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

The Rotterdam Study

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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

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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 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 of ≥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 in case of a confirmed self-report of myocardial infarction or a combination of calcified and noncalcified material. A plaque score ranging from 0 to 1 was computed by dividing the number of sites with a detectable plaque by the total number of sites for which an ultrasonographic image was available (with a maximum of 6). Subjects for whom data on the presence of plaques were not available for at least 2 of the 6 sites examined were excluded. Progression of carotid atherosclerosis was defined as an increase in plaque score of >0.17 (or one sixth). Participants (15.1%) with a decrease in plaque score were added to the group with no progression, because we considered this to be due mainly to measurement error. Exclusion of these subjects from analyses did not substantially change the results. Because of the limited availability of ultrasonographers at the end of 1992 and in 1993, not all subjects who visited the research center could be examined for the presence of carotid plaques.

Aortic and Iliac Atherosclerosis
Aortic and iliac atherosclerosis were diagnosed by radiographic detection of calcified deposits in the abdominal aorta and iliac arteries on a lateral abdominal film.16 The extent of aortic atherosclerosis was scored according to the length of the involved area. Aortic and iliac atherosclerosis was scored as absent, present either left or right, or present on both sides. For progression of aortic and iliac atherosclerosis, baseline and follow-up films were examined in pairs. Progression of aortic atherosclerosis was scored on a graded scale (with scores of 0 through 4 corresponding to progression of 0, ≤1, 1 to 2.5, 2.5 to 4.9, and ≥5.0 cm, respectively) and considered present if the score was >1; progression of iliac atherosclerosis was scored as either absent or present. None of the participants showed a decrease in the extent of aortic and iliac atherosclerosis. All films were read by 1 observer who was aware of the date of the radiographs. Before the scoring, a sample of the films was read by 2 observers simultaneously to reach agreement on the interpretation of the scoring protocol. Interobserver agreement on progression scoring (absent versus present), as previously determined at our department for 758 pairs of lateral radiographic films of the lumbar spine, reached an agreement on atherosclerotic change of 88% and a k statistic of 0.74.16 Progression of aortic and iliac atherosclerosis could not be evaluated for 19 and 29 participants, respectively, because the aorta or iliac arteries were not clearly depicted on the radiograph at baseline or follow-up.

Lower Extremity Atherosclerosis
We computed the ratio of systolic blood pressure at the ankle to systolic blood pressure at the arm to obtain the AAI.17 The AAI is susceptible to measurement error; therefore, we considered a decline in AAI to be real if it was >15%.18 In addition, because it is not likely that an AAI that is still in the upper range of the distribution at phase 3 reflects a true difference in lower extremity atherosclerosis, we considered progression of lower extremity atherosclerosis to be present if the decline in AAI resulted in an AAI at phase 3 of <0.9. Because arterial rigidity prevents arterial compression and thus leads to spuriously high values of the AAI, an AAI >1.50 was considered invalid.

Composite Progression Score
Finally, we computed a composite progression score by adding 1 point for each measure of atherosclerosis that had shown progression during follow-up. Generalized progression of atherosclerosis was defined as a composite progression score ≥3. For logistic reasons, data were not complete for all subjects. Data on progression of carotid, aortic, iliac, and lower extremity atherosclerosis were available for 88.2%, 81.9%, 80.6%, and 80.3% of the study population, respectively. For subjects who had a missing value on 1 (n=269) or 2 (n=180) of the 4 measures, a weighted score was computed that gave equal weight to each measure.

Measurement of CRP
A venipuncture was performed by application of minimal stasis with a 21-gauge Butterfly needle with tube (Surflo winged infusion set, Terumo). Nonfasting blood was collected in tubes containing 0.129 mol L sodium citrate at 4°C. The ratio of blood to sodium citrate was 9:1. Plasma was collected after centrifugation for 10 minutes at 3000 rpm. Subsequently, platelet-free plasma was obtained by centrifugation for 10 minutes at 10 000 rpm, immediately frozen in liquid nitrogen, and stored at –80°C. All tubes were stored on ice before and after blood sampling. CRP was measured by sensitive immunological methods by use of an in-house enzyme immunoassay (n=381 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).
Several cross-sectional studies have shown that CRP is related to atherosclerotic disease.\textsuperscript{4,5,7} Furthermore, CRP predicted PAD in apparently healthy men,\textsuperscript{6} and in Japanese outpatients, it was associated with progression of carotid plaques.\textsuperscript{12} 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.

### 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></td>
<td>1 (â‰£0.78)</td>
</tr>
<tr>
<td>Carotid atherosclerosis</td>
<td>Model 1</td>
</tr>
<tr>
<td></td>
<td>Model 2</td>
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<tr>
<td>Aortic atherosclerosis</td>
<td>Model 1</td>
</tr>
<tr>
<td></td>
<td>Model 2</td>
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<tr>
<td>Iliac atherosclerosis</td>
<td>Model 1</td>
</tr>
<tr>
<td></td>
<td>Model 2</td>
</tr>
<tr>
<td>Lower extremity atherosclerosis</td>
<td>Model 1</td>
</tr>
<tr>
<td></td>
<td>Model 2</td>
</tr>
<tr>
<td>Generalized progression of atherosclerosis\footnote{As indicated by a composite progression score â‰¥3.}</td>
<td>Model 1</td>
</tr>
<tr>
<td></td>
<td>Model 2</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.

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.
ment of atherosclerosis. Subjects with the most severe ath-
erosclerosis at baseline were more likely to die. Although this
may have somewhat limited the range of baseline levels of
atherosclerosis, it does not affect the validity of the risk
estimates presented in the study. Second, we used different
measures of atherosclerosis. Carotid, aortic, and lower ex-
tremity atherosclerosis have been shown to be associated with
cardiovascular risk factors and cardiovascular disease
risk.\textsuperscript{14,21,22} The use of iliac atherosclerosis is not yet very
common, and more research is needed to determine its value
as an indicator of atherosclerosis. Moreover, we did not study
the association of CRP with the progression of carotid
intima-media thickness, because evaluation of intima-media
thickness as a measure of progression of atherosclerosis
suggested that—at least within the Rotterdam Study—it is
substantially influenced by measurement error. Third, not all
subjects in our study population had complete data for all 4
measures of atherosclerosis. Because missing data are pre-
dominantly 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 con-
cluded that CRP appeared to be tightly regulated, with few
short-term fluctuations.\textsuperscript{23} Furthermore, intra-individual vari-
a tion in CRP would likely result in underestimation of the true
relationship.

Sudden plaque rupture, thrombus formation, and subse-
quently incorporation of the thrombus into the atherosclerotic
plaque are thought to cause rapid progression of atheroscle-
rosis.\textsuperscript{11} Not only CRP but multiple inflammatory mediators
regulate a variety of pathophysiological processes that have
been shown to be involved in atherosclerotic plaque rupture.\textsuperscript{8,10} 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 athero-
sclerotic disease. This idea is supported by the prospective data
presented in this study.

Many studies have shown that CRP predicts myocardial
infarction and stroke.\textsuperscript{1} 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 progres-
sion of carotid atherosclerosis is consistent with reports about
the predictive value of CRP for stroke.\textsuperscript{1,2} 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,\textsuperscript{24}
and the predictive value of CRP for progression of extracor-
onary atherosclerosis may be in line with the predictive value
of CRP for myocardial infarction.

The association of CRP with progression of aortic athero-
sclerosis, represented by the extent of abdominal aortic
calcification, was weaker than the association of CRP with
progression of atherosclerosis measured at other sites. Like-
wise, several cross-sectional studies reported a lack of asso-
ciation of CRP with coronary calcification measured by
electron beam tomography.\textsuperscript{25} 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,\textsuperscript{26} 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,\textsuperscript{27} 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 pro-
gression of atherosclerosis associated with CRP were as high
as those associated with traditional cardiovascular risk fac-
tors. As expected in an elderly population,\textsuperscript{28} the risk estimates
for progression of atherosclerosis associated with traditional
risk factors were relatively low; however, this was especially
true for hypertensive and progression of carotid athero-
sclerosis.\textsuperscript{29} 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\textsuperscript{30} and that statin treatment positively influences
clinical outcome in persons with low cholesterol but high
CRP levels.\textsuperscript{31} Although more research is necessary to deter-
mine the value of CRP in everyday clinical practice, our study
indicates that CRP is an important risk factor for cardiovas-
cular disease progression.

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

\textbf{Figure 2.} Geometric mean level and 95\% CI of CRP for each
value of the composite progression score. Means are adjusted
for baseline levels of traditional cardiovascular risk factors.
Acknowledgments

This study was supported by the Netherlands Organization for Scientific Research (NWO), grant 980-10-005. We thank Toos Stehmann and Inge Haumersen 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


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