Should C-Reactive Protein Be a Target of Therapy?

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Multiple studies have demonstrated that elevated levels of high-sensitivity C-reactive protein (hs-CRP) are associated with increased cardiovascular (CV) risk. Newer CV risk stratification strategies incorporating hs-CRP (e.g., Reynolds Risk Score) have also been shown to improve risk stratification better than algorithms incorporating only traditional risk factors. There is also evidence from several landmark statin trials that on-treatment hs-CRP levels predict the likelihood of CV events. Although there is increasing evidence that CRP may be directly involved in the pathogenesis of atherosclerosis, the question of whether reduction in CRP levels and/or its associated downstream effects will provide novel therapeutic avenues to reduce CV risk requires further investigation.

CV diseases are the number one cause of mortality worldwide (1). Recent efforts directed at primary prevention of atherosclerosis have significantly reduced the incidence of initial clinical presentation of atherosclerosis. It is well established that patients with known diabetes or other traditional CV risk factors have an increased risk of atherosclerosis. Nonetheless, 15–20% of major CV events occur in patients with no major traditional CV risk factors (2). As a result, recent preventive efforts have been directed at finding newer, nontraditional, biomarkers to improve risk stratification,

particularly in otherwise apparently low-risk individuals.

hs-CRP, previously considered to be an indicator of systemic inflammation, has recently received much attention in the scientific literature, not only as a potential marker of increased atherosclerotic risk, but also as a potential target of therapy for the prevention of atherosclerotic CV disease. In this review, we discuss whether hs-CRP should indeed be a target for therapy in the prevention of CV disease as well as other potential clinical implications related to hs-CRP.

BACKGROUND/RATIONALE—

Multiple studies have demonstrated that elevated levels of hs-CRP are clearly associated with increased CV risk (3,4). However, considerable controversy exists on whether CRP itself is actually pathogenic versus an "innocent bystander" (marker) for CV disease and coronary heart disease (CHD).

Evidence derived mainly from statin trials, as outlined below, supports the potential value of CRP as a therapeutic target for both primary and secondary prevention of CV disease and CHD. However, this notion remains controversial.

The earliest evidence came from a post hoc analysis of the AFCAPS/TexCAPS (Air Force/Texas Coronary Atherosclerosis Prevention Study) trial, a landmark primary prevention study of statin therapy in individuals with "normal" LDL cholesterol

levels but low HDL cholesterol. In this study of 6,605 patients with an LDL cholesterol between 152 and 191 mg/dL (3.9 and 5.0 mmol/L) and a low HDL cholesterol (<47 mg/dL [1.2 mmol/L]), 20–40 mg lovastatin daily versus placebo resulted in a 37% reduction in CV events (myocardial infarction [MI], CV death) (5). Post hoc analysis of the data revealed that subjects with high LDL cholesterol (>149.1 mg/dL [3.86 mmol/L]) on treatment had high event rates with statin therapy associated with the number needed to treat (NNT) to prevent one major CV event ranging between 33 and 58. Interestingly, however, subjects with a low LDL cholesterol (<149.1 mg/dL [3.86 mmol/L]) and an elevated hs-CRP (>1.6 mg/L) had high event rates and benefited to a similar degree from statin therapy with an NNT of 48. In contrast, subjects with low levels of both LDL cholesterol and hs-CRP had extremely low event rates and no clinical benefit, despite similar lowering of LDL cholesterol (6) (Table 1).

Further support for CRP as a potential target for therapy in CV disease arose from secondary prevention trials using statin therapy. The Aggrastat-to-Zocor (A to Z) trial compared an early and intensive 80 mg simvastatin versus a delayed and less intensive statin regimen (20 mg simvastatin) in 3,813 patients (7). In a post hoc analysis, hs-CRP was assessed at 30 days and 4 months to predict prognosis. Patients with hs-CRP >3 mg/L at 30 days had significantly higher 2-year mortality rates than patients with hs-CRP 1–3 mg/L or hs-CRP <1 mg/L (6.1 vs. 3.7 vs. 1.6%, respectively; P < 0.0001). Similar results were reported at 4 months. Patients subjected to early intensive statin therapy (40 mg simvastatin for 1 month, then 80 mg simvastatin) were slightly more likely to achieve hs-CRP levels <1 mg/L at 30 days (22 vs. 18%; P < 0.03) and at 4 months (30 vs. 22%; P < 0.0001) compared with patients who were administered 20 mg simvastatin (8).

Analyses of another secondary prevention statin trial, the Pravastatin or Atorvastatin Evaluation and Infection Therapy (PROVE-IT)—Thrombolysis in Myocardial Infarction study, demonstrated the "dual target hypothesis." In

Table 1—Dual targets (hs-CRP and LDL cholesterol) as a method to target statin therapy for primary prevention of CV disease: evidence from AFCAPS/TexCAPS

Study group	Placebo	Statin	NNT
Low LDL cholesterol/low hs-CRP	0.022	0.025	—
Low LDL cholesterol/high hs-CRP	0.051	0.029	48
High LDL cholesterol/low hs-CRP	0.050	0.020	33
High LDL cholesterol/high hs-CRP	0.055	0.038	58

Median LDL cholesterol = 3.86 mmol/L; median hs-CRP = 1.6 mg/L. Post hoc analysis of AFCAPS/TexCAPS demonstrated a potential role for using hs-CRP as a target for statin therapy. Patients with low LDL cholesterol but elevated hs-CRP had high event rates and benefitted from statin therapy with an NNT of 48. Adapted from Ridker et al. (6).

this study, 4,162 patients with acute coronary syndrome were also randomized to "standard" therapy with 40 mg pravastatin daily versus "intensive" therapy with 80 mg atorvastatin daily (9). Patients who achieved either LDL cholesterol <70 mg/dL (1.8 mmol/L) or hs-CRP <2 mg/L had substantial reductions in major CV events during the 2-year follow-up period. Furthermore, patients who achieved both the LDL cholesterol and the hs-CRP goals were about half as likely to have an event as those who did not achieve either the LDL cholesterol or the hs-CRP goals. Hence, by attaining the "dual targets" of both LDL cholesterol and hs-CRP, subjects had a more substantial reduction in major CV events compared with achieving only one of the "targets" of therapy (10).

In an intravascular ultrasound secondary prevention statin study, the Reversal of Atherosclerosis with Aggressive Lipid Lowering (REVERSAL) trial, the effects of "standard" therapy (40 mg pravastatin) versus "intensive" therapy (80 mg atorvastatin) were studied in 502 patients with angiographically proven coronary artery disease randomized to each group. Effects were studied using intravascular ultrasound performed at baseline and after 18 months of therapy. The 80 mg atorvastatin group demonstrated reductions in LDL cholesterol and hs-CRP of 46 and 36%, respectively. In contrast, the 40 mg pravastatin group demonstrated less substantial reductions in LDL cholesterol and hs-CRP of 25 and 5%, respectively. Univariate analysis demonstrated that the percent change in both LDL cholesterol and hs-CRP related to the rate of progression of atherosclerosis on intravascular ultrasound. Even after adjusting for lipid levels, it was determined that reductions in hs-CRP were independently and significantly correlated with the rate of atherosclerosis progression ($r = 0.09$, $P = 0.04$). In addition,

patients with reductions in both LDL cholesterol and hs-CRP to levels greater than the median reductions achieved had significantly lower rates of atherosclerosis progression on intravascular ultrasound than did patients with reductions in only one marker or patients with reductions in both markers that were less than the median reduction achieved ($P < 0.001$) (11).

CRP AS A TARGET FOR THERAPY—The largest study to suggest an integral role for CRP as a target for therapy in primary prevention of CV disease is the recent Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER) trial. The investigators randomized 17,802 men ≥50 years of age and women

≥60 years of age with low LDL cholesterol levels <130 mg/dL and hs-CRP ≥2 mg/L and no history of CV disease or diabetes to 20 mg rosuvastatin daily or placebo. The primary end point was the first occurrence of MI, stroke, hospitalization for unstable angina, arterial revascularization, or CV death (12).

JUPITER was terminated early because of evidence of a reduction in CV morbidity and mortality in patients treated with rosuvastatin compared with placebo. During the 1.9-year median follow-up duration (maximum follow-up period 5 years), rosuvastatin reduced LDL cholesterol by 50% and hs-CRP by 37%, and this result was associated with a 44% reduction in the JUPITER primary trial end point ($P < 0.00001$; 95% CI 0.46–0.69). Moreover, the NNT extrapolated out to 5 years to prevent one major event was only 25, a value that is less than that associated with the use of statin therapy for primary prevention among individuals with more overt hyperlipidemia (Fig. 1). Thus, despite targeting a population outside current guidelines and with low levels of LDL cholesterol, JUPITER demonstrated a magnitude of effect larger than that of almost all prior statin trials (13). Based on the results of the JUPITER study, the U.S. Food and Drug Administration (FDA) in February 2010 agreed to broader labeling for rosuvastatin. As per
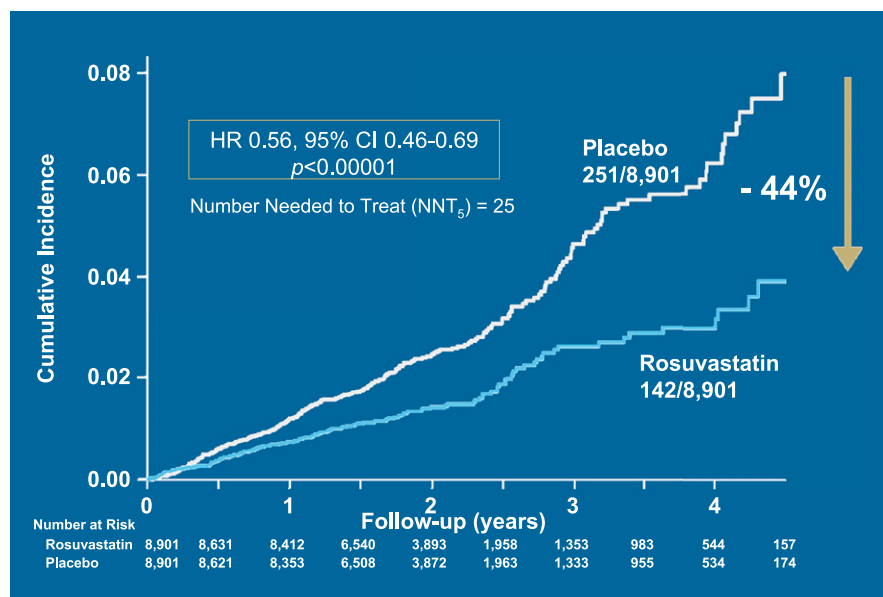
Figure 1—*Cumulative incidence of CV events according to study group. Significant relative risk reduction of the primary composite end point of nonfatal MI, nonfatal stroke, unstable angina, revascularization, and CV death in JUPITER is shown. Hazard ratio for rosuvastatin compared with placebo group was 0.56 (95% CI 0.46–0.69, $P < 0.00001$). Adapted from Ridker et al. (12).*

the FDA, rosuvastatin is currently approved for the reduction of risk for stroke, MI, and revascularization procedures in individuals who have normal LDL cholesterol levels and no clinically evident CHD but who do have an increased risk based on age, CRP levels, and the presence of at least one additional CVD risk factor.

JUPITER also provided further credence for the "dual target" hypothesis. In a prespecified analysis, a 65% reduction in major events was noted in patients who achieved LDL cholesterol <70 mg/dL and hs-CRP <2 mg/L versus a 33% reduction in patients who realized only one or neither target ($P < 0.0001$ across treatment groups). Accordingly, JUPITER not only demonstrated that hs-CRP successfully identified a population with "hidden risk" for CV disease but also provided additional evidence for the potential utility of hs-CRP as a target for therapy in primary prevention of CV disease.

Is there any basic science rationale for hs-CRP as a target for therapy?

There is an accumulating body of literature demonstrating a role for CRP as a putative mediator of atherosclerosis, independently and in synergy with other traditional risk factors, such as LDL cholesterol. The mechanisms through which CRP is thought to potentiate atherosclerosis are numerous and diverse. Our understanding of atherosclerosis over the last 3 decades has undergone a paradigm shift from that of a lipid-centric disease to one that is centered on inflammation (14). Cumulative evidence indicates that

inflammation is an active component in all phases of atherosclerosis, from early plaque initiation, to plaque development, rupture, and ultimately acute coronary occlusions (15) (Fig. 2). The results of the hs-CRP blood test are thought to reflect the inflammatory process. Evidence from in vitro and in vivo models of CV disorders including hypertension and thrombosis support this contention (16,17) and, consequently, there is much interest in CRP as a potential therapeutic target for atherosclerosis. Under physiological conditions, the monolayer endothelium releases an array of substances that interact to promote overall vascular health (18). A disturbance in endothelial integrity triggers disturbances in the release and activity of these factors, resulting in endothelial dysfunction, a critical initial step in atherosclerosis that often occurs in the absence of angiographic evidence of coronary artery disease and is a common feature of hypertension- and thrombosis-associated complexities (19).

CRP has been associated with several mechanisms that are well known to potentiate atherosclerosis. Nitric oxide (NO) plays a fundamental role in endothelial function and decreased NO production, and its activity includes events well associated with atherosclerosis. CRP, at concentrations known to predict adverse vascular events, appreciably decreased NO release and bioactivity in human endothelial cell cultures (20). Furthermore, CRP inhibited NO-linked angiogenesis, an important compensatory mechanism in chronic ischemia (20). Reduced aortic NO bioavailability has also

been reported in various animal models, including human CRP-overexpressing transgenic (hCRPtg) mice (21). The negative vasoregulatory effects of CRP are substantiated by observations that secretion of the vasodilator prostacyclin was attenuated in CRP-treated human aortic endothelial cells (22), whereas release of the potent vasoconstrictor endothelin-1 in human saphenous vein endothelial cells (17) was significantly elevated after chronic CRP exposure. CRP may therefore facilitate the development of diverse CV diseases via its influence on the events mediated by NO, prostacyclin, and endothelin-1.

Whether or not CRP directly affects endothelial function, however, is still unknown. Sternik et al. (23) reported that physiologically relevant concentrations of CRP directly relaxed human internal thoracic artery segments, but a recent clinical study found that bolus administrations of CRP aggravated endothelial dysfunction in hypercholesterolemic subjects (24). Other groups have found a negative correlation between endothelial function and hs-CRP levels in patients (25) and impaired endothelial function in hCRPtg mice (21) and that CRP abrogates 5-hydroxytryptamine–induced vasorelaxation in porcine coronary arterioles (26). Perhaps more thought-provoking is the finding that hs-CRP levels were the only independent correlate of human saphenous vein graft endothelial function, thereby suggesting that preoperative CRP levels may predict the functionality and patency of saphenous vein grafts after coronary artery bypass graft procedures (27).

Recent findings indicating that CRP is not exclusively produced in the liver but is also present in normal coronary artery smooth muscle (28), endothelial cells (29), and diseased coronary artery bypass grafts (30) support the concept that CRP exerts paracrine and autocrine effects. This is reinforced by documentation of translesional CRP concentration gradients in patients with acute coronary syndromes (31). Local deposits of CRP likely occur before plaque formation, and elegant in vivo work has demonstrated that CRP escalates aortic atherosclerotic plaque formation in atherosclerosis-prone ApoE$^{-/-}$ mice (32). CRP also upregulates expression of interleukin-6, macrophage chemoattractant protein-1, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1, all of which promote monocyte-macrophage-endothelium interactions (21,32). Moreover, CRP is

NO: Nitric oxide, VSMC: Vascular Smooth Muscle Cell, TF: Tissue factor
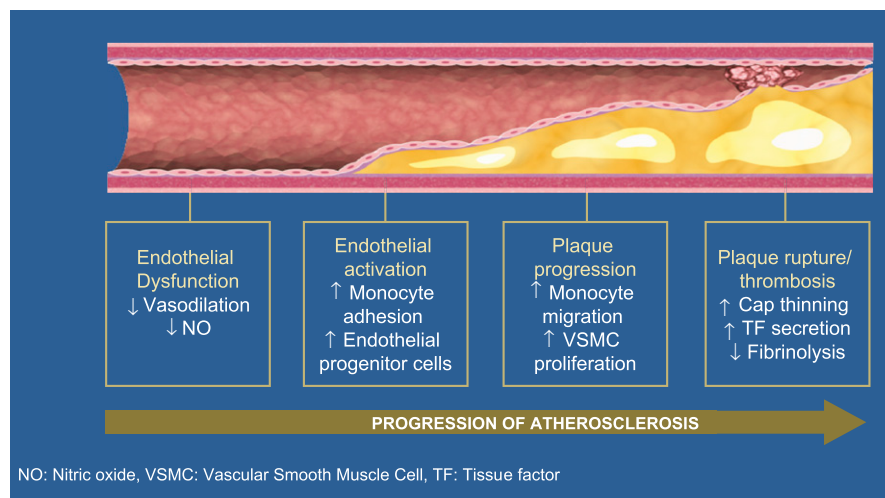
Figure 2—*Inflammation drives many stages of the atherosclerotic process and the major mechanisms are illustrated here. Adapted from Bisoendial et al. (19) and Packard and Libby (53).*

chemotactic for human monocytes (33) and facilitates oxidized LDL uptake by macrophages, resulting in greater foam cell formation (34) and exacerbation of the detrimental endothelial effects of oxidized LDL via increased levels of the lectin-like oxidized LDL receptor-1 protein (35).

Aside from its potential role in the earlier stages of atherogenesis, there is also some evidence that CRP may also contribute to later phases of atherosclerosis. The latter outcomes appear to involve inducing matrix metalloproteinase expression and collagenase activity in monocyte-macrophages (35,36) and exaggerated vascular remodeling in response to experimental modeling, as demonstrated in hCRPtg mice (21,36).

Endothelial cell apoptosis results in not only endothelial denudation but also in plaque destabilization with subsequent thrombosis. CRP appears to facilitate apoptosis of endothelial cells (20) and endothelial progenitor cells (37). It also increases the bioavailability and bioactivity of plasminogen activator inhibitor-1 and decreases tissue plasminogen activator content and effects in endothelial cells (38), suggesting that CRP has prothrombic and antifibrinolytic properties. Indeed, hypercholesterolemic patients administered CRP exhibited augmented procoagulant responses (24), and hCRPtg mice demonstrated higher incidence of the prothrombotic phenotype (39).

Some investigators have directly delved into the potential of CRP to cause myocardial ischemia and for a CRP antibody to decrease myocardial ischemia. Injection of human CRP into a rat model of coronary artery disease reproducibly enhanced infarct size by ~40% (40). Similarly, injection of human CRP into a rat model of middle cerebral artery occlusion produced significantly larger cerebral infarcts compared with controls (41). Interestingly, therapeutic inhibition of CRP using a small-molecule inhibitor of CRP in a rat acute MI model abrogated the increase in infarct size and cardiac dysfunction produced by injection of human CRP. Further studies are warranted to better define the potential clinical utility of directly targeting CRP for the prevention and treatment of CV disease (42).

The collective basic and translational evidence to date therefore support a mechanistic association between CRP and atherosclerosis, but do not provide direct evidence that CRP is a causative factor of atherosclerosis or that it should

be a target for therapy. In fact, there is also important evidence to the contrary.

The strongest evidence to date comes from Mendelian randomization studies (43,44). Recently, Elliott et al. (45) performed a genome-wide association and replication study to identify genetic loci associated with plasma hs-CRP concentrations. Specifically, these investigators completed a Mendelian randomization study of the most closely associated single-nucleotide polymorphism in the CRP locus and published data on other CRP variants involving a total of 28,112 case subjects and 100,823 control subjects to determine the association of CRP variants with CHD. In congruence with previous reports (43,44,46), this group demonstrated that genetic variations in the CRP gene are associated with lifelong increased hs-CRP levels and confirmed earlier findings (46) that there is a significant association between CRP levels and CHD. Of note, however, the authors of this study (45) were unable to demonstrate a significant association between CRP gene variants with the risk of CHD.

Consequently, while there is substantial evidence supporting an association between hs-CRP levels and the development as well as progression of atherosclerosis, the verdict remains out on whether variants of the CRP gene are causally linked with an increased risk of CHD (45). In the meantime, further investigations into the potential non–genetic-based role(s) played by CRP in the inflammatory system remain under investigation and are warranted in view of the novel therapeutic avenue.

CLINICAL UTILITY OF CRP—
Whereas the potential of hs-CRP as a therapeutic target for the prevention of CV disease remains unresolved, hs-CRP has been demonstrated to have other clinical utilities.

In particular, hs-CRP may be useful in cardiac risk prediction. Traditional CV risk prediction algorithms such as the Framingham Risk Score fail to take into account family history of premature CV disease and nontraditional risk biomarkers such as hs-CRP. The Reynolds Risk Score, in contrast, is a recently developed CV risk prediction algorithm (47) that incorporates the hs-CRP value and family history in addition to the traditional risk factors.

The Reynolds Risk Score was initially developed and validated in women and

was shown to improve global CV risk prediction. For example, it reclassified 40–50% of the women who were at intermediate risk (5–20% 10-year risk) into either higher- or lower-risk categories. In addition, global CV risk prediction with the Reynolds Risk Score more accurately matched actual event rates (47). The Reynolds Risk Score has subsequently been validated in men, again with improved CV global risk prediction. A total of 15–20% of men at intermediate risk were reclassified into higher- or lower-risk categories (48).

Beyond global risk prediction of CV events, an association between higher hs-CRP levels and new onset of future diabetes has been demonstrated (49). In the West of Scotland Coronary Prevention Study (WOSCOPS), another primary prevention statin trial, which studied the effects of pravastatin on 6,595 men with high LDL cholesterol levels, it was found that high hs-CRP levels (defined as >3 mg/L) (in combination with the presence of metabolic syndrome) demonstrated greatest prognostication for both CHD as well as future onset of diabetes (50,51). Another study found that hs-CRP was independently associated with insulin levels in nondiabetic women (52), strengthening the previously elucidated association between hs-CRP levels and increased risk of future diabetes.

CONCLUSIONS—There is much recent evidence demonstrating a strong association between elevated hs-CRP values and atherosclerosis. There remains, however, controversy as to whether CRP itself is pathogenic. Although animal studies suggest that CRP may play a role in the development of atherosclerosis, recent Mendelian randomization studies have failed to demonstrate a causal role between CRP levels and atherosclerosis, suggesting that CRP may more likely be a marker than an actual pathogenic component of atherosclerosis.

Ongoing studies should provide important additional information. The Cardiovascular Inflammation Reduction Trial aims to further assess the role of CRP as a target for therapy, as well as confirm the inflammatory hypothesis of atherothrombosis, in the setting of very-low-dose methotrexate therapy in 7,000 patients with stable coronary artery disease and persistent elevations of CRP.

In any case, hs-CRP has been demonstrated to improve global cardiac risk estimation compared with traditional

cardiac risk factor assessment. The Reynolds Risk Score, which takes into account hs-CRP readings, has been shown to improve global CV risk prediction compared with previous assessment of traditional CV risk factors and represents a practical and simple method of risk assessment in the clinical setting.

Further studies are clearly warranted to elucidate the potential pathogenic role of CRP in atherothrombosis and to determine whether CRP is a useful target of therapy. In addition, it remains to be determined if routine use of hs-CRP in risk calculation will definitively improve CV risk estimation.

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