Is it important to measure or reduce C-reactive protein in people at risk of cardiovascular disease?

Aroon D. Hingorani1,2†, Reecha Sofat2†, Richard W. Morris3, Peter Whincup4, Gordon D. Lowe5, Jennifer Mindell1, Naveed Sattar5, Juan P. Casas1,6, and Tina Shah1,2*

1Genetic Epidemiology Group, Research Department of Epidemiology and Public Health, Division of Population Health, University College London, Torrington Place, London WC1E 6BT, UK; 2Centre for Clinical Pharmacology, Division of Medicine, University College London, Rayne Institute, 5 University Street, London WC1E 6JF, UK; 3Department of Primary Care and Population Health, University College London Medical School, Rowland Hill Street, London NW1 2PF, UK; 4Division of Population Health Sciences and Education, Cranmer Terrace, St George’s, University of London, London SW17 0RE, UK; 5Institute of Cardiovascular and Medical Sciences, University of Glasgow, 126 University Place, Glasgow G12 8TA, UK; and 6Faculty of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK

Received 11 July 2011; revised 3 May 2012; accepted 4 May 2012; online publish-ahead-of-print 5 July 2012

Introduction

Systemic and local vascular inflammation is implicated in atherogenesis. High-sensitivity (hs) assays detecting low concentrations of C-reactive protein (CRP) in healthy individuals have delineated associations of this inflammation marker with cardiovascular events years later. A 160 309-person participant level meta-analysis of 54 prospective studies found the relationship between log-CRP concentration and cardiovascular disease (CVD) events to be linear, with a 2.5-fold risk difference in individuals at opposite extremes of the CRP distribution.1 In vitro studies and animal experiments point to potentially atherogenic actions of CRP,2 and statins lower both CRP and low-density lipoprotein cholesterol (LDL-C).3 The Justification for the Use of statins in Primary prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER),4 designed to ‘assess the effect of rosuvastatin on first ever cardiovascular events in apparently healthy men and women who do not qualify for statin therapy due to low levels of LDL-C, but who are at increased cardiovascular risk due to elevated levels of hs-CRP’, was halted because of ‘unequivocal evidence of a reduction in cardiovascular morbidity and mortality among patients who received CRESTOR (rosuvastatin) when compared to placebo’ (http://clinicaltrials.gov/ct2/show/NCT00239681). Consequently, the US Food and Drug Administration approved an amended license for rosvastatin for the primary prevention of CVD events in men and women over 50 and 60 years, respectively, with one other risk factor and a CRP concentration of >2 mg/L.

Even before the publication of this trial, the estimated 5 million ‘hs-CRP’ tests were being ordered annually in the USA,5 and updated Canadian guidance on CVD prevention6 now recommends the consideration of ‘hs-CRP’ measurement in those at intermediate risk. The 2010 American College of Cardiology Foundation/American Heart Association Guidelines stated that in ‘men 50 years of age or older and women 60 years of age or older with an LDL-C < 130 mg/dL (3.38 mmol/L) . . . CRP can be useful in selection of patients for statin therapy’.7 However, the European view has been more cautious. European Society of Cardiology Guidelines considered the measurement of CRP for CVD risk prediction ‘was premature’.8 The European Medicines Evaluation Agency (EMEA) opted to license rosvastatin in individuals at a high risk of CVD based on the ‘established’ risk factors but was criticized for its failure to incorporate recommending CRP measurement in the licensing decision.9

Here, we critically appraise evidence on the measurement of CRP for CVD risk assessment and targeting of statins and the role, if any, of CRP lowering in the prevention of CVD events.

Why the current interest in measuring or targeting C-reactive protein for cardiovascular disease prevention?

Despite the known causal association of blood pressure (BP) and LDL-C with CVD, and their inclusion in multivariable risk prediction models, it is known that such models are poor at discriminating between individuals who do, or who do not, eventually suffer events.10,11 About 50% of all coronary events occur among individuals with an average level of cholesterol (or at intermediate abso-
Moreover, many CVD events occur even in statin-treated populations. Therefore, interest in reducing the burden of events among those at intermediate risk and those already treated with statins provoked interest in new biomarkers like CRP as predictive tests, therapeutic targets, or both. Three distinct proposals can be distilled from the literature on CRP: first, that CRP measurement aids risk prediction; second, that CRP plays a causal role in CVD, like LDL-C; and third, that CRP measurement identifies an otherwise concealed subgroup of individuals who gain more from statin treatment. But how well does the available evidence support these assertions?

**First proposal: C-reactive protein measurement aids risk prediction**

**Supporting evidence: C-reactive protein is associated with later CVD events in observational studies**

The association of a marker with coronary disease fails to guarantee performance as a predictor, even if causal. The association of CRP and coronary heart disease (CHD) events is log-linear in shape, and the distribution of CRP in populations is log-normal. Thus, as for LDL-C, two systematic reviews found that the majority of CHD events occur among the many individuals with intermediate values of CRP, with a wide overlap in CRP concentration among later cases of CVD and those remaining disease free (Figure 1). The overall sensitivity (or the disease detection rate) was estimated to be 11% for a 5% false-positive rate. The summary area under the receiver operating characteristic (ROC) curve (an index of the ability of a marker to discriminate cases) for a range of CRP cut-points was 0.57, close to the 0.5 value that indicates no discrimination.

Two further limitations to the wider implementation of risk prediction based on a single CRP cut-point value are: first, that population average CRP levels differ according to ethnicity, with the differences not being fully explained by differences in cardiovascular risk; and second, that two individuals with the same CRP value, but with a different constituency of risk factors, can have a very different absolute risk CVD (Figure 2). Although CRP is included in the multivariable Reynolds risk score, adding CRP to scores that already include the established risk factors only marginally improves the area under the ROC curve, partly because CRP is itself associated with these risk factors. Using calibration as a metric, risk models with and without CRP perform almost equivalently. CRP also provides little useful reclassification when added to models based on the established risk factors (see Supplementary material online, Appendix) if reclassification tables are presented appropriately and interpreted on the basis of effect size rather than P-values.

Thus, judicious interpretation of the evidence indicates that CRP on its own does not usefully discriminate CHD events and, at best, only marginally improves discrimination, calibration or reclassification when included in the established risk models.

**Second proposal: the benefit of statin treatment is partly attributable to C-reactive protein-lowering**

**Supporting evidence: statins lower C-reactive protein**

Statins reduce both LDL-C and CRP. In statin-treatment trials, on-treatment CRP concentration is associated with the CVD event rate. These observations have been interpreted as indicating: (i) that CRP lowering contributes mechanistically to the prevention of CVD events and (ii) the effect is independent of the lowering of LDL-C. But are these interpretations consistent with the evidence?

That CRP lowering contributes mechanistically to the benefit of statins implies that CRP itself is ‘causally’ involved in atherosclerosis and its complications. However, convergent evidence suggests that this is not the case. The reportedly deleterious effects of CRP on vascular cells and tissues, used in evidence of a proatherogenic
effect, are likely to have been artefactual and due to sodium azide preservative and endotoxin in commercial CRP preparations. Experiments using pure CRP, free from azide or endotoxin, did not reproduce these effects. Reports of atherosclerosis in mouse models engineered to overexpress human CRP were also not reproduced.

The association of CRP with CHD in observational studies in humans could be explained by confounding, because CRP is also associated with a very large number of established or suspected risk factors or by reverse causation because preclinical atheroma (known to be present from the second decade of life) could provide an inflammatory stimulus for hepatic CRP synthesis. In the individual participant-level meta-analysis of CRP, progressive adjustment for lipids, smoking, and fibrinogen, progressively attenuated the association of CRP with vascular events.

Single-nucleotide polymorphisms (SNPs) in the CRP gene associated with differences in the CRP level of ≈15–20% per allele are fixed throughout life and unaffected by disease (overcoming reverse causation). As a consequence of their naturally randomized allocation at conception, such SNPs also have no association with confounders, in contrast to CRP itself, akin to a natural trial of a CRP-modifying treatment. However, CRP SNPs are not associated with carotid atheroma, or CVD events, suggesting that CRP does not influence these outcomes.

What of statin-induced CRP reductions? A participant-level meta-regression analysis from the Cholesterol Treatment Trialists (CTT) indicates that the benefits of statins can be explained by the LDL-C reduction. Although statin-induced reductions in CRP correlate poorly with LDL-C reductions within trials, the reduction in CRP and LDL-C is strongly associated when examined ‘across’ trials ($r = 0.80, P < 0.001$); the poor correlation within study being explained by the measurement error of the two analytes. The statin effect on CRP could operate through rather than independently of LDL-C or may be an off-target consequence of statin treatment. Non-statin LDL-C-lowering interventions also reduce the CHD risk to an extent consistent with their LDL-lowering effect, casting doubt on the clinical relevance of the CRP-lowering action of statins.

What then accounts for the reported association of the ‘on-treatment’ CRP concentration with clinical outcome in the statin trials? A potential explanation is that subgroups defined post hoc according to the achieved CRP value differ in other ways, including the mean on-treatment values of the other risk factors with which CRP is associated and which may not have been adjusted for. Such non-randomized comparisons may be as prone to confounding as non-randomized observational studies. A recent substudy from the Anglo Scandinavian Outcomes Trial, in which the appropriate adjustments were made, failed to find evidence for an association between on-treatment CRP and cardiovascular outcomes.

Third proposal: C-reactive protein measurement could identify an otherwise concealed high-risk group particularly responsive to statin treatment

Supporting evidence: subgroup analysis in the AFCAPS/TexCAPs primary prevention trial

In a post hoc, subgroup analysis of the AFCAPS/TexCAPs primary prevention trial of lovastatin, the relative risk reduction (RRR) was statistically significant in subgroups with a high starting value of CRP, even if the LDL-C value was low, but was non-significant in a group with a low value of LDL-C as well as low CRP. The
interpretation was that individuals with a high CRP might be targeted as a distinct high-risk group particularly responsive to statins. However, tests of significance within trial subgroups can be misleading. The preferred analysis, a statistical test for a treatment–subgroup interaction (Figure 3), did not corroborate the original interpretation. When the same question was addressed in another statin trial (PROSPER),32 the correct analysis provided no evidence for a CRP subgroup by treatment interaction (Table 1). Among 20,536 participants from the Heart Protection Study, the proportional reduction in the incidence of a first major cardiovascular event was also consistent in patients from six different categories of baseline CRP values from 1.25 to ≥8 mg/L.29 A prior analysis also showed that age, gender, BP, diabetes, smoking, or starting value of high-density lipoprotein cholesterol (all associated with CRP concentration) do not modify the treatment effect of statins.27

In summary, critical scrutiny of the evidence prior to the JUPITER trial reduces confidence in the role of CRP as a predictive test, therapeutic target, or to guide statin therapy. What then could explain the apparently dramatic findings of the JUPITER trial in people with a high CRP but low LDL-C?

Reinterpretation of the JUPITER trial

In the JUPITER trial, screening of 90,000 individuals was required to identify 17,802 with the low LDL/high CRP profile necessary for inclusion.33 This placebo-controlled trial lacked a comparator arm of individuals with a similar CVD risk but with an average or low CRP. Scheduled to last 4 years, the trial was halted early by the independent Data and Safety Monitoring Board because of unequivocal evidence of a reduction in cardiovascular morbidity and mortality in patients treated with rosuvastatin compared to placebo.

Prior statin trials had supported the prediction of observational epidemiology that LDL-lowering should produce a similar ‘proportional’ reduction in the CVD risk at all levels of the absolute risk, even among those with below-average LDL values. So did the findings of the JUPITER trial actually deviate from expectation?

Figure 3  Re-analysis for a treatment—subgroup interaction in the AFCAPS/TexCAPs study comparing the RRR for individuals with a high starting C-reactive protein value and individuals with low levels of low-density lipoprotein and C-reactive protein.21

Table 1  Effect of pravastatin on cardiovascular events by tertile of baseline C-reactive protein in the PROSPER trial32

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Lower third</th>
<th>Middle third</th>
<th>Top third</th>
<th>P-value for interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td>–13</td>
<td>–2</td>
<td>–22</td>
<td>0.36</td>
</tr>
<tr>
<td>History of CVD</td>
<td>–15</td>
<td>–19</td>
<td>–28</td>
<td>0.71</td>
</tr>
<tr>
<td>No CVD history</td>
<td>–15</td>
<td>29</td>
<td>–17</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Secondary (coronary events)

| All subjects | –14 | –15 | –22 | 0.81 |
| History of CVD | –20 | –35 | –22 | 0.86 |
| No CVD history | –11 | 27 | –24 | 0.22 |

HR, hazard ratio. Negative numbers represent a lower event rate in pravastatin- vs. placebo-treated subjects. Data are presented for all subjects and are subdivided into those with and without a history of vascular disease. The HR was adjusted for treatment allocation, age, sex, country, LDL-cholesterol, HDL-cholesterol, systolic BP, smoking status, history of diabetes, and history of hypertension. Probability values are the significance of interaction term for tertile-by-treatment effect.

*CHD death, nonfatal myocardial infarction, or fatal or nonfatal stroke.
Evidence from a meta-regression analysis of statin trials

The perception of a larger than expected RRR was supported by a graph in the Supplementary material online of the trial report, reproduced here as Figure 4A, in which the JUPITER trial appears as an apparent outlier. However, in a formal meta-regression analysis, in which each trial contributes a separate data point, the JUPITER trial looks less like an outlier (Figure 4B). The meta-regression analysis also concurs with the updated CTT Collaboration meta-analysis that added a further seven trials (including JUPITER) to the previous overview. In the updated analysis, a larger LDL-C reduction was associated with a greater RRR in major cardiovascular events but there was no significant residual variation between trials after adjustment for LDL-C differences. Indeed, in JUPITER itself, the LDL reduction and relative hazard of a CVD event during rosuvastatin treatment did not differ by the stratum of CRP, or by stratum of Framingham risk score, with which CRP is highly correlated.  

Evidence from simulation

We previously simulated the JUPITER outcome based on established risk assessment methods and the predicted LDL-C-lowering, without consideration of CRP, and submitted the findings as a British Medical Journal rapid response, 24 h before the outcome of the JUPITER trial was first reported (http://www.bmj.com/cgi/eletters/337/aug28_2/a1371). At the time, we indicated that it would be 'of interest to recalculate the expected treatment effect in the simulation “post-JUPITER”, based on the achieved LDL reduction in the JUPITER trial…without measuring CRP'. The results of both simulations (Table 2) very closely approximate the observed event rates. Notably, the simulations predicted a highly significant difference in event rates between treatment arms after just 2 years. The JUPITER trial was terminated prematurely after a median follow-up of 1.9 years.

These analyses support the view that the RRR observed in JUPITER was within the bounds expected from the degree of LDL-C reduction. Any residual deviation might be adequately explained by the premature termination of the trial which is known to contribute to overestimation of treatment effects.  

Are there more equitable and cost-effective ways of targeting statins for primary prevention?

If correct, individuals with a similar absolute risk but with a different constellation of risk factors should derive the same risk reduction from cholesterol lowering with statins as the JUPITER participants, but would be overlooked if the high CRP/low LDL-C criteria were adopted as a means of population screening.

Eligibility for statins based on the absolute risk (rather than CRP) but with a lower threshold for intervention is likely to be more inclusive and cost-effective. Only one-fifth of men over 50 and women over 60 screened would be expected to have a high CRP and a low LDL-C, based on screening and enrolment to the JUPITER trial. Therefore, the 5-year number needed to screen (NNS; NNS = NNT/proportion of screen positive individuals) would be ~100. In contrast, based on the Health Survey for England 2006 (http://www.esds.ac.uk/findingData/snDescription.asp?sn=5809), ~2–3 men for every five screened over age 50,
and 1–2 women for every five screened above the age of 60 would be eligible for statins based on a 20% absolute risk threshold, estimated using established risk factors, a higher screen positive rate than using the JUPITER criterion. Moreover, the CRP measurement with an hs-assay is patent-protected in some countries, so CRP-based risk assessment is likely to have a larger acquisition cost than risk assessment based on the established risk factors. Lastly, an age threshold has been proposed as an even simpler criterion for statin treatment. About 95% of all CHD events occur beyond the age of 50 in men and 55 in women, and age alone discriminates later cases of CHD nearly as well any of the available risk factors or risk equations.37 As well as modelling the cost-effectiveness of these alternative screening strategies, it would be important to evaluate their acceptability to healthcare providers, clinicians, and, most important of all, individuals being targeted for prevention.

Conclusions

Despite two decades of observational and experimental research, a large clinical trial, and early adoption in some regions, careful scrutiny of the available evidence does not provide compelling evidence for the CRP measurement for risk prediction or the targeting of statins, nor that CRP lowering is a valid therapeutic goal for the primary prevention of CVD. The lessons learnt with CRP should now inform the future evaluation of other biomarkers of CVD risk.

Acknowledgements

We acknowledge the NHS Information Centre who funded the HSE 2006, which was not involved in any way with this paper.

### Funding

A.D.H. was supported by a British Heart Foundation Senior Fellowship (FS 05/125). R.S. was supported by a British Heart Foundation (Schillfingford) Clinical Training Fellowship (FS/07/011). J.M. is funded by the NHS Information Centre to conduct the HSE but was not funded for this paper.

### Conflict of interest

A.D.H. has received honoraria for speaking at educational meetings relating to risk factor management and primary prevention, donated in whole or part to charity.

### References


3. Mora S, Ridker PM. Justification for the Use of Statins in Primary Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER)—Can C-reactive protein be used to target statin therapy in primary prevention? Am J Cardiol 2006; 97(2, Suppl. 1):33–41.


### Table 2

<table>
<thead>
<tr>
<th>Year</th>
<th>Pre-JUPITER simulation</th>
<th>Post JUPITER simulation</th>
<th>JUPITER trial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Predicted events (treatment arm)</td>
<td>Predicted events (placebo arm)</td>
<td>Predicted events (treatment arm)</td>
</tr>
<tr>
<td>1</td>
<td>146</td>
<td>174</td>
<td>104</td>
</tr>
<tr>
<td>2</td>
<td>226</td>
<td>348</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>3</td>
<td>305</td>
<td>522</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

The values are based on an average baseline CVD risk similar to the JUPITER trial, estimated using the Framingham risk equation without consideration of CRP, and on the known relationship between LDL-C-lowering and CVD risk reductions.

*See [http://www.bmj.com/cgi/eletters/337/aug28_2/a1371](http://www.bmj.com/cgi/eletters/337/aug28_2/a1371).

*We estimated the annual average risk of CVD and CHD in JUPITER participants (weighted by the proportion of males and smokers) using published information on baseline characteristics at enrolment.12 using an online Framingham risk calculator ([http://www.myycrisk.co.uk](http://www.myycrisk.co.uk)). The annual CHD risk was estimated to be 1.21% (10-year risk 12.1%) and the annual CVD risk 1.95% (10-year risk 19.5%). The risk reduction from 20 mg rosuvastatin was based on an estimated long-term LDL-C reduction of 1.6 mmol/L. A meta-analysis of short-term trials of cholesterol-lowering drugs estimated rosuvastatin 20 mg daily reduces LDL-C by 2.32 mmol/L,28 but LDL-C reductions may be attenuated in the longer term. We therefore estimated that LDL-C would be reduced by 1.6 mmol/L, with this dose of rosuvastatin. The corresponding expected relative reduction in the CVD risk of 16% in Year 1, 35% in Year 2, and 42% in Year 3 was derived using information from Baigent et al. The absolute number of events is based on 8901 individuals in each arm and the simplifying assumption of equal follow-up and that all participants are at risk throughout the trial. The post-JUPITER simulation was repeated using a baseline CVD risk equal to the annual CVD event rate reported in the JUPITER trial itself and the LDL-C reduction of 1.4 mmol/L observed in the trial.

A meta-analysis of short-term trials of cholesterol-lowering drugs estimated rosuvastatin 20 mg daily reduces LDL-C by 2.32 mmol/L,28 but LDL-C reductions may be attenuated in the longer term. We therefore estimated that LDL-C would be reduced by 1.6 mmol/L, with this dose of rosuvastatin. The corresponding expected relative reduction in the CVD risk of 16% in Year 1, 35% in Year 2, and 42% in Year 3 was derived using information from Baigent et al.29 The absolute number of events is based on 8901 individuals in each arm and the simplifying assumption of equal follow-up and that all participants are at risk throughout the trial. The post-JUPITER simulation was repeated using a baseline CVD risk equal to the annual CVD event rate reported in the JUPITER trial itself and the LDL-C reduction of 1.4 mmol/L observed in the trial.


