Elevated C-Reactive Protein Levels and Impaired Endothelial Vasoreactivity in Patients With Coronary Artery Disease

Stephan Fichtlscherer, MD; Gunter Rosenberger, MD; Dirk H. Walter, MD; Susanne Breuer, MD; Stefanie Dimmeler, PhD; Andreas M. Zeiher, MD

Background—Elevated C-reactive protein (CRP) serum levels, an exquisitely sensitive objective marker of inflammation, relate to long-term prognosis in patients with coronary artery disease and in apparently healthy men. Because abnormalities of endothelial regulation of vascular function may contribute to the occurrence of coronary events, we tested the hypothesis that elevated CRP levels are associated with an abnormal systemic endothelial vascular reactivity.

Methods and Results—Endothelium-dependent (10 to 50 μg/min acetylcholine) and endothelium-independent (2 to 8 μg/min sodium nitroprusside) forearm blood flow responses were measured with venous occlusion plethysmography in 60 male patients with angiographically documented coronary artery disease. Forearm blood flow responses to acetylcholine were inversely correlated with CRP serum levels (r = −0.46, P = 0.001). With multivariate analysis that included the classic risk factors for coronary artery disease, elevated CRP serum level remained a statistically significant independent predictor of a blunted endothelial vasodilator capacity. Most important, normalization of elevated CRP levels over time was associated with a normalization of endothelium-mediated forearm blood flow responses after 3 months.

Conclusions—Thus, elevated CRP serum levels indicative of a systemic inflammatory response are associated with a blunted systemic endothelial vasodilator function. The identification of elevated CRP levels as a transient independent risk factor for endothelial dysfunction might provide an important clue to link a systemic marker of inflammation to atherosclerotic disease progression. (Circulation. 2000;102:1000-1006.)

Key Words: endothelium ▪ proteins ▪ blood flow ▪ coronary disease ▪ angina ▪ inflammation

Observational studies in humans have provided intriguing hints that relate infection or acute systemic inflammation to a temporarily increased risk for acute cardiovascular events. The transition from stable to unstable angina has been shown to be associated with increased plasma levels of C-reactive protein (CRP), serum amyloid A protein, or interleukin-6 indicative of a systemic inflammatory response. Importantly, not only is the presence of systemic markers of inflammation associated with an unfavorable short-term prognosis in patients with acute coronary syndromes, but also elevated CRP levels are related to long-term prognosis in patients with documented coronary artery disease and in apparently healthy men. The mechanisms that relate the level of acute-phase proteins to short- and long-term prognosis in coronary artery disease are unclear.

The endothelium exerts potent antithrombotic and vasodilator effects on the vascular wall. The exposure of endothelial cells to proinflammatory cytokines induces procoagulant activity, leads to the expression of cell surface adhesion molecules, and impairs endothelium-dependent vascular relaxation. All of these alterations in endothelial cell function, collectively termed “endothelial activation,” have been implicated to promote acute events in atherosclerotic vascular disease. These experimental data suggested that the impairment of normal endothelial function by inflammatory responses may provide a link between systemic inflammation and ischemic coronary syndromes. Therefore, we tested the hypothesis that elevated CRP levels, an exquisitely sensitive objective marker of inflammation, are associated with an abnormal systemic endothelial vascular reactivity in patients with coronary artery disease.

Methods

Patients
A total of 60 male patients were studied. Twenty-six patients had stable angina, defined as angiographically documented coronary artery disease and stable effort angina for ≥3 months before forearm blood flow (FBF) measurements. Thirty-four patients were studied within 5 days of an acute coronary syndrome defined as angina at rest with ST-segment alterations (Braunwald class IIIIB).
Because myocardial necrosis may induce an increase in CRP serum levels, patients with troponin T levels of $>0.2 \text{ng/mL}$ were excluded. Additional exclusion criteria were inflammatory disease or malignancy, ejection fraction of $<0.45$, clinical evidence of heart failure, and Q-wave myocardial infarction within 3 months before the study.

All patients had documented coronary artery disease with identification of the culprit lesion on coronary angiography. The clinical characteristics of these patients are summarized in Table 1. Vasoactive medications, including calcium channel blockers, ACE inhibitors, and long-acting nitrates, were withheld for $\geq 24$ hours before the study. All patients were taking aspirin (100 mg/d) and $\beta$-blocker therapy (metoprolol in most cases) on a long-term basis. All patients gave written informed consent. The study protocol was approved by the Ethical Committee of the Johann Wolfgang Goethe University of Frankfurt/Main.

### Study Protocol

FBF measurements were performed in the morning in a quiet, temperature-controlled room at $\sim 22^\circ \text{C}$ (72°F). Patients were asked to refrain from drinking alcohol or caffeine and from smoking for $\geq 12$ hours before the examination. With the patient under local anesthesia ($<1.5 \text{ mL of 2% mepivacaine; Astra Pharmaceuticals}$) and with sterile conditions, a 22-gauge catheter (Braun-Melsungen) was inserted into the brachial artery of the nondominant arm (left in most cases) for the infusion of drugs or saline. This arm was elevated ($\sim 30^\circ$) (Braunwald 5) and with sterile conditions, a 22-gauge catheter (Braun-Melsungen) was inserted into the brachial artery of the nondominant arm (left in most cases) for the infusion of drugs or saline. This arm was elevated ($\sim 30^\circ$) (Braunwald 5)

### Laboratory Analysis

In all patients, serum was collected at the time of the FBF study for the measurement of plasma CRP levels (turbidimetric test; Boehringer Mannheim), and serum lipid levels (Boehringer Mannheim). CRP levels were measured with a commercially available kit; the measurement range is 0.3 to 24 mg/dL, with coefficients of variation within assays ranging from 0.6% to 1.3% and between-assay coefficients ranging from 1.3% to 6.0% at different levels of CRP.

In 37 of the 60 patients, CRP levels were also determined with an ultrasensitive CRP test (N Latex CRP mono; Behring). The measurement range is 0.02 to 1.1 mg/dL (for 1:20 dilution; higher

### Follow-Up Studies

To investigate the natural course of systemic vascular reactivity, 29 patients underwent repeated FBF measurements under identical conditions after 4 weeks and after 3 months. Lipid-lowering or ACE inhibitor therapy or other vasoactive substances were not initiated in any of these patients during this 3-month period to avoid any potentially confounding effects of concomitant therapy, but all patients were continually treated with aspirin (100 mg/d) and $\beta$-blockers.

### Table 1. Baseline Clinical Characteristics of the Study Population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CRP &lt;0.5 mg/dL</th>
<th>CRP $\geq$0.5 to &lt;1.3 mg/dL</th>
<th>CRP $\geq$1.3 mg/dL</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>53.8±9.2</td>
<td>54.8±12.4</td>
<td>58.7±9.3</td>
<td>NS</td>
</tr>
<tr>
<td>Extent of disease, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 vessel</td>
<td>9 (15.0)</td>
<td>13 (21.7)</td>
<td>9 (15.0)</td>
<td>NS</td>
</tr>
<tr>
<td>2 vessels</td>
<td>8 (13.3)</td>
<td>2 (3.3)</td>
<td>5 (8.3)</td>
<td>NS</td>
</tr>
<tr>
<td>3 vessels</td>
<td>3 (5.0)</td>
<td>5 (8.3)</td>
<td>6 (10.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Total serum cholesterol, mg/dL</td>
<td>187.4±37.1</td>
<td>201.9±35.5</td>
<td>194.4±21.7</td>
<td>NS</td>
</tr>
<tr>
<td>LDL-cholesterol, mg/dL</td>
<td>114.7±27.7</td>
<td>126.9±31.8</td>
<td>125.8±19.4</td>
<td>NS</td>
</tr>
<tr>
<td>Mean arterial blood pressure, mm Hg</td>
<td>90.8±9.6</td>
<td>88.2±11.2</td>
<td>91.0±14.6</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>7 (11.7)</td>
<td>5 (8.3)</td>
<td>8 (13.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>3 (5.0)</td>
<td>2 (3.3)</td>
<td>3 (5.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Current smoking, n (%)</td>
<td>4 (6.7)</td>
<td>7 (11.7)</td>
<td>5 (8.3)</td>
<td>NS</td>
</tr>
<tr>
<td>CRP levels, mg/dL</td>
<td>0.20±0.15</td>
<td>0.82±0.27</td>
<td>3.08±1.33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Stable angina (N=26), n (%)</td>
<td>17 (65.4)</td>
<td>5 (19.2)</td>
<td>4 (15.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Unstable angina (N=34)</td>
<td>3 (8.8)</td>
<td>15 (44.1)</td>
<td>16 (47.1)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are mean±SD or n (%).
concentrations were determined after appropriate dilution) with intra-assay coefficients of variation of 1.7% to 2.5% and interassay coefficients of variation of 1.7% to 3.6%) to ensure that the impossibility of detecting very low levels with the turbidimetric test did not affect the results. In addition, tumor necrosis factor-α (TNF-α) and soluble intercellular adhesion molecule-1 (sICAM-1) serum levels were determined in these 37 patients, for whom frozen plasma samples were available. TNF-α and s-ICAM serum levels were measured with ELISA with commercially available kits (range 0.5 to 32 pg/mL for TNF-α and 2 to 46 ng/mL for sICAM; R and D Systems Europe).

Statistical Analysis
Data are expressed as mean±SD value. Continuous variables were tested for normal distribution with the Kolmogorov-Smirnov test and compared by 1-way ANOVA. Categorical variables were compared by the χ² test and the Fisher exact test. In the case of non-normal distribution, nonparametric tests were used (Mann-Whitney U test or Kruskal-Wallis ANOVA on ranks). Differences between the group FBF and vascular resistance measurements are presented as mean±SEM. Differences in forearm vascular reactivity were examined by repeated-measures ANOVA followed by post hoc 1-tailed t tests adjusted with a Bonferroni correction for multiple comparisons. FBF responses to acetylcholine and sodium nitroprusside were calculated as area under the curve and expressed in arbitrary units. Linear regression analysis and nonparametric bivariate correlation (Spearman rank correlation coefficient [r]) were used to compare FBF responses with CRP values. Because CRP levels have a skewed distribution, logarithmically transformed CRP values were also used to relate CRP levels with FBF data. Multivariate analysis was performed with the logistic regression model. Statistical significance was assumed if a null hypothesis could be rejected at P=0.05. All statistical analysis was performed with SPSS for Windows 7.0 (SPSS Inc).

Results
All patients were taking β-blockers to exclude the potential effects of β-adrenergic receptor stimulation, which has been shown to elicit endothelium-dependent, nitric oxide–mediated vasodilation in the human forearm.17 Blood flow in the noninfused control arm (measured in 15 patients) did not change significantly in response to drug infusions in the contralateral arm, confirming the results of a number of previous studies that, at the doses used, the drugs did not cause systemic effects when infused into the brachial artery.15,17

CRP levels ranged from 0.0 to 4.5 mg/dL (mean±SD 1.2±1.2 mg/dL). Figure 1 illustrates that FBF responses to acetylcholine, expressed as area under the curve, were inversely correlated with CRP serum levels. Log-transformation of CRP values to account for the skewed distribution gave almost identical results (r=-0.39, P=0.006). In addition, an identical inverse correlation was observed between CRP serum levels and acetylsalicylic-induced FBF response (r=-0.43, P<0.01), when only those 37 patients were analyzed, in whom CRP levels were determined with the ultrasensitive measurement method. In contrast, neither TNF-α (P=0.29) nor sICAM-1 (P=0.96) serum levels were significantly associated with acetylsalicylic-induced FBF responses.

Subsequently, patients were stratified into 3 groups according to tertiles of CRP serum levels. Table 1 illustrates that the 3 groups of patients did not differ with respect to age, extent of coronary artery disease, serum cholesterol levels, mean arterial blood pressure, or the presence of classic risk factors for coronary artery disease. As expected, however, the patients in the lowest tertile of CRP serum levels significantly less frequently presented with an acute coronary syndrome compared with the patients in the upper 2 tertiles.

Figure 2 (top) illustrates that patients with CRP serum

Figure 1. Relation between FBF dose-response curves to acetylcholine (ACH) expressed as area under the curve (AUC) and CRP serum levels.

Figure 2. FBF dose-response curves to acetylcholine (top) and sodium nitroprusside (bottom) according to tertiles of CRP levels. Values represent mean±SEM.
levels of <0.5 mg/dL (lowest tertile) demonstrated a significantly increased acetylcholine-induced FBF response compared with patients in the upper 2 tertiles. Importantly, not only were acetylcholine-induced FBF responses dose-dependently attenuated but also baseline FBF (1.6±0.5 versus 1.9±0.9 mL min⁻¹ 100 mL of forearm tissue⁻¹, P<0.05) was significantly reduced and baseline vascular resistance (51.2±2.8 versus 62.5±4.7 mm Hg mL⁻¹ min⁻¹ 100 mL of forearm tissue⁻¹, P<0.05) was significantly elevated in patients in the upper tertile compared with those in the lowest tertile.

FBF increases in response to sodium nitroprusside were also slightly, but significantly, reduced in patients with elevated CRP levels of ≥0.5 mg/dL (upper 2 tertiles) (Figure 2, bottom). However, when FBF responses are expressed as percent change in forearm vascular resistance to account for the significantly increased basal tone in patients with elevated CRP levels, only the vasodilator response to acetylcholine (Figure 3, top), not the response to sodium nitroprusside (Figure 3, bottom), was significantly blunted. Thus, elevated plasma CRP levels are associated with a significantly reduced forearm vasodilator response, which is in large part due to a blunted endothelium-dependent component.

To investigate whether CRP serum level is an independent predictor of acetylcholine-induced FBF responses, a multivariate analysis was performed in which LDL-cholesterol serum levels, CRP levels, extent of coronary artery disease, the presence of unstable angina, smoking, diabetes, and hypertension were entered as independent variables. Table 2 illustrates that CRP serum level remained a statistically significant independent predictor of acetylcholine-induced FBF responses in addition to LDL serum levels. In contrast, the presence of unstable angina was not an independent predictor of a blunted FBF response. Indeed, even in the group of patients with stable angina, CRP levels were significantly inversely correlated with acetylcholine-induced FBF responses (P<0.03). Thus, independent of classic risk factors for coronary artery disease, elevated CRP serum levels are associated with a blunted endothelium-mediated systemic vasodilator capacity in patients with coronary artery disease regardless of the presence or absence of an episode of unstable angina.

To investigate whether the normalization of elevated CRP levels over time is associated with improved FBF responses, 29 patients were reexamined with the identical FBF study protocol 4 weeks and 3 months after the initial study. Thirteen of these patients had CRP levels within the lowest tertile (<0.5 mg/dL) throughout the 3 months. In 7 patients, CRP levels were initially in the upper 2 tertiles (mean±SD 1.44±0.9 mg/dL) but returned to the lowest tertile (<0.5 mg/dL) after 4 weeks (0.31±0.24) and remained <0.5 mg/dL (0.28±0.26) until the 3-month follow-up study. Nine patients had CRP levels of ≥0.5 mg/dL (upper 2 tertiles) continuously during the follow-up period (1.78±0.98 at the initial study, 0.78±0.62 after 4 weeks, and 0.91±0.45 after 3 months). Serum cholesterol levels were essentially identical at the initial study (192.8±38.5 mg/dL), after 4 weeks (187.9±40.5 mg/dL), and at the 3-month follow-up (193.4±39.7 mg/dL).

Figure 4 illustrates that in patients with CRP levels in the lowest tertile, FBF responses to acetylcholine as well as to sodium nitroprusside were virtually identical after 4 weeks and after 3 months (Figure 4, top). In the 9 patients with continually elevated CRP levels, the initially blunted FBF responses remained depressed throughout the study period (Figure 4, middle). In contrast, however, in the 7 patients with...
Initially elevated CRP levels, the blunted FBF response to acetylcholine was slightly increased after 4 weeks and significantly ($P < 0.03$) improved after 3 months (Figure 4, bottom). Indeed, FBF responses to acetylcholine did not significantly differ anymore after 3 months between patients with initially elevated CRP levels, which returned to levels in the lowest tertile, compared with the patients with CRP levels in the lowest tertile throughout the study period. FBF responses to SNP also slightly increased over time in patients with initially elevated CRP levels, but this improvement did not achieve statistical significance (Figure 4, bottom). Multivariate analysis revealed that the recurrence of CRP levels to $\leq 0.5$ mg/dL (lowest tertile) was an independent predictor of improved endothelial vasoreactivity over time, whereas the
presence of unstable angina before the initial study did not independently predict an improvement in FBF responses to acetylcholine over time. Thus, the impairment in systemic endothelial vascular reactivity appears to be a transient phenomenon associated with increased plasma CRP levels.

Discussion
The results of the present study demonstrate for the first time that elevated CRP levels are associated with a profound impairment in systemic endothelial vascular reactivity in patients with coronary artery disease. The blunted systemic endothelial vasodilator function related to elevated plasma CRP levels is independent of classic risk factors for coronary artery disease. Most importantly, normalization of CRP levels over time is associated with a significant improvement in endothelium-dependent FBF responses. These findings support the concept that alterations in endothelial cell function may provide a link between systemic inflammation and ischemic coronary events.

It is well established that atherosclerosis impairs endothelial vasodilator function in both the coronary and the forearm circulation. Moreover, chronic risk factors for coronary artery disease have been shown to adversely affect endothelium-dependent blood flow responses in the human forearm circulation. However, the present study demonstrates that the impairment in systemic endothelial vasodilator function associated with elevated CRP levels is superimposed on the effects of atherosclerosis itself and its risk factors.

Numerous studies have confirmed the independent prognostic relevance of CRP for the risk of coronary artery disease not only in patients with stable or unstable coronary artery disease but also in apparently healthy men. Likewise, endothelial dysfunction is believed to importantly contribute to coronary artery disease progression and cardiovascular event rates. Thus, the identification of elevated CRP levels as an independent predictor of endothelial dysfunction might provide an important clue to link a systemic marker of inflammation to atherosclerotic disease progression.

Importantly, the present study also demonstrates that the normalization of CRP levels over time is associated with a significant improvement in endothelium-mediated FBF responses. Thus, the impairment in systemic endothelial vascular reactivity appears to be a transient phenomenon associated with an enhanced systemic inflammatory response. Indeed, experimental studies have shown that bacterial endotoxin and certain proinflammatory cytokines can inhibit agonist-stimulated release of nitric oxide and vasodilator prostanooids. Moreover, recent studies in healthy volunteers have documented that even a very brief exposure to endotoxin or certain cytokines impairs endothelium-dependent relaxation for many days, a phenomenon termed “endothelial stunning.”

Surprisingly, however, TNF-α, which stimulates the expression of interleukin-6, which in turn may provoke the augmented expression of CRP in the liver, did not show any relation with forearm vascular reactivity in the present study. Likewise, serum levels of another distal indicator of inflammation, sICAM-1, were unrelated to acetylcholine-induced FBF responses. Although it is beyond the scope of the present study, it can be speculated that the exquisitely sensitive, but nonspecific, marker of low-grade systemic inflammation, CRP, is more informative than are serum levels of cytokines such as TNF-α or soluble forms of leukocyte adhesion molecules like sICAM-1 to disclose a systemic impairment in vascular reactivity. Indeed, although prospective data are very limited for sICAM or even absent for TNF-α, the clinical use of CRP serum levels is well established and remarkably consistent with respect to prediction of the risk of future cardiovascular events.

In line with numerous previous studies, patients with unstable angina exhibited significantly elevated CRP serum levels. However, by multivariate analysis, it was the elevated CRP level that independently predicted a blunted endothelial vasodilator capacity, not the presence of unstable angina. Moreover, improvement of endothelium-mediated blood flow responses over time was related to normalization of initially elevated CRP levels, whereas the presence of unstable angina did not independently predict subsequent improvement of endothelial vasodilator function. Thus, endothelial vasodilator dysfunction clearly appears to be associated with a marker of systemic inflammation and not secondary to the presence of unstable angina. Taken together, the data of the present study strongly support the hypothesis that the temporary endothelial activation with ensuing vasodilator dysfunction in response to systemic inflammatory stimuli might play a role as a transient risk factor for acute ischemic events in patients with coronary artery disease.

Thus, the combination of the present observations with experimental and epidemiological data suggests that the altered vasoreactivity associated with systemic inflammation might represent a transient risk factor for coronary events in patients with coronary artery disease. However, further studies are required to explore the mechanisms that underlie endothelial vascular dysfunction associated with systemic inflammation and, more importantly, to establish blunted systemic endothelial vasoreactivity as an independent risk factor for coronary events rather than a phenomenon of no pathological importance.

Acknowledgments
We gratefully acknowledge Margret Müller-Ardogan for expert technical assistance. We also acknowledge Susanne Ficus for measurement of high sensitivity CRP, TNF-α, and s-ICAM levels.

References


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_Circulation_. 2000;102:1000-1006
doi: 10.1161/01.CIR.102.9.1000

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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