Inflammatory Markers in Men with Angiographically Documented Coronary Heart Disease

Nader Rifai,1* Rana Joubran,2 Harry Yu,1 Mohamad Asmi,3 and Mohidien Jouma2

Background: Recent evidence suggests that atherosclerosis is a chronic inflammatory process. In this study, we examined several markers of inflammation in men with coronary heart disease (CHD) and appropriate controls.

Methods: The concentrations of C-reactive protein (CRP), serum amyloid A (SAA), interleukin-6 (IL-6), and soluble intracellular adhesion molecule (sICAM-1) were examined in 100 men with angiographically documented CHD and 100 age-, gender-, and smoking-matched controls with no history of CHD. We assessed the association of these markers with severity of disease as indicated by >50% obstruction in one vessel (n = 30), two vessels (n = 39), or three vessels (n = 31).

Results: Significant increases were noted in serum CRP (median for cases vs controls, 3.4 vs 1.5 mg/L; P < 0.0001), SAA (5.9 vs 3.7 mg/L; P < 0.005), and IL-6 (2.3 vs 1.7 ng/L; P < 0.013) in patients with CHD compared with controls. These differences remained significant after correction for age, smoking, hypertension, diabetes, and lipid and homocysteine concentrations. Plasma sICAM-1 was not significantly different between the two groups (335 vs 339 μg/L). No significant correlation was seen between these markers and the severity of coronary disease.

Conclusions: Concentrations of CRP, SAA, and IL-6 were increased in patients with CHD but failed to correlate with severity of coronary disease. These markers might reflect the diffuse atherosclerotic process in the vascular system rather than the degree of localized obstruction from coronary lesions.

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The contribution of inflammation to the initiation and progression of atherosclerosis is receiving increasing attention. Several acute phase reactants, cytokines, and soluble cellular adhesion molecules have been implicated in this process, with their plasma concentrations increased in a variety of atherosclerotic diseases. C-reactive protein (CRP) is a marker of systemic inflammation that is increased in patients with unstable angina and myocardial infarction (MI) compared with those with chronic stable angina (1, 2). Recently, it has been shown that CRP and serum amyloid A (SAA), another nonspecific acute phase reactant, have a useful prognostic utility in patients with MI (3), unstable angina, or non-Q wave MI (4–6). Furthermore, several prospective studies have shown that CRP is a predictor of increased risk for MI, stroke, or peripheral vascular disease in asymptomatic individuals with no known coronary heart disease (CHD) (7–11).

Interleukin-6 (IL-6) is produced mainly by activated monocytes and smooth muscle cells. This cytokine causes the de novo hepatic synthesis of acute phase reactants (12), and its concentration is increased in patients with unstable angina compared with those with stable angina (13). Soluble cellular adhesion molecules such as soluble intercellular adhesion molecule-1 (sICAM-1) are expressed on the surface of activated endothelial cells, in response to several inflammatory cytokines, to capture circulating leukocytes and increase the migration of white cells into the vascular intima. Recently, it has been shown that sICAM-1 concentrations are associated with ultrasound-based carotid intimal-medial thickness, an early index of atherosclerosis, thus providing additional support for the role of systemic inflammation in the development of the atherosclerotic lesion (14). Furthermore, increased sICAM-1 concentrations have been associated

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4 Nonstandard abbreviations: CRP, C-reactive protein; MI, myocardial infarction; SAA, serum amyloid A; CHD, coronary heart disease; IL-6, interleukin-6; sICAM-1, soluble intercellular adhesion molecule-1; HDL-C, HDL-cholesterol; and LDL-C, LDL-cholesterol.
with increased risk of future MI in apparently healthy men (15).

To further explore the role of the systemic markers of inflammation and coronary disease, we examined the plasma concentrations of CRP, SAA, IL-6, and sICAM-1 in men with angiographically documented CHD and compared them with appropriate controls. In addition, we studied the possible association of these markers with the severity of coronary stenosis.

**Materials and Methods**

**Subjects**

One hundred men (age range, 25–75 years) who underwent cardiac catheterization at the University of Damascus Hospitals from January to March of 1997 for clinical suspicion of CHD and who had >50% occlusion in at least one coronary artery were recruited for this study. Those who had any acute coronary syndrome in the past 6 months were not included in the study. The control group, which consisted of 100 men with similar age and smoking habits, was recruited randomly from the same neighborhoods in Damascus as the case subjects. Potential participants were identified from the phone book for the desired residential areas. Of the subjects contacted and interviewed, 100 met the requirement for inclusion in this study. Although the controls were not subjected to coronary catheterization, they underwent a comprehensive physical examination by a physician and completed a questionnaire developed by a cardiologist (M.A.). None of the controls had angina pectoris or a prior history of CHD. All controls had normal resting electrocardiograms. The protocol for this study was approved by the Committee on the Protection of Human Subjects of the University of Damascus, and informed consents were signed by all participants.

Prior medical histories and personal characteristics and habits were obtained from all participants via a questionnaire. Smoking was characterized by the current smoking status and the number of cigarettes smoked per day. Hypertension was defined as resting systolic blood pressure >160 mmHg and/or diastolic blood pressure >95 mmHg; diabetes mellitus was established by preexisting diagnosis, a fasting blood glucose concentration >1.20 g/L, or glycosylated hemoglobin concentrations >6%.

Blood samples were collected after 12 h fast for the determination of cholesterol, high density lipoprotein cholesterol (HDL-C), triglycerides, homocysteine, CRP, SAA, IL-6, and sICAM-1 concentrations. In cases, blood samples were obtained on the day before the angiographic procedure. Blood samples were also collected in the morning from all controls after 12 h fast, as has been done in cases. Serum and plasma samples were separated immediately after collection by centrifugation at 2000 g for 15 min and stored at −70 °C until analysis. Serum samples were used for the measurement of lipids and lipoproteins at the University of Damascus Hospitals and plasma EDTA samples were used for the determination of homocysteine and the inflammatory markers at Children’s Hospital in Boston.

**Laboratory Measurements**

Cholesterol and triglycerides were determined on a Hitachi 911 automated analyzer (Roche Diagnostics) according to the manufacturer’s recommendations. Triglyceride measurements were corrected for endogenous glycerol. HDL-cholesterol (HDL-C) was determined after precipitation of the apolipoprotein B-containing particles by phosphotungstic acid-MgCl₂ (Roche Diagnostics). LDL-cholesterol (LDL-C) was estimated using the Friedewald equation (16). The total homocysteine concentration was measured by HPLC with fluorometric detection as reported previously (17). The concentrations of CRP and

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**Table 1. Baseline patient characteristics.**

<table>
<thead>
<tr>
<th></th>
<th>Controls a (n = 100)</th>
<th>Cases a (n = 100)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>51.9 ± 10.3</td>
<td>50.9 ± 9.7</td>
<td>NS b</td>
</tr>
<tr>
<td>Current smoker</td>
<td>41</td>
<td>39</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension</td>
<td>2</td>
<td>12</td>
<td>0.005</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0</td>
<td>17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>History of MI</td>
<td>0</td>
<td>2</td>
<td>NS b</td>
</tr>
<tr>
<td>Cholesterol, mg/L</td>
<td>2084 ± 379</td>
<td>2325 ± 547</td>
<td>0.0004</td>
</tr>
<tr>
<td>LDL-C, mg/L</td>
<td>1409 ± 360</td>
<td>151.6 ± 469</td>
<td>NS</td>
</tr>
<tr>
<td>HDL-C, mg/L</td>
<td>338 ± 109</td>
<td>323 ± 67</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides, mg/L</td>
<td>1505 (922, 2210)</td>
<td>2130 (1242, 3166)</td>
<td>0.001</td>
</tr>
<tr>
<td>Cholesterol/HDL-C</td>
<td>6.67 ± 2.29</td>
<td>7.36 ± 1.66</td>
<td>0.020</td>
</tr>
<tr>
<td>Homocysteine, µmol/L</td>
<td>10.9 (8.5, 15.6)</td>
<td>13.5 (9.6, 18.1)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Angiography</th>
<th>Cases a (n = 100)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-VD</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>2-VD</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>3-VD</td>
<td>31</td>
<td></td>
</tr>
</tbody>
</table>

a Values given in mean ± SD, or median (20th, 80th percentiles).

b NS, not significant; -VD, number of coronary vessel(s) diseased.
SAA were determined using the BN II nephelometer (Dade Behring) (18), and the concentrations of IL-6 and sICAM-1 were measured by ELISA (R&D Systems), according to the manufacturers’ specifications. The run-to-run imprecision of the CRP assay at concentrations of 0.47, 54.9, and 138 mg/L was 6.4%, 2.9%, and 3.6%, respectively; for SAA at concentrations of 11.5, 81.6, and 301 mg/L it was 7.3%, 7.0%, and 8.5%, respectively; for IL-6 at concentrations of 2.6 and 4.1 ng/L it was 5.8% and 6.9%, respectively; and for sICAM-1 at concentrations of 257.1 and 320.2 μg/L it was 8.7% and 4.7%, respectively.

Statistical Analysis
All data are presented as the mean ± SD, with the exception of triglycerides, the inflammatory markers, and homocysteine, which are presented as median (20th, 80th percentiles). The CRP, IL-6, sICAM-1, and SAA concentrations were treated as continuous variables and as quintiles defined by the distributions in the control group. Because triglycerides, CRP, IL-6, sICAM-1, SAA, and homocysteine were skewed rightward, a natural log transformation was applied to normalize the data for analysis. The unpaired Student t-test, Pearson correlation, and analysis of variance were performed on normally distributed and transformed data. Case-control differences in nominal data (diabetes, hypertension, and smoking) were evaluated with the $\chi^2$ test. A general linear model was used to consider case-control differences after adjustment for other CHD risk factors and covariates. All $P$ values were two-tailed, and $P < 0.05$ was deemed statistically significant. All analyses were performed with SPSS 8.0 (SPSS Inc.).

Results
The baseline characteristics of the study participants are presented in Table 1. Because of matching criteria, no statistically significant difference was noted in age and smoking between the two groups. The incidence of hy-
pertension and diabetes, however, was higher in the case group. Furthermore, the total cholesterol, triglycerides, and homocysteine concentrations and the total cholesterol/HDL-C ratio were higher in the case group compared with the control group.

The frequency distributions of CRP, SAA, IL-6, and sICAM-1 are presented in Fig. 1. The median concentrations of CRP, SAA, and IL-6 were significantly higher in the case group than in the control group (Table 2). These differences remained statistically significant after correction for age, smoking, diabetes, hypertension, cholesterol, LDL-C, HDL-C, triglycerides, and homocysteine. Two-thirds of the cases had CRP, SAA, and IL-6 concentrations in the fourth or fifth quintile, as defined by the distributions in controls (Fig. 2). No significant difference was seen in sICAM-1 concentrations between the two groups. Fewer than 50% of the cases had sICAM-1 concentrations in the fourth or fifth quintile (Fig. 2). For both cases and controls, the concentrations of all four inflammatory markers were higher in smokers and hypertensives. However, these differences reached statistical significance only for CRP in cases with hypertension (6.8 vs 3.3 mg/L; \( P < 0.04 \)) and for sICAM-1 in control smokers (366 vs 318 \( \mu \)g/L; \( P < 0.001 \)).

No significant correlation was seen between any of the inflammatory markers and measured lipids or homocysteine. In contrast, modest but statistically significant correlations were noted between IL-6 and SAA (\( r = 0.480; P \)

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**Table 2. Plasma inflammatory markers.**

<table>
<thead>
<tr>
<th></th>
<th>Controls*</th>
<th>Cases*</th>
<th>( P^b )</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP, mg/L</td>
<td>1.5 (0.6, 4.2)</td>
<td>3.4 (1.5, 8.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IL-6, ng/L</td>
<td>1.71 (1.07, 3.10)</td>
<td>2.30 (1.61, 3.86)</td>
<td>0.013</td>
</tr>
<tr>
<td>SAA, mg/L</td>
<td>3.7 (1.8, 8.1)</td>
<td>5.9 (2.7, 11.7)</td>
<td>0.005</td>
</tr>
<tr>
<td>sICAM-1, ( \mu )g/L</td>
<td>338.5 (268.7, 397.1)</td>
<td>334.9 (274.6, 412.4)</td>
<td>NS^c</td>
</tr>
</tbody>
</table>

* Values given as median (20th, 80th percentiles).

^b Controlling for age, diabetes, hypertension, smoking, and lipids.

^c NS, not significant.

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Fig. 2. Distribution of the inflammatory markers concentrations of cases in quintiles defined by controls.
The strongest correlations were seen between CRP and IL-6 ($r = 0.643; P < 0.0001$) and CRP and SAA ($r = 0.697; P < 0.0001$; Fig. 3).

The relationship between the inflammatory markers and the severity of coronary disease was examined. Case subjects, who have undergone coronary angiography, were divided into three categories: those with >50% obstruction in one vessel ($n = 30$), two vessels ($n = 39$), or three vessels ($n = 31$). Plasma CRP, SAA, IL-6, and sICAM-1 concentrations were examined in these three subgroups and compared with the control group (Table 3). None of these markers showed any significant correlation with the severity of disease. Similar findings were also noted for the measured lipids. In contrast, homocysteine showed a significant association ($P < 0.002$).

**Discussion**

The data from this study demonstrate that plasma concentrations of CRP, SAA, and IL-6 are increased in men with angiographically documented CHD. The differences seen in these markers between cases and controls remained highly significant after adjustment for age, smoking, hypertension, diabetes, total cholesterol, LDL-C, HDL-C, triglycerides, and homocysteine. These increases, however, failed to reflect the severity of coronary disease as assessed by angiography.

Evidence from laboratory, clinical, and epidemiological studies regarding the role of inflammation in atherogenesis is accumulating rapidly. These findings suggest that atherosclerosis is a chronic inflammatory condition that evolves as a result of a combination of biochemical, physical, and possibly infectious processes. Biochemical measures, such as acute phase reactants, have been used for detecting and assessing severity of systemic inflammation. Of the four inflammatory markers examined in this report, CRP has been studied the most in a variety of atherosclerotic diseases because of the availability of sensitive and automated analytical methods for its measurement. The de novo hepatic synthesis of CRP is triggered by the pleiotropic cytokine IL-6 (12). Therefore, CRP acts as a reliable surrogate marker for IL-6 and other mediators such as tumor necrosis factor. Data from prospective studies have shown that plasma CRP concentra-

![Fig. 3. Correlations among inflammatory markers.](image)

### Table 3. Plasma inflammatory markers and severity of coronary artery disease.

<table>
<thead>
<tr>
<th></th>
<th>Controls*</th>
<th>One vessel*</th>
<th>Two vessels</th>
<th>Three vessels</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP, mg/L</td>
<td>1.5 (0.6, 4.2)</td>
<td>3.2 (1.4, 7.5)</td>
<td>3.0 (1.4, 9.7)</td>
<td>4.6 (1.6, 9.1)</td>
</tr>
<tr>
<td>IL-6, ng/L</td>
<td>1.71 (1.07, 3.10)</td>
<td>2.63 (1.54, 4.57)</td>
<td>2.15 (1.32, 3.45)</td>
<td>2.58 (1.82, 4.14)</td>
</tr>
<tr>
<td>SAA, mg/L</td>
<td>3.7 (1.8, 8.1)</td>
<td>5.1 (2.3, 11.0)</td>
<td>6.0 (3.2, 11.8)</td>
<td>6.5 (2.6, 19.4)</td>
</tr>
<tr>
<td>sICAM-1, µg/L</td>
<td>338.5 (268.7, 397.1)</td>
<td>341.8 (292.5, 388.4)</td>
<td>334.2 (260.6, 434.6)</td>
<td>332.9 (265.8, 411.3)</td>
</tr>
</tbody>
</table>

* Values given in median (20th, 80th percentile).

* Number of vessels with >50% obstruction.
tions are increased many years in advance of the first coronary and cerebrovascular events in healthy (7–9, 11) and high-risk (10, 19, 20) individuals. In addition, sICAM-1 has been reported to predict MI and stroke many years before these events in asymptomatic healthy men (15). Studies have also shown that concentrations of CRP, SAA, and IL-6 are increased in patients with MI or unstable angina (1, 13, 21). In fact, in patients with unstable angina, CRP and SAA are good predictors of mortality (4–6) and IL-6 is associated with poor in-hospital prognosis (13), which seems to be independent of plaque rupture or myocardial necrosis (22).

In this study of men with angiographically documented CHD, plasma concentrations of CRP, SAA, and IL-6 were significantly increased when compared with age-, gender-, and smoking-matched apparently healthy controls. The distributions of CRP, SAA (7, 18), and IL-6 (Dr. Paul Ridker, personal communication) of our controls were comparable to those reported earlier. The CRP and SAA medians of our cases were lower than those seen in subjects with unstable angina (CRP, 3.4 vs 5.8 mg/L; SAA, 5.9 vs 7.6 mg/L) when the same noncompetitive immunoassays were used (4, 5), but CRP and IL-6 medians were higher than those reported in asymptomatic men who later developed MI [CRP, 3.4 vs 1.5 mg/L; IL-6, 2.3 vs 1.8 ng/L; Ref. (7), and personal communication from Dr. Paul Ridker for IL-6]. The median concentrations of sICAM-1 did not differ significantly among cases and controls (335 vs 339 μg/L). This adhesion molecule is thought to mediate the binding of circulating leukocytes to the endothelial cell and the subsequent transendothelial migration, a pivotal process in atherogenesis. An increased sICAM-1 concentration has been shown to be significantly associated with risk of future MI in men (15). In the latter study, sICAM-1 values in controls were ~30% lower, over a wide range of encountered concentrations, than those seen in our controls. Whether this difference could be attributed to methodological or physiological causes is unclear at present.

The high correlation seen between CRP, SAA, and IL-6 in this study raises the question of whether additional information would be obtained by measuring more than one marker. We previously have shown that CRP and SAA are both equal predictors of mortality in patients with unstable angina and non-Q-wave MI (4, 5) and of recurrent coronary events in patients who suffered acute MI (3). The addition of SAA to CRP in those studies did not improve its predictive power. In this report, when IL-6 and SAA values were adjusted for CRP, their expected differences among cases and controls fell short of statistical significance. In contrast, the difference in CRP values between the two groups remained significant (P <0.01) after correcting for SAA and IL-6, thus suggesting that CRP may be a stronger discriminator between patients with CHD and controls than SAA or IL-6.

The severity of coronary disease may be assessed angiographically by examining the number of vessels with >50% stenosis. In this study, none of the inflammatory markers, however, showed a significant correlation with severity of disease. The increases seen in CRP, SAA, and IL-6 of cases may, therefore, reflect the diffuse atherosclerotic process in the vascular system rather than the degree of localized obstruction from lesions. The small sample size in this study could not also be ruled out as the cause of this negative finding.

The potential limitations of this study merit consideration. The control group did not include diabetics, which potentially could influence the differences in measured inflammatory markers. In the case group, diabetics had higher CRP (median, 7.8 vs 2.9 mg/L; P <0.004) and IL-6 (2.7 vs 2.2 ng/L; P <0.024) than nondiabetics. However, all measured changes in biochemical markers were similar and remained statistically significant when nondiabetics were considered separately or when the data were adjusted for diabetes. Further work is required to investigate inflammatory markers in diabetics with and without CHD. Because all participants in this study were men, the effect of gender could not be ascertained. Lipids and CRP were shown to be predictors of future coronary events in both men and women. However, the literature supports gender-specific differences in these biochemical markers (11). Therefore, it is unclear at present whether it is appropriate to extrapolate these findings to women.

In conclusion, in this case-control study, concentrations of CRP, SAA, and IL-6 are increased in patients with CHD but failed to correlate with severity of coronary disease. These findings suggest that biochemical markers of inflammation might reflect the diffuse atherosclerotic process in the vascular system rather than the degree of localized obstruction from coronary plaques.

References


