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REVIEW

*Controversies in cardiovascular medicine*

# C-reactive protein is a bystander of cardiovascular disease

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**Abstract** A large number of studies have evaluated the association of hs-C-reactive protein with atherosclerosis and coronary heart disease (CHD) in mechanistic, genetic, population-based studies, as well as clinical trials. This paper reviews the collective evidence to determine if hs-C-reactive protein is part of the causal pathway of atherosclerosis and CHD or whether it is a bystander.

**Keyword** C-reactive protein

## Introduction

For the past three decades, there has been interest in the role of inflammation in the pathogenesis of atherosclerosis and vascular disease. C-reactive protein is an acute phase reactant and is synthesized by the liver. C-reactive protein production from the liver increases in response to a variety of systemic events such as infection, trauma, or autoimmune inflammatory diseases. This elevation in C-reactive protein is non-specific and parallels the elevation of other inflammatory markers such as the erythrocyte sedimentation rate. The upstream factors which influence C-reactive protein secretion from the liver are inflammatory cytokines such as interleukin 6, interleukin 1, and tumour necrosis alpha. In the presence of atherosclerosis, these cytokines are produced by macrophages, as they are converted into lipid-laden foam cells.[1] Assays for C-reactive protein have evolved from a crude measure of a large protein to a highly sensitive assay (hs-C-reactive protein) which can detect mild inflammation. High-sensitivity C-reactive protein assays measure levels close to zero and up to 10 mg/L. Values of >3 mg/L have been used as the threshold above which the risk of coronary heart disease (CHD) is believed to be increased.[2] While modest elevations in hs-C-reactive protein may reflect inflammation associated with atherosclerosis, they may also be present in other conditions including abdominal obesity, type 2 diabetes, cancer, and acute (usually self limited) events such as a dental infections and minor trauma. Individual variations in hs-C-reactive protein over time are typical and the with-in person correlation in apparently healthy populations is estimated to be 0.58 (95% CI: 0.52–0.63) which is similar to total cholesterol (0.59; 95% CI: 0.51–0.58).[3] Other cardiovascular risk factors which are correlated with circulating hs-C-reactive protein include LDL cholesterol, cigarette smoking, increased body mass index, increased triglyceride concentration, and other markers of inflammation such as fibrinogen and von Willebrand factor.[4] Ethnic variations in hs-C-reactive protein also exist, but they are mostly explained by differences in the frequency of cardio-metabolic risk factors.[5]

Since the widespread availability of hs-C-reactive protein, a large number of studies have investigated whether C-reactive protein is associated with atherosclerosis and vascular disease in animals and large human cohorts to determine whether hs-C-reactive protein is a crucial causal mediator or a non-specific marker (bystander) of vascular disease.[3] More recently, genetic studies have applied the principle of Mendelian randomization to distinguish whether genetic variations in the C-reactive protein gene are associated with circulating C-reactive protein levels and CHD. Furthermore, a recent clinical trial posits that lowering hs-C-reactive protein is associated with a reduction in CHD outcomes.[6] Below we will critically review four types of studies (mechanistic studies, observational epidaemiologic studies, genetic studies, and experimental randomized trials) to determine whether the collective evidence supports whether hs-C-reactive protein is part of the

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causal pathway of atherosclerosis and CHD or whether it is a bystander.[7]

## Mechanistic studies

Since the observation was made that C-reactive protein is found in atherosclerotic plaques,[8] numerous studies *in vitro*, *in vivo*, and in animals have been conducted to determine whether C-reactive protein is simply a marker of atherosclerosis, or plays an inherent role in its pathogenesis. However, the results of these studies have been conflicting.[9–14] Some of the early studies evaluating C-reactive protein *in vitro* were invalidated due to contaminants in the C-reactive protein preparations.[15] Some studies suggest that C-reactive protein is a mediator[9,10,16] of atherosclerosis, whereas others finding no evidence that C-reactive protein is a proatherogenic or prothrombotic factor.[11,14,17] For example, in a 2004 study, Paul *et al.*[10] showed that transgenic apolipoprotein E (ApoE) deficient mice which expressed high serum levels of human C-reactive protein (100 mg/L) developed an acceleration of atherosclerotic lesion formation in comparison with control 7-month-old male ApoE deficient mice. A more recent study by Tennent *et al.*[11] performed a similar experiment using ApoE deficient mice with and without transgenic expression of human C-reactive protein. However, in this study, the C-reactive protein levels were similar to those found in humans (<10 mg/L) and these investigators found that after 18 months there was no impact of C-reactive protein on atherosclerosis burden or plaque size. In addition, Koike *et al.*[14] recently used transgenic rabbits which expressed human C-reactive protein and concluded that C-reactive protein in low or high levels did not promote the development of atherosclerosis. Thus, these contradictory results cast doubt on the role of C-reactive protein as a mediator of atherosclerosis.

## Observational studies

Epidaemiologic studies have attempted to characterize the association between hs-C-reactive protein and cardiovascular outcomes in an attempt to determine whether hs-C-reactive protein is in the causal pathway of vascular disease or is simply a by-product of atherosclerosis or atherosclerosis risk factors. In 2004, a meta-analysis of 22 prospective studies (involving >200 000 subjects and 7689 CHD events) characterized the association of C-reactive protein and CHD and reported an overall odds ratio of 1.58 (95% CI: 1.48–1.68) for the highest compared with the lowest tertile of C-reactive protein after adjustment for smoking and some other CHD risk factors.[4] However, summary data from smaller studies (i.e. 18 studies of <500 patients each; 2961 cases of CHD) were associated with a higher odds ratio, i.e. approximately 1.8, whereas summary data from the four larger studies (≥500 patients; 4107 CHD cases) suggested a more modest relationship; OR = 1.49 (95% CI: 1.37–1.62) which suggests that small studies are unable to control for a substantial degree of confounding.[4] Since that time, other studies have been conducted to determine whether C-reactive protein adds additional predictive value to CHD over and above traditional CHD risk factors, and have reported only weak relative risks of less than two between C-reactive protein and CHD.[18,19] However, given the strong interdependence between C-reactive protein levels and conventional CV risk factors, most observational studies using conventional epidaemiologic approaches are unable to fully adjust for the effect of confounding and this lack of 'full adjustment' leads to overestimation of the relationship between C-reactive protein and outcomes. To address this limitation, recently the Emerging Risk factor Collaboration (ERC) tried to overcome this issue of confounding by conducting a meta-analysis using individual level data which enabled direct adjustment for the effect of possible confounders.[3] They conducted a meta-analysis using individual level data on 160 309 people (27 769 fatal or non-fatal CVD) from 54 prospective studies who did not have a history of vascular disease. The ERC reported the risk ratio for CHD per 1-SD higher log of C-reactive protein concentration was 1.63 (95% CI: 1.51–1.76) when adjusted for age and sex only, which was reduced to 1.37 (95% CI: 1.27–1.48) when additionally adjusted for conventional risk factors, and further reduced to 1.23 (95% CI: 1.07–1.42) when adjusted for fibrinogen. Furthermore, in this analysis, the risk ratios for a 1 SD higher log of C-reactive protein (equivalent to a three-fold higher C-reactive protein level) for vascular deaths (1.55, 95% CI: 1.37–1.76) and non-vascular deaths (1.54, 95% CI: 1.40–1.68) after adjustment for conventional risk factors were very consistent with each other. In addition, significant associations were also observed between C-reactive protein and cancer deaths and even for external causes (i.e. injuries), which raises the issue whether C-reactive protein is simply a marker of overall poor prognosis and not a specific cause of any single condition. Such non-specific associations always suggest that the degree of 'adjustment' is likely to be incomplete and that the residual odds ratio suggested could well be an artefact.

In addition to studies conducted among healthy individuals, evaluations of the predictive value of hs-C-reactive protein on recurrent CV events among patients with established vascular disease or multiple CV risk factors do not lend support an independent association of C-reactive protein separate from other markers of inflammation. In the HOPE trial, the association of multiple inflammatory markers with the risk of MI, stroke, or CV death ($n = 501$ events) over 4.5 years of follow-up was assessed.[20] Multiple inflammatory markers including hs-C-reactive protein, Nt-proBNP, soluble intercellular adhesion molecule-1, soluble interleukin-1 receptor antagonist, and fibrinogen were correlated with each other, and independently associated with recurrent CV events after adjustment for conventional risk factors and drug allocation. However, most inflammatory markers, including C-reactive protein, provided little or no additional predictive information over that obtained from assessment of traditional risk factors. In contrast, only the inclusion of Nt-proBNP improved the model accuracy [i.e. area under the curve (AUC) increased from 0.65 to 0.69; $P < 0.001$].

A recent systematic review of 31 prospective studies ($n =$ 84 063 individuals and 11 252 incident CHD events) evaluated the predictive performance of hs-C-reactive protein on CHD.[21] The ROC curve or C statistic is the traditional method used to describe the clinical utility for prediction models. This curve assesses how well a test or model separates individuals into two classes, such as diseased and non-diseased. More recently, the concept of the net reclassification improvement has gained

popularity as a method to directly compare whether the addition of a new biomarker or test improves the reclassification of people who develop and who do not develop events.[22] Among 13 studies that reported on the effect on the ROC curve or C-statistic of adding C-reactive protein to the Framingham-based models, in 5 studies there was no change with the addition of hs-C-reactive protein, and in 8 studies only minimal improvement in the CHD prediction measured by change in AUC (range: 0.01–0.15) was observed. Specifically, in two prospective cohorts (NPHS-2 and EAS: 3441 individuals, 309 coronary events), the C statistics with C-reactive protein alone were 0.61 in NPHS-2 and 0.62 in EAS, and increased the discrimination modestly when added to the Framingham risk score in NPHS-2 (+0.04 in AUC)), and decreased the discrimination when added to the Framingham risk score in EAS (−0.01 in AUC). The net reclassification of people predicted to have CHD with the addition of hs-C-reactive protein to the Framingham risk factors has also been evaluated and is small (8.5% in NPHS-2 and 8.8% in EAS), suggesting that in only about 9% of individuals, does C-reactive protein lead to a change in risk category above that of the Framingham-based risk score. The authors concluded that hs-C-reactive protein does not perform significantly better than the Framingham risk equation to discriminate between who will and who will not develop CHD.[21] More recently, the Framingham investigators studied 3006 offspring subjects in the Framingham Heart Study who were free of CVD. After 12 years of follow-up, 129 CHD events occurred and 286 total CVD events occurred. They evaluated whether or not hs-C-reactive protein added to the prediction of CHD and CVD over and above traditional risk factors. The net reclassification improvement with C-reactive protein added to traditional Framingham risk factors was 11.6% for CHD and 5.6% for CVD.[23] Taken together, these studies demonstrate that the increase in predictive value of hs-C-reactive protein added to other known CHD risk factors is little or modest at best.[18]

## Genetic variants and C-reactive protein

Classical epidaemiologic studies have limitations which include the incomplete adjustment for confounders, especially when all confounders all not measured or when they are measured with some degree of imprecision (which is almost always the case even with repeated measures). These factors lead to under adjustments and therefore overestimation of the impact of C-reactive protein on any outcome such as CHD. Recent advances in genetics offer novel approaches to conduct relatively unconfounded explorations as to whether any biomarker is likely to be causally related to an outcome as an individual's genetic profile is not altered by other behaviours (e.g. smoking or obesity) or subsequent conditions. Therefore, individuals can be considered to be randomly allocated by genotype.[24,25] Second, given that genetic influences are likely to exert their effects over a lifetime, then genetic associations are likely to be stronger (if real) compared with associations with a biomarker that is potentially influenced by other factors (e.g. smoking or obesity) i.e. confounded. Therefore, Mendelian randomization studies offer a new line of investigation to assess the life-long influence of elevated C-reactive protein levels on CHD, even if such associations are modest in magnitude. Mendelian randomization takes advantage of the random assortment of alleles at the time of gamete formation, and posits that in the presence of a risk allele which is associated with C-reactive protein plasma concentration, if C-reactive protein is part of the causal pathway of CHD, the risk allele should increase the CHD risk in proportion to the change in C-reactive protein attributed to this allele. This approach is based upon the understanding that confounders of the C-reactive protein levels should be distributed evenly between the genotypes thereby avoiding the reverse causation bias. The early genetic studies were limited because they often were retrospective, and generally of small size. A meta-analysis of studies including 4659 European men from six studies by Casas *et al.*[26] assessed the association between the 1444C>T polymorphism in the C-reactive protein gene with hs-C-reactive protein levels and CHD events. They observed that although this SNP is strongly associated with C-reactive protein level [C-reactive protein was 0.68 mg/L (95% CI: 0.31–1.10; $P = 0.0001$) higher among subjects homozygous for the +1444-T allele], it was not associated with the expected increase in coronary events estimated by the increase in plasma C-reactive protein (adjusted-OR for non-fatal MI among TT subjects = 1.01) (95% CI: 0.74–1.38). A meta-analysis of SNPs in the C-reactive protein gene, plasma C-reactive protein, and CHD is currently underway 37 000 CHD outcomes and about 120 000 controls with the intention of performing a Mendelian randomization experiment.[27] Recently, a genome wide association study revealed that multiple genes with presumably varying functions (i.e. C-reactive protein, LEPR, IL6R, HNF1A, APOE-CI-CII) are strongly associated with plasma C-reactive protein levels. Furthermore, the C-reactive protein SNP was not associated with CHD (OR = 0.98; 95% CI: 0.94–1.01) per a 20% lower C-reactive protein level. Thus this Mendelian randomization analysis showed no association between the C-reactive protein variants with CHD per 20% lower C-reactive protein level (OR = 1.00; 95% CI: 0.97–1.02) which differed from the prediction of CHD risk from epidaemiologic studies.[28] These findings do not support a causal association between C-reactive protein and CHD, and also provide further evidence that C-reactive protein is influenced by a number of other factors. While it may be a good marker of generalized inflammation, it may not be superior to other inflammatory markers, and is likely not part of the causal pathway.

## Experimental randomized clinical trials

To our knowledge, no clinical trial has tested whether hs-C-reactive protein lowering, independent of lipid-lowering will reduce vascular disease. To date, three randomized clinical trials, all involving a statin, have reported the relation between hs-C-reactive protein lowering with a statin and impact on cardiovascular events.[6,28,29] The largest clinical trial JUPITER randomly assigned 17 802 men and women with LDL cholesterol levels <3.4 mmol/L and hs-C-reactive protein ≥2.0 mg/L to rosuvastatin (20 mg/day) or placebo and followed them for the occurrence of a combined primary outcome of cardiovascular outcomes (myocardial infarction, stroke, arterial revascularization, hospitalization for unstable angina, or death from cardiovascular causes).[6] In this trial after a median follow-up of 1.9 years, use of rosuvastatin vs. placebo was associated with a 50% reduction in LDL, a 37% reduction in C-reactive protein, and a large reduction in the

primary outcome (0.77/100 person-years vs. 1.36/100 person-years of follow-up; hazard ratio 0.56; 95% CI: 0.46–0.69; $P < 0.00001$) was also observed. However, this trial did not include individuals with lower C-reactive protein levels and it is unclear whether the benefits observed in JUPITER are confined to individuals with elevated C-reactive protein. Two other trials using rosuvastatin (10 mg) have been conducted in which C-reactive protein was measured. CORONA ($n = 5011$)[29] enrolled patients with ischaemic heart disease and heart failure, and observed a 45% reduction in LDL and a 37% reduction in hs-C-reactive protein, effects which were very similar to JUPITER, and GISSI-HF ($n = 4574$) included patients with congestive heart failure and observed a 32% reduction in LDL and a 17% reduction in hs-C-reactive protein.[30] Yet neither of these trials observed a significant reduction in cardiovascular events. Thus the JUPITER trial provides evidence that use of rosuvastatin reduces cardiovascular events in those initially free of CVD, and it has now been approved for use by the FDA among patients who meet the JUPITER inclusion criteria. However, there are inconsistent effects of rosuvastatin on C-reactive protein across all trials, and it is difficult to separate whether and to what extent the benefits are due to the large LDL lowering effect of rosuvastatin, or C-reactive protein lowering, or both.

Recently, the JUPITER investigators reported a subgroup analysis of the trial using the post-randomization variables of LDL and C-reactive protein levels 1 year after trial entry. The investigators divided patients who received rosuvastatin into groups depending on if they achieved a C-reactive protein $<2$ or $\geq 2$ mg/L or LDL $<1.8$ vs. $\geq 1.8$ mmol/L, and compared the CV events in these four subgroups to the placebo event rates.[31] They reported that trial participants who achieved an LDL $<1.8$ mmol/L and C-reactive protein $<2$ mg/L had the lowest risk of a CV events compared with placebo (0.38), whereas those with C-reactive protein $<2$ and LDL $\geq 1.8$ the risk ratio was 0.54, with C-reactive protein $\geq 2$ mg/L and LDL $<1.8$ mmol/L the risk ratio was 0.62, and for C-reactive protein $\geq 2$ mg/L and LDL $\geq 1.8$ mmol/L the CV events were higher than that of the placebo group (risk ratio = 1.11), P across four groups $<0.0001$. However, this type of subgroup analysis is hard to interpret as similar groups cannot be constituted in the placebo group with comparable pre-randomization characteristics and such analyses can be potentially misleading.[32] Furthermore, if C-reactive protein is causally associated with atherosclerosis and CHD, one would expect to see a greater treatment effect of rosuvastatin with increasing C-reactive protein concentration. However, the opposite was observed, with a relative risk reduction of 59% among subjects with a baseline C-reactive protein $\leq 4$ compared with a relative risk reduction of 30% ($P_{\text{interaction}} = 0.014$) among patients with a baseline C-reactive protein of $>4$ mg/L (*Table 1*).[33] Thus, because C-reactive protein is so highly correlated with LDL cholesterol, for which there is strong and consistent evidence that LDL is part of the causal pathway of atherosclerosis and CHD, the reduced rate of CHD events in the JUPITER trial among patients who were treated with a potent LDL lowering agent does not add to the evidence in support of C-reactive proteins role in the causal pathway of atherosclerosis or CHD. Trials of therapeutic interventions that directly lower C-reactive protein without altering LDL

**Table 1 Treatment effect of rosuvastatin by baseline C-reactive protein value in JUPITER**

| | Baseline C-reactive protein ≤4 mg/L | | Baseline C-reactive protein >4 mg/L | |
|---|---|---|---|---|
| | Rosuvastatin | Placebo | Rosuvastatin | Placebo |
| Number per group | 4211 | 4113 | 4689 | 4788 |
| CHD events per group (rate/1000 patient-year) | 50 (5.6) | 119 (13.8) | 92 (9.5) | 133 (13.5) |
| Risk ratio rosuvastatin vs. placebo | 0.41 (95% CI: 0.30–0.57) | | 0.70 (95% CI: 0.54–0.91) | |
| Relative risk reduction per subgroup | 59% (95% CI: 43–70%) | | 30% (95% CI: 9–46%) | |

*P*-interaction = 0.014.

**Table 2 Key summary points comparing the robustness and coherence of LDL cholesterol vs. C-reactive protein as a 'causal' marker for coronary heart disease**

| Features to prove causality | LDL Cholesterol and CHD | C-reactive protein and CHD |
|---|---|---|
| Consistency of observational data | Yes; summary relative risk per 1 SD = 1.38 (95% CI: 1.09–1.73) | Yes; summary relative risk per 1 SD = 1.23 (95% CI: 1.07–1.42) |
| Temporality | Yes | Yes |
| Dose response | Yes | Yes |
| Mechanistic data consistent with observational | Yes; consistent across multiple model systems | No |
| Genetic data supporting causality | Yes | No |
| Experimental Evidence: including RCTs | Yes, multiple RCTs | Unclear |

cholesterol are required to definitively evaluate whether lowering C-reactive protein *per se* leads to a reduction in CHD.

## Summary

The factors which are used to determine causality in observational studies were outlined by Hill[34] in 1965. These include consistency, temporality, dose-response, coherence of data across studies, and experimental evidence, and they are often used by epidaemiologists to test 'causality' of a marker. Considering the evidence from several studies, hs-C-reactive protein meets the criteria of consistency of association [summary relative risk = 1.23 (95% CI: 1·07–1·42)], temporality (hs-C-reactive protein measured prior to onset of CHD), and biologic gradient (increased risk with increasing levels of hs-C-reactive protein). However, hs-C-reactive protein does not meet the criteria of coherence of the data across study types (i.e. unclear association in animal and mechanistic studies, and no evidence from genetic studies), and hs-C-reactive protein does not meet the criteria of experimental evidence in human populations (i.e. null results from Mendelian randomization, and no randomized trial showing C-reactive protein lowers CHD events independent of other CHD risk factors). Thus, while hs-C-reactive protein meets *some* of the criteria for causality, unlike LDL cholesterol, it does not meet the two critical factors of *coherence* and *experimental evidence* (*Table 2*). Moreover, the effect after adjustment of other factors is so modest that residual confounding is impossible to exclude and its role as an incremental predictor of CHD is weak.

# Conclusion

hs-C-reactive protein is weakly associated with CHD and to date the collective evidence does not support its role as a causal factor. Instead it is likely to be a bystander (marker), but given that the incremental predictive value is modest, it adds little to prediction of CVD compared with other information and is therefore likely of little clinical value.

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