

Review

High-sensitivity C-reactive protein and atherosclerotic disease: From improved risk prediction to risk-guided therapy

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ABSTRACT

There is compelling experimental and clinical evidence suggesting a crucial role for inflammation in the initiation and also the progression of atherosclerosis. Numerous biomarkers involved at various levels of the inflammation cascade have been shown to be associated with adverse cardiovascular outcomes. Yet, to date, it is not clear whether inflammation simply accompanies the atherosclerotic process or represents a major driver. Among all blood biomarkers, C-reactive protein (CRP), the classical acute phase reactant that can be measured with high-sensitivity (hs) assays seems to be the most promising candidate. It has already found its way into the guidelines in primary prevention. Hs-CRP can also be used to identify a high-risk group for recurrent events in patients with manifest atherosclerosis. Several post hoc analyses of large-scale randomized clinical trials testing various statins have indicated that, besides low density lipoprotein (LDL) cholesterol, hs-CRP levels might also further aid in tailoring statin treatment. The large JUPITER trial has prospectively confirmed these findings in primary prevention in patients with elevated hs-CRP but normal LDL cholesterol levels. Still, statin therapy is not a specific anti-inflammatory regime acting on the inflammation cascade. Thus, to directly test the inflammation hypothesis, a novel, more proximally located cytokine-based approach is needed. Canakinumab, a fully human monoclonal antibody against interleukin-1 β , might represent a promising compound in this regard and provide a proof of concept. If successful, this may become a novel strategy to treat high-risk patients with stable atherosclerotic disease to prevent recurrent events on top of standard medical care.

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Abbreviations: ACS, acute coronary syndrome; AHA, American Heart Association; ASCOT, Anglo-Scandinavian Cardiac Outcomes Trial; A-to-Z, Aggrastat-to-Zocor; CAD, coronary artery disease; CARE, Cholesterol and Recurrent Events; CHD, coronary heart disease; CI, confidence interval; CRP, C-reactive protein; CVD, cardiovascular disease; ECAT, European Concerted Action on Thrombosis and Disabilities; FRS, Framingham Risk Score; GPx-1, glutathione peroxidase; GRACE, Global Registry of Acute Coronary Events; GWAS, genome-wide association and replication study; HPS, Heart Protection Study; Hs-CRP, high-sensitivity C-reactive protein; HDL, high-density lipoprotein; HAECs, human aortic endothelial cells; IL, interleukin; JUPITER, Justification for the Use of Statins in Primary Prevention: an Intervention Trial Evaluating Rosuvastatin; LDL, low-density lipoprotein; Lp-PLA₂, lipoprotein associated phospholipase A₂; LOX-1, lectin-type oxidized LDL receptor 1; MCP-1, monocyte chemoattractant protein-1; MMPs, matrix metalloproteinases; MPO, myeloperoxidase; oxLDL, oxidized LDL; PAPP-A, pregnancy-associated plasma protein-A; PlGF, placental growth factor; PROVE-IT TIMI 22, Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis in Myocardial Infarction 22; PROCAM, Prospective Cardiovascular Münster; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule; SAA, serum amyloid A protein; SCORE, Systematic COronary Risk Evaluation; SOLID-TIMI, Stabilization of pLaques using Darapladib-Thrombolysis in Myocardial Infarction; SNP, single-nucleotide polymorphism; STABILITY, STabilization of Atherosclerotic plaque By Initiation of darapladib Therapy; THROMBO, Thrombotic Risk Factor; TIMI, Thrombolysis in Myocardial Infarction; TNF- α , tumor necrosis factor- α ; ULSAM, Uppsala Longitudinal Study of Adult Men; VILCAD, the Vienna and Ludwigshafen Coronary Artery Disease.

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1. Introduction

Inflammation plays a critical role in the atherosclerotic process in various vascular beds, starting from endothelial dysfunction through all stages of plaque build-up until its detrimental clinical ischemic complications [1,2]. Considerable evidence on the significant contribution of inflammation in the initiation and progression of atherosclerosis has been gathered over many years and there is an abundance of experimental and clinical studies that support this concept. Yet, they have not translated into clinical practice [3,4]. This review attempts to summarize the evidence on hs-CRP, the classical acute-phase protein and an extremely sensitive marker of systemic inflammation, as an important molecule for improved risk prediction in patients at risk of developing CHD, after an ACS, and long term during the stable phase of CHD. A number of retrospective analyses of large-scale trials in ACS and prospective analyses in the JUPITER suggest that the potential of hs-CRP may even go beyond improved risk prediction and may in fact be an important marker in deciding on statin therapy besides low density lipoprotein cholesterol (LDL-C) [5]. Ultimately, the inflammation hypothesis needs to be adequately tested in a large trial, with an intervention that directly interferes with the inflammation cascade.

2. Risk assessment in patients at risk for CHD or with recurrent complications after an acute event

Cardiovascular disease (CVD) is prevalent in industrialized countries and accounts for the majority of deaths in these societies [6]. Frequently, myocardial infarction (MI) represents the first manifestation of an atherosclerotic complication. Therefore, primary prevention based on the detection and treatment of traditional risk factors is crucial in avoiding such deleterious events. Yet, despite the availability of various global risk assessment scores like the Framingham Risk Score [7], the PROCAM Score [8] and the European Society of Cardiology SCORE [9], prediction of cardiovascular events is incomplete and a considerable number of patients at risk go unidentified on the basis of traditional risk factors alone. This has prompted the search for novel markers of cardiovascular risk in apparently healthy subjects.

Similarly, in patients with manifest CVD, in particular after an ACS, a number of clinical variables have been proven useful for risk assessment. Based on these variables, various scores have been generated like the TIMI Risk Score [10], the GRACE Score [11], and the recently published VILCAD Risk Score [12]. These scores measure several important clinical variables such as history of previous MI or congestive heart failure (CHF); resting heart rate on admission to the hospital; systolic blood pressure; electrocardiographically based variables like ST segment depression; biochemical variables collected during hospitalization like creatinine and cardiac enzymes; and other laboratory variables like HbA1c and information on in-hospital procedures like percutaneous coronary intervention (PCI). Based on such variables, fairly reliable prediction can be made not only in the short term but even over a period of more than 5 years [13]. In addition, other variables such as global left ventricular (LV) function [12], results of exercise stress testing, and indicators of atherosclerotic burden like carotid plaque and the ankle-brachial index (ABI) have been proven useful. However, despite such routinely available variables, further improvement of risk assessment in this high-risk group is of paramount importance because available treatment options should be tailored to the individual patient's risk. Hence, in this regard, numerous biomarkers have been tested in various populations. Such markers include blood biomarkers relevant to the pathophysiology of atherosclerosis, for example, markers of the coagulation cascade (e.g. fibrinogen; [14], plasminogen activator inhibitor (PAI)-1; [15], markers of platelet aggregation [16]), the measurement of specific lipoproteins like lipoprotein (a) [17], apolipoprotein B and small dense LDL-C as additional markers of the lipoprotein profile. Other candidates under consideration include markers of hemodynamic stress (BNP and NT-proBNP) [18], which could reflect the functional consequences of damage to the myocardium after MI or through uncontrolled hypertension; markers of a chronically elevated inflammatory response, for example, hs-CRP and more recently sensitive markers of myocardial damage that have been already proven useful in the diagnosis of ACS, like high-sensitivity troponins (troponin T or I) [19,20].

3. CRP: Biochemistry and function

CRP is a classical acute-phase protein, which is a highly sensitive systemic marker of inflammation and tissue damage. It belongs to the pentraxin family and is composed of five identical non-glycosylated polypeptide sub-units [21]. In acute phase reactions such as in the case of an infection or extensive tissue injury, CRP rises with a dynamic range of 10,000-fold within 6 h and peaks at 48 h. Its half-life is constant at approximately 19 h; therefore, its level is mainly determined by the rate of hepatic production [22]. The gene responsible for its synthesis is located on the proximal long arm of chromosome 1. A significant association between CRP genotypes and CRP concentrations has been documented, and heritability estimates for CRP baseline concentrations are between 35% and 40% [23]. CRP binds to phosphocholine residues expressed on the surface of microbes and other autologous and extrinsic ligands such as native and modified plasma lipoproteins,

damaged cell membranes, different phospholipids and apoptotic cells; through these ways it may assist in complement binding to foreign and damaged cells and enhance phagocytosis [22,24]. Yet, the precise *in vivo* role of CRP is not completely understood.

4. CRP – more than a bystander?

Although there is still an ongoing debate on whether or not CRP may be directly involved in the atherosclerotic process, a number of *in vitro* and animal studies suggest a pro-atherogenic role for CRP. Mechanisms that have been elucidated cover a role for CRP in decreasing endothelial nitric oxide synthase mRNA protein expression as well as bioactivity in HAEcs [25], an increase in LOX-1 expression which is crucial for oxidized LDLs having a detrimental effect on endothelial function [26] and enhancement of angiotensin II-induced pro-inflammatory effects [27]. It has also been shown to impair the number and function of endothelial progenitor cells by promoting apoptosis and attenuating their migration and adherence capacities. Moreover, mechanistic approaches have related CRP to a pro-thrombotic state by stimulating tissue factor (TF) release from mononuclear endothelial smooth muscle cells, increase (PAI)-1 activity with a concomitant reduction in tissue type plasminogen activator (t-PA) activity resulting in overall impaired fibrinolysis [28]. In addition, it has been ascribed to increase MMPs synthesis with a consecutive increase in collagen degradation in monocyte-macrophages. Finally, CRP activates the complement system, resulting in a significant increase in infarct size in various MI models [28], as well as in humans [29]. Additional mechanistic support comes from data that demonstrate an acceleration of the progression of atherosclerosis in apolipoprotein E-deficient mice suggesting direct involvement of CRP in atherogenesis and its presence in atherosclerotic lesions but not in the normal vessel wall. Finally, it has been shown that CRP might be involved in the uptake of LDL-C by macrophages, turning them into foam cells, a most critical step in atherogenesis [30,31].

Although, some of the aforementioned experimental studies suggest a role for CRP in atherogenesis, an ongoing controversy exists regarding their validity. One important argument consists in the notion that the majority of experimental data retrieved with CRP might be a consequence of contamination. However, several carefully designed controlled experiments by various groups have demonstrated that CRP itself is a major contributor. For example, Devaraj et al. and Singh et al. showed that heat-induced CRP denaturation abolished the pro-atherogenic effects [32,33]. Furthermore, Bisoendial et al. demonstrated that infusion of CRP induces inflammation and activates coagulation in humans [34]. They also showed that small amounts of endotoxin that may be present in recombinant human CRP do not contribute to its pro-atherogenic effects [35,36]. Such data seem to be in favor of CRP to exert pro-atherogenic effects both *in vitro* and in humans.

On the other hand, recently published data from genetic studies do not support a causal role of CRP in the pathogenesis of atherosclerosis. Mendelian randomization in four large independent Danish cross-sectional studies and prospective cohorts have demonstrated that elevated CRP is associated with increased risk of CVD and that genetic variation in the CRP gene associates with increased CRP levels, but this genetic variation in the CRP gene does not associate with increased risk of CVD [37]. Using a GWAS study to identify genetic variants associated with CRP levels, Elliot et al. identified a SNP in the CRP gene associated with a 20% decrease in the CRP level. A systematic review of 35 observational studies predicted that CHD risk would be reduced by 6% for this degree of CRP reduction. However, in the Mendelian randomization experiment this SNP was not associated with CHD in pooled studies nor were two other CRP SNPs associated with CHD in another meta-analysis [38]. However, there are several limitations regarding inferences about causality using Mendelian randomization: it can be influenced by linkage disequilibrium, pleiotropy of CRP SNPs that influence other

biomarkers that cause or prevent CHD, gene–gene interactions, and canalization.

Karakas et al. investigated the association of the CRP receptor FC γ RIIIa genotype with risk of CHD in two large population-based samples [39]. The results from this study did not suggest that the HH131 genotype of FC γ RIIIa which is associated with strongly decreased binding capacity for CRP, and therefore, expected to be less susceptible to atherosclerotic disease, is associated with a lower risk of CHD. Taken together, observed associations between serum CRP and CHD are unlikely to be causal. Inflammation may rather play a causal role by upstream effects than through the downstream marker CRP. Yet, the ultimate proof is still missing because of the absence of adequate animal models and a lack of direct CRP intervention studies in humans.

5. Predictive value of hs-CRP in subjects at risk for CHD

Among all emerging blood biomarkers, so far the largest database exists for CRP measured by hs-assays. As early as in 1997, Ridker et al. demonstrated a strong association between elevated hs-CRP levels and cardiovascular outcome in the Physicians' Health Study [40]. This was followed by numerous well-controlled prospective epidemiological studies and the majority of those could replicate this initial finding. In a first meta-analysis by Danesh et al. based on 22 prospective studies with an average follow-up of 12 years, those in the top tertile vs. the bottom tertile of the hs-CRP distribution showed a 58% increased risk (odds ratio [OR] 1.58; 95% CI: 1.48–1.68) [41]. In addition to MI as a primary endpoint, other cardiovascular outcomes like sudden death, stroke, and peripheral arterial disease also showed the same association. More recently, newer statistical tools to assess discrimination, calibration, and reclassification have been introduced and in some studies no incremental value of hs-CRP over and above the Framingham Risk Score was seen. In the large ULSAM study, the authors demonstrated that even in the presence of other robust biomarkers like troponin I, NT-proBNP and cystatin C, hs-CRP was an independent predictor of major fatal cardiovascular event and total mortality [42]. In this study, CRP clearly showed an incremental value over and above a score based on traditional risk factors. However, in another study, Melander et al. suggested very little incremental value of any newer biomarker investigated including hs-CRP [43]. Meanwhile, a further large meta-analysis has been published by the Emerging Risk Factors Collaboration and in multivariable analysis based on individual patient data that controlled for age, sex plus study type, and in addition to classical risk factors like systolic blood pressure, smoking, history of diabetes, body mass index, triglycerides, non-HDL-C, HDL-C, and alcohol intake, hs-CRP turned out as an independent predictor of major vascular outcomes of similar magnitude as for example systolic blood pressure and it was even superior to non-HDL-C in predicting future events. The hazard ratio for coronary events in the fully adjusted model was 1.37 (95% CI: 1.27–1.48) for a one standard deviation increase in hs-CRP. For systolic blood pressure, the HR was 1.33 (95% CI: 1.23–1.45), and for non-HDL-C it was 1.28 (95% CI: 1.16–1.40) [44]. Thus, without any doubt, hs-CRP as a predictor of future cardiovascular outcomes performed equally well or better than several traditional risk factors. Based on such evidence, the most recent ACCF/AHA Guidelines in 2010 gave hs-CRP a Class IIb Level of Evidence B indication as an additional risk marker in those at intermediate risk based on the Framingham Risk Score [45]. Furthermore, a Class IIa Level of Evidence B was given to subjects with criteria based on the JUPITER study. The recently published European Society of Cardiology Guidelines on Prevention also gave hs-CRP a Class IIb Level of Evidence B recommendation [46].

The largest meta-analysis looking simultaneously into hs-CRP and fibrinogen as predictors of CVD has been published in October 2012 [47]. This meta-analysis was based on 52 prospective studies and included almost 250,000 participants without prior history of CVD and studied the predictive value of adding hs-CRP or fibrinogen to conventional risk factors using state-of-the-art performance measures of discrimination and

reclassification during follow-up. Measurement of hs-CRP resulted in a better discrimination as assessed by the C-index of a model that already contained age, sex, smoking status, blood pressure, history of diabetes, total cholesterol, and HDL-C. Similar findings could be demonstrated for fibrinogen. There was no such association with stroke. Subgroup analysis showed a better performance of hs-CRP in males compared with females and in smokers compared with non-smokers. Hs-CRP performed equally well in subjects at intermediate risk for CVD based on a Framingham Risk Score of between 10% and 20% or in those at high risk based on $\geq 20\%$ risk over 10 years. These results suggest that based on current guidelines, determination of hs-CRP may prevent one additional event over a period of 10 years for every 400–500 people screened with a similar effect seen for fibrinogen. Thus, although in the primary prevention setting, the incremental value of hs-CRP is modest in nature, it still remains the only blood biomarker that has undergone such in depth research over recent years and has shown fairly consistent results in meta-analyses. Unfortunately, fibrinogen, due to the lack of a commonly accepted standard, has a major analytical draw-back for its translation into clinical practice, despite a similar predictive value as demonstrated for hs-CRP.

Apart from the issue of an incremental contribution for improved risk stratification in patients at intermediate risk, a second role of hs-CRP may be even more important. CRP can be considered as an integrative biomarker and indicator of the body's total inflammatory burden as it is correlated with many cardiovascular risk factors that are not contained in traditional scores. Thus, a simple measurement of hs-CRP in the setting of cardiovascular risk assessment may provide a more comprehensive view of the patient's overall risk profile.

6. Predictive value of hs-CRP in patients with ACS

In 1994, Liuzzo et al. published their seminal paper demonstrating for the first time the prognostic utility of hs-CRP and serum amyloid A (SAA) protein in unstable angina [48]. Both parameters together with cardiac troponin were measured in 31 patients with severe unstable angina and 29 patients with acute MI. Although at the time of hospital admission, the markers of myocyte necrosis were still normal, a significant proportion already showed increased levels of these two markers of inflammation. The 20 patients with unstable angina who had levels of hs-CRP ≥ 3 mg/L had more ischemic episodes in-hospital than those with levels < 3 mg/L. By contrast, no deaths or MI occurred among 11 patients with hs-CRP < 3 mg/L and only 2 of them required coronary revascularization. Among patients admitted with a diagnosis of acute MI, unstable angina preceded infarction in 14 out of 22 patients with levels of hs-CRP ≥ 3 mg/L but in none of the 7 patients with levels < 3 mg/L.

These initial data were confirmed by a large observational study comprising 1030 patients with unstable angina enrolled in the ECAT Angina Pectoris Study [49]. All patients underwent coronary angiography and had extensive clinical and laboratory assessment at study entry and were followed up for 2 years. Concentrations of hs-CRP at study entry were associated with coronary events in these patients with an approximately 2-fold increase in risk in patients with an hs-CRP above the fifth quintile (> 3.6 mg/L) compared with the first four quintiles. About a third of all events occurred among patients in this group. The long-term predictive value of hs-CRP could also be demonstrated in the Fragmin during Instability in Coronary Artery Disease trial [50]. Besides troponin T and fibrinogen, hs-CRP collected during the first 24 h after enrollment in 917 patients was clearly related to the long-term risk of death from cardiac causes over a 3-year period independent of other measures of risk. In another study involving 1042 patients, who underwent a very early invasive strategy after a non-ST-elevation ACS, elevated hs-CRP on admission was found to be a significant predictor of short- and long-term mortality. In-hospital mortality in those with hs-CRP > 10 mg/L was three times higher compared with those with levels < 3 mg/L and in multivariable analysis, patients with hs-CRP

>10 mg/L had a >4 times increased risk of death independent of other risk variables [51].

Apple et al. studied 457 patients on admission and measured 7 biomarkers including MPO, soluble CD40 ligand, PIGF, MMP-9, cardiac troponin I, NT-proBNP, and hs-CRP [52]. In multivariable analysis, only NT-proBNP and cardiac troponin levels were significantly related to cardiac events (MI, PCI, CABG and cardiac death), whereas hs-CRP was not a significant predictor for cardiac events but still predicted total mortality. Bogaty et al. [53] reported on a large cohort of 1210 patients admitted with an ACS and hs-CRP was measured repeatedly on hospital admission, discharge, and one month later, and at outcome evaluation after one year. In univariate analysis, hs-CRP collected on admission modestly predicted the composite endpoint which, however, after adjusting for common clinical variables was no longer seen. Tello-Montoliu et al. [54] studied 358 consecutive patients with non ST-elevation ACS, besides troponin, NT-proBNP, and fibrin D-dimer, hs-CRP was also measured. Troponin T, NT-proBNP and hs-CRP were all predictors of adverse events in multivariate analysis, but not d-dimer levels. After adjustment for baseline characteristics and ECG changes, elevated levels of troponin T, NT-proBNP, and hs-CRP were significantly associated with adverse events at 6 months with an almost 2-fold increased risk in those with elevated biomarkers. The incremental value of these three markers was even seen when added to the TIMI Risk Score. In another study by Schaub et al. [55] in 398 consecutive patients presenting with acute chest pain novel biomarkers like MPO, MRP-8/14 and hs-CRP, although not being helpful in the diagnosis of acute MI, provided incremental value in the risk stratification of these patients. A formal meta-analysis carried out by He et al. [56] comprising 9787 patients with 1364 events demonstrated an approximately 2-fold increased long-term risk of recurrent cardiovascular events or death in those patients with hs-CRP >10 mg/L as measured within 72 h after onset of symptoms. Table 1 summarizes the essential information of these studies.

7. Predictive value of hs-CRP in patients with stable CHD

Further clinical studies demonstrated that hs-CRP concentrations that remained elevated for at least 3 months after an acute event were related to recurrent instability. Thus, to improve risk stratification and consider possible adjustments of therapy, it may be important to detect those patients demonstrating a prolonged inflammatory response [57,58]. The positive predictive value of hs-CRP was also shown by Haverkate et al. in 743 patients with stable CHD in the large ECAT study [49]. A one standard deviation increase in hs-CRP was associated with a 50% increase in the risk for a coronary event. In the ULSAM [42], a community-based cohort of elderly men with a follow-up of 10 years, hs-CRP, NT-proBNP, and cystatin C were measured in addition to classical risk factors of CVD. The addition of hs-CRP significantly improved the area under the curve, but this was particularly true when all three biomarkers were analyzed in one model. The incremental value of this biomarker combination was not only seen for death from cardiovascular

causes but also for total mortality. Thus, measurement of these biomarkers may substantially improve risk stratification among elderly men.

8. Hs-CRP and risk-guided therapy

The aforementioned studies during the past 15 years clearly provide substantial evidence to suggest that elevated hs-CRP may be an important prognostic factor in patients with manifest CHD. Yet, a question arises as to whether there is any evidence that this information might change clinical practice, in particular therapeutic strategies (Table 2).

8.1. The CARE trial

Initial data from the Cholesterol and Recurrent Event (CARE) study [59], which tested the effect of pravastatin on recurrent events in patients after MI, suggested that this indeed might be the case. In a nested case-control design, hs-CRP and SAA protein were measured in 391 incident cases and 391 non-cases, and the results showed that the placebo group with elevated hs-CRP and SAA had the highest risk for recurrent coronary events (relative risk [RR] = 2.81, $p = 0.007$). The RR for a recurrent event was non-significant (RR = 1.29, $p = 0.5$) in the pravastatin group suggesting that treatment with pravastatin decreased risk in these patients. Furthermore, baseline lipid levels among patients with and without evidence of inflammation were comparable, suggesting that this observation may be consistent with a non-lipid effect of pravastatin.

8.2. The PROVE-IT-TIMI 22 trial

In the PROVE-IT-TIMI 22 trial [60], hs-CRP was measured in 3745 ACS patients at randomization, 30 days, 4 months, and at the end of the study (24 months). Measurement of LDL-C and hs-CRP levels at 30-day follow-up was taken and related to recurrent events. Events were lower in patients with decreased LDL-C levels <70 mg/dL due to statin therapy. Comparable lower event rates were seen in patients with hs-CRP levels <2 mg/L. The most pronounced effect with lowest cardiovascular risk, however, was seen in patients who had both an LDL-C <70 mg/dL, and an hs-CRP <1 mg/L.

8.3. The A-to-Z trial

Similar observations were made in the A-to-Z trial [61] which demonstrated that early intensive therapy with statins resulted in reduced hs-CRP levels and lowest 2-year mortality rates.

8.4. The MIRACL study

In the MIRACL study [62], hs-CRP, SAA and interleukin (IL)-6 were measured in 2926 subjects. The levels of these markers were related

Table 1
Overview of major clinical studies of hs-CRP in ACS.

Author	Study	Subjects	CRP cut point	Follow-up	End point, relative risk (RR) or odds ratio (OR) for hs-CRP (95% CI, p -value)
Haverkate et al. [49]	Observational	2121	≥ 3 mg/L	2 years	Coronary events, 1.45 (1.5–1.83, $p = 0.002$)
Lindahl et al. [50]	Substudy of RCT	917	≥ 10 mg/L	>3 years	Death, 2.6 (1.5–4.5)
Mueller et al. [51]	Observational	1042	>10 mg/L	20 months	Death, 3.8 (2.3–6.2)
Apple et al. [52]	Observational	457	≥ 3 mg/L	4 months	All-cause mortality, 3.0 (1.2–7.7)
Bogaty et al. [53]	Observational	1210	≥ 3 mg/L	1-year	Death, nonfatal myocardial infarction (MI), and unstable angina (UA), 1.12 (0.93–1.34, $p = 0.24$)
Tello-Montoliu et al. [54]	Observational	358	Q4	6 months	Death, recurrent ACS, PCI/CABG and/or admission for acute heart failure, 1.90 (1.24–2.92, $p = 0.0034$)
Schaub et al. [55]	Observational	398	>3.8 mg/L	27 months	Death, 8.98 (4.13–19.56)
He et al. [56]	Meta-analysis	9787	>10 mg/L	1–96 months	Cardiovascular events, death, 2.18 (1.77–2.88)
Biasucci et al. [57]	Observational	53	≥ 3 mg/L	1 year	Recurrent instability, 8.57 (1.66–44.2, $p < 0.01$)
Niccoli et al. [58]	Observational	97	–	6 months	Death, MI and recurrent instability, 1.8 (1.1–3.0, $p = 0.017$)

Table 2
Overview of clinical studies: hs-CRP and risk guided therapy.

Author	Clinical trial	Subjects	Follow-up	Statins	CRP levels predicted response to treatment
Ridker et al. [5]	JUPITER	17,802	Median follow-up of 1.9 years (maximum follow-up 5 years)	Rosuvastatin 20 mg once daily vs. placebo	Yes
Ridker et al. [59]	CARE trial	782	5 years	Pravastatin 40 mg vs. placebo	Yes
Ridker et al. [60]	PROVE-IT-TIMI 22	3745	24 months	Atorvastatin 80 mg vs. pravastatin 40 mg	Yes
Morrow et al. [61]	A-to-Z trial	3813	30 days/4 months	Simvastatin 80 mg vs. simvastatin 20 mg	Yes
Kinlay et al. [62]	MIRACL	2926	16 weeks	Atorvastatin 80 mg once daily vs. placebo	Yes
Nissen et al. [63]	REVERSAL	502	18 months	Pravastatin 40 mg or atorvastatin 80 mg	Yes
Heart Protection Study Collaborative Group et al. [64]	HPS	20,536	5 years	Simvastatin 40 mg vs. placebo	No
Sever et al. [65]	ASCOT (subset)	155 cases, 488 controls	5.5 years	Atorvastatin 10 mg vs. placebo	No
Ridker et al. [68]	AFCAPS/TexCAPS	5742	5.2 years	Lovastatin 20/40 mg once daily vs. placebo	Yes

to risk of stroke over the 16 week study period. Risk for an adverse cerebrovascular event was related to higher hs-CRP, SAA, and IL-6 levels in the placebo group only, but was not seen in the atorvastatin treatment group. Thus, treatment with atorvastatin abrogated the risk associated with elevated markers of inflammation in this study, extending the findings from earlier trials to the cerebrovascular bed.

8.5. The REVERSAL study

More data suggesting that hs-CRP may be an important aid to guide statin therapy in patients with CAD came from the REVERSAL study [63]. In this trial, 502 patients with angiographically documented CAD were randomly assigned to either 40 mg of pravastatin or 80 mg of atorvastatin. Intravascular ultrasound (IVUS) was done at baseline and at 18 months to measure progression of atherosclerosis. Similarly, lipoproteins and hs-CRP levels were measured at both baseline and 18 months follow-up. Both LDL-C and hs-CRP levels decreased from a mean of 150 mg/dL and 2.9 mg/L at baseline to 94.5 mg/dL and 2.3 mg/L at 18 months, respectively. The group with significant reductions in both LDL-C and hs-CRP levels greater than the median showed regression of atherosclerosis.

However, results from two recent analyses, the large HPS study and the ASCOT trial did not report an association between lower hs-CRP levels and improved cardiovascular outcome [64,65].

8.6. The HPS

In the HPS [64], 20,536 patients aged between 40 and 80 years at high risk of vascular events were given 40 mg of simvastatin daily versus placebo during a 5-year follow-up. Simvastatin treatment resulted in a 24% reduction in the incidence of a first major vascular event versus placebo and the authors reported no evidence for an association between baseline hs-CRP levels and the effect of statins on the incidence of vascular events. However, the HPS trial has recruited many high-risk patients with already manifest atherosclerosis or diabetes as a CHD equivalent, and, thus cannot be considered as a primary prevention trial, the setting in which hs-CRP has been shown to add incremental information to risk prediction similar to total and HDL-C [66].

8.7. The ASCOT trial

In the ASCOT trial [65], a subset of 4853 patients was assigned to either atorvastatin or placebo. In 155 cases and 488 controls the on-statin hs-CRP association with cardiovascular outcomes was evaluated. The authors reported a reduction in LDL-C by 40% at 6 months and a median hs-CRP reduction by 27%. In patients on atorvastatin, lower on-treatment LDL-C at 6 months was associated with a significant reduction in subsequent cardiovascular events compared with those above and below the median hs-CRP levels. However, there are several arguments that question the negative results reported from this study

[67]. Firstly, hs-CRP and LDL-C baseline levels were comparable in identifying subjects at CVD risk. Secondly, the addition of hs-CRP to the Framingham Risk Score improved risk classification. Thirdly, there was no interaction between the baseline values of either hs-CRP or LDL-C and the relative effect of statin therapy on cardiovascular events. Lastly, the effect point estimates in ASCOT are consistent with the hypothesis that lowering hs-CRP concentrations by a statin predicts greater reduction in relative risk. In patients on atorvastatin with a less than median reduction in hs-CRP, a 14% reduction in vascular events was observed, whereas among those with a greater than median hs-CRP reduction, a 39% reduction was seen. Thus, a 25% greater relative risk reduction was seen in patients with lower levels of on-treatment hs-CRP, a value that is similar to results achieved in the other abovementioned studies.

8.8. The Air Force/Texas Coronary Atherosclerosis Prevention (AFCAPS/TexCAPS) study

Evidence from the AFCAPS/TexCAPS Study [68] conducted in 5742 hyperlipidemic men and women suggested that the therapeutic benefit of lovastatin was similar in individuals with high LDL-C above the median of 149.1 mg/dL and normal or increased hs-CRP above the median of 1.62 mg/L compared with those with increased hs-CRP but an LDL-C below the median. By contrast, no effect was seen in those patients with both LDL-C and hs-CRP below the median.

8.9. The JUPITER trial

The JUPITER trial [5] was conducted in 17,802 apparently healthy subjects including men aged ≥ 50 years and women aged ≥ 60 years without a prior history of CVD. This study prospectively tested the hypothesis whether treatment with rosuvastatin (20 mg) would decrease the rate of a first major cardiovascular event in individuals with LDL-C < 3.37 mmol/L (130 mg/dL), below the current treatment threshold for a statin on the basis of ATP III Guidelines but who had signs of a low-grade inflammatory response indicated by a hs-CRP ≥ 2 mg/L after repeated measurements. Baseline characteristics showed an average LDL-C of 2.8 mmol/L (108 mg/dL) and an hs-CRP of 4.2 mg/L. The trial was stopped prematurely after a median follow-up of 1.9 years (maximum 5.0 years). Rosuvastatin reduced LDL-C levels by 50% and hs-CRP by 37%. Rates of the primary endpoint were 0.77 and 1.36 per 100 person-years of follow-up in the rosuvastatin and placebo groups, respectively [5]. In subsequent analyses, reductions in both LDL-C and/or hs-CRP were shown to be an indicator of successful treatment with rosuvastatin leading to the dual target hypothesis. The achieved hs-CRP concentrations were predictive of event rates irrespective of the lipid endpoint used, including the apolipoprotein B to apolipoprotein A1 ratio. Furthermore, rosuvastatin significantly reduced the occurrence of symptomatic venous thromboembolism by 43% when participants were followed for the first occurrence of pulmonary embolism or deep vein thrombosis [69].

Thus, in light of several studies in which post hoc analyses have clearly shown that hs-CRP may be useful in guiding statin therapy, and the positive outcome of the JUPITER trial, which formally tested this hypothesis prospectively, there seems to be abundant evidence for the role of hs-CRP as both a predictor of excess risk in patients after ACS and stable CHD and also in primary prevention among high risk subjects. Furthermore, hs-CRP levels may represent a guide to tailor statin therapy in addition to LDL-C levels. The fact that the absolute risk in JUPITER was similar to the West of Scotland Coronary Prevention Study (WOSCOPS) [70], where baseline LDL-C was 192 mg/dL, might be at least in part due to a more pro-inflammatory phenotype in the latter study. Lowering of LDL-C levels by 50% was the most pronounced reduction seen in all primary prevention studies so far, and a median of 50 mg/dL had not been achieved in any other study before. However, one might argue that LDL-C still may solely be responsible for the results. The fact that rosuvastatin was equally effective in those without any cardiovascular risk factors but with elevated hs-CRP as in those with cardiovascular risk factors and the fact that it reduced thromboembolic events to a similar extent as atherosclerotic events do not suggest that this is the only explanation. In particular, the reduction of venous thromboembolisms rather suggests a role for pleiotropic effects of statins. Hs-CRP may have simply been an excellent indicator of the total inflammatory burden of the individual as it is associated with a number of risk factors that are not being considered traditional risk factors and which are not part of any major risk score. Therefore, increased inflammatory burden in patients with and without CVD seems to be associated with adverse outcome independent of traditional risk factors and a number of clinically important variables. Still, because of the controversy of a causal role of inflammation in atherosclerosis, formal testing of the inflammation hypothesis is clearly needed. Only a large randomized clinical trial with sufficient power selecting patients with manifest CVD and an extended inflammatory response will finally provide the definite answer of whether or not inflammation plays a causal role or simply represents a bystander in the atherosclerotic process.

9. Other markers of inflammation

There is a large number of further inflammatory biomarkers, e.g., various cytokines like TNF- α , IL-1, IL-6, and IL-18, chemokines like MCP-1, cellular adhesion molecules like sICAM-1 and sVCAM-1, and markers possibly more closely related to plaque destabilization and plaque rupture, namely oxLDL, Lp-PLA₂, GPX1, MPO, MMPs, PIGF, PAPP-A [3], and neopterin, a marker of macrophage activation, that have been tested prospectively. For most of them, positive associations with various cardiovascular outcomes could be demonstrated and the predictive value for several molecules, like neopterin, was at least as good as for hs-CRP [71]. However, most of these markers are also unspecific (e.g., neopterin) and are mainly available in ACS or stable CHD (e.g., neopterin) [72–76]. All of these markers do lack such a broad basis of evidence as hs-CRP including a randomized controlled clinical trial like JUPITER, and many of them, because of analytical issues, do not seem to be suitable for routine clinical application. However, this list necessarily must be incomplete. Finally, several of these molecules have already been identified as potential targets for intervention (e.g., inhibition of Lp-PLA₂ activity) and are currently being tested in clinical trials like STABILITY and SOLID [77,78].

10. Testing the inflammation hypothesis: the CANTOS trial

In light of the compelling evidence of a fundamental role of inflammation in all stages of the atherosclerotic process, an obvious logical next step would be to directly test the inflammation hypothesis. Yet inflammation represents an important defense mechanism during infections and other injuries. On the other hand, circumstantial evidence from several chronic inflammatory diseases like rheumatoid arthritis, psoriasis, or more rare inflammatory diseases such

as the Muckle–Wells syndrome clearly demonstrates that long-term inflammation is associated with adverse outcome in many ways. In particular for rheumatoid arthritis and psoriasis, it could be shown that those with more active and more aggressive disease do have the highest risk for adverse cardiovascular events. Such evidence from various inflammatory diseases lends further support to intervene with the inflammatory process. However, as outlined above, we certainly do not want to impair host defense, and thus put the individuals at an increased risk for infections. Therefore, the selection of the appropriate target for anti-inflammatory therapy is of utmost importance. Looking at the inflammatory cascade there may be several such targets. Yet, for some of the more downstream markers including CRP, there is no data suggesting any of them as suitable candidates. Thus, more upstream mediators of inflammation like various cytokines may be more appropriate. Recently, large meta-analyses and Mendelian randomization studies [79,80] have suggested that for example the IL-6 receptor might lie in the causal pathway of inflammation-mediated atherosclerosis. Another possible candidate is IL-1 β which is also an inflammatory molecule implicated in atherothrombosis. It exerts pro-inflammatory effects by binding to the IL-1 type 1 receptor, and the IL-1 receptor antagonist (IL-1 RA) blocks the binding of IL-1 α and β to the IL-1 type 1 receptor. Thus, in healthy conditions, there is a balance of pro- and anti-inflammatory effects mediated through IL-1 and the IL-1 RA. IL-1 β is produced by monocytes and macrophages which are key cells in atherosclerotic plaque biology. More recently, the importance of the nucleotide-binding leucine-rich repeat containing pyrin receptor 3 (NLRP3) inflammasome has been stressed. The NLRP3 inflammasome represents an intra-cellular multiprotein platform that activates caspase-1 to convert the inactive form of IL-1 β to its active form which then directly exerts pro-inflammatory effects and also stimulates the acute phase reaction in the liver. The activation of the NLRP3 inflammasome can be triggered by uric acid crystals as well as cholesterol crystals in plaque [81]. Thus, there is a good evidence of a direct link between atherosclerotic changes in the vascular wall and consecutive inflammatory responses that can be measured via IL-1 β . The fact that IL-1 β does not play a crucial role in host defense, its neutral effects on various metabolic parameters like the lipid profile including total cholesterol, LDL-C, and HDL-C as well as triglycerides and chylomicrons, makes it an interesting candidate for further study. Pre-clinical evidence suggests that IL-1 β is among the most potent pro-inflammatory vascular cytokines. IL-1 β and the IL-1 RA are both expressed in human atherosclerosis [82]. There is also reduced atherosclerosis in mouse knock-out models for both IL-1 β and the type 1 IL-1 receptor [83,84]. Conversely, increased atherogenesis has been reported in IL-1 receptor deficient mice [85,86]. In another mouse model, the ApoE knockout, IL-1 β exposure has been found to promote experimental atherogenesis in quantitative analysis in aortic lesion areas [87]. And finally, fatty streak formation is reduced in ApoE mice by direct IL-1 RA administration [88], the ablation of the IL-1 receptor inhibits atherosclerosis in ApoE mice inoculated with *Porphyromonas gingivalis* [89] and in a porcine coronary model increased neo-intimal formation with administration of IL-1 β was reduced in the presence of IL-1 RA [90,91].

Human studies have shown that IL-1 β levels are increased in atherosclerotic arteries as compared with normal coronary arteries [92] and IL-1 RA concentrations are higher among those with ACS as compared with asymptomatic patients or those with chronic stable CAD [93]. Furthermore, polymorphisms in the IL-1 RA are associated with density of coronary lesions seen on angiography and rates of atherosclerotic lesions [94,95]. In rheumatoid arthritis, the IL-1 RA anakinra is associated with reduced plasma levels of IL-6, hs-CRP, and the vasoconstrictor endothelin-1, as well as improvement in coronary flow reserve, LV function, and flow mediated brachial artery dilation [96].

Canakinumab is a high-affinity human monoclonal IL-1 β antibody currently indicated for the treatment of IL-1 β -driven inflammatory diseases. It is designed to bind to human IL-1 β and functionally neutralize

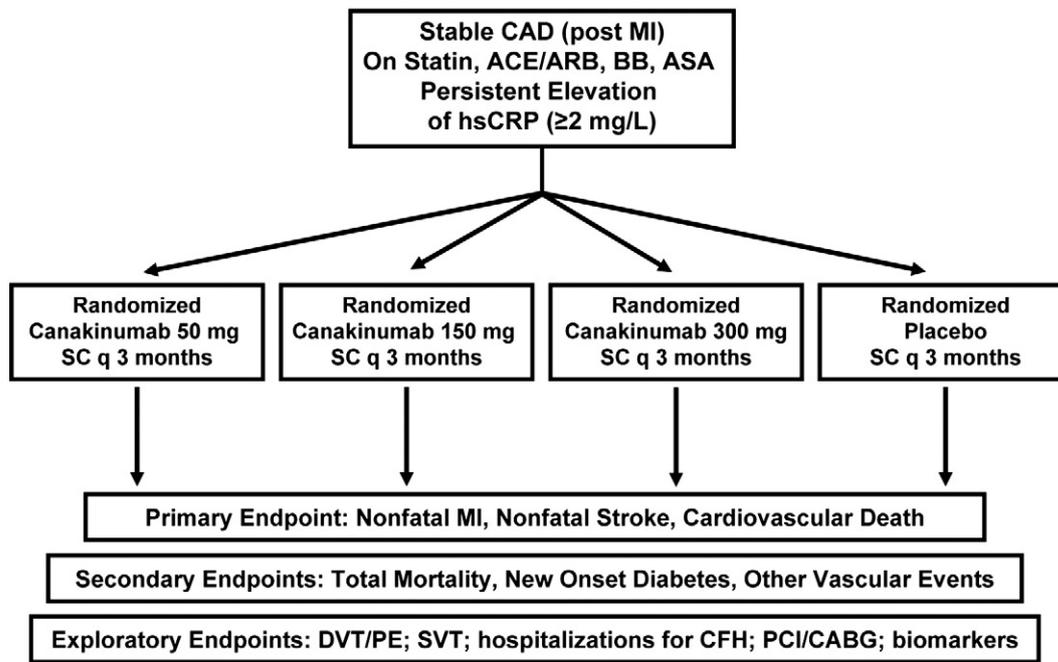


Fig. 1. CANTOS study design. ASA, acetylsalicylic acid; CAD, coronary artery disease; MI, myocardial infarction; ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker; hs-CRP, high-sensitivity C-reactive protein; DVT, deep vein thrombosis; SVT, supraventricular tachycardia; CHF, congestive heart failure; PCI, percutaneous coronary intervention; CABG, coronary artery bypass graft.

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the bioactivity of this pro-inflammatory cytokine with a long half-life of 48 weeks and consistent hs-CRP and IL-6 reductions for up to 3 months.

Canakinumab, therefore, will be used in the Canakinumab ANti-inflammatory Thrombosis Outcome Study (CANTOS) to directly test the inflammatory hypothesis of atherothrombosis (Fig. 1). The main aim of the study is to determine whether the long-term inhibition of IL-1 β with canakinumab (50 mg, 150 mg or 300 mg every 3 months) compared with placebo will reduce the rates of recurrent cardiovascular events among stable post-MI patients who remain at elevated vascular risk due to increased levels of hs-CRP (≥ 2 mg/L) despite usual care including statin therapy. Altogether, 17,200 patients will be randomized to 3 different doses of canakinumab or placebo. The number of primary endpoints should provide an approximately 90% power to detect the superiority of at least one dose of canakinumab compared with placebo assuming a hazard ratio of 20%. Besides the usual composite endpoint consisting of non-fatal MI, non-fatal stroke, and cardiovascular death, secondary endpoints like total mortality, new onset diabetes, and other vascular events will be looked at. Furthermore, several exploratory endpoints like deep vein thrombosis, pulmonary embolism, supraventricular tachycardia hospitalizations for congestive heart failure, and the need for urgent interventions like PCI, and CABG will also be analyzed.

Thus, CANTOS will be the first randomized trial to formally address the inflammatory hypothesis of atherothrombosis [97]. If positive, CANTOS will provide a proof of concept and present a novel cytokine-based therapy for the secondary prevention of high-risk patients with CVD.

Conflict of interest

Member of the Executive Steering Committee of CANTOS.

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