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## Inflammatory mediators and cell adhesion molecules as indicators of severity of atherosclerosis: the Rotterdam Study

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# Arteriosclerosis, Thrombosis, and Vascular Biology

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Irene M. van der Meer, Moniek P.M. de Maat, Michiel L. Bots, Monique M.B. Breteker, John Meijer, Amanda J. Kiliaan, Albert Hofman and Jacqueline C.M. Witteman

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# Inflammatory Mediators and Cell Adhesion Molecules as Indicators of Severity of Atherosclerosis

## The Rotterdam Study

Irene M. van der Meer, Moniek P.M. de Maat, Michiel L. Bots, Monique M.B. Breteler, John Meijer, Amanda J. Kiliaan, Albert Hofman, Jacqueline C.M. Witteman

**Abstract**—Inflammatory mediators and soluble cell adhesion molecules predict cardiovascular events. It is not clear whether they reflect the severity of underlying atherosclerotic disease. Within the Rotterdam Study, we investigated the associations of C-reactive protein (CRP), interleukin-6 (IL-6), soluble intercellular adhesion molecule-1, and soluble vascular cell adhesion molecule-1 with noninvasive measures of atherosclerosis. Levels of CRP were assessed in a random sample of 1317 participants, and levels of IL-6 and soluble cell adhesion molecules were assessed in a subsample of 714 participants. In multivariate analyses, logarithmically transformed CRP (regression coefficient  $[\beta] = -0.023$ , 95% CI  $-0.033$  to  $-0.012$ ) and IL-6 ( $\beta = -0.025$ , 95% CI  $-0.049$  to  $-0.001$ ) were inversely associated with the ankle-arm index. Only CRP was associated with carotid intima-media thickness ( $\beta = 0.018$ , 95% CI  $0.010$  to  $0.027$ ). Compared with the lowest tertile, the odds ratio for moderate to severe carotid plaques associated with levels of CRP in the highest tertile was 2.0 (95% CI 1.3 to 3.0). Soluble intercellular adhesion molecule-1 levels were strongly associated with carotid plaques (odds ratio 2.5, 95% CI 1.5 to 4.4 [highest versus lowest tertile]). Soluble vascular cell adhesion molecule-1 was not significantly associated with any of the measures of atherosclerosis. This study indicates that CRP is associated with the severity of atherosclerosis measured at various sites. Associations of the other markers with atherosclerosis were less consistent. (*Arterioscler Thromb Vasc Biol.* 2002;22:838-842.)

**Key Words:** atherosclerosis ■ inflammation ■ C-reactive protein ■ interleukins ■ cell adhesion molecules

Over the past years, it has been recognized that inflammation may contribute to all stages of the atherosclerotic process.<sup>1</sup> C-reactive protein (CRP), an acute-phase protein that is mainly regulated by the cytokine interleukin-6 (IL-6), is a sensitive marker of inflammation. In several prospective studies, high levels of CRP and IL-6 predicted coronary events in patients with stable or unstable angina and in healthy subjects.<sup>2-4</sup> Other molecules that have been suggested to contribute to atherosclerosis and its clinical outcomes are the endothelial cell adhesion molecules (CAMs), which facilitate the emigration of leukocytes into the vessel wall, an important feature of atherosclerotic plaque formation.<sup>5</sup> Indeed, high levels of soluble intercellular adhesion molecule-1 (sICAM-1) have been shown to predict myocardial infarction in the Physicians' Health Study and the Atherosclerosis Risk in Communities study.<sup>6,7</sup>

The presence of CRP, IL-6, ICAM-1, and vascular cell adhesion molecule-1 (VCAM-1) has been demonstrated in atherosclerotic lesions.<sup>8-10</sup> Moreover, levels of CRP and IL-6 have been positively related to peripheral and coronary artery disease in selected patient populations,<sup>11,12</sup> although Folsom et al<sup>13</sup> recently concluded that

CRP is not a strong marker of prevalent atherosclerosis. sICAM-1 has shown a positive relationship with peripheral arterial disease,<sup>14</sup> established by angiography or ultrasound, whereas soluble VCAM-1 (sVCAM-1) has been related to the extent of atherosclerosis, established angiographically in multiple vascular beds in patients with peripheral arterial disease.<sup>15</sup> In various populations, soluble CAMs (sCAMs) have been positively related to common carotid intima-media thickness (IMT).<sup>6,16,17</sup>

Most of the studies involving inflammatory mediators or adhesion molecules and atherosclerosis have been based on patient series. In the Rotterdam Study, a population-based cohort study of men and women aged  $\geq 55$  years, we investigated whether plasma levels of CRP, IL-6, sICAM-1, and sVCAM-1 are associated with the severity of atherosclerosis, measured at various sites of the arterial tree.

## Methods

### Population

The Rotterdam Study is a prospective population-based cohort study composed of 7983 men and women aged  $\geq 55$  years. Its overall aim

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is to investigate the incidence of and risk factors for chronic disabling diseases. From 1990 until 1993, all inhabitants of a suburb of the city of Rotterdam aged  $\geq 55$  years were invited to participate in an extensive home interview and 2 visits to the research center. The overall response rate was 78%. The Medical Ethics Committee of the Erasmus University Rotterdam approved the Rotterdam Study, and written informed consent was obtained from all participants. For the present study, levels of CRP were assessed in a randomly selected age- and sex-stratified sample of 1317 participants. Levels of IL-6, sICAM-1, and sVCAM-1 were assessed in a random subsample of 714 participants. A more detailed description of the Rotterdam Study and of the collection of data has been provided elsewhere.<sup>18,19</sup>

### Measures of Atherosclerosis

By use of a random zero sphygmomanometer, sitting blood pressure was measured at the right upper arm. The average of 2 measurements obtained on 1 occasion was used. Systolic blood pressure at the ankles (posterior tibial artery) was measured with subjects in the supine position with a random zero sphygmomanometer and an 8-MHz continuous-wave Doppler probe (Huntleigh 500D, Huntleigh Technology). The ratio of the systolic blood pressure at the ankle to the systolic blood pressure at the arm was computed to obtain the ankle-arm index (AAI). For the analyses, we used the lowest value of the 2 legs. Because arterial rigidity prevents arterial compression and will therefore lead to spurious high values of the AAI, an AAI  $>1.50$  was considered invalid.<sup>19</sup>

Ultrasonography of both carotid arteries was performed with a 7.5-MHz linear-array transducer and a duplex scanner (ATL Ultra-Mark IV). Common carotid IMT was determined as the average of near-wall and far-wall measurements, and the average of left and right common carotid IMT was computed.<sup>20</sup>

Carotid plaques were defined as a focal widening relative to adjacent segments, with the protrusion into the lumen composed of only calcified deposits or of a combination of calcification and noncalcified material.<sup>20</sup> A plaque score ranging from 0 to 1 was derived by dividing the number of sites (left and right side of the common carotid artery, carotid bifurcation, and internal carotid artery) with a detectable plaque by the total number of sites (with a maximum of 6) for which an ultrasonographic image was available. To indicate the presence of moderate or severe carotid plaques, we used cutoff points of 0.5 and 0.75, respectively.

Aortic atherosclerosis was diagnosed by radiographic detection of calcified deposits in the abdominal aorta on a lateral abdominal film. The extent of abdominal aortic atherosclerosis was scored according to the length of the involved area (with scores 0 to 5 corresponding to 0,  $\leq 1$  cm, 1 to 2.5 cm, 2.5 to 4.9, 5.0 to 9.9, and  $\geq 10.0$  cm).<sup>21</sup> A calcification score of 3 was considered moderate, and scores 4 and 5 were considered severe.

### Inflammatory Mediators and CAMs

For logistic reasons, CRP was measured by 2 sensitive immunological methods, an in-house enzyme immunoassay ( $n=603$  subjects, DAKO) and a nephelometric method ( $n=714$ , Dade-Behring). These 2 methods demonstrate a high level of agreement.<sup>22</sup> CRP was measured by both methods in 134 subjects. For each of these subjects with values of CRP  $\leq 10$  mg/L, we plotted the difference between the logarithmically transformed results of the 2 methods against the mean of the 2 methods.<sup>23</sup> 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 0.036 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 by using the standardized data and found results similar to the ones reported in the present study.

IL-6, sICAM-1, and sVCAM-1 were measured by using commercially available ELISA (IL-6, Quantikine HS; sICAM-1 and sVCAM-1, Parameter; all assays were from R&D Systems Europe). Outliers (values  $>3$  SDs of the population distribution) of logarith-

**TABLE 1. Baseline Characteristics of the Study Population**

Variable	All Subjects (n=1317)	Subsample (n=714)
Age, y	71.2 $\pm$ 8.9	71.6 $\pm$ 9.0
Sex, % men	46.3	47.2
Body mass index, kg/m <sup>2</sup>	26.3 $\pm$ 3.6	26.4 $\pm$ 3.6
Systolic blood pressure, mm Hg	138.8 $\pm$ 21.5	138.4 $\pm$ 21.0
Total cholesterol, mmol/L	6.6 $\pm$ 1.2	6.6 $\pm$ 1.2
HDL-cholesterol, mmol/L	1.3 $\pm$ 0.4	1.3 $\pm$ 0.4
Current smokers, %	19.3	19.9
Diabetes mellitus, %	9.5	8.3
History of myocardial infarction, %	15.1	14.9
Carotid plaques, %*	27.2	25.0
Aorta calcifications, %†	32.3	30.9
C-reactive protein, mg/L‡	1.82 (0.85 to 3.68)	1.73 (0.81 to 3.68)
Interleukin-6, pg/mL‡	...	1.91 (1.25 to 3.13)
sICAM-1, ng/mL	...	223.7 $\pm$ 69.9
sVCAM-1, ng/mL	...	542.6 $\pm$ 184.5

Data are means  $\pm$  SD for continuous variables and percentages for dichotomous variables.

\*Defined as a plaque score (range, 0 to 1) larger than 0.5.

†Defined as a calcification score (range, 0 to 5) larger than 3.

‡For data with a skewed distribution, the median and interquartile range are shown.

mically transformed CRP ( $n=4$ ) and IL-6 ( $n=9$ ) and of sICAM-1 ( $n=13$ ) and sVCAM-1 ( $n=13$ ) were excluded because they may indicate the presence of an active inflammatory disease. In the study population, 5.2% had levels of CRP  $>10$  mg/L.

### Statistical Analysis

We used logarithmically transformed values of CRP and IL-6 to normalize the distribution of these variables. Cutoff points for tertiles were 1.11 and 2.77 mg/L for CRP, 1.44 and 2.43 pg/mL for IL-6, 190.1 and 231.4 ng/mL for sICAM-1, and 451.8 and 574.8 ng/mL for sVCAM-1. We estimated partial correlations between inflammatory mediators and sCAMs. Mean values of the AAI and IMT were computed for increasing tertiles of CRP, IL-6, sICAM-1, and sVCAM-1 by means of ANCOVA. We tested linearity by using linear regression analysis with the AAI and IMT as dependent variables. Logistic regression analysis was used to compute odds ratios (ORs, for the higher tertiles of inflammatory mediators and sCAMs compared with the lowest tertile) of having moderate to severe carotid plaques and abdominal aorta calcifications. Analyses involving CRP were adjusted for the method used to measure CRP levels. All analyses were adjusted for age, sex, smoking status (current, past, or never smokers), and pack-years of smoking and additionally for body mass index (BMI) and diabetes mellitus. Analyses were performed by using SPSS 9.0 for Windows.

### Results

Baseline characteristics of the study population are shown in Table 1. Baseline characteristics of the subsample of 714 participants were similar to those of the total sample.

CRP and IL-6, as well as sICAM-1 and sVCAM-1, were moderately correlated ( $r=0.56$  and  $r=0.27$ , respectively;  $P<0.001$ ; Table 2). CRP and IL-6 were both correlated with sICAM-1 ( $r=0.20$  and  $r=0.21$ , respectively;  $P<0.001$ ).

Increasing tertiles of CRP and IL-6 were inversely associated with the AAI. In linear regression analysis, the regression coefficients ( $\beta$  values) were  $-0.026$  (95% CI  $-0.036$  to

**TABLE 2. Partial Correlations Between Inflammatory Mediators and Soluble Cell Adhesion Molecules**

	IL-6*	sICAM-1	sVCAM-1
CRP*	0.56†	0.20†	0.04
IL-6*	...	0.21†	0.09‡
sICAM-1	...	...	0.27†

Correlations are adjusted for age, sex, smoking status, and pack-years of smoking.

\*For CRP and IL-6 log-transformed values are used.

† $P < 0.001$ . ‡ $P = 0.02$ .

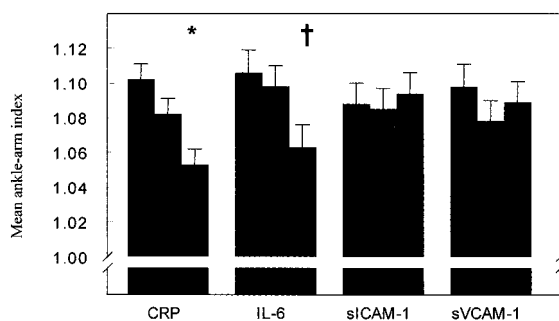
−0.016) and −0.034 (95% CI −0.057 to −0.011) for logarithmically transformed values of CRP and IL-6, respectively. After adjustment for BMI and diabetes mellitus, the associations of CRP ( $\beta = -0.023$ , 95% CI −0.033 to −0.012) and IL-6 ( $\beta = -0.025$ , 95% CI −0.049 to −0.001) with the AAI remained. The data presented in Figure 1 are based on the fully adjusted model. sCAMs were not linearly associated with the AAI.

CRP was significantly linearly associated with IMT before ( $\beta = 0.021$ , 95% CI 0.013 to 0.029) and after ( $\beta = 0.018$ , 95% CI 0.010 to 0.027; Figure 2) additional adjustment for BMI and diabetes mellitus. IL-6 and sCAMs were not associated with IMT.

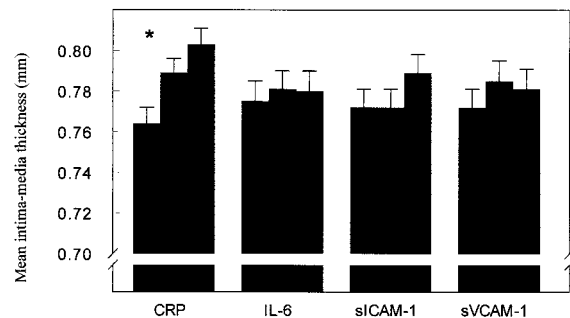
Compared with the lowest tertile, ORs for carotid plaques associated with levels of CRP and sICAM-1 in the highest tertile were 2.0 (95% CI 1.3 to 2.9) and 2.6 (95% CI 1.5 to 4.5), respectively (Table 3). For IL-6 and sVCAM-1, there was a comparable, but not statistically significant, trend. Associations of CRP and sICAM-1 with carotid plaques were very robust; the association between IL-6 and carotid plaques was somewhat weaker after adjustment for BMI and diabetes mellitus (OR 1.5, 95% CI 0.8 to 2.7).

Only sICAM-1 was of borderline statistical significance in its association with abdominal aorta calcifications (OR 1.7, 95% CI 1.0 to 3.1; Table 3). For IL-6, there was a similar trend. After adjustment for BMI and diabetes mellitus, the association of sICAM-1 with aortic calcifications (OR 1.6, 95% CI 0.9 to 2.9) was slightly weaker.

To take possible changes in lifestyle and medication use in participants with a clinically manifest myocardial infarction in the past into account, we repeated all analyses with



**Figure 1.** Mean AAI by tertiles of CRP, IL-6, sICAM-1, and sVCAM-1. Bars represent means, and lines represent SEM. Means are adjusted for age, sex, smoking status, pack-years of smoking, BMI, and diabetes mellitus. \* $P$  for trend  $< 0.001$ ; † $P$  for trend = 0.04.



**Figure 2.** Mean IMT (mm) of the common carotid artery by tertiles of CRP, IL-6, sICAM-1, and sVCAM-1. Bars represent means, and lines represent SEM. Means are adjusted for age, sex, smoking status, pack-years of smoking, BMI, and diabetes mellitus. \* $P$  for trend  $< 0.001$ .

additional adjustment for past myocardial infarction and for aspirin and statin use at baseline. Associations of inflammatory mediators and adhesion molecules with measures of atherosclerosis were not meaningfully different (data not shown).

## Discussion

This population-based study in elderly subjects shows that CRP is related to the severity of atherosclerosis at various sites of the arterial tree. Although the results of the present study suggest that IL-6 and sICAM-1 are also associated with atherosclerosis, associations of these markers with measures of atherosclerosis were less consistent.

In the present study, we examined multiple inflammatory mediators in relation to multiple measures of atherosclerosis in a population-based setting. There are several methodological issues that need to be discussed before interpreting these data. First, we used different methods and different parts of the vascular tree to assess the severity of atherosclerosis. The AAI represents the amount of atherosclerotic disease distal to the aortic bifurcation, but it may also be influenced by hemodynamic factors and vascular stiffness. Carotid IMT and plaques reflect atherosclerosis in the carotid vessel wall, although it cannot be excluded that nonatherosclerotic processes, such as fibromuscular hypertrophy, cause modest degrees of intima-media thickening.<sup>24</sup> Radiographically assessed calcifications of the abdominal aorta have been shown to be highly specific, representing advanced intimal atherosclerosis.<sup>25</sup> Second, we have presented our results with and without additional adjustment for BMI and diabetes mellitus. It has recently been reported that adipose tissue is an important source of cytokine production<sup>26</sup> and adjusting for BMI will substantially lower the variation in levels of inflammatory mediators. Furthermore, inflammation has been suggested to be a triggering factor in the origin of diabetes mellitus,<sup>27</sup> which implies that diabetes mellitus may be an intermediate factor rather than a confounder. Adjusting for BMI and diabetes mellitus is indicated only if one wants to examine whether the association of inflammation with atherosclerosis is independent of these variables. Third, levels of CRP, IL-6, sICAM-1, and sVCAM-1 were measured only once. Therefore, intraindividual variation, as has been reported for CRP and IL-6,<sup>28,29</sup> cannot be taken into account.

**TABLE 3. Odds Ratios for Carotid Plaques and Abdominal Aorta Calcifications Associated With Increasing Tertiles of Inflammatory Mediators and Cell Adhesion Molecules**

	Tertiles of Plasma Level			Tertiles of Plasma Level*		
	1	2	3	1	2	3
<b>Carotid plaques†</b>						
CRP	1.0	1.5 (1.0 to 2.3)	2.0 (1.3 to 2.9)	1.0	1.6 (1.0 to 2.4)	2.0 (1.3 to 3.0)
IL-6	1.0	1.4 (0.8 to 2.5)	1.7 (0.9 to 3.0)	1.0	1.4 (0.8 to 2.5)	1.5 (0.8 to 2.7)
sICAM-1	1.0	1.5 (0.9 to 2.5)	2.6 (1.5 to 4.5)	1.0	1.4 (0.8 to 2.4)	2.5 (1.5 to 4.4)
sVCAM-1	1.0	1.4 (0.8 to 2.3)	1.5 (0.9 to 2.6)	1.0	1.4 (0.8 to 2.4)	1.6 (0.9 to 2.7)
<b>Abdominal aorta calcifications‡</b>						
CRP	1.0	1.3 (0.8 to 1.9)	1.3 (0.9 to 2.0)	1.0	1.2 (0.8 to 1.9)	1.2 (0.8 to 1.9)
IL-6	1.0	1.4 (0.8 to 2.6)	1.7 (0.9 to 3.1)	1.0	1.4 (0.8 to 2.5)	1.5 (0.8 to 2.8)
sICAM-1	1.0	1.6 (0.9 to 2.8)	1.7 (1.0 to 3.1)	1.0	1.6 (0.9 to 2.9)	1.6 (0.9 to 2.9)
sVCAM-1	1.0	1.6 (0.9 to 2.9)	1.1 (0.6 to 1.9)	1.0	1.6 (0.9 to 2.9)	1.1 (0.6 to 1.9)

All analyses are adjusted for age, sex, smoking status, and pack-years of smoking.

\*Additionally adjusted for body mass index and diabetes mellitus.

†Defined as a plaque score (range, 0 to 1) larger than 0.5.

‡Defined as a calcification score (range, 0 to 5) larger than 3.

However, such variation will likely result in an underestimation of the true relationship.

Many studies have found a relationship between CRP and the risk of myocardial infarction in the general population.<sup>3</sup> In addition, levels of CRP and IL-6 were positively related to peripheral and coronary artery disease in selected patient populations.<sup>11,12</sup> The present study is the first to show a positive association between inflammation, represented by levels of CRP and IL-6, and severity of atherosclerosis in a population-based study in the elderly.

IL-6 is the principal determinant of the hepatic synthesis of CRP. In the present study, CRP is linearly associated with carotid IMT, whereas IL-6 is not. A possible explanation for the discrepancy between CRP and IL-6 is the substantial intraindividual variation in levels of IL-6.<sup>29</sup> Although associations with different measures of atherosclerosis are more consistent for CRP than for IL-6, it is likely that levels of CRP reflect general inflammatory activity. However, specific actions of CRP itself may also be involved in atherogenesis.<sup>30,31</sup>

CRP was not associated with the presence of abdominal aortic calcifications. This suggests that once atherosclerotic plaques have reached the stage in which they are calcified, inflammation is no longer one of the main features of these plaques. Similarly, several cross-sectional studies have reported a lack of association of CRP with coronary calcifications as measured by electron-beam tomography.<sup>32,33</sup>

Because the acute-phase response may be induced by damage to the vascular endothelium caused by atherosclerotic processes, plasma levels of inflammatory mediators may simply represent the extent of atherosclerotic disease. Alternatively, however, increased local or systemic inflammation due to chronic infection, chronic inflammatory diseases, smoking, obesity, or impaired glucose tolerance<sup>26,34,35</sup> may precede (the progression of) atherosclerosis.<sup>36</sup> Because of the cross-sectional design of the present study, it is not possible to draw conclusions about the direction of the associations between inflammatory mediators and atherosclerosis.

CAMs may be important in atherosclerosis, inasmuch as they facilitate the emigration of leukocytes into the vessel wall.<sup>5</sup> Pathological studies have shown that CAMs are expressed on atherosclerotic plaques,<sup>10</sup> and in mice, a deficiency of CAMs appeared to protect against atherosclerosis.<sup>37</sup> sCAMs are likely to be the result of shedding from atherosclerotic plaques,<sup>38</sup> and they are thought to represent the severity of atherosclerosis. However, reports on adhesion molecules and atherosclerosis are not consistent. Several studies have reported significant associations of only sICAM-1,<sup>6,14</sup> only sVCAM-1,<sup>15,16</sup> or both sCAMs<sup>17</sup> with measures of atherosclerosis.

It is not clear what accounts for the diversity of the associations found between sCAMs and atherosclerosis. The populations that have been studied varied substantially in the degree of atherosclerosis, and atherosclerosis has been measured at different sites of the arterial tree. In the present study, sICAM-1, but not sVCAM-1, was significantly correlated with CRP and IL-6, and the ORs for carotid plaques and aortic calcifications associated with high levels of sICAM-1 were increased. sVCAM-1 was not related to atherosclerosis. The present study adds to the understanding that sICAM-1 and sVCAM-1 are likely to have different roles in atherogenesis or the progression of atherosclerosis. Moreover, whereas CRP was strongly associated with the AAI and IMT, sICAM-1 was not. More research, at the molecular level and at the population level, is necessary to elucidate the role of CAMs in atherosclerosis.

In the present study, associations with measures of atherosclerosis were more consistent for CRP than for IL-6. Because relatively cheap, automated, high-sensitivity methods for CRP measurement have become commercially available,<sup>39</sup> measurement of CRP will be the most useful once inflammatory markers are routinely assessed in clinical settings.

We conclude that CRP is associated with the severity of atherosclerosis at various sites of the arterial tree. Associations of IL-6 and sCAMs with atherosclerosis are less consistent.

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