Clinical Investigation and Reports

Elevated Levels of C-Reactive Protein at Discharge in Patients With Unstable Angina Predict Recurrent Instability

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Background—In a group of patients admitted for unstable angina, we investigated whether C-reactive protein (CRP) plasma levels remain elevated at discharge and whether persistent elevation is associated with recurrence of instability.

Methods and Results—We measured plasma levels of CRP, serum amyloid A protein (SAA), fibrinogen, total cholesterol, and Helicobacter pylori and Chlamydia pneumoniae antibody titers in 53 patients admitted to our coronary care unit for Braunwald class IIIB unstable angina. Blood samples were taken on admission, at discharge, and after 3 months. Patients were followed for 1 year. At discharge, CRP was elevated (>3 mg/L) in 49% of patients; of these, 42% had elevated levels on admission and at 3 months. Only 15% of patients with discharge levels of CRP <3 mg/L but 69% of those with elevated CRP (P<0.001) were readmitted because of recurrence of instability or new myocardial infarction. New phases of instability occurred in 13% of patients in the lower tertile of CRP (≥2.5 mg/L), in 42% of those in the intermediate tertile (2.6 to 8.6 mg/L), and in 67% of those in the upper tertile (≥8.7 mg/L, P<0.001). The prognostic value of SAA was similar to that of CRP; that of fibrinogen was not significant. Chlamydia pneumoniae but not Helicobacter pylori antibody titers significantly correlated with CRP plasma levels.

Conclusions—In unstable angina, CRP may remain elevated for at least 3 months after the waning of symptoms and is associated with recurrent instability. Elevation of acute-phase reactants in unstable angina could represent a hallmark of subclinical persistent instability or of susceptibility to recurrent instability and, at least in some patients, could be related to chronic Chlamydia pneumoniae infection. (Circulation. 1999;99:855-860.)

Key Words: angina ■ prognosis ■ Chlamydia pneumoniae

In patients with unstable angina, the common persistence of instability for weeks,1 together with evidence of a rebound phenomenon after reduction of the dose of heparin treatment,2 raises the possibility that a subclinical component of instability may persist after the waning of symptoms. In unstable patients, elevated levels of C-reactive protein (CRP), a nonspecific but sensitive marker of inflammation, were found to have a short-term prognostic value unrelated to myocardial cell damage, myocardial ischemia, or episodes of activation of the hemostatic system.3–5 More recently, evidence of a long-term prognostic value of elevated CRP levels was reported in patients with coronary artery disease6–8 and in healthy individuals with high9 and low10 levels of coronary risk factors. Evidence of persisting inflammation at the time of hospital discharge could represent either a subclinical hallmark of persistent instability or a marker of susceptibility to recurrent unstable phases, possibly related to Chlamydia pneumoniae (CP) or Helicobacter pylori (HP) infection as recently proposed.11–14 We have found that serum CRP levels may remain elevated at the time of discharge and at a 3-month follow-up in a substantial proportion of patients admitted with Braunwald class IIIB unstable angina and that such an elevation is associated with frequent hospital readmission for recurrent instability.

Patients

Of 164 patients admitted to our institute with a diagnosis of unstable angina from November 1992 to March 1994, 53 were included in the study, 10 patients were also part of our previous study,4 and the remaining 111 patients were excluded because they did not fulfill the inclusion criteria on admission or at discharge. Inclusion criteria on admission were angina at rest with >2 ischemic episodes or 1 episode lasting >20 minutes in the last 24 hours with diagnostic ST-segment shift. Patients were excluded if they had any ECG abnormalities that could affect the recognition of ST-segment ischemic changes (10) and elevation of creatine kinase or troponin T (32). Exclusion criteria were malignancy or inflammatory disease (4), surgery or major trauma in the previous month (5), known thrombotic disorders, dilated cardiomyopathy (1), valvular heart disease (5), previous myocardial infarction within 3 weeks or during the index hospitalization (28), and ejection fraction <40% (10) or age >75 years (16) because these latter conditions carry an additional risk independent of the severity of instability. Because of

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the severity of their symptoms, 55% of patients were secondary referrals. This study was approved by the Ethics Committee of the university, and all patients gave written informed consent.

**Study Protocol and Laboratory Assays**

Blood samples were taken from all patients at entry to the study, at discharge (mean, 12.5 ± 5 days after admission), and 3 months after admission (≥15 days after any acute event); all patients were followed up regularly as outpatients for 1 year. In case of cardiac surgery, discharge samples were collected before surgery. Plasma concentrations of CRP and serum amyloid A protein (SAA) were measured in a single batch at the end of the study (to avoid bias in the evaluation of symptoms and in clinical decision making) except for the first 10 patients; in all an automated monoclonal antibody solid-phase sandwich enzyme immunoassay was used.15,16 Plasma concentrations of fibrinogen were measured by use of the Clauss method, and HP antibody titers were measured in all patients on admission with a commercial ELISA for specific IgG (Dade-Behring). A titer >10 U/mL was considered a sign of HP infection. CP antibody titers were also measured in all patients on admission with a microimmunofluorescence assay (MRK Diagnostics Chlamydia MIF kit).17,18 Samples were diluted from 1:8 to 1:64. Seropositivity to CP was defined as presence of specific antibodies at a dilution of 1:16; however, seropositivity at a dilution of 1:32 was also considered. Coronary angiography was performed before discharge in all but 2 patients (who refused) and during follow-up when clinically indicated. Patients were classified into 2 groups according to CRP levels at the time of discharge: individuals with normal values of CRP (<3 mg/L) and those with levels >3 mg/L (ie, above the upper value found in 90% of healthy individuals). Refractory angina was defined as angina persisting despite full medical therapy, including intravenous nitrates and heparin (at least 2 episodes within 48 hours from institution of full medical therapy), requiring urgent revascularization. During follow-up, the recurrence of instability was defined as a new phase of unstable angina, with documented ischemic changes on the ECG, requiring readmission to hospital.

**Statistical Analysis**

Because CRP values were not normally distributed, data are presented as median and range. Nonparametric tests were used for comparison of CRP levels between groups (Mann-Whitney U test) and correlations (Spearman’s ρ test); discontinuous variables were tested by a contingency χ² test. Event-free survival was analyzed by the Kaplan-Meier method, and the log-rank test was used for comparison among curves. By logistic regression analysis, we calculated the relative odds ratio (OR) and 95% confidence intervals (CIs) for CRP and the following confounding variables: occurrence of in-hospital events, family history of ischemic heart disease, cholesterol level >200 mg/dL, fibrinogen level >400 mg/dL, smoking, age, sex, and diabetes or systemic hypertension. For comparison with recent studies,6,11 patients were also grouped into tertiles according to CRP, fibrinogen, and cholesterol levels, and differences in events among tertiles were calculated by a contingency χ² test.

**Results**

At discharge, CRP levels were normal (<3 mg/L) in 27 of 53 patients (51%) (group 1) and elevated (>3 mg/L) in 26 patients (group 2). SAA levels at discharge were elevated in 28 of 53 patients and correlated closely with those of CRP (r=0.76, P<0.001). Because the results of SAA analysis were similar to those of CRP, the SAA data are not presented. The demographic data of patients are reported in Table 1. Therapy before hospitalization, during hospitalization in the coronary care unit, and after discharge was not different between the 2 groups because all patients took aspirin or ticlopidine and a various combination of β-blocking agents, calcium antagonists, and nitrates; in the coronary care unit, all patients were treated with intravenous heparin (Table 2). No patient was discharged with lipid-lowering drugs, but in 18 patients, these drugs were added at the 1-month follow-up visit. These patients were equally distributed between the 2 groups.

**Group 1**

In group 1, median CRP levels at discharge were 2.4 mg/L (range, 0.6 to 2.9 mg/L) and were normal on admission in 15 of 27 patients and in 23 of 27 at 3 months. A total of 11 of the 27 patients had CRP levels within the normal range throughout the initial 3-month follow-up period. During hospitalization, coronary revascularization was undertaken in 33% of patients on an urgent basis and in 22% on an elective basis; 45% of patients had an uneventful course. In this group, 20 of 27 patients (75%) were positive to HP, and HP antibody titers were 35.3 ± 79 U/I; 10 of 27 patients (37%) were positive to CP, and 6 of 27 (22%) were positive at a dilution ≥1:32. At discharge, blood levels of fibrinogen and cholesterol were 319 ± 164 and 220 ± 49 mg/dL, respectively. During follow-up, 4 of 27 patients (15%) had coronary events: myocardial infarction in 1 and a new phase of instability requiring readmission to hospital.

**Group 2**

In group 2, median CRP levels at discharge were 9.9 mg/L (range, 3.3 to 9 mg/L; P<0.001 versus group 1) and were elevated in 24 of 26 patients on admission and in 22 of 26 at 3 months. A total of 21 of 26 patients (81%) had elevated CRP levels throughout the initial 3-month follow-up period. They represent 40% of the group of 53 total patients. During hospitalization, 58% of group 2 patients had coronary events and underwent urgent coronary revascularization; 19% underwent elective revascularization. Of these patients, 23% (versus 45% in group 1) were treated medically, but the difference was not significant. In group 2, 21 of 26 patients (81%) were positive to HP (P=NS versus group 1); HP antibody titers were 28.9 ± 53 U/I (P=NS versus group 1). In addition, 21 of 26 patients (81%) were positive to CP

**Table 1. Clinical Findings**

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n=27)</th>
<th>Group 2 (n=26)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>58±9.4</td>
<td>63.3±7</td>
<td>NS</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>27/0</td>
<td>17/9</td>
<td>0.005</td>
</tr>
<tr>
<td>Coronary angiography, n (%)</td>
<td>17 (63)</td>
<td>12 (46)</td>
<td>NS</td>
</tr>
<tr>
<td>1-vessel disease</td>
<td>5 (18)</td>
<td>6 (23)</td>
<td>NS</td>
</tr>
<tr>
<td>2-vessel disease</td>
<td>5 (18)</td>
<td>8 (30)</td>
<td>NS</td>
</tr>
<tr>
<td>Previous MI, n (%)</td>
<td>11 (41)</td>
<td>8 (30)</td>
<td>NS</td>
</tr>
<tr>
<td>Family history of IHD, n (%)</td>
<td>11 (41)</td>
<td>13 (50)</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma cholesterol &gt;200 mg/dL, n (%)</td>
<td>10 (37)</td>
<td>11 (42)</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>7 (26)</td>
<td>17 (65)</td>
<td>0.009</td>
</tr>
<tr>
<td>Cigarette smoking, n (%)</td>
<td>9 (33)</td>
<td>12 (46)</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>3 (11)</td>
<td>4 (15)</td>
<td>NS</td>
</tr>
</tbody>
</table>

MI indicates myocardial infarction; IHD, ischemic heart disease.
(P = 0.0014 versus group 1), and 13 of 26 (50%) were positive at a dilution $\geq$ 1:32 (P = 0.035 versus group 1). At discharge, blood levels of fibrinogen and cholesterol were 395 ± 152 and 207 ± 51 mg/dL, respectively (P = NS versus group 1 for both).

During follow-up, 18 of 26 patients (69%, versus 15% in group 1, P < 0.001) had coronary events: myocardial infarction in 4 (1 patient died after the infarction) and a new phase of instability requiring readmission in 14. The 1-year survival free from readmission, myocardial infarction, and death was significantly higher in group 1 than group 2 as assessed by the log-rank test (P = 0.0001, Figure 1A) and was independent of in-hospital revascularization during the first admission (Figure 1B). CRP values $>3$ mg/L at discharge had an adjusted OR for recurrent instability of 8.57 (95% CI, 1.66 to 44.2; Table 3). In all patients, we carefully considered whether or not recurrent ischemia or infarction was at the same site as the index event, and we found that the site of the recurrent event was the same as the culprit site in 16 patients (73%) but was different in 6. Culprit site was assessed by the analysis of ECG and echocardiographic data; in 11 patients, this was confirmed by coronary angiography.

Recurrence of instability was due to post-PTCA restenosis in 5 of 22 patients (23%) and to saphenous vein graft stenosis in 2 of 22 (9%). PTCA was performed with the same technique in the 2 groups, and no stents were used. Procedure and vessel characteristics were similar; all but 2 patients were treated on a single vessel. In only 1 group 1 patient, a severe complication caused by abrupt vessel occlusion was observed and treated with emergency CABG. The procedure was successful in all other patients without acute or subacute coronary occlusion. The only difference that we observed was a more severe preprocedural stenosis in group 2 than in group 1 (82.3 ± 10.5% versus 78.5 ± 5%, P = 0.03); however, residual stenosis after the procedure was not different in the 2 groups (33.4 ± 9% in group 2 versus 29 ± 7.9% in group 1, P = NS), with TIMI grade III flow in all patients.

Tertile Distribution of CRP, Fibrinogen, and Cholesterol
We also assessed the relation between the recurrence of instability in the whole group and the distribution of patients into tertiles according to levels of CRP, fibrinogen, and cholesterol. A new phase of instability occurred in 13% of patients in the lower tertile of CRP (with values $\leq$ 2.5 mg/L), in 42% of those in the intermediate tertile (with values from 2.5 to 8.6 mg/L), and in 67% of those in the upper tertile (with values $\geq$ 8.7 mg/L, P < 0.01, Figure 2A). A similar trend,

<table>
<thead>
<tr>
<th>Table 2. Therapy</th>
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<tbody>
<tr>
<td>Before Hospital Admission</td>
</tr>
<tr>
<td>Group 1, n (%)</td>
</tr>
<tr>
<td>Heparin</td>
</tr>
<tr>
<td>Nitrates</td>
</tr>
<tr>
<td>Calcium blockers</td>
</tr>
<tr>
<td>$\beta$-Blockers</td>
</tr>
<tr>
<td>ACE inhibitors</td>
</tr>
<tr>
<td>Antiplatelet agents</td>
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<tr>
<td>Anticoagulant</td>
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<tr>
<td>Lipid-lowering drugs</td>
</tr>
</tbody>
</table>
CRP and Recurrence of Instability

although not significant, was observed for fibrinogen levels (22% versus 44% versus 59%, Figure 2B) but not for total cholesterol. For patients in the upper tertile of cholesterol but in the lower tertile of CRP, readmission rate was lower than for those in the intermediate and upper tertiles of CRP, but the difference was of borderline significance \((P=0.05, \text{Figure } 3A)\). A similar trend was observed by a comparison of tertiles of cholesterol with those of CRP and fibrinogen according to the following distribution: low-low (first tertile), high-high (third), and any different combination (intermediate) (Figure 3B).

No correlation was found between CRP levels and HP antibody titers or seropositivity, but a significant correlation was found between seropositivity to CP and CRP at any time \((P<0.005)\), and a trend was observed between CP dilution titers and CRP levels at entry \((P=0.06)\). Seropositivity to CP was associated with recurrence of instability because seropositivity to CP was found in 17 of 22 patients (77%) with recurrent instability but in only 14 of 31 patients (45%) without events \((P<0.05)\).

TABLE 3. ORs for Recurrent Instability at 1 Year

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OR</th>
<th>95% CI</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.03</td>
<td>0.9–1.14</td>
<td>NS</td>
</tr>
<tr>
<td>Male sex</td>
<td>0.05</td>
<td>0.001–1.9</td>
<td>NS</td>
</tr>
<tr>
<td>Family history of IHD</td>
<td>1.64</td>
<td>0.3–8.9</td>
<td>NS</td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td>5.00</td>
<td>0.6–40</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma cholesterol &gt;200 mg/dL</td>
<td>3.00</td>
<td>0.5–17.1</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension</td>
<td>2.10</td>
<td>0.38–12.1</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.42</td>
<td>0.1–19.6</td>
<td>NS</td>
</tr>
<tr>
<td>Multivessel disease</td>
<td>2.04</td>
<td>0.7–5.6</td>
<td>NS</td>
</tr>
<tr>
<td>Fibrinogen &gt;400 mg/dL</td>
<td>4.90</td>
<td>0.88–27.9</td>
<td>NS</td>
</tr>
<tr>
<td>CRP &gt;3 mg/L</td>
<td>8.57</td>
<td>1.66–44.2</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

IHD indicates ischemic heart disease.

CRP Admission Levels and Follow-Up Prognosis

On admission, CRP levels were elevated in most group 2 patients (24 of 26). Patients with elevated CRP levels at entry had significantly more events at follow-up by the log-rank test compared with patients with normal CRP levels \((P=0.037)\), but the difference was weaker than that observed for CRP discharge levels. Positive predictive value of CRP at entry was 50%, but that of CRP at discharge was 69%. Negative predictive value was 86% for CRP levels at entry and 85% at discharge; diagnostic accuracy was 60% for CRP levels at entry and 77% at discharge.

Discussion

Our study demonstrates that serum levels of CRP may remain elevated above the normal range after the waning of symptoms in nearly one half of patients admitted to hospital with a diagnosis of Braunwald class IIIB unstable angina and that such an elevation is associated with a significantly higher incidence of new phases of instability within 1 year. The recurrence of instability appears to be independent of coronary revascularization procedures performed during the initial hospitalization and cholesterol levels, which were of borderline significance only when associated with elevated CRP levels. Thus, recurrent instability may be associated with persisting inflammatory stimuli, the cause of which is still unknown. This observation, if confirmed, would have important implications for clinical practice and research because it could provide a useful marker for the separation of patients at low risk of recurrent instability from those at high risk for whom more careful clinical monitoring and more aggressive treatment are required. Moreover, the understanding of the causes of persistent inflammation may provide novel additional therapeutic tools.

Our study shows that although entry levels of CRP predict recurrence of instability at 1 year, this predictive value is lower than that of CRP levels at discharge because entry values may be affected by the acute phase of the disease and are better predictors of short-term occurrence of new ischemic episodes.\(^3,20\) Our findings confirm the primary role of an inflammatory component in unstable angina by showing that CRP levels remained elevated in 42% of our patients 3 months after hospital discharge, when causal roles of coronary thrombosis, myocardial necrosis, or ischemia and of in-hospital revascularization procedures are unlikely.

Elevated CRP levels are a marker of increased production of IL-1 and IL-6,\(^21,22\) 2 proinflammatory cytokines that also have prothrombotic properties, and CRP itself can activate monocytes to produce tissue factor\(^23\) and induce monocyte and endothelial cell release of IL-1 and IL-6.\(^22\) However, the mechanisms that can lead to initiation of such inflammatory reaction may be multiple and to date are largely unexplained. The mechanisms that can lead to the persistence of this “acute-phase” reaction also are unexplained.\(^24\) It is possible that oxidized LDL\(^25\) or infections caused by CP, HP, and Cytomegalovirus (CMV) may cause a low-grade chronic inflammatory condition.\(^26\) Preliminary findings from our group failed to detect mRNA of CMV in endoarterectomy specimens of coronary plaques in unstable patients.\(^27\) We also failed to detect an association between CMV and HP serum...
antibody titers and acute myocardial infarction and unstable angina, although such an association was found with chronic atherosclerotic syndromes. In the present study, HP antibody titers were not correlated with the levels of CRP at discharge or with the recurrence of instability. Only seropositivity for CP correlated with CRP levels and was associated with recurrence of instability during follow-up. Thus, CP infection could be a stimulus responsible for the persistent inflammatory state or for the recurrence of instability. Although our study does not allow us to draw definitive conclusions on the role of CP in unstable angina, this possibility is supported by recent reports of a reduction in cardiovascular events (recurrence of angina, myocardial infarction, and death) in unstable angina and in survivors of myocardial infarction after antibiotic treatment for CP.

We have also recently shown that patients with persistently elevated CRP levels have an exaggerated production of IL-6 after angioplasty, acute myocardial infarction, and lipopolysaccharide challenge. Collectively, these observations, together with the stronger correlation with recurrence of instability and myocardial infarction of elevated levels of CRP than of seropositivity to CP, suggest that the intensity of the individual inflammatory response may be an independent pathogenic component of unstable angina.

Conclusions
Our study has included a relatively small number of patients; however, we adopted strict enrollment criteria to have a homogeneous population: Not only were all patients in Braunwald class IIIB, but they also were selected to avoid patients in whom severity of angina was not necessarily the major determinant of prognosis. Thus, our findings may not apply to the whole spectrum of unstable patients.

If confirmed in larger studies, our findings may have relevant practical implications because low serum levels of CP at the time of hospital discharge identify a group of patients at low risk, even in patients with severe unstable angina. Conversely, elevated CRP levels at discharge identify patients at high risk even after successful revascularization who therefore may benefit from a more careful clinical follow-up and appropriate antithrombotic and possibly anti-inflammatory treatment. Precise knowledge of the possible triggers of the inflammation and the determinants of its individual response may open novel therapeutic avenues.

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References


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