C-reactive Protein and Coronary Artery Disease

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SUMMARY

Evidence suggests that inflammation plays a key role in the pathogenesis of atherosclerosis. The chronic inflammatory process can develop to an acute clinical event by the induction of plaque rupture and therefore cause acute coronary syndromes.

The aim of this study was to determine the serum levels of the circulating acute-phase reactant C-reactive protein (CRP), which is a sensitive indicator of inflammation, in patients with chronic stable coronary artery disease (CAD) and acute coronary syndromes (ACS).

We studied 56 subjects: 1) 25 consecutive patients (18 men, 7 women; mean age, 68.5±14.3 years, range, 40-86) with unstable angina (UA) or acute myocardial infarction (AMI); 2) 31 consecutive patients (25 men, 6 women; mean age 64±12.7; range, 47-83, years) with signs and symptoms of clinically stable CAD. High-sensitivity-C-reactive protein (hs-CRP) levels were determined with a commercially available enzyme-linked immunoassay method.

In patients with unstable angina and AMI before reperfusion therapy, CRP levels were not significantly different to those in patients with stable CAD (5.96±2.26 versus 4.35±2.6 mg/L; P=0.12), but tended to be higher in patients with unstable angina and AMI. Baseline CRP levels in the subgroup of patients with AMI (6.49±2.28 mg/L) were significantly higher than levels in patients with stable CAD (4.35±2.6 mg/L; P=0.02).

CRP levels in patients with unstable angina and AMI were measured four times during a 72-hour period (0, 12, 24, and 72 hours). The lowest value was observed at baseline and differed significantly from values measured at any other time of the observation period (P<0.001; 5.96±2.26; 9.5±9.04, 18.25±11.02; 20.25±10.61). CRP levels after 12, 24, and 72 hours were also significantly different to the initial values for patients with stable CAD (P<0.01). There was no correlation between CRP and creatine kinase (CK), CK-MB isoenzyme, or troponin I positivity as markers for the extent of the myocardial injury during the observation period.

Baseline levels of serum CRP tended to be higher in patients with unstable angina or AMI but were not significantly different from levels in patients with chronic stable CAD. In the subgroup of patients with AMI, baseline CRP levels were significantly higher than the levels in patients with stable CAD. CRP as a marker of inflammation is significantly increased in patients with AMI and unstable angina shortly after the onset of symptoms (after a period of 12 hours), supporting the hypothesis of an activation of inflammatory
mechanisms in patients with an acute coronary syndrome or AMI. (Jpn Heart J 2002; 43: 607-619)

**Key words:** Coronary artery disease, Acute coronary syndromes, Ischemia, Inflammation, C-reactive protein

In patients with unstable angina, persistent or worsening symptoms and signs of ischemia despite full medical therapy indicate a poor prognosis. However, at the time of hospital admission, it is not possible to predict whether unstable angina will remit or progress to myocardial infarction, because the causes of instability and the mechanisms underlying its evolution are not known.

The presence of inflammatory infiltrates in unstable coronary plaques suggests that inflammatory processes may contribute to the pathogenesis of these syndromes. In patients with unstable angina, coronary atherosclerotic plaques are characterized by the presence of macrophages, and to a lesser extent, T-lymphocytes, at the immediate site of either plaque rupture or superficial erosion; moreover, the rupture-related inflammatory cells are activated, indicating ongoing inflammation at the site of plaque disruption. These observations are confirmed by clinical studies demonstrating activated circulating neutrophils, lymphocytes and monocytes, and increased concentrations of pro-inflammatory cytokines, such as interleukin (IL)-1 and 6, and of acute phase reactants in patients with unstable angina and myocardial infarction.

A role for inflammation in unstable angina is suggested by histologic studies of unstable coronary plaques, evidence of the systemic release of thromboxanes and leukotrienes, and the presence of activated circulating leukocytes. Furthermore, increased concentrations of plasma C-reactive protein, the prototypal acute-phase reactant, have been reported in some patients with unstable angina, in patients with coronary artery disease and other types of angina, and in 20 percent of patients who have an acute myocardial infarction within six hours after the onset of symptoms, before any elevation of myocardial enzyme levels in serum. The acute-phase reactants are very sensitive, although nonspecific, markers of inflammation. Acute-phase response observed in unstable angina patients may be a primary component of instability because it is not due to myocardial cell necrosis, since it is unrelated to the elevation of troponin, to ischemia because it is normal in patients with severe variant angina, or to activation of the hemostatic system because it does not increase after its activation. The levels may remain elevated for months after waning of the symptoms. In patients with severe unstable angina, elevated plasma levels of C-
reactive protein (CRP) are associated with an unfavorable short-term prognosis.\textsuperscript{23}

A long-term predictive value of elevated CRP levels was found in patients with documented coronary artery disease and angina\textsuperscript{24,25} and in individuals with multiple risk factors.\textsuperscript{26} Moreover, in the Physicians' Health Study, among low-risk individuals, high-sensitivity (hs)-CRP levels within the normal range were linearly related to the incidence of myocardial infarction over a follow-up period of 8 years.\textsuperscript{27}

The risk of plaque rupture depends more on the number and activation status of macrophages, the principal inflammatory cells in atherosclerotic plaques, than on plaque size.\textsuperscript{28} The mechanisms that relate the level of acute-phase proteins to short- and long-term prognoses in acute coronary syndromes are unclear. The aim of this investigation was to determine whether “active” coronary plaque disruption could be detected by a systemically measurable inflammatory response reflected by CRP levels in unstable angina and acute myocardial infarction. Furthermore, temporal variations in plasma levels of CRP were also examined to investigate whether ischemia-reperfusion injury causes this acute-phase response.

We compared hs-CRP levels in patients with unstable angina (UA)\textsuperscript{29} or acute myocardial infarction (AMI) with those in patients with stable coronary artery disease (CAD). A commercially available enzyme-linked immunoassay method was used. Additionally, we examined whether CRP levels increase during UA or AMI, thus serving as a marker for inflammation, and whether this variable is correlated with noninvasive indexes for the extent of myocardial necrosis, that is, creatine kinase (CK), CK-MB isoenzyme (CK-MB), or myocardial tropinin I (TnI).\textsuperscript{30}

\section*{Materials and Methods}

\textbf{Patients:} We studied 56 patients. Group 1 was comprised of 25 consecutive patients (18 men, 7 women; mean age, 68.5\pm14.3 years, range, 40-86) admitted to our coronary care unit with unstable angina (UA; \( n = 14 \)) or acute myocardial infarction (AMI; \( n = 11 \)). Inclusion criteria were typical chest pain and either ST-segment elevation \( \geq 0.1 \) mV in at least two contiguous electrocardiographic leads in patients with AMI and ST-segment depression \( \geq 0.1 \) mV in at least two contiguous electrocardiographic leads, elevated cardiac tropinin levels, or angina at rest following myocardial infarction within 2 weeks in patients with UA (defined according to Braunwald’s classification).\textsuperscript{29} In the subgroup of patients with AMI, we excluded patients with the usual thrombolytic contraindications, those with previous myocardial infarction at the same site and those with previous coronary
artery bypass surgery. Patients in group 1 were treated with front loaded recombinant tissue-type plasminogen activator \(^{31}\) (\(n=2\)), percutaneous coronary intervention [PCI] (\(n=16\); coronary stenting in 14 cases), or coronary artery bypass surgery (CABG; \(n=5\)). Two (8\%) patients were not suitable for coronary revascularisation. Additionally, they received co-medication like aspirin, heparin (low molecular-weight heparin in usual doses or unfractionated heparin according to activated partial thromboplastin time), analgesic drugs, diazepam, nitroglycerin (when systolic blood pressure was \(\geq 90\) mmHg) and glycoprotein(GP)-IIb-IIIa-receptor antagonists (\(n=16\)) as usual. Blood samples were taken initially after hospital admission (0 hours), and 12, 24, and 72 hours thereafter from a separate cannula in the forearm. Separation of the serum and analysis were performed immediately.

Group 2 was comprised of 31 consecutive patients (25 men, 6 women; mean age, 64±12.7; range, 47-83 years) with signs and symptoms of clinically stable CAD. Stable angina was defined as typical exertional chest pain relieved by rest, glyceryl trinitrate administration, or both, with positive responses to exercise ECG stress testing, abnormal myocardial perfusion scintigraphy or abnormal stress echocardiography. In all patients symptoms were stable for at least 10 weeks before study entry. None of the patients in this group had experienced a recent (<10 weeks) myocardial infarction, previous PCI, CABG, malignant arrhythmias, cardiac valve disease, apparent acute or chronic liver disease, renal failure, or apparent inflammatory disease. Blood samples were taken after a rest period of 12 hours in the morning before coronary angiography from a separate cannula in the forearm. Separation and analysis of the serum were performed immediately.

**Measurements and methods:** Serum CK and CK-MB isoenzyme were measured with an autoanalyzer (Hitachi 917, Roche, Germany) and troponin I was measured by enzyme immunoassay (OPUS, Behring, Marburg, Germany). Values >1.6 ng/mL were considered to be positive.\(^{32}\) CRP was assayed by rate nephelometry (Behring NA latex CRP; Behring, Germany).\(^{33}\)

**Statistical analysis:** Continuous variables between groups were analyzed by the unpaired \(t\) test or the Mann-Whitney rank sum test where appropriate in case of not normally distributed groups. In case of dichotomous variables, the chi-square test was used. Data within groups were compared with the Friedman test; differences between groups were analysed by the Student-Newman-Keuls test. Correlations were tested by the Spearman rank correlation coefficient,\(^{34}\) and linear regression analysis was performed. Differences were considered to be significant if the null hypothesis could be rejected with >95% confidence (\(P\) values <0.05 [two-tailed] were considered to indicate statistical significance). Results for normally distributed continuous variables are expressed as the mean (± SD) and con-
Continuous variables with non-normal distribution are presented as the median (± interquartile interval).

**RESULTS**

Comparisons among patient groups: The two patient groups were similar with respect to gender and age (Table I). A box plot graph of hs-CRP values is shown in the Figure. Patients with AMI/UA (after 72 hours) showed the highest CRP

| Table I. Characteristics of the Two Patients Groups (Group1 and Group2) |
|-----------------|-----------------|-----------------|
| **Group 1**     | **Group 2**     |                 |
| **Patients with AMI (n=11)** | **Patients with stable CAD (n=31)** |
| **Age (years)** | 68.5±14.3       | 64±12.7         |
| **Male/female** | 18/7            | 25/6            |
| **AMI site (anterior/inferior)** | 7/4            |                 |
| **Delay from onset of symptoms (min)** | 244±112        |                 |
| **rt-PA/PCI/CABG** | 2/16/5        |                 |
| **CK-MB (U/L) * | 107±84          |                 |
| **Troponin I positive (≥1.6 ng/mL)** | 18 (Patients) | (11 [all] with AMI and 7 with UA) |

* Mean ± standard deviation (SD) of the highest individual values. Other values are expressed as mean±SD or number of patients. AMI=acute myocardial infarction; CAD=coronary artery disease; CK-MB=creatine phosphokinase, MB isoenzyme; rt-PA=recombinant tissue-type plasminogen activator.

**Figure.** Box plot graph of baseline hs-CRP values in the two groups (values of patients with AMI/UA are baseline values measured at hospital admission). Values presented are median (center rule in the box) with 25th percentile (lower rule of box), and the 75th percentile (upper rule in box). *AMI=acute myocardial infarction; sCAD=stable coronary artery disease.
levels (20.25±10.61 mg/L; *P*<0.001 versus baseline levels in patients with AMI/UA (5.96±2.26) and *P*<0.001 versus baseline levels in patients with stable CAD (4.35±2.6). No significant difference was observed between baseline values in patients with AMI/UA and patients with stable CAD (*P*=0.12), but hs-CRP values tended to be higher in patients with AMI/UA.

**Patients in group 1 were classified into two subgroups:** Group 1a comprised 11 patients with AMI (8 men, 3 women; mean age, 65.4±11.2 [range, 40-78]). Group 1b comprised 14 patients (10 men, 4 women; mean age 69.5±15.5 [range, 44-86]) with unstable angina. Patients in subgroup 1a tended (not significant; NS) to have higher baseline hs-CRP values (6.49±2.28) than patients in group 1b (5.47±1.77). Baseline CRP levels in the subgroup of patients with AMI (group 1a; (6.49±2.28 mg/L) were significantly higher than levels in patients with stable CAD (4.35±2.6 mg/L; *P*=0.02).

Smoking status did not differ significantly between the two subgroups (7 smokers and 4 nonsmokers in group 1a, and 9 smokers and 5 nonsmokers in group 1b).

**AMI/UA group [group 1]:** Patients with AMI or UA were followed up over a 72-hour period. Within-group comparison showed significant differences over time (Friedman test, *P*<0.001; [Table II]). The lowest hs-CRP level (5.96±2.26) was observed at baseline and was significantly different from the level after 12, 24, and 72 hours (9.5±9.04; 18.25±11.02; 20.25±10.61; *P*<0.001). In group 1a patients, no correlation was found between CRP and the duration of symptoms before the first measured CRP values, CK or CK-MB as markers for the extent of myocardial infarction at any time during this observation period (hs-CRP versus duration of symptoms before first measured hs-CRP values, *r* = −0.188, *P*=NS.; hs-CRP versus maximal CK, *r* = −0.243, *P*=NS.; hs-CRP versus CK-MB, *r* = −0.208, *P*=NS). In group 1b patients the hs-CRP values were not significantly different between troponin I (TnI) positive and troponin I (TnI) negative individuals (6.23±2.82 in TnI positive [*n*=7] and 5.84±1.73 in TnI negative patients [*n*=7]; *P*=NS).

### Table II. Time Course of C-reactive Protein (CRP) Levels in 25 Patients with Acute Myocardial Infarction (AMI) or Unstable Angina (UA)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>12 h</th>
<th>24 h</th>
<th>72 h</th>
<th><em>P</em> for trend across time</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-reactive protein (mg/L)*</td>
<td>5.96±2.26</td>
<td>9.5±9.04</td>
<td>18.25±11.02</td>
<td>20.25±10.61</td>
<td>&lt;0.01</td>
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</table>

*Mean ± standard deviation (SD)
DISCUSSION

The results of the present study show that CRP, a marker of inflammation, is significantly increased in patients with AMI or UA shortly after the onset of symptoms. Our data do not demonstrate significantly elevated CRP levels in patients with acute coronary syndromes at baseline compared with patients with chronic coronary artery disease, although CRP levels tended to be higher in patients with AMI/UA.

The results of the present study expand upon previous investigations\(^ {17,23}\) which showed that CRP is raised in patients with AMI and unstable angina shortly after the onset of symptoms compared to patients with stable CAD.

Our data do not support previous reports\(^ {17,23,35}\) which found significantly higher baseline levels of hs-CRP in patients with UA or AMI compared with levels in patients with stable CAD. The present results are consistent with the findings from Berk and colleagues\(^ {17}\) and from Liuzzo and colleagues\(^ {23}\) who reported that baseline hs-CRP concentrations were similar in patients with unstable angina and in those with chronic stable angina, suggesting that hs-CRP levels may be a valid prognostic marker, although it fails to differentiate patients with stable CAD from patients with acute coronary syndromes. A proportion close to 13% of patients with elevated hs-CRP was observed in chronic stable angina patients during the symptom-free periods.

A possible explanation for this finding is that because the affected coronary vessels are small, the total number of activated macrophages involved in unstable coronary plaques is too small to be detected by increased peripheral serum hs-CRP concentrations.

In patients with UA or AMI, the most fatal consequence of plaque rupture, CRP levels changed significantly within 72 hours (Table II). The CRP values shortly after admission to the coronary care unit in patients with UA or AMI were significantly higher than the baseline levels in this patient group and baseline levels in patients with stable CAD. The lowest level was observed at baseline and was significantly different from the levels measured during the following 72 hours. The CRP increase reflects the pronounced activation of inflammation as a cause and a consequence of plaque instability. The CRP increase shortly after admission from AMI/UA observed in our study corresponds with the increase in the white blood cell count\(^ {36}\) and the increase in serum neopterin levels\(^ {37}\) immediately after myocardial infarction. It may also be that the late rise in CRP is partly an indirect consequence of reperfusion or revascularisation therapy.\(^ {38}\)

Our results, together with the evidence of an inflammatory component documented in previous studies,\(^ {7-15,17}\) have important pathophysiologic implications regarding acute-phase response and C-reactive protein levels in patients with
acute coronary syndromes. However, it is not known whether the elevated levels of acute-phase proteins are related to the type of inflammatory stimuli or to the intensity of the individual response. It is also not known whether the stimuli triggering the production of acute-phase proteins arise from the heart or other parts of the body. Ischemia-induced endothelial damage, oxidized low-density lipoprotein, immune complexes, and reactivation of dormant cytomegalovirus or chlamydia infection are all potential causes of vascular injury and an acute-phase response. In addition to their practical clinical importance, the present observations point to new avenues of investigation into the causes of unstable angina and myocardial infarction.

Thus, accumulating evidence suggests that inflammation may cause local endothelial activation, and possibly plaque fissure, leading to unstable angina and infarction. Although no information is yet available on the causes of the inflammation or its localization, these novel lines of research may open the way to a different approach to the patient with acute coronary syndromes. Increased concentrations of hs-CRP, a sensitive marker of inflammation, have been reported in patients with unstable angina. It is well known that myocardial necrosis is an established cause of the acute-phase response. Thus, in addition to plaque rupture, acute phase markers of inflammation may also be elevated because of the presence of necrotic myocardial cells or due to reperfusion injury caused by abrupt closure of the infarct related artery and by initiation of thrombolysis or revascularisation procedure. In patients with AMI, no correlation was found between CRP and the duration of symptoms before the first measured hs-CRP values and CK or CK-MB as markers for the extent of myocardial infarction at any time during this observaton period. Additionally, in patients with unstable angina, CRP values were not significantly different between troponin I (TnI) positive and troponin I (TnI) negative individuals. Therefore, CRP is not a marker for the extent of myocardial damage but indicates inflammation associated with myocardial damage.

In a rat model of myocardial infarction and reperfusion, an early increase in tumor necrosis factor (TNF)-alpha messenger ribonucleic acid (m-RNA) expression in rat hearts with induced myocardial infarction with and without reperfusion has been found. These data support the hypothesis that cytokine gene expression is primarily induced in myocardial cells in response to ischemia. Increased secretion of TNF-alpha in the peripheral blood was found in patients with acute transmural myocardial infarction with a peak of about 24 hours after initiation of reperfusion therapy, reflecting an inflammatory response following ischemia and reperfusion. CRP concentrations in patients with AMI are most probably the result of immune activation related to the atherogenic process, the inflammatory mechanisms that lead to acute coronary events, and the inflamma-
tory response associated with the presence of necrotic myocardial cells in the postischemic or reperfused myocardium. CRP is known to be a marker of stimulation of the cellular immune system.

Plasma levels of CRP start to rise about 6 hours after an acute stimulus, reaching a peak within about 48 hours, and with abrupt cessation of the stimulus, the values then decrease exponentially at a rate close to the measured plasma half-life of CRP of about 19 hours. Thus, after ischemia-reperfusion triggering an acute-phase response, the peak values of CRP are observed after 48 to 72 hours. Our finding, that in the small subgroup of patients with AMI baseline CRP levels were significantly higher than the levels in patients with stable CAD, supports this concept.

The acute-phase response of C-reactive protein is a nonspecific phenomenon reflecting cytokine-mediated hepatic production triggered by most forms of inflammation, infection, and tissue injury. Our patients were carefully selected to eliminate intercurrent disorders likely to be associated with an acute-phase response, and similar attention to intercurrent processes will be essential for the practical application of our findings.

The results of our study confirm the observation that the plasma concentration of C-reactive protein is elevated shortly after admission in the majority of patients with unstable angina, and we also found that C-reactive protein is elevated in the time course after hospital admission in patients with myocardial infarction.

The acute-phase reaction cannot be attributed simply to the disruption of particularly “active” coronary plaques. The magnitude of the acute-phase response is determined to a greater extent by the individual responsiveness than by the type of provocative stimuli.

Experimental studies have shown that periods of ischemia as short as 15 minutes followed by reperfusion elicit a cascade of proinflammatory reactions that include production of oxygen-derived free radicals, activation of the complement system, adherence of neutrophils to the coronary endothelium, leukocyte-mediated injury of the myocardial cells, and production of cytokines, including interleukin (IL)-6 and IL-1, which are the major determinants of acute-phase protein production. In patients, neutrophil activation with signs of endothelial injury and release of proinflammatory cytokines have been demonstrated in acute myocardial infarction and after coronary angioplasty. Furthermore, in unstable angina patients a significantly increased urinary concentration of leukotriene E4 was observed immediately after ischemia compared with 2 days later. Melchiar and colleagues found an increase in urinary neopterin during the first week after myocardial infarction. CRP represents a more practical clini-
cal marker of inflammation than IL-6, the major determinant of their production\textsuperscript{59} because of its much shorter half-life (4 hours).\textsuperscript{60}

Our finding that CRP levels increase in patients with AMI/UA shortly after the onset of symptoms supports previous reports, which have shown that acute coronary syndromes are associated with inflammatory mechanisms.\textsuperscript{61,62} The results of the present study suggest that Hs-CRP levels fail to differentiate patients with stable CAD from patients with acute coronary syndromes.

**Limitations of the Study:** Hs-CRP levels may be a valid prognostic marker but fail to differentiate patients with stable CAD from patients with acute coronary syndromes. We did not investigate the prognostic value of hs-CRP measurement in this study.

Hs-CRP levels tended to be higher in patients with AMI/UA compared with patients with stable coronary artery disease. We cannot rule out the possibility that the detected difference could reach statistical significance when much larger patient groups are included in future investigations.

CRP concentrations in patients with AMI are most probably the result of immune activation related to the atherogenic process, the inflammatory mechanisms that lead to acute coronary events, and the inflammatory response associated with the presence of necrotic myocardial cells in the postischemic or reperfused myocardium. The contribution of each of these factors needs to be determined in future trials.

The results of our study should be confirmed by measurements of other sensitive acute-phase reactants, for example serum amyloid A protein. Indeed, serum amyloid A protein may be more useful in routine practice than C-reactive protein, because it has an even wider dynamic range and because most of the commercially available automated assays for C-reactive protein are not sufficiently precise in the low range, as compared with the assay for serum amyloid A protein.

**Conclusions:** The present data suggest an activation of the inflammatory system in the initial time course in patients with AMI or UA, reflected by increased CRP levels, and extend prior observations concerning acute coronary syndromes to be associated with inflammatory mechanisms.\textsuperscript{15,17,61,62} The chronic inflammatory process during the period of stable atherosclerotic disease and in the initial period of plaque instability with only a few inflammatory cells involved, induces CRP production that reaches levels not high enough to detect statistically significant differences in baseline hs-CRP values between patients with stable CAD and acute coronary syndromes. The increase in CRP levels shortly after admission in patients with AMI/UA seems to express the result of immune activation related to the atherogenic process, the inflammatory mechanisms that lead to acute coronary events, and the inflammatory response associated with myocardial injury and myocardial necrosis and reflects an inflammation-mediated process.
reaction could be affected by other proinflammatory cytokines and possibly by therapeutic interventions. CRP does not represent a marker for the extent of myocardial damage but indicates inflammation associated with myocardial damage.

Baseline hs-CRP may be a valid prognostic marker but is not suitable for distinguishing between patients with stable CAD and patients with acute coronary syndromes. It reflects an activation of the inflammatory system in the initial time course in patients with AMI or UA.

REFERENCES


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