C-reactive protein: risk assessment in the primary prevention of atherosclerotic disease.
Has the time come for including it in the risk profile?

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About half of patients presenting with myocardial infarction do not have the "classic" risk factors. This has stimulated a search for other factors that may be responsible and, when present, may help to predict which patients are at greatest risk for myocardial infarction and other cardiovascular events. With improved understanding of the pathogenesis of ischemic cardiovascular disease, we have gleaned new insights into potential markers of underlying atherosclerosis and cardiovascular risk. In recent years, data suggesting that certain markers of inflammation – both systemic and local – play a key role in the development and progression of atherosclerosis, and in its final clinical complications have accumulated. Specifically, elevated levels of one systemic marker of inflammation, C-reactive protein (CRP), are associated with an increased risk of cardiovascular disease events. Among several markers of systemic inflammation, CRP shows the strongest associations with vascular events, and the addition of CRP to total cholesterol dramatically improves risk prediction. CRP fulfils most of the requirements needed to serve as a new risk factor, but still several issues await further confirmation and clarification before this marker can ultimately be included in the routine risk profile. Moreover, potentially important associations have been established between elevated CRP levels and increased efficacy of established therapies, in particular lipid-lowering therapy with statins; CRP testing may enable us to tailor expensive cardiovascular medication to the individual patient. Such an improved prescription strategy might be especially valuable in the primary care setting where the absolute cardiovascular risk is considerably lower compared to that in secondary prevention.

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Introduction

Approximately 50% of patients who present with acute myocardial infarction or unstable angina pectoris do not have classical cardiovascular risk factors[1]. Increasing evidence from basic research suggests that atherosclerosis is an inflammatory disease and represents much more than simple accumulation of lipids in the vessel wall[2]; cholesterol is fundamentally a poor predictor of cardiovascular risk. This is shown in the Framingham Heart Study 26-year follow-up data, which reveal that more than one third of patients with coronary heart disease (CHD) have total cholesterol levels < 200 mg/dl[3].

Such evidence has prompted the search for new factors that might complement our current knowledge and improve risk stratification. Within the last decade, it has become clear that a variety of other factors, such as hemostatic and thrombotic mechanisms, inflammatory markers, and genetic factors, may also have a great influence[4-6].

This review attempts to summarize the data in favor of the notion that atherosclerosis is an inflammatory disease, focusing on the evidence from prospective studies regarding C-reactive protein (CRP), a sensitive systemic marker of inflammation, in initially healthy subjects. Furthermore, on the basis of the increasing evidence for CRP as a mediator in the pathophysiology of atherothrombosis, the potential to modify the inflammatory response will be briefly discussed. Finally, practical implications for the risk assessment in clinical routine will be outlined and, since CRP is a completely unspecific marker, pertinent problems in risk assessment are pointed out.

Pathogenesis of atherothrombosis

Any discussion of how coronary atherosclerosis produces symptoms and cardiovascular events must begin with plaque. The arterial wall is not a static unchanging structure; it can remodel itself by increasing its external diameter to accommodate plaque without narrowing the lumen[7]. It is not necessarily the size of the plaque, but rather its impaired stability that renders it vulnerable to an atherothrombotic event[8].

Several factors may lead to instability of a plaque and a subsequent acute coronary event[8-11]. Some of the proposed mechanisms, such as impaired endothelial function, can result in loss of endothelial cells, exposure of collagen and tissue factor, and superficial thrombosis over a plaque[12]. If the area of cell loss is great enough, a large, clinically significant thrombus may form. Impaired plaque stabilization is characterized by the presence of a large lipid core and a thin fibrous cap with few smooth muscle cells. Inflammatory activity of T lymphocytes, macrophages and mast cells contributes to plaque rupture. Exposure of platelets to the lipid core initiates thrombus formation. All of these events together may contribute to the risk of coronary events.

Not surprisingly, the risk of vascular events in patients with stable atherosclerotic plaques is lower than for those with unstable plaques[9]. Stable plaques are characterized by a small lipid core protected by a thick fibrous cap. Overexpression of smooth muscle cells helps prevent damage to the lipid core and reduces the risk of plaque rupture and thrombus formation. Blood flow through the atherosclerotic area is uninhibited and platelets do not accumulate.

Atherogenesis: evidence for systemic inflammation

Atherogenesis is usually a focal disease and it is not obvious that local inflammation in any vascular bed should ultimately lead to a systemic response. However, the leukocyte count, a number of inflammatory proteins, like fibrinogen[13], plasminogen-activator inhibitor-1[14,15], von Willebrand factor[16,17], albumin[18], and more recently CRP, and various cytokines and adhesion molecules have been found to be independently associated with future cardiovascular endpoints.

CRP, the classical acute phase protein, represents a highly sensitive marker of inflammation, increasing by several hundred-fold in response to acute injury, infection, or other inflammatory stimuli[19]. Its plasma half-life (~19 hours) is rapid but is identical under all conditions, so that the synthesis rate of CRP is the sole determinant of its plasma concentration. Excellent anti-CRP antibodies and a well established WHO International Reference Standard for CRP are available so that precise, sensitive and robust clinical plasma/serum assays can be readily undertaken[20,21]. The measurement of CRP thus has several advantages for detection and monitoring of the acute phase response in general and particularly in relation to atheroma formation and its complications. Indeed, high-sensitivity (hs) CRP assays have recently been used in several prospective studies in initially clinically healthy subjects and strong and consistent associations have been established between baseline levels of CRP and various future cardiovascular endpoints.

C-reactive protein and risk of coronary heart disease

Kuller et al.[22] reported the first study in healthy, but high risk, middle-aged male individuals, in whom they had prospectively investigated the relation between baseline levels of CRP and the future risk of myocardial infarction and CHD death. In a nested case-control design within MRFIT, 98 incident cases of myocardial infarction and 148 CHD deaths were identified and compared to 491 controls. The minimum follow-up was 6-7 years. Compared to the bottom quartile, the risk of dying from CHD in the top quartile of the CRP distribution was increased almost 3-fold with an odds ratio (OR) of 2.8 (95% confidence interval-CI 1.4-5.4). In smokers, this risk was even more pronounced with an OR of 4.3 (95% CI 1.7-10.8). All analyses were controlled for potential confounders. For non-fatal myocardial infarction, however, no significant association was observed.

Tracy et al.[23] carried out a prospective nested case-control study in 146 men and women from the Cardiovascular Health Study aged 65 or older with either angina, non-fatal and fatal myocardial infarction or CHD death and compared them to 146 subjects matched for sex and subclinical cardiovascular disease. The mean follow-up was 2.4 years. For men and women with subclinical disease in the top quartile of the CRP distribution compared to those in the three lower quartiles, the OR for incident myocardial infarction was 2.67 (95% CI 1.04-6.81), with the association being somewhat stronger in women. Similar results were obtained in an independent sample from the Rural Health Promotion Project.

As part of the Physicians' Health Study, Ridker et al.[24] examined CRP levels by means of an hs-assay in 543 apparently healthy men in whom myocardial infarction, venous thrombosis, or stroke subsequently developed and in 543 men in whom vascular disease did not develop over a follow-up of at least 8 years. These subjects were assigned to receive placebo or aspirin at the beginning of the trial. Baseline plasma CRP levels were higher in men who subsequently had a myocardial infarction. The men in the quartile with the highest CRP levels had nearly 3 times the relative risk for myocardial infarction compared with men in the lowest quartile. The increased risk remained stable over at least 6 years of follow-up.

In order to confirm these observations in large, unselected populations in the MONICA-Augsburg co-

hort, serum CRP levels were measured at baseline in 936 initially healthy men aged 45-64 years drawn from a random sample of the general population. Prevalent cases of myocardial infarction had been excluded at baseline as were subjects with concurrent infections or evidence of malignant disease. Based on an 8-year follow-up, an almost 3-fold increase in the risk of a first major coronary event was observed for individuals in the top quintile of the CRP distribution compared to those in the bottom quintile, even after adjustment for potential confounders[25].

Results of the largest cohort studied so far have recently been published by Danesh et al.[26]. They identified 506 middle-aged men from the British Regional Heart Study with major coronary events during a mean follow-up of 9.5 years and compared them to 1026 controls who remained free of disease during follow-up. Compared with men in the bottom third of baseline measurements of CRP, men in the top third had an OR for the occurrence of CHD of 2.13 (95% CI 1.38-3.28), after adjustment for other covariates.

Such associations could also be demonstrated in the female population. In the Women's Health Study, Ridker et al.[27] found that slight elevations in CRP levels were associated with an increased risk for cardiovascular events. Those in the highest quartile were 5 times as likely to suffer a cardiovascular event compared with those in the lowest quartile ($p = 0.0001$).

Even in women considered to be at very low risk for a vascular event, CRP levels measured with an hs-assay were predictive[27]. In fact, risk estimates were independent of other risk factors, and prediction models that included CRP provided a better method of predicting risk than models that excluded it (all $p < 0.01$). In stratified analysis, CRP was a predictor among women at low risk as well as among those at high risk. Most recent analyses from the same cohort showed CRP to be the strongest univariate predictor of cardiovascular risk among 12 markers including inflammation, lipids and lipoproteins, and homocysteine[28].

In formal meta-analysis[26] summarizing the results of 11 prospective studies with a total of 1953 cases, a relative risk (RR) of 2.0 (95% CI 1.6-2.5) for CHD was found after adjustment for various confounders, if individuals in the top third were compared to the bottom third of the CRP distribution.

C-reactive protein and risk of stroke

On an epidemiological level, cholesterol concentrations are not predictive of stroke. However, CRP levels were also predictive of stroke in the Physicians' Health Study[24]. Men in the highest quartile of CRP values had nearly 2 times the risk for stroke compared with men in the lowest quartile. This is supported by two more recent studies in elderly populations.

In the Leiden 85-Plus Study[29], baseline CRP levels and the occurrence of fatal strokes were analyzed prospectively in a population-based cohort. During follow-up lasting 5 years, 82 subjects died of stroke and were compared to 82 controls and 83 participants who died of non-cardiovascular causes. The risk of death from stroke as well as from non-cardiovascular causes increased linearly up to 10-fold in subjects with the highest levels of CRP at baseline. The authors concluded that CRP was a strong but unspecific risk factor for fatal stroke. In the population-based Helsinki Ageing Study[30], 10-year mortality was investigated in three elderly cohorts (aged 75, 80, and 85 years). Only in the 75-year-old cohort, a 10 mg/l increase in CRP levels predicted total (RR 1.20, 95% CI 1.08-1.32) and cardiovascular mortality (RR 1.22, 95% CI 1.10-1.35) in age and sex adjusted analyses. In the Women's Health Study, the association between CRP levels and incident stroke was even more striking (RR 5.5, 95% CI 1.8-16.6) when the risk in the top quartile of the CRP distribution was compared to that in the bottom quartile[27].

C-reactive protein and risk of peripheral vascular disease

The association between CRP and progression of peripheral vascular disease is not well studied; but again, in the Physicians' Health Study, CRP levels were shown to predict the development of peripheral vascular disease[31]. Using a prospective, nested case-control design, the investigators measured baseline CRP levels in 144 apparently healthy men in whom peripheral vascular disease subsequently developed and in an equal number of control cases matched by age and smoking habit who remained free from peripheral vascular disease over 5 years. Median baseline CRP levels were significantly higher among those in whom peripheral vascular disease subsequently developed. Further, the risk increased significantly with each increasing quartile of CRP levels. Patients who had disease severe enough to require revascularization surgery had the highest CRP levels.

Pathophysiological implications of elevated C-reactive protein levels

Although the underlying mechanisms that trigger the low-grade inflammatory response in atherosclerosis are essentially unknown, elevation of CRP levels in the context of this disorder is biologically plausible and indeed, it may be causally involved in the pathophysiology of atherosclerosis and its complications. At present, CRP must be regarded primarily as a surrogate marker for cytokine-mediated inflammation and there is sound experimental and pathological evidence that these molecules are directly involved in various processes of atherogenesis[2]. Besides this, CRP may act as a pro-

coagulant since it is known to induce the expression of tissue factor in monocytes. Further, there are accumulating data in favor of a number of direct vascular/endothelial effects: CRP is found in the vessel wall, even in the very early stages of plaque formation[32]. CRP is chemotactic for monocytes[33] and avidly binds to human neutrophils. It facilitates the uptake of enzymatically modified LDL by macrophages[34]; it induces complement activation, and may enhance tissue injury via this mechanism[35]. CRP peptides are involved in cellular adhesion molecule shedding and finally, only recently a strong association between increased plasma CRP levels and impaired endothelial function could be demonstrated[36].

Modifying the inflammatory response by drug treatment

Evidence indicating an interaction between certain preventive therapies and baseline CRP levels raises the possibility that inflammatory markers may also have a role in targeting specific therapeutic and preventive interventions[37].

Data from the Physicians' Health Study cited earlier indicate that cardiovascular risk can be modified by anti-inflammatory therapy with aspirin[24]. The investigators measured plasma CRP levels in 543 apparently healthy men in whom cardiovascular events subsequently developed, and in 543 controls who did not report vascular disease during a follow-up period lasting longer than 8 years. Subjects were randomly assigned to receive aspirin or placebo at the beginning of the trial. The men in the quartile with the highest levels of CRP had 3 times the risk of myocardial infarction ($p < 0.001$) and 2 times the risk of ischemic stroke compared to those in the lowest quartile. The use of aspirin was associated with an overall 44% reduction in the risk of a first myocardial infarction. While the risk was reduced by 56% in patients with the highest levels of CRP, aspirin use minimally and non-significantly reduced the risk (only 14%) of future myocardial infarction in patients with the lowest CRP levels.

The Cholesterol and Recurrent Events (CARE) study evaluated whether long-term therapy with pravastatin, which reduces cardiovascular risk, might alter levels of CRP[38]. This inflammatory marker was measured at baseline and at 5 years in 472 randomly selected participants who remained free from recurrent coronary events during follow-up. Overall, CRP levels at baseline and at 5 years were moderately correlated ($r = 0.60$, $p < 0.001$). However, the level of CRP was even better correlated than standard cholesterol screening in this study. In the entire CARE study, random assignment to treatment with pravastatin was associated with a significant 24% reduction in the risk of recurrent myocardial infarction or coronary death. Similar to results for aspirin therapy in the Physicians' Health Study, the magnitude of the risk reduction due to therapy in this substudy was greater in those with evidence of inflammation (risk reduction attributable to pravastatin therapy, 54%) compared to those without evidence of inflammation (risk reduction 25%). Moreover, the association between inflammation and risk for recurrent events was significant among those randomized to placebo but was attenuated and no longer significant among those treated with pravastatin[39].

Most recent data from the AFCAPS/TexCAPS trial in primary prevention have shown that lovastatin effectively reduced cardiovascular endpoints in those with LDL levels > 150 mg/dl, with or without elevated CRP levels (> 1.65 mg/l). Interestingly, lovastatin was equally effective in those with high CRP concentrations but with LDL levels < 150 mg/dl[40]. This finding is clearly in support of a central role of inflammation in cardiovascular complications and underlines the importance of CRP measurements in these patients.

Ridker et al.[41] also examined the relationship between levels of CRP and hormone replacement therapy (HRT, estrogen alone or estrogen plus progesterone) in 493 postmenopausal women from the Women's Health Study. Median CRP levels in women using HRT were twice as high as in women not using HRT ($p = 0.001$). In addition, there was no difference in CRP levels between women not using HRT and healthy, middle-aged men included in the Physicians' Health Study[24]. Similar results have been reported by Fröhlich et al.[42] in 749 postmenopausal women randomly drawn from the general population who participated in the MONICA-Augsburg survey 1994/1995, and by Cushman et al.[43] in participants of the PEPI trial. Thus, the use of HRT is associated with elevated levels of CRP and may increase cardiovascular risk in this population.

Distribution of C-reactive protein in the general population

Before CRP can be used for the screening of subjects at risk for cardiovascular complications, we need to know its distribution in various populations. Data from the MONICA-Augsburg and the Glasgow MONICA surveys including several thousand men and women aged 25-74 years show the well known distribution skewed to the right. A significant trend to higher CRP values with increasing age is seen in both areas, but in Augsburg, women had slightly increased values compared to those in Glasgow. This may be explained by differences in the frequency of HRT. Combining men and women, tertile cut-points in these populations were 1 mg/l and approximately 3 mg/l respectively[21].

Improving risk prediction and persistent problems in risk assessment

Because serum concentrations of CRP predict cardiovascular risk, it has been suggested that this inflam-

matory marker may have a role in routine cardiovascular risk assessment.

CRP fulfils most of the requirements needed to serve as a new risk factor for cardiovascular disease:

• the consistency of results from 11 prospective population-based studies in initially healthy subjects is remarkable;

• the association between CRP and future coronary events is strong. The combined risk ratio for CHD from meta-analysis is 2 if subjects with baseline CRP in the upper tertile of the population distribution are compared with those in the lower tertile. This holds true in men and in women, in the short term, but even for periods lasting more than 10 years. Compared with a variety of other inflammatory markers and lipid variables, on univariate analyses CRP turned out to be the best predictor for future coronary events[28];

• the association between CRP and coronary risk has proved to be independent of a wide variety of potential confounders in prospective studies, including social class in some of them;

• to date there are two studies that demonstrate that the addition of CRP determination to that of total cholesterol dramatically improves risk prediction[28,44]. On multivariate analysis, models incorporating CRP and lipid parameters provided a significantly better method to predict risk than those using lipids alone. This was seen in men and also in women;

• CRP is relatively stable, it can be measured in plasma or serum and therefore pre-analytical handling of the samples is easy compared to that of other inflammatory markers such as cytokines and adhesion molecules. The measurement procedure is standardized and automated hs-CRP assays with low analytical intra- and interassay variability are available[45]. An excellent internationally accepted standard that enables comparison between various laboratories is available and widely used;

• the reproducibility over time is however only moderate. Clearly, this is to be expected from a protein that is part of the acute phase response and which increases unspecifically in reaction to many different stimuli. The intersubject variability is sufficiently large compared to the intraindividual variation and an index of individuality derived from these components compares favorably with total cholesterol in one study[20];

• elevation of CRP in the context of atherothrombotic disease is biologically plausible. However, the available evidence is indirect, and thus causality has not been proven yet;

• the costs involved in CRP testing are reasonable, in particular if its measurement would enable us to tailor expensive cardiovascular medication to the individual patient, the one who has elevated cholesterol levels and signs of systemic inflammation. Such improved prescription strategies might be especially valuable in the primary care setting where the overall cardiovascular risk is considerably lower compared to that of secondary prevention.

However, there are several issues which await further confirmation and clarification: despite the consistency of results from prospective studies and the strength of the association with CHD, the causal relevance of CRP remains uncertain; residual bias is still an issue. The additive predictive value of CRP measured with hs-assays in the context of routine total cholesterol measurements must be replicated in other populations, especially since the reproducibility of total cholesterol levels was unusually low in CARE[38]. These further studies should also provide data on interaction analyses between CRP and lipoproteins. Until then it cannot be considered a robust finding, but rather an interesting hypothesis. At present, there are no data showing that CRP testing performs adequately in daily clinical routine in general medical practice. Proper exclusion criteria for interpretation have yet to be defined, and finally, to date there is no agreement on which cut-points should be used in the primary and the secondary care setting. Quartiles (tertiles, quintiles) are sample and population-dependent and therefore are not universally applicable. More data from general populations are needed to resolve this issue.

How to proceed in daily practice?

Despite the above mentioned limitations, based on the solid and consistent information available on the association between CRP and atherosclerotic complications and the increasing evidence for its potential pathophysiological role, the measurement of CRP with an hs-assay is recommended in subjects at risk for cardiovascular complications for further risk stratification, provided that other causes of elevated CRP levels have been excluded. If values are found to be low (e.g. < 3 mg/l), no further measurements are needed. However, if they are elevated, several serial measurements would be desirable.

In 696 initially healthy middle-aged men, the variability of CRP concentrations over 3 years was considerable with a reliability coefficient of 0.54 and practically no difference in the mean levels. Based on these reliability estimates, three serial determinations of the CRP level (with a single assay in each) should be done to reach a reliability of 0.75, the reliability we found for total cholesterol which still represents the clinical standard to which a new risk marker should be compared (Koenig et al., unpublished data). Correcting the hazards rate ratio in our original analysis on the prediction of CHD by CRP[25] for the variability in this marker leads to a considerable improvement in the risk estimate.

Future directions

Despite the increasing number of new risk markers for cardiovascular disease, it is still not clear which represents the best one and at which stage in the natur-

al history of disease. The combination of various markers reflecting different aspects of the disease process should be studied in more detail. Our improved understanding of the role of inflammation in atherogenesis may offer specific targets for novel therapeutic interventions. For example, interruption of the interaction of key intercellular adhesion molecules might inhibit atherogenesis at its earliest stage: the response to endothelial injury. Pharmacologic interventions targeted at reducing smooth muscle cell proliferation might slow or prevent progression of the fatty streak to the intermediate atheromatous plaque. Lowering serum CRP levels or interfering with its binding to the monocyte receptor might constitute additional promising strategies. Well conducted clinical and laboratory studies are needed to evaluate the multiple interdependent effects of modulating inflammatory mediators.

Conclusions

Inflammation is an important contributor to atherothrombosis. Prospective observational studies show that moderately elevated levels of CRP, measured at baseline, are associated with an adverse cardiovascular prognosis among healthy individuals. The availability of hs-assays for CRP should lead to better prediction of the cardiovascular risk – and early institution of preventive or secondary therapy for atherosclerosis.

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