C-Reactive Protein Predicts Progression of Atherosclerosis Measured at Various Sites in the Arterial Tree

The Rotterdam Study

Irene M. van der Meer, MSc; Moniek P.M. de Maat, PhD; A. Elisabeth Hak, MD, PhD; Amanda J. Kiliaan, PhD; Antonio Iglesias del Sol, MD, PhD; Deirdre A.M. van der Kuip, MD, PhD; Rogier L.G. Nijhuis, MD; Albert Hofman, MD, PhD; Jacqueline C.M. Witteman, PhD

Background and Purpose—C-reactive protein (CRP) predicts myocardial infarction and stroke. Its role as a predictor of the progression of subclinical atherosclerosis is not yet known. We investigated whether CRP predicts progression of atherosclerosis measured at various sites in the arterial tree.

Methods—CRP levels were measured in a random sample of 773 subjects ≥55 years of age who were participating in the Rotterdam Study. Subclinical atherosclerosis was assessed at various sites at 2 points in time, with a mean duration between measurements of 6.5 years.

Results—After adjustment for age, sex, and smoking habits, odds ratios (ORs) associated with CRP levels in the highest compared with the lowest quartile were increased for progression of carotid (OR, 1.9; 95% CI, 1.1 to 3.3), aortic (OR, 1.7; 95% CI, 1.0 to 3.0), iliac (OR, 2.0; 95% CI, 1.2 to 3.3), and lower extremity (OR, 1.9; 95% CI, 1.0 to 3.7) atherosclerosis. The OR for generalized progression of atherosclerosis as indicated by a composite progression score was 4.5 (95% CI, 2.3 to 8.5). Except for aortic atherosclerosis, these estimates hardly changed after additional adjustment for multiple cardiovascular risk factors. In addition, ORs for progression of atherosclerosis associated with high CRP levels were as high as those associated with the traditional cardiovascular risk factors high cholesterol, hypertension, and smoking. Geometric mean levels of CRP increased with the total number of sites showing progression of atherosclerosis (P=0.002 for trend).

Conclusions—CRP predicts progression of atherosclerosis measured at various sites in the arterial tree. (Stroke. 2002;33:2750-2755.)

Key Words: atherosclerosis ■ C-reactive protein ■ inflammation ■ risk factors

In recent years, C-reactive protein (CRP) has become established as a risk factor for cardiovascular disease. Increased levels of CRP predict future myocardial infarction and stroke independently of other cardiovascular risk factors, and it has been suggested that measurement of CRP, in addition to traditional risk factors, may improve our ability to predict cardiovascular disease. In selected patient groups, CRP levels were positively associated with angiographically established coronary artery disease. In addition, CRP has been related both cross-sectionally and prospectively to peripheral arterial disease (PAD). In the Rotterdam Study, we found that CRP is strongly associated with atherosclerosis measured at various sites in the arterial tree. Several mechanisms have been described by which CRP and other inflammatory mediators may be actively involved in atherogenesis. However, not all studies found a clear association between CRP and atherosclerosis.

The inflammatory activity within atherosclerotic plaques is one of the main determinants of the vulnerability of plaques to rupture. Because plaque rupture, thrombus formation, and subsequent organization and incorporation of the thrombus in the plaque are thought to be the most important cause of rapid progression of atherosclerotic plaques, CRP may be a good predictor of progression of atherosclerosis. Until now, only 1 small study has reported an association between CRP and progression of carotid atherosclerosis.

In the Rotterdam Study, a population-based cohort study of men and women ≥55 years of age, we investigated whether CRP levels are associated with the progression of atherosclerosis measured at various sites in the arterial tree.

Materials and Methods

Population
The Rotterdam Study is a prospective, population-based cohort study composed of 7983 men and women ≥55 years of age. Its overall aim...
is to investigate the incidence and determinants of chronic disabling diseases. The first phase lasted from 1990 until 1993, when all inhabitants of a suburb of Rotterdam ≥55 years of age were invited to participate in an extensive home interview and 2 visits to the research center. The overall response rate was 78%. Phase 3 was held from 1997 until 1999. Between phases 1 and 3, 25% of the participants died, and 0.4% were lost to follow-up. The Medical Ethics Committee of the Erasmus University Rotterdam approved the Rotterdam Study, and written, informed consent was obtained from all participants. The population used for the present study was an age- and sex-stratified, randomly selected sample of 773 participants. Given the age and sex distributions, the prevalence of cardiovascular risk factors in the whole Rotterdam Study population was similar to the prevalence of these risk factors in the whole Rotterdam Study population. A more detailed description of the Rotterdam Study has been given elsewhere.13

Clinical Characteristics
A trained investigator visited all participants at home and collected information using a computerized questionnaire. The obtained information included current health status, medical history, drug use, and smoking behavior. Additionally, during 2 visits to the research center, blood samples were drawn, and established cardiovascular risk factors were measured, as described previously.14 We defined hypertension as systolic blood pressure ≥160 mm Hg and/or diastolic blood pressure ≥100 mm Hg and/or use of antihypertensive medication. Diabetes mellitus was defined as the use of blood glucose lowering medication and/or a nonfasting serum glucose level ≥11.1 mmol/L. A 12-lead resting ECG was recorded and analyzed by the Modular ECG Analysis System.15 A history of myocardial infarction before entering the study was considered present if the decline in AAI at phase 3 reflects a true difference in lower extremity atherosclerosis. We measured CRP using an in-house enzyme immunoassay (n = 334 subjects; DAKO) or a nephelometric method (n = 439; Dade Behring). These 2 methods demonstrate a high level of agreement.19 CRP was measured by both methods in 70 subjects. For each of these subjects with values of CRP ≥10 mg/L, we plotted the difference between the logarithmically transformed results of the 2 methods against the mean of the 2 methods.20 The plot showed no systematic relationship between the difference and the mean of the paired measurements, and the 2 methods showed good agreement. The mean difference in CRP was 0.01 mg/L. To ascertain that differences in the distribution of CRP for the 2 methods had not influenced the results, we standardized the 2 distributions of CRP by computing z scores (value minus mean divided by the SD of the mean). We repeated all analyses using the standardized data and found results similar to those reported in the present study. In the study population,
Results

Baseline characteristics of the study population are shown in Table 1. The geometric mean level of CRP was 1.54 mg/L (interquartile range, 0.78 to 2.93).

ORs associated with levels of CRP in the highest quartile of the population distribution compared with the lowest quartile were clearly increased for progression of carotid (OR, 1.9; 95% CI, 1.1 to 3.3), aortic (OR, 1.7; 95% CI, 1.0 to 3.0), iliac (OR, 2.0; 95% CI, 1.2 to 3.3), and lower extremity (OR, 1.9; 95% CI, 1.0 to 3.7) atherosclerosis after adjustment for age, sex, smoking behavior, and duration of follow-up (Table 2, model 1). For iliac and lower extremity atherosclerosis, ORs for progression increased across quartiles of CRP, whereas for carotid and aortic atherosclerosis, the increase was present only for subjects with CRP levels in the highest quartile. Of the population, 26.4% had generalized progression of atherosclerosis as indicated by a composite progression score ≥3. The OR for generalized progression of atherosclerosis associated with levels of CRP in the highest quartile was 4.5 (95% CI, 2.3 to 8.5).

To investigate whether the associations of CRP with progression of atherosclerosis were independent of other cardiovascular risk factors, we additionally adjusted for baseline ratio of total cholesterol to HDL cholesterol, BMI, presence of diabetes mellitus, systolic blood pressure, aspirin and statin use, and history of myocardial infarction (Table 2, model 2). ORs for carotid (OR, 1.7; 95% CI, 1.0 to 3.1), iliac (OR, 2.2; 95% CI, 1.3 to 3.8), lower extremity (OR, 1.9; 95% CI, 0.9 to 4.1), and generalized (OR, 4.6; 95% CI, 2.2 to 9.5) progression of atherosclerosis associated with CRP levels in the highest quartile were still clearly increased. The OR for progression of aortic atherosclerosis was attenuated.

Analyses were repeated in subjects without atherosclerosis at the baseline examination. In subjects with a carotid plaque score of 0, no PAD (AAI ≥0.9), or no generalized atherosclerosis at baseline, associations of CRP with progression of carotid plaques, lower extremity atherosclerosis, and generalized atherosclerosis, respectively, did not meaningfully change (data not shown). However, in subjects without aortic atherosclerosis at baseline, the association of CRP with progression of aortic atherosclerosis disappeared (OR for the highest quartile, 0.5; 95% CI, 0.1 to 1.6). In subjects without iliac atherosclerosis at baseline, associations with progression of iliac atherosclerosis were attenuated (OR for the highest quartile, 1.5; 95% CI, 0.7 to 3.2).

We compared ORs for progression of atherosclerosis associated with high levels of CRP with the ORs for progression associated with a high total cholesterol (highest quartile of the population distribution), hypertension, and current smoking. Figure 1 shows that the age- and sex-adjusted ORs for progression of atherosclerosis associated with high levels of CRP are comparable to those associated with traditional cardiovascular risk factors. For example, the ORs for progression of carotid atherosclerosis associated with high levels of CRP, high cholesterol, hypertension, and smoking are 2.1 (95% CI, 1.2 to 3.5), 2.5 (95% CI, 1.4 to 4.3), 1.3 (95% CI, 0.9 to 1.9), and 2.2 (95% CI, 1.2 to 3.8), respectively.

Finally, we computed geometric mean levels of CRP for increasing scores of the composite progression score. Figure 2 shows that mean levels of CRP increase linearly with the number of sites at which progression of atherosclerosis is present (P=0.002 for trend).

Discussion

In this population-based study, we show that CRP predicts progression of atherosclerosis measured at various sites in the arterial tree.
Several cross-sectional studies have shown that CRP is related to atherosclerotic disease. Furthermore, CRP predicted PAD in apparently healthy men, and in Japanese outpatients, it was associated with progression of carotid plaques. The strength of the present study is that we investigated the progression of atherosclerosis at multiple sites in a relatively large, population-based study. Our study shows that CRP predicts the progression of atherosclerosis, as indicated by various noninvasive measures, and that the risk estimates associated with CRP are as high as those associated with more traditional cardiovascular risk factors.

Several methodological issues need to be discussed before these data are interpreted. First, it should be kept in mind that all study participants survived until the follow-up measurement.

### Table 2. Association of C-Reactive Protein With Progression of Atherosclerosis Measured at Various Sites in the Arterial Tree

<table>
<thead>
<tr>
<th>Progression of Atherosclerosis</th>
<th>Quartiles of C-Reactive Protein (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carotid atherosclerosis</td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>1.0 (0.78)</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.4 (0.8-2.4)</td>
</tr>
<tr>
<td>Model 3</td>
<td>0.9 (0.5-1.6)</td>
</tr>
<tr>
<td>Model 4</td>
<td>1.9 (1.1-3.3)</td>
</tr>
<tr>
<td>Aortic atherosclerosis</td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>1.0 (0.78)</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.3 (0.7-2.2)</td>
</tr>
<tr>
<td>Model 3</td>
<td>0.8 (0.4-1.4)</td>
</tr>
<tr>
<td>Model 4</td>
<td>1.7 (1.0-3.1)</td>
</tr>
<tr>
<td>Iliac atherosclerosis</td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>1.0 (0.78)</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.0 (0.5-1.6)</td>
</tr>
<tr>
<td>Model 3</td>
<td>0.7 (0.4-1.3)</td>
</tr>
<tr>
<td>Model 4</td>
<td>1.5 (0.8-2.8)</td>
</tr>
<tr>
<td>Lower extremity atherosclerosis</td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>1.0 (0.78)</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.0 (0.5-1.6)</td>
</tr>
<tr>
<td>Model 3</td>
<td>0.7 (0.4-1.3)</td>
</tr>
<tr>
<td>Model 4</td>
<td>1.5 (0.8-2.8)</td>
</tr>
<tr>
<td>Generalized progression of atherosclerosis*</td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>1.0 (0.78)</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.0 (0.5-1.6)</td>
</tr>
<tr>
<td>Model 3</td>
<td>0.7 (0.4-1.3)</td>
</tr>
<tr>
<td>Model 4</td>
<td>1.5 (0.8-2.8)</td>
</tr>
</tbody>
</table>

Estimates are ORs with 95% CIs. Model 1 is adjusted for age, gender, and smoking status at baseline, and duration of follow-up. Model 2 is additionally adjusted for baseline total cholesterol:HDL cholesterol ratio, body mass index, diabetes mellitus, systolic blood pressure, aspirin and statin use, and a history of myocardial infarction.

*As indicated by a composite progression score ≥3.

**Figure 1.** ORs and 95% CIs for progression of atherosclerosis associated with traditional cardiovascular risk factors and CRP. ORs were adjusted for age, sex, and duration of follow-up. For CRP and total cholesterol (TC), the highest quartile is compared with the lowest quartile. Hypertension (HT) is defined as systolic blood pressure ≥160 mm Hg, diastolic blood pressure ≥100 mm Hg, or use of antihypertensive medication. Smokers are compared with nonsmokers.
CRP levels were measured only once. However, a study in which CRP was measured regularly over a 6-month period concluded that CRP appeared to be tightly regulated, with few measurement error. Third, not all subjects in our study population had complete data for all 4 measures of atherosclerosis. Because missing data are predominantly the result of logistics and therefore random, it is not likely that they have affected our results. Fourth, CRP levels were measured only once. However, a study in which CRP was measured regularly over a 6-month period concluded that CRP appeared to be tightly regulated, with few short-term fluctuations. Furthermore, intra-individual variation in CRP would likely result in underestimation of the true relationship.

Sudden plaque rupture, thrombus formation, and subsequent incorporation of the thrombus into the atherosclerotic plaque are thought to cause rapid progression of atherosclerosis. Not only CRP but multiple inflammatory mediators regulate a variety of pathophysiological processes that have been shown to be involved in atherosclerotic plaque rupture. It is likely that elevated levels of CRP reflect the total amount of inflammatory activity within, and therefore the vulnerability of, the atherosclerotic plaque, suggesting that CRP can be a valuable predictor of progression of atherosclerotic disease. This idea is supported by the prospective data presented in this study.

Many studies have shown that CRP predicts myocardial infarction and stroke. Part of the predictive value of CRP for these events may be explained by the association between CRP and progression of atherosclerosis as reported in the present study. Clearly, the association of CRP with progression of carotid atherosclerosis is consistent with reports about the predictive value of CRP for stroke. Although no data were available on the progression of coronary atherosclerosis, there is a strong relationship between the various measures of extracoronary atherosclerosis and coronary atherosclerosis, and the predictive value of CRP for progression of extracoronary atherosclerosis may be in line with the predictive value of CRP for myocardial infarction.

The association of CRP with progression of aortic atherosclerosis, represented by the extent of abdominal aortic calcification, was weaker than the association of CRP with progression of atherosclerosis measured at other sites. Likewise, several cross-sectional studies reported a lack of association of CRP with coronary calcification measured by electron beam tomography. It is possible that, once the atherosclerotic plaque is in the process of being calcified, it is protected against progression caused by (inflammation-induced) rupture. Evidence for such a mechanism has been reviewed by Doherty et al., who argued that calcification may stabilize plaques and diminish the risk of rupture. However, this hypothesis cannot explain why the association with progression of iliac atherosclerosis was attenuated and the association with progression of aortic atherosclerosis disappeared in subjects without calcification at the baseline examination. Although calcification is an indicator of the total atherosclerotic burden, calcification and inflammation represent very distinct processes within the atherosclerotic plaque, which may well explain the modest associations between CRP and progression of calcification in the present study.

The present study shows that risk estimates for the progression of atherosclerosis associated with CRP were as high as those associated with traditional cardiovascular risk factors. As expected in an elderly population, the risk estimates for progression of atherosclerosis associated with traditional risk factors were relatively low; however, this was especially the case for hypertension and progression of carotid atherosclerosis. The latter may be due to the fact that more than half of the hypertensive subjects in our study received antihypertensive treatment and thus were at a lower risk of progression of atherosclerosis or to the relatively high age of the population.

Because a substantial part of incident myocardial infarction and stroke is unaccounted for by traditional cardiovascular risk factors, there is a great need to find novel and preferably modifiable factors that can identify subjects at high risk. CRP is a serious candidate, especially because it has recently been reported that measuring CRP levels may improve clinical risk prediction and that statin treatment positively influences clinical outcome in persons with low cholesterol but high CRP levels. Although more research is necessary to determine the value of CRP in everyday clinical practice, our study indicates that CRP is an important risk factor for cardiovascular disease progression.

We conclude that CRP predicts progression of atherosclerosis measured noninvasively at various sites in the arterial tree.
Acknowledgments

This study was supported by the Netherlands Organization for Scientific Research (NWO), grant 980-10-005. We thank Toos Stehmann and Inge Hauersen for collecting the data on aortic and iliac calcification and carotid plaques; we also thank Piet Meijer and Numico Research for conducting the laboratory analyses of CRP.

References

C-Reactive Protein Predicts Progression of Atherosclerosis Measured at Various Sites in the Arterial Tree: The Rotterdam Study

Irene M. van der Meer, Moniek P.M. de Maat, A. Elisabeth Hak, Amanda J. Kiliaan, Antonio Iglesias del Sol, Deirdre A.M. van der Kuip, Rogier L.G. Nijhuis, Albert Hofman and Jacqueline C.M. Witteman

Stroke. 2002;33:2750-2755; originally published online November 21, 2002; doi: 10.1161/01.STR.0000044168.00485.02

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/33/12/2750

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/