in ischemic stroke) and in all-cause mortality (20 %), as well as in need for bypass surgery or angioplasty (46 %), with an overall 44 % relative risk reduction for the primary endpoint of major arterial vascular events. Results were identical between several subpopulations in all ethnic groups, including women vs. men, elderly, as well as with and without arterial hypertension, obesity, or MetS [42–44]. As recently commended [45], JUPITER trial also showed that there was a significant reduction in venous thromboembolism (43 %), and the maximum levels of risk reduction were found in those who achieved low hsCRP levels. Magnitude of hsCRP reduction could not be predicted on the basis of the magnitude of LDL-C reduction, and the reduction of absolute risk of events for both the rosuvastatin-treated and placebo-treated (control) groups was greater among those with higher levels of CRP at study entry, an effect not observed

for LDL-C. Genetic determinants of rosuvastatin-induced LDL-C reduction were found to differ from the genetic determinants of rosuvastatin-induced CRP reduction, altogether suggesting that at least part of the benefits of statin therapy were due to anti-inflammatory effects independent of LDL-C reduction. All those strong evidences coming from JUPITER trial, including the smaller number needed to treat (NNT) found for subjects with low LDL-C levels and elevated hsCRP concentrations (when compared with primary prevention patients under treatment of dyslipidemia or arterial hypertension) [46], had impacted the spectrum of patients candidate for statin therapy according to the FDA, as well as to other several national authorities, now including patients with elevated hsCRP levels and at least one additional risk factor, independently of high or average LDL-C levels.

Despite several strong indications coming from that trial, highlighting a statin benefit that goes beyond the effect on LDL-C reduction, additional studies were recommended to clearly test the hypothesis that directly targeting inflammation will improve vascular outcomes. The Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS) and the Cardiovascular Inflammation Reduction Trial (CIRT) have recently started and will evaluate, respectively, a human monoclonal antibody that targets human interleukin-1β (IL-1β) and low-dose methotrexate, in order to reduce cardiovascular event rates, due to direct anti-inflammatory effects [47, 48]. The results of these trials are expected with great curiosity, as they could be essential to define new algorithms to improve CV risk prediction and to gather information that could serve as basis to define new drugs targeting the machinery of inflammation.

Emergent Biomarkers

Other Acute-Phase Reactants

Serum amyloid A (SAA) protein and fibrinogen, like CRP, are acute-phase reactants generated downstream of IL-6 in the liver, as part of the acute-phase response, reflecting the intensity of cytokine activation.

Fibrinogen

Fibrinogen influences endothelial function, thrombosis, and inflammation and has been indicated as an independent variable contributing to CV risk. In brief, fibrinogen forms the substrate for thrombin (leading to platelet aggregation), modulates endothelial function, and promotes SMC proliferation and migration [49]. Several epidemiologic studies demonstrate that fibrinogen concentrations predict future risk of MI and stroke. However, it seems to be a less potent predictor of CV events than CRP [50]. Whether or not fibrinogen is causally involved in atherothrombogenesis remains to be elucidated.

Serum Amyloid A (SAA)

SAA protein, like CRP, is an acute-phase protein synthesized in the liver in response to infection, inflammation, injury, or stress. It has been linked to atherosclerosis, namely, because it is secreted as the predominant apolipoprotein on plasma HDL cholesterol particles, where it seems to replace apolipoprotein A-I, thus changing HDL-mediated cholesterol delivery to cells [51].

The more rapid response of SAA than other nonspecific inflammatory markers, such as CRP, has suggested that it could be a better marker of disease. SAA has also been shown to be a predictor of CV events [52]. However, some studies suggest that this relationship may be dependent on other risk factors [32], indicating that the independent predictive value of SAA for CAD and CV events remains unclear, deserving further studies.

Cytokines

Cytokines are key in regulating inflammatory and immune responses and have a pivotal role in controlling the innate and the adaptive immunity. Pathogenesis of atherosclerosis involves a complex interplay between cytokines, chemokines, and adhesion molecules, leading to monocyte infiltration and multiple other leukocyte responses within the arterial wall. A variety of plasma inflammatory markers have been shown to predict future CV risk. In addition, they may be useful for risk stratification and also to identify patients who might benefit from targeted therapy. Cytokines are classified according to their pro- or anti-inflammatory activities. The balance between pro- and anti-inflammatory cytokines has emerged as a major determinant of plaque stability [53].

Pro-inflammatory Cytokines

Several inflammatory cytokines have also been investigated as markers of CV risk, including TNF-α, IL-6, and CD40/CD40 ligand.

Tumor Necrosis Factor Alpha (TNF-α)

TNF-α is a cytokine primarily produced by macrophages, endothelial cells, and SMCs of atherosclerotic arteries and has been shown to have several pro-inflammatory properties, including induction of expression of cellular adhesion molecules, surface leukocyte adhesion molecules, chemokines, other cytokines, and growth factors, as well as proangiogenic activity [54]. TNF-α activities affect atherosclerotic process and have been implicated in metabolic disorders, such as obesity and insulin resistance [54, 55]. Increased plasma concentrations of TNF-α have been associated with increased risk of CV events, namely, in stable patients after MI, as it was demonstrated in the Cholesterol and Recurrent Events (CARE) trial [56].

Interleukin-6 (IL-6)

IL-6 is produced by hepatocytes, endothelial cells, fibroblasts, phagocytes, neutrophils, and lymphocytes, among other cell types. This pleiotropic cytokine has a broad range of functions and regulates several cellular processes, including growth, differentiation, angiogenesis, and healing. The precise role of IL-6 in the evolution of atherosclerosis lesions remains uncertain, but several important activities/effects of IL-6 have been described, namely, in ApoE knockout mice, including stimulation of synthesis and secretion of CRP and enhancement of fatty lesion development [57]. Increased levels of IL-6 seem to be predictive of future CV events, as suggested in the Physicians' Health Study (PHS) [58].

CD40/CD40 Ligand

CD40 ligand (CD40L) is a transmembrane protein of the TNF family that links to its receptor (CD40) and has a role in the inflammatory processes underlying atherosclerosis, plaque destabilization, and thrombosis. In fact, CD40/CD40L, expressed in a variety of immune and vascular cells, regulates platelet-dependent responses that contribute to atherothrombosis, activate endothelial cells, and in vitro promote expression of adhesion molecules, pro-inflammatory cytokines, and chemokines [59]. Soluble CD40L levels have been indicated as predictive of CV events (MI and stroke) and death in some populations [60].

Anti-inflammatory Cytokines

Interleukin-4 (IL-4) and IL-10

IL-4 and IL-10 are pleiotropic cytokines produced by Th2 lymphocytes and by other types of immune cells that have been associated with anti-inflammatory activities, mostly in mouse models of atherosclerosis. While decreased IL-10 levels have been reported in patients with acute CV events [61], the association of IL-4 levels with CVD is debatable as IL-4 may also play a role in atherosclerosis through induction of inflammatory responses (it is worth to say that increased IL-4 levels were found in patients with CAD) [62].

Transforming Growth Factor Beta (TGF-β)

TGF-β is a potent anti-inflammatory cytokine that plays a pivotal role in the maintenance of normal blood vessel wall architecture and protects against vulnerability to atherosclerosis. TGF-β isoforms 1, 2, and 3 are mainly expressed by SMCs and modulate vascular development and remodeling and determine the extent to which developing atherosclerotic lesions are stabilized [63]. Decreased levels of TGF-β1, as well as genetic polymorphisms and defective TGF-β signaling, have been reported in patients with CVD [64, 65].

Adiponectin

Adiponectin is an adipocytokine produced by adipocytes that exerts anti-inflammatory and antiatherogenic effects, having a protective role in CV terms [66]. It reduces TNF-α-stimulated expression of E-selectin, NF-κB, VCAM-1, and IL-8 and regulates monocyte adhesion to endothelium and endothelial nitric oxide synthase (eNOS) activity. Adiponectin also has insulin-sensitizing effects, and its secretion diminishes as adipose tissue mass increases. It is suggested that adiponectin contributes to the relationship between obesity, insulin resistance, and CV disease. Its concentrations are inversely associated with CVD incidence in most of the studies. In the PHS, there was a robust inverse relationship between total adiponectin and incident CHD, even after adjustment for traditional risk factors, while high levels of adiponectin have been associated with lower risk for CV events [67, 68].

These observations suggest that there is promise for the application of adiponectin and other cytokines as predictors of CVD risk. However, since the associations are complex, a more complete understanding of the exact role played by these emergent biomarkers in disease's pathophysiology is required, as well as stronger evidences from larger clinical studies without confounding factors and after proper adjustment for traditional risk factors.

Cell Adhesion Molecules

Due to their central role in the recruitment of inflammatory cells to the site of atheroma development, the cell adhesion molecules (CAMs) are promising candidates to reflect underlying vascular inflammation. E-selectin, vascular cell adhesion molecule-1 (VCAM-1), and intercellular cell adhesion molecule-1 (ICAM-1) are all members of the cellular adhesion molecule family, each having a plasma-soluble form, which can serve as a surrogate marker for increased expression of CAMs on vascular endothelial cells, reflecting inflammation and activation of endothelial cells [69].

E-Selectin

E-selectin is the most interesting form of the selectin family, which also includes L and P selectins. E-selectin promotes the interaction between endothelial cells, where it is expressed, to leukocytes. While increased E-selectin levels have been observed in some studies with CVD populations, other reports showed divergent results; at the moment, the prognostic value of E-selectin remains to be clearly defined [69, 70].

Intercellular Cell Adhesion Molecule-1 (ICAM-1)

ICAM-1 mediates attachment of circulating leukocytes to the endothelium and their subsequent transmigration and accumulation in the arterial intima, thus promoting progression of atherosclerosis. The circulating soluble form of ICAM-1, which is

expressed on the surface of endothelial cells, leukocytes, and SMCs in response to a variety of stimuli (such as shear stress and pro-inflammatory cytokines), is released from endothelial cell membranes and may be viewed as a marker of atherosclerosis. Increased levels of ICAM-1 have been shown to predict future CV events and are associated with death due to CV events in distinct populations [71, 72].

Vascular Cell Adhesion Molecule-1 (VCAM-1)

VCAM-1 expression in vessels is increased when endothelial cells are stimulated by cytokines (namely, TNF-α and IL-1), facilitating adhesion and migration of leukocytes across the endothelial barrier. sICAM-l is viewed as a general marker of a pro-inflammatory status and correlates with CRP in primary prevention studies. While ICAM-1 predicts symptomatic disease in healthy individuals, VCAM-1 seems to be a better marker of the extent and severity of atherosclerosis in patients with established disease. VCAM-1 has been reported to predict future cardiovascular events in patients with CAD and CHD [71, 73] and proved to be a better predictor than ICAM and E-selectin in a study that evaluated these three cell adhesion molecules [73].

E-selectin, VCAM-1, and ICAM-1 could be viewed as markers of inflammation and activation of endothelial cells, but their prognostic value remains unclear, deserving further elucidation.

Chemokines

Chemokines are pro-inflammatory chemotactic cytokines present in circulation and in atherosclerotic lesions and cause leukocyte migration into vascular-inflamed tissue, being also involved in SMC migration and growth and platelet activation [74].

Interleukin-8 (IL-8)

IL-8 is mainly produced by monocytes and macrophages and acts as a chemoattractant for neutrophils and T lymphocytes. While most of the current knowledge was obtained from experimental animal studies, clinical data showed that IL-8 might have a predictive value for CV events, namely, in patients presenting with acute MI [75].

Migration Inhibitory Factor (MIF)

MIF is expressed in a wide variety of tissues, where it is able to promote the synthesis of other pro-inflammatory mediators. During the progression of atherosclerosis, MIF is overexpressed in endothelial cells, SMCs, and macrophages [76]. Preclinical and clinical data have been showing divergent data, but increased circulating levels

of MIF were associated to future CV events in patients with stable CAD and type 2 diabetes mellitus, even after adjusting for the traditional risk factors [77].

Monocyte Chemoattractant-1 (MCP-1)

MCP-1 is one of the key chemokines that regulate migration and infiltration of monocytes and macrophages and appears to play a relevant role in atherosclerotic lesions. Elevated levels of MCP-1 have been suggested as direct markers of inflammation for populations of CVD risk [78].

Other Molecules

Matrix Metalloproteinases (MMPs)

MMPs are a family of endopeptidases predominantly expressed in macrophages but also in vascular SMCs, lymphocytes, and endothelial cells, playing a role in vascular remodeling, progression of atherosclerosis, and plaque destabilization [79]. By degrading the extracellular matrix, MPPs promote increment of plaque vulnerability when submitted to mechanical stresses, thus increasing risk of acute CV events. In fact, increased levels of MMPs were found in distinct risk populations, namely, in those with acute coronary syndromes [80].

Myeloperoxidase (MPO)

MPO is a heme protein mainly secreted by activated neutrophils and monocytes, which has also been found in human plaques and exerts proatherogenic effects, including oxidation of LDL and reduction of NO bioavailability [81]. Elevated levels of MPO have been indicated as an independent predictor of mortality in acute MI patients and of future CV events, even after correction for traditional risk factors and CRP; however, it seems to be a weaker predictor than these established CV risk factors and CRP [82, 83].

Unanswered Questions and Challenges

The revolution in the understanding of the pathophysiology of atherosclerosis has focused interest on inflammation and provided new insight into mechanisms of disease. Several lines of evidence illustrate the remarkable data that associate inflammation with risk of future CV events and emphasize the pivotal relevance of inflammatory mechanisms in determining plaque vulnerability. Clinical application of the concept that inflammation is crucial in the initiation and progression of atherosclerosis illustrates the translation of basic science understanding to clinical practice.

During the last years, several putative biomarkers of inflammation have been tentatively adopted to improve diagnostic capacity, to monitor disease activity and efficacy of therapy, as well as to improve prognosis. However, evaluation of clinical use of biomarkers in the context of atherosclerotic CV disease requires considerable attention, starting from proper distinction between risk factor and risk marker, which depends whether (risk factor) or not (biomarker) it has a causal role in the pathology. Additionally, the putative utility of the biomarker should be clearly established, defining if it will be useful for risk stratification of healthy individuals or diseased populations, if it could be used per se or in addition to traditional accepted risk factors, or if it could be used to monitor efficacy of therapy. These questions will determine the type of validation needed. Before a novel marker reaches clinical application, important conditions must be met: there should be robust data coming from several large-scale prospective studies; the marker must improve knowledge upon traditional risk evaluation; there should be a standardized assay to its feasible quantification, and it should potentially assist in therapeutic interventions.

Although the circulating concentrations of several inflammatory mediators correlate with increased CV risk or were able to predict future events, few have been able to be considered as really candidates for clinical use. Despite the controversies, hsCRP has been viewed as the strongest candidate to clinically act as a biomarker. hsCRP has proved to be robust because it is a stable protein (analyte), with a standardized and high-sensitivity assay, it has minor diurnal variations and a long plasma half-life, and it is independent of food intake. One of the major problems is that elevated levels of hsCRP, despite strongly associated with risk, do not allow to infer directly the presence of a disease, but of an inflammatory state, acting as a biomarker rather than a risk factor. The causality can only be considered after properly excluding the contribution of confounders. A wide collection of studies shows that epidemiologic associations between hsCRP and CVD outcomes are independent of other risk factors. However, several of them have not properly adjusted all the modifiable (obesity, insulin resistance, or physical inactivity) and non-modifiable (genetic or ethnic characteristics) risk factors, which is obviously relevant, because several risk factors for atherosclerosis, such as smoking habits, obesity, insulin resistance, diabetes, or dyslipidemia, are themselves associated with increased inflammation.

Emerging evidence has shown a strong relationship between hsCRP and various characteristics of MetS. The addition of hsCRP measurement to the actual definition of MetS may help in identifying patients at high risk for future diabetes and CVD. Further research is required to clarify the precise role of hsCRP in MetS pathogenesis and whether it is able to improve prediction of CV events in patients with elevated hsCRP concentrations.

Accumulated evidence of improved CV risk prediction using hsCRP levels independently of LDL-C led to the development of the Reynolds Risk Score, which adds hsCRP to the FRS and improves global CV risk prediction in women by reclassifying up to 50 % of subjects considered at intermediate risk into higher- or lower-risk categories. An expert panel assembled by CDC and AHA has recommended that global risk prediction in asymptomatic individuals deemed at intermediate risk for

CVD by classical risk factors should include hsCRP measurement using the cutoff points of <1 mg/L for low-risk and >3 mg/L for high-risk individuals.

If hsCRP is a risk factor with a causal role, interventions targeted towards lowering its levels should improve outcomes. JUPITER study showed a clear benefit of aggressive statin (rosuvastatin) therapy in patients with hsCRP greater than 2 mg/L that do not meet formal criteria for statin prescription (given the low/average LDL-C concentrations). The remarkable reduction in CV events and in all-cause mortality, among several other positive indications, in the JUPITER trial, suggested that at least part of the benefits of statin therapy were due to anti-inflammatory effects independent of LDL-C reduction. That strong evidence coming from the trial had impacted on the spectrum of statin clinical usage according to the FDA, as well as several national authorities, now considering for treating patients with elevated hsCRP levels and at least one additional risk factor, independently of high or average LDL-C levels. Two clinical trials (CANTOS and CIRT) are ongoing, aiming to test the hypothesis that directly targeting inflammation will improve vascular outcomes and the results might bring new light on the issue.

With the exception of hsCRP, until now, none of the emerging/novel inflammation biomarkers for CV risk has demonstrated additive value to the FRS, and few have available commercial assays that achieve adequate levels of standardization and accuracy for clinical use. Like CRP, SAA and fibrinogen are acute-phase reactants that seem to be involved in several steps of atherosclerosis mechanisms, despite that an undoubtedly causal role in atherothrombogenesis remains to be elucidated. In addition, fibrinogen seems to be a less potent predictor of CV events than hsCRP, and the predictive value of SAA for CAD and CV events seems to be dependent on other risk factors, deserving further research.

The balance between pro- and anti-inflammatory cytokines may be important for risk stratification and also to identify patients who might benefit from targeted therapy. Measurement of pro-inflammatory (namely, IL-6, IL-1, TNF-α, and INF-γ) and anti-inflammatory cytokines (namely, IL-4, IL-10, TGF-β, and adiponectin) may be useful for indicating the complex interplay between inflammatory and anti-inflammatory processes. Despite preclinical and clinical evidences suggesting that pro-inflammatory cytokines (namely, TNF-α, IL-6, and sCD40L), but also INF-γ and IL-1, have a role in atherosclerosis development and that their levels could be predictive of future CVD events and CV deaths, more data are needed, in order to validate these molecules as biomarkers of CVD. The clinical utility of adiponectin is based on its strong epidemiologic relationships with obesity, inflammation, and diabetes, strengthened by its established biological actions in blood vessels and immune cells. Adiponectin levels have been widely evaluated as epidemiologic markers of diabetes and CVD risk, and increased concentrations of adiponectin are being studied as indicators of treatment need, as predictors of response to therapy, and as markers of therapeutic effectiveness, in order to feasibly translate adiponectin measurements into clinical practice.

E-selectin, VCAM-1, and ICAM-1, all belonging to the cellular adhesion molecule family, have been tested as markers of early onset of inflammation, but their prognostic value remains uncertain. VCAM-1 seems to be unable to act as risk factor in healthy individuals but as strong predictor of risk in patients.

The role of chemokines (including IL-8, MCP-1, and MIF) as biomarkers remains also unclear. While IL-8 and MCP-1 have shown ability to act as markers of inflammation in subpopulations of CVD-diagnosed patients, MIF seems to show ability to predict future CV events in patients with stable CAD or type 2 diabetes. MMPs may be useful for prognosis in patients with ACS, while elevated MPO levels have been suggested to predict future risk of CAD in healthy subjects, but for both of them, available data are insufficient to dispose of a direct clinical application.

Studies including a larger number of patients are needed to confirm some data already available. However, some strategies must be previously considered to be successful, such as include serial determinations in protocols (and not only baseline measurements) and recruit preferably patients with preexisting CAD or ACS, instead of healthy populations. In addition, the quality of the trials and the power of the evidence will depend on the evaluation of the relationship between the concentrations of these molecules and the degree of atherosclerosis and plaque instability, which is better estimated using new technical approaches, namely, molecular imaging.

Due to the complexities of CVD pathogenesis, a single biomarker cannot be used to estimate absolute risk of future CV events. Furthermore, particular biomarkers are more suited for prognosis of particular events and for a given stage of a given CVD. It should also be recognized that the biological functions of many biomarkers may overlap. Therefore, they should be selected for a specific stage of a given disease, and a particular biomarker should not be considered in isolation. Simultaneous measurements of disease appropriate biomarkers over time can provide a more detailed picture of the specific nature of the CV event.

It is also important to underscore that the lack of value as a biomarker does not exclude an important pathogenic role of these molecules in atherogenesis and plaque destabilization and, accordingly, does not negate the potential value as novel targets for therapy in atherosclerotic disorders.

Further studies, both in progress and on the horizon, will help to evaluate the role of novel and emerging biomarkers in the clinical management of atherosclerosis and targeting of therapies. Until then, while measurement of inflammatory biomarkers is a valuable adjunct to CVD risk assessment, the emphasis should not digress from lowering the burden of conventional modifiable risk factors.

Take-Home Messages

- Even though conventional risk factors in the FRS account for most of the risk of CV events, a substantial percentage of subjects die from CHD. In addition, many of those events occur in patients treated with statins and presenting with cholesterol levels below population average. Thus, an improved ability to predict CV risk and decrease the high level of residual risk of further CV events is mandatory.
- A major shift in the paradigm of our understanding of atherosclerosis pathogenesis has been seen in the last decade, which is now recognized as having a clear inflammatory signature, since inflammatory mechanisms play a crucial role in all

phases of the disease, from initial recruitment of circulating leucocytes to the arterial wall to eventual rupture of the unstable plaque.

- The ominous presence of inflammation in atherosclerosis allows the consideration of a number of emergent biomarkers as potentially useful tools to help in identifying patients at high risk for future CV events, including acute-phase reactants, pro- and anti-inflammatory cytokines, cell adhesion molecules, chemokines, as well as other mediators involved in the pathogenesis of atherosclerosis.
- While experimental and/or clinical data have been shown the possibility of several emergent biomarkers to have predictive value in determining future CV events and/or death, until now only hsCRP has demonstrated additive value to the FRS. Therefore, an expert panel assembled by the CDC and the AHA recommended that global risk prediction in asymptomatic individuals deemed at intermediate risk for CVD by classical risk factors should include hsCRP measurement and the cutoff points of <1 mg/L for low-risk and >3 mg/L for high-risk individuals.
- Further studies, some of them already ongoing and others on the horizon, will define the precise value of all these novel/emerging biomarkers of inflammation in the clinical management of CVD risk prediction and therapy.

Acknowledgments/Support The authors thank Portuguese Foundation for Science and Technology (FCT), COMPETE-FEDER and POPH/FSE, through the following support: PTDC/SAU-TOX/114253/2009, UID/NEU/04539/2013 (CNC.IBILI).

References

1. Stavitsky AB. The innate immune response to infection, toxins and trauma evolved into networks of interactive, defensive, reparative, regulatory, injurious and pathogenic pathways. Mol Immunol. 2007;44(11):2787–99.
2. Roda JM, Eubank TD. Chapter 2: The cellular component of chronic inflammation. In: Roy S, Bagchi D, Raychaudhuri SP, editors. Chronic inflammation: molecular pathophysiology, nutritional and therapeutic interventions. Boca Raton: CRC Press, Taylor & Francis Group; 2013. p. 21–33.
3. World Health Organization. Global status report on non-communicable diseases 2010. Geneva: World Health Organization; 2011.
4. Ghazalpour A, Doss S, Yang X, Aten J, Toomey EM, Van Nas A, et al. Thematic review series: the pathogenesis of atherosclerosis. Toward a biological network for atherosclerosis. J Lipid Res. 2004;45(10):1793–805.
5. Khot UN, Khot MB, Bajzer CT, Sapp SK, Ohman EM, Brener SJ, et al. Prevalence of conventional risk factors in patients with coronary heart disease. JAMA. 2003;290(7):898–904.
6. Smith Jr SC. Current and future directions of cardiovascular risk prediction. Am J Cardiol. 2006;97(2A):28A–32.
7. Baigent C, Keech A, Kearney PM, Blackwell L, Buck G, Pollicino C, et al. Efficacy and safety of cholesterol lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. Lancet. 2005;366(9493):1267–78.
8. Cannon CP, Braunwald E, McCabe CH, Rader DJ, Rouleau JL, Belder R, et al. Intensive versus moderate lipid lowering with statins after acute coronary syndromes. N Engl J Med. 2004;350(15):1495–504.

9. Ross R. Atherosclerosis — an inflammatory disease. N Engl J Med. 1999;340(2):115–26.
10. Shah PK. Mechanisms of plaque vulnerability and rupture. J Am Coll Cardiol. 2003;41(4 Suppl 1):S15–22.
11. Nissen SE, Yock P. Intravascular ultrasound: novel pathophysiological insights and current clinical applications. Circulation. 2001;103(4):604–16.
12. Libby P, Ridker PM, Hansson GK. Progress and challenges in translating the biology of atherosclerosis. Nature. 2011;473(7347):317–25.
13. Galkina E, Ley K. Immune and inflammatory mechanisms of atherosclerosis. Annu Rev Immunol. 2009;27:165–97.
14. Pai JK, Pischon T, Ma J, Manson JE, Hankinson SE, Joshipura K, et al. Inflammatory markers and the risk of coronary heart disease in men and women. N Engl J Med. 2004;351(25):2599–610.
15. Libby P, Aikawa M. Stabilization of atherosclerotic plaques: new mechanisms and clinical targets. Nat Med. 2002;8(11):1257–62.
16. Alberti KG, Zimmet P, Shaw J. The metabolic syndrome – a new worldwide definition. Lancet. 2005;366(9491):1059–62.
17. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults: Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III.) JAMA. 2001;285(19):2486–97.
18. Després JP. Abdominal obesity and cardiovascular disease: is inflammation the missing link? Can J Cardiol. 2012;28(6):642–52.
19. Haffner S, Cassells HB. Metabolic syndrome – a new risk factor of coronary heart disease? Diabetes Obes Metab. 2003;5(6):359–70.
20. Galassi A, Reynolds K, He J. Metabolic syndrome and risk of cardiovascular disease: a meta-analysis. Am J Med. 2006;119(10):812–9.
21. Gami AS, Witt BJ, Howard DE, Erwin PJ, Gami LA, Somers VK, et al. Metabolic syndrome and risk of incident cardiovascular events and death: a systematic review and meta-analysis of longitudinal studies. J Am Coll Cardiol. 2007;49(4):403–14.
22. Després JP, Lemieux I. Abdominal obesity and metabolic syndrome. Nature. 2006;444(7121):881–7.
23. Vasan RS. Biomarkers of cardiovascular disease: molecular basis and practical considerations. Circulation. 2006;113(19):2335–62.
24. Ge Y, Wang TJ. Circulating, imaging, and genetic biomarkers in cardiovascular risk prediction. Trends Cardiovasc Med. 2011;21(4):105–12.
25. Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther. 2001;69(3):89–95.
26. Rao M, Jaber BL, Balakrishnan VS. Inflammatory biomarkers and cardiovascular risk: association or cause and effect? Semin Dial. 2006;19(2):129–35.
27. Devaraj S, Singh U, Jialal I. The evolving role of C-reactive protein in atherothrombosis. Clin Chem. 2009;55(2):229–38.
28. Verma S, Szmitko PE, Ridker PM. C-reactive protein comes of age. Nat Clin Pract Cardiovasc Med. 2005;2(1):29–36.
29. Verma S, Devaraj S, Jialal I. Is C-reactive protein an innocent bystander or proatherogenic culprit? C-reactive protein promotes atherothrombosis. Circulation. 2006;113(17):2135–50.
30. Devaraj S, Yun JM, Adamson G, Galvez J, Jialal I. C-reactive protein impairs the endothelial glycocalyx resulting in endothelial dysfunction. Cardiovasc Res. 2009;84(3):479–84.
31. Libby P, Ridker PM, Hansson GK. Inflammation in atherosclerosis: from pathophysiology to practice. J Am Coll Cardiol. 2009;54(23):2129–38.
32. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med. 2000;342(12):836–43.
33. Ridker PM, Rifai N, Rose L, Buring JE, Cook NR. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. N Engl J Med. 2002;347(20):1557–65.

34. Koenig W, Lowel H, Baumert J, Meisinger C. C-reactive protein modulates risk prediction based on the Framingham Score: implications for future risk assessment: results from a large cohort study in southern Germany. Circulation. 2004;109(11):1349–53.
35. Kaptoge S, Di Angelantonio E, Lowe G, Pepys MB, Thompson SG, Collins R, et al. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. Lancet. 2010;375(9709):132–40.
36. Ridker PM, Buring JE, Cook NR, Rifai N. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14 719 initially healthy American women. Circulation. 2003;107(3):391–7.
37. Ridker PM, Buring JE, Rifai N, Cook NR. Development and validation of improved algorithms for the assessment of global cardiovascular risk in women: the Reynolds risk score. JAMA. 2007;297(6):611–9.
38. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon 3rd RO, Criqui M, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation. 2003;107(3):499–511.
39. Ridker PM, Rifai N, Clearfield M, Downs JR, Weis SE, Miles JS, et al. Measurement of C-reactive protein for the targeting of statin therapy in the primary prevention of acute coronary events. N Engl J Med. 2001;344(26):1959–65.
40. Ridker PM, Cannon CP, Morrow D, Rifai N, Rose LM, McCabe CH, et al. C-reactive protein levels and outcomes after statin therapy. N Engl J Med. 2005;352(1):20–8.
41. Braunwald E. Creating controversy where none exists: the important role of C-reactive protein in the CARE, AFCAPS/TexCAPS, PROVE IT, REVERSAL, A to Z, JUPITER, HEART PROTECTION, and ASCOT trials. Eur Heart J. 2012;33(4):430–2.
42. Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto Jr AM, Kastelein JJ, et al. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. N Engl J Med. 2008;359(21):2195–207.
43. Mora S, Glynn RJ, Hsia J, Macfadyen JG, Genest J, Ridker PM. Statins for the primary prevention of cardiovascular events in women with elevated high-sensitivity C-reactive protein or dyslipidemia. Results from the Justification for the Use of Statins in Prevention: An intervention trial evaluating rosuvastatin (JUPITER) and meta-analysis of women from primary prevention trials. Circulation. 2010;121(9):1069–77.
44. Glynn RJ, Koenig W, Nordestgaard BG, Shepherd J, Ridker PM. Rosuvastatin for primary prevention in older persons with elevated C-reactive protein and low to average low-density lipoprotein cholesterol levels: exploratory analysis of a randomized trial. Ann Intern Med. 2010;152(8):488–96.
45. Ridker PM. Moving beyond JUPITER: will inhibiting inflammation reduce vascular event rates? Curr Atheroscler Rep. 2013;15(1):295.
46. Ridker PM, MacFadyen JG, Fonseca FA, Genest J, Gotto AM, Kastelein JJ, et al. Number needed to treat with rosuvastatin to prevent first cardiovascular events and death among men and women with low low-density lipoprotein cholesterol and elevated high-sensitivity C-reactive protein: justification for the use of statins in prevention: an intervention trial evaluating rosuvastatin (JUPITER). Circ Cardiovasc Qual Outcomes. 2009;2(6):616–23.
47. Ridker PM, Thuren T, Zalewski A, Libby P. Interleukin-1beta inhibition and the prevention of recurrent cardiovascular events: rationale and design of the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS). Am Heart J. 2011;162(4):597–605.
48. Ridker PM. Testing the inflammatory hypothesis of atherothrombosis: scientific rationale for the cardiovascular inflammation reduction trial (CIRT). J Thromb Haemost. 2009;7 Suppl 1:332–9.
49. Koenig W. Fibrinogen in cardiovascular disease: an update. Thromb Haemost. 2003;89(4):601–9.
50. Ridker PM, Stampfer MJ, Rifai N. Novel risk factors for systemic atherosclerosis: a comparison of C-reactive protein, fibrinogen, homocysteine, lipoprotein(a), and standard cholesterol screening as predictors of peripheral arterial disease. JAMA. 2001;285(19):2481–5.

51. Artl A, Marsche G, Lestavel S, Sattler W, Malle E. Role of serum amyloid A during metabolism of acute-phase HDL by macrophages. Arterioscler Thromb Vasc Biol. 2000;20(3):763–72.
52. Morrow DA, Rifai N, Antman EM, Weiner DL, McCabe CH, Cannon CP, et al. Serum amyloid A predicts early mortality in acute coronary syndromes: a TIMI 11A substudy. J Am Coll Cardiol. 2000;35(2):358–62.
53. Tedgui A, Mallat Z. Cytokines in atherosclerosis: pathogenic and regulatory pathways. Physiol Rev. 2006;86(2):515–81.
54. McKellar GE, McCarey DW, Sattar N, McInnes IB. Role for TNF in atherosclerosis? Lessons from autoimmune disease. Nat Rev Cardiol. 2009;6(6):410–7.
55. Nieto-Vazquez I, Fernández-Veledo S, Krämer DK, Vila-Bedmar R, Garcia-Guerra L, Lorenzo M. Insulin resistance associated to obesity: the link TNF-alpha. Arch Physiol Biochem. 2008;114(3):183–94.
56. Ridker PM, Rifai N, Pfeffer M, Sacks F, Lepage S, Braunwald E. Elevation of tumor necrosis factor-alpha and increased risk of recurrent coronary events after myocardial infarction. Circulation. 2000;101(18):2149–53.
57. Schuett H, Luchtefeld M, Grothusen C, Grote K, Schieffer B. How much is too much? Interleukin-6 and its signalling in atherosclerosis. Thromb Haemost. 2009;102(2):215–22.
58. Ridker PM, Rifai N, Stampfer MJ, Hennekens CH. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. Circulation. 2000;101(15):1767–72.
59. Lutgens E, Lievens D, Beckers L, Donners M, Daemen M. CD40 and its ligand in atherosclerosis. Trends Cardiovasc Med. 2007;17(4):118–23.
60. Heeschen C, Dimmeler S, Hamm CW, van den Brand MJ, Boersma E, Zeiher AM, et al. Soluble CD40 ligand in acute coronary syndromes. N Engl J Med. 2003;348(12):1104–11.
61. Heeschen C, Dimmeler S, Hamm CW, Fichtlscherer S, Boersma E, Simoons ML, et al. Serum level of the antiinflammatory cytokine interleukin-10 is an important prognostic determinant in patients with acute coronary syndromes. Circulation. 2003;107(16):2109–14.
62. Jha HC, Divya A, Prasad J, Mittal A. Plasma circulatory markers in male and female patients with coronary artery disease. Heart Lung. 2010;39(4):296–303.
63. Cipollone F, Fazia M, Mincione G, Iezzi A, Pini B, Cuccurullo C, et al. Increased expression of transforming growth factor-β1 as a stabilizing factor in human atherosclerotic plaques. Stroke. 2004;35:2253–7.
64. Stefoni S, Cianciolo G, Donati G, Dormi A, Silvestri MG, Colì L, et al. Low TGF-β1 serum levels are a risk factor for atherosclerosis disease in ESRD patients. Kidney Int. 2002;61:324–35.
65. Koch W, Hoppmann P, Mueller JC, Schomig A, Kastrati A. Association of transforming growth factor-β1 gene polymorphisms with myocardial infarction in patients with angiographically proven coronary heart disease. Arterioscler Thromb Vasc Biol. 2006;26:1114–9.
66. Ouchi N, Shibata R, Walsh K. Cardioprotection by adiponectin. Trends Cardiovasc Med. 2006;16(5):141–6.
67. Pischon T, Girman CJ, Hotamisligil GS, Rifai N, Hu FB, Rimm EB. Plasma adiponectin levels and risk of myocardial infarction in men. JAMA. 2004;291(14):1730–7.
68. Frystyk J, Berne C, Berglund L, Jensevik K, Flyvbjerg A, Zethelius B. Serum adiponectin is a predictor of coronary heart disease: a population-based 10-year follow-up study in elderly men. J Clin Endocrinol Metab. 2007;92(2):571–6.
69. Miwa K, Igawa A, Inoue H. Soluble E-selectin, ICAM-1 and VCAM-1 levels in systemic and coronary circulation in patients with variant angina. Cardiovasc Res. 1997;36(1):37–44.
70. Roldan V, Marin F, Lip GY, Blann AD. Soluble E-selectin in cardiovascular disease and its risk factors. A review of the literature. Thromb Haemost. 2003;90(6):1007–20.
71. Luc G, Arveiler D, Evans A, Amouyel P, Ferrieres J, Bard JM, et al. Circulating soluble adhesion molecules ICAM-1 and VCAM-1 and incident coronary heart disease: the PRIME Study. Atherosclerosis. 2003;170(1):169–76.

72. Jenny NS, Arnold AM, Kuller LH, Sharrett AR, Fried LP, Psaty BM, et al. Soluble intercellular adhesion molecule-1 is associated with cardiovascular disease risk and mortality in older adults. J Thromb Haemost. 2006;4(1):107–13.
73. Blankenberg S, Rupprecht HJ, Bickel C, Peetz D, Hafner G, Tiret L, et al. Circulating cell adhesion molecules and death in patients with coronary artery disease. Circulation. 2001;104(12):1336–42.
74. Braunersreuther V, Mach F, Steffens S. The specific role of chemokines in atherosclerosis. Thromb Haemost. 2007;97(5):714–21.
75. Prondzinsky R, Unverzagt S, Lemm H, Wegener NA, Schlitt A, Heinroth KM, et al. Interleukin-6, −7, −8 and −10 predict outcome in acute myocardial infarction complicated by cardiogenic shock. Clin Res Cardiol. 2012;101:375–84.
76. Burger-Kentischer A, Goebel H, Seiler R, Fraedrich G, Schaefer HE, Dimmeler S, et al. Expression of macrophage migration inhibitory factor in different stages of human atherosclerosis. Circulation. 2002;105(13):1561–6.
77. Herder C, Illig T, Baumert J, Muller M, Klopp N, Khuseyinova N, et al. Macrophage migration inhibitory factor (MIF) and risk for coronary heart disease: results from the MONICA/KORA Augsburg case-cohort study, 1984–2002. Atherosclerosis. 2008;200(2):380–8.
78. Martinovic I, Abegunewardene N, Seul M, Vosseler M, Horstick G, Buerke M, et al. Elevated monocyte chemoattractant protein-1 serum levels in patients at risk for coronary artery disease. Circ J. 2005;69(12):1484–9.
79. Lijnen HR. Plasmin and matrix metalloproteinases in vascular remodeling. Thromb Haemost. 2001;86(1):324–33.
80. Inokubo Y, Hanada H, Ishizaka H, Fukushi T, Kamada T, Okumura K. Plasma levels of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 are increased in the coronary circulation in patients with acute coronary syndrome. Am Heart J. 2001;141:211–7.
81. Nicholls SJ, Hazen SL. Myeloperoxidase and cardiovascular disease. Arterioscler Thromb Vasc Biol. 2005;25:1102–11.
82. Vita JA, Brennan M-L, Gokce N, Mann SA, Goormastic M, Shishehbor MH, et al. Serum myeloperoxidase levels independently predict endothelial dysfunction in humans. Circulation. 2004;110(9):1134–9.
83. Baldus S, Heeschen C, Meinertz T, Zeiher AM, Eiserich JP, Munzel T, et al. Myeloperoxidase serum levels predict risk in patients with acute coronary syndromes. Circulation. 2003;108(12):1440–5.