Coronary Spasm is Associated With Chronic Low-Grade Inflammation

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Background  Coronary spasm plays an important role in the pathogenesis of ischemic heart disease and it may be associated with low-grade inflammation.

Methods and Results  Intracoronary injection of acetylcholine was done in 199 patients (99 men, 100 women, mean age, 64.5±11.0 years) with chest pain and normal coronary angiograms. According to the results of the provocation test, the study subjects were divided into 2 groups: the spasm group of 112 patients and the non-spasm group of 87 patients. Clinical data including high-sensitivity C-reactive protein (hs-CRP) and other coronary risk factors were compared between the 2 groups. Serum levels of hs-CRP were significantly higher in the spasm group than in the non-spasm group (median: 1.2 mg/L vs 0.5 mg/L, p<0.005). Multivariate analysis showed that hs-CRP and smoking history were independently associated with coronary spasm with an odds ratio of 2.28 (p=0.027) and 2.25 (p=0.037), respectively, with a hs-CRP value of ≥2 mg/L as cutoff point.

Conclusions  Minor elevations of the serum hs-CRP level are significantly associated with coronary spasm, suggesting that chronic low-grade inflammation may be involved in the pathogenesis of coronary spasm.  (Circ J 2007; 71: 1074–1078)

Key Words:  Coronary spasm; C-reactive protein; Endothelial dysfunction; Inflammation; Smoking

C oronary (artery) spasm plays an important role in the pathogenesis of not only variant angina, but also ischemic heart disease in general, including effort angina, unstable angina, acute myocardial infarction and sudden death. However, the precise mechanism underlying coronary spasm still remains unknown. We have shown that endothelial dysfunction is involved in the pathogenesis of coronary spasm and that endothelial nitric oxide (NO) activity is reduced in the spastic coronary arteries. There is accumulating evidence that inflammation is involved in endothelial dysfunction and atherothrombosis, but it is not yet clear whether inflammation is also involved in the pathogenesis of coronary spasm.

C-reactive protein (CRP) is an exquisitely sensitive systemic marker of inflammation and tissue damage and the recent development of high-sensitivity CRP (hs-CRP) revealed that increased CRP values, even within the range previously considered normal, strongly predict coronary events in apparently healthy individuals and in patients with established coronary artery disease. A previous study reported that the levels of serum CRP were within normal range in patients with variant angina, whereas the levels were elevated in unstable angina. However, a more recent and larger study reported that the levels were substantially elevated in patients with coronary spasm as compared with those without.

Thus, it is not yet clear whether inflammation is involved in the pathogenesis of coronary spasm. The present study was designed to determine whether serum CRP levels, as well as those of other coronary risk factors, are associated with coronary spasm.

Methods

Study Subjects  One hundred and ninety-nine patients (99 men, 100 women; mean age 64.5±11.0 years) consecutively referred for diagnostic catheterization for evaluation of chest pain and/or ECG abnormalities and who had normal coronary angiograms were the subjects of this study. All patients revealed normal wall motion and left ventricular ejection fraction. Patients with acute coronary syndrome defined as chest pain associated with ischemic ST changes (>0.1 mV) and/or elevated concentration of creatine kinase-MB, or troponin, congestive heart failure, severe hypertension (>160/110 mmHg), valvular heart disease, malignant diseases or endocrine, hepatic, or inflammatory disease were excluded. Also, patients with elevated white blood cell counts (>9,000) and/or serum CRP (>10 mg/L) were excluded to avoid the possible confounding effects of occult infection or other systemic inflammatory diseases on CRP levels. None of the patients had had more than 2 episodes of chest pain within the past 48 h. Vasoactive drugs, including Ca-antagonists, nitrates, ACE inhibitors, angiotensin-receptor blockers, and nitrates were withdrawn for at least 5 days before the study, except sublingual nitroglycerin, which was withdrawn within 24 h of the study. None of the patients was on hormonal replacement therapy. Risk factors assessed included hypertension (>140/90 mmHg), history of smoking (>2 pack-years), hypercholesterolemia, hypertriglyceridemia, low HDL-cholesterol, diabetes mellitus,
body mass index, and uric acid, and previous history of cardiovascular disease. In all of the study subjects, intracoronary infusion of acetylcholine (ACh) was done to determine the occurrence of coronary spasm. Coronary spasm was defined as an abnormal contraction of an epicardial coronary artery associated with ischemic ECG changes (ST depression >0.1 mV or ST elevation >0.2 mV in more than 2 leads), which resolved promptly with intracoronary injection of nitroglycerin. The details of these methods have been previously reported. Coronary spasm was induced in 112 of the patients (designated as the spasm group) and not induced in the remaining 87 patients (non-spasm group). The clinical data, including the serum levels of hs-CRP and other coronary risk factors, were compared between the 2 groups. The protocol of this study was approved by the institutional ethics committee and written informed consent was given by each patient.

### Laboratory Methods

Fasting blood samples were drawn by venipuncture 1–2 days before coronary angiography for hematological and biochemical analysis using standard laboratory procedures. Serum hs-CRP was measured in duplicate by automated immunoturbidimetric assay using the Synchron LX20 Pro system (Beckman/Coulter). The lower limit of this assay was 0.2 mg/L, with intra- and inter-assay coefficients of variation of <5.0% at 0.25 mg/L of CRP.

### Statistical Analysis

As CRP values do not follow a normal distribution, the Mann-Whitney test and the Kruskal-Wallis test were used for comparison between groups. The remaining continuous variables were compared using unpaired t-test and non-continuous variables with the chi-square test. CRP values were expressed as median and range, and the remaining variables were expressed as mean ± SD or proportion (%). Logistic regression analysis was used to identify the independent risk factors for coronary spasm. All variables with a p-value of <0.05 were entered into multivariate analysis and were considered statistically significant.

### Results

Clinical data for the 2 groups are presented in Table 1. The serum hs-CRP levels were significantly higher in the spasm group than in the non-spasm group, using either the continuous values or proportional values with a cutoff point

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**Table 1 Clinical Characteristics of the Study Subjects**

<table>
<thead>
<tr>
<th></th>
<th>Non-spasm group (n=87)</th>
<th>Spasm group (n=112)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>64.4±1.2</td>
<td>64.5±1.0</td>
<td>0.9265</td>
</tr>
<tr>
<td>Men</td>
<td>34/87 (39%)</td>
<td>65/112 (58%)</td>
<td>0.0080</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.1±0.4</td>
<td>24.3±0.4</td>
<td>0.1470</td>
</tr>
<tr>
<td>Hypertension</td>
<td>52/86 (60%)</td>
<td>52/111 (47%)</td>
<td>0.0576</td>
</tr>
<tr>
<td>DM</td>
<td>14/86 (16%)</td>
<td>21/111 (19%)</td>
<td>0.6307</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>197.4±3.4</td>
<td>195.0±3.7</td>
<td>0.6418</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>110.6±2.9</td>
<td>108.7±3.2</td>
<td>0.6562</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>58.7±1.5</td>
<td>57.7±1.4</td>
<td>0.6580</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>142.1±11.6</td>
<td>150.2±10.7</td>
<td>0.6103</td>
</tr>
<tr>
<td>WBC count (×10⁹/μl)</td>
<td>5,836.8±187.1</td>
<td>6,300.0±203.8</td>
<td>0.1045</td>
</tr>
<tr>
<td>RBC count (×10¹²/μl)</td>
<td>418.6±5.2</td>
<td>425.8±4.4</td>
<td>0.2900</td>
</tr>
<tr>
<td>Platelet count</td>
<td>22.7±0.6</td>
<td>24.1±0.8</td>
<td>0.1676</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.9±0.03</td>
<td>3.85±0.04</td>
<td>0.4986</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>5.2±0.1</td>
<td>5.8±0.1</td>
<td>0.0056</td>
</tr>
<tr>
<td>BNP (pg/dl)</td>
<td>56.9±11.2</td>
<td>84.0±14.3</td>
<td>0.1658</td>
</tr>
<tr>
<td>hs-CRP (mg/L)</td>
<td>0.5 (0.2–7.8)</td>
<td>1.2 (0.2–9.0)</td>
<td>0.0005</td>
</tr>
<tr>
<td>2≤ (mg/L)</td>
<td>14/87 (16.1%)</td>
<td>41/112 (36.6%)</td>
<td>0.0013</td>
</tr>
<tr>
<td>3≤ (mg/L)</td>
<td>9/87 (10.3%)</td>
<td>23/112 (20.5%)</td>
<td>0.0522</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>74.0±0.9</td>
<td>72.5±0.9</td>
<td>0.2617</td>
</tr>
</tbody>
</table>

BMI, body mass index; DM, diabetes mellitus; TC, total cholesterol; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; TG, triglyceride; WBC, white blood cell; RBC, red blood cell; BNP, B-type natriuretic peptide; hs-CRP, high-sensitivity C-reactive protein; LVEF, left ventricular ejection fraction.

**Table 2 Multivariate Analysis of Variables Associated With Coronary Spasm**

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>1.39 (0.70–2.78)</td>
<td>0.348</td>
</tr>
<tr>
<td>Uric acid</td>
<td>1.19 (0.95–1.51)</td>
<td>0.137</td>
</tr>
<tr>
<td>Smoker</td>
<td>2.25 (1.05–4.84)</td>
<td>0.037</td>
</tr>
<tr>
<td>hs-CRP ≥2 mg/L</td>
<td>2.28 (1.10–4.74)</td>
<td>0.027</td>
</tr>
</tbody>
</table>

OR, odds ratio; CI, confidence interval. Other abbreviation see in Table 1.
of 2.0 mg/L, but the difference did not reach the significant level (p=0.052) with a cutoff point of 3.0 mg/L (Table 1, Fig 1). The prevalence of men, smoking history, and serum levels of uric acid were also higher in the spasm group than in the non-spasm group (Table 1). Multivariate analysis showed that serum hs-CRP, as well as smoking history, was significantly and independently associated with coronary spasm with an odds ratio of 2.28 (p=0.027) and 2.25 (p=0.037), respectively, when the cutoff point of hs-CRP >2.0 mg/L was used (Table 2). Of the 112 patients in the spasm group, 31 (27.7%) had chest pain within the past week and 40 (35.7%) within the past month before the study, while the remaining 35 (31.3%) patients had no chest pain for more than 1 month at the time of the study. However, there were no significant differences in the levels of hs-CRP among the time intervals from chest pain to the study time; that is, <1 week, >1 week, <1 month, and >1 month, (median value: 1.1, 0.9, and 1.9 mg/L, respectively, p=0.3883). Coronary spasm induced by the infusion of ACh was associated with ischemic chest pain in 89 (79.5%) of the patients, but was silent or asymptomatic in the remaining 23 (20.5%) patients.

Discussion

The present study shows that plasma levels of hs-CRP, a highly sensitive marker of inflammation, are higher in patients with coronary spasm than in those without, although the values in both groups were within the range previously considered normal.13,14 The incidences of a history of smoking, male gender, and elevated serum uric acid levels were also higher in the patients with coronary spasm than in those without, in agreement with the results of previous studies13,14. There were, however, no differences between the 2 groups in the other coronary risk factors including age, lipid profiles, hypertension, body mass index, diabetes mellitus, and previous cardiovascular history.

The multivariate analysis revealed that only a history of smoking and elevated CRP with a cutoff point of 2 mg/L15,16 were independently and significantly associated with coronary spasm. However, the association of CRP levels with coronary spasm did not reach the significant level when the cutoff point of 3 mg/L was used, as recommended by the American Heart Association/the Centers for Disease Control and Prevention7 reflecting the fact that most of the CRP levels are within the normal range in patients with coronary spasm. Smoking is a well-known risk factor for coronary spasm13,14 and we have shown that it was strongly associated with markers of inflammation, including CRP.17 In the present study also the hs-CRP levels tended to be higher in the smokers than in the non-smokers. Thus, even though smoking and high hs-CRP levels were independent correlates of coronary spasm, higher hs-CRP levels in smokers could partly explain why smoking is related to coronary spasm.

A previous study reported that the serum CRP levels were normal in patients with variant angina, whereas they were elevated in those with unstable angina.2 However, the number of the patients was small and comparison was not made with the group without demonstrable coronary spasm in that study. In contrast, a more recent study of a larger number of patient reported that serum levels of hs-CRP were highly significantly elevated in patients with coronary spasm as compared with those without.18 However, 27% of the patients had CRP levels above the range considered normal (>10 mg/L) and had increased WBC counts as well. Thus, it is possible that a number of those patients may have had overt inflammation or tissue damage or unstable angina and the high CRP levels could have been a consequence of unstable angina. In the present study, patients with CRP levels >10 mg/L were excluded from the study, none of the study patients had more than 2 episodes of chest pain within 48 h of CRP measurement, and most (70%) of them were free from chest pain for more than 1 week prior, although it must be kept in mind that spontaneous coronary spasm is more often silent than symptomatic!

CRP was formerly considered solely as a biomarker for inflammation5,7 but with the advent of high-sensitivity assays, a number of studies have demonstrated that minor elevations of baseline CRP levels are associated with the risk of developing cardiovascular disease in apparently healthy populations and adverse outcomes in patients with established coronary artery disease.6–8 The present study adds that minor elevations of serum CRP levels within the normal range are also associated with coronary spasm in patients with normal coronary angiograms, suggesting that chronic low-grade inflammation is involved in the genesis of coronary spasm. Indeed, we and others have shown that coronary spasm is associated with elevation of other inflammatory markers, including adhesion molecules such as soluble P-selection and intercellular adhesion molecule-1, activation of A2 phospholipase and T-cells18–21. We have also shown that angiographically normal coronary arteries involved in spasm have diffuse thickening of the intima on intravascular ultrasound scanning.22 Indeed, coronary arteries in patients with coronary spasm show neointimal hyperplasia with infiltration by inflammatory cells on histological examination.23

The underlying mechanisms linking chronic low-grade inflammation to coronary spasm remain to be elucidated. We have shown that endothelial dysfunction is involved in the pathogenesis of coronary spasm and that endothelial NO activity is reduced in the spastic coronary arteries.12,14 Recent evidence reveals that chronic inflammation suppresses endothelial NO activity and impairs endothelial function, and thereby leads to atherothrombosis.3,5,24–26 Therefore, chronic low-grade inflammation may be involved in the pathogenesis of coronary spasm and may reflect reduced endothelial NO activity and impaired endothelial function.

Emerging evidence indicates that hypercontraction of vascular smooth muscle is caused by enhanced Ca2+ sensitization through the activation of the RhoA/Rho-kinase (ROCK) pathway27–30 and that inflammatory cytokines, such as tumor necrosis factor-Î² and interleukin-1 Î², enhance the expression and activity of RhoA.29,31 Indeed, Shimokawa's group has developed a swine model of coronary spasm by chronic application of IL-1 Î² to a segment of coronary artery, thereby activating ROCK pathway.32 It has also been shown recently that the activity of the NO/cGMP pathway reduces Ca2+ sensitization by suppressing RhoA activity.35,36 It is therefore quite reasonable to hypothesize that chronic low grade inflammation may cause coronary spasm by enhancing Ca2+ sensitization in the vascular smooth muscle through activation of the ROCK pathway. The plasma levels of inflammatory cytokines have a circadian variation, with the peak occurring from midnight to early morning37 and it is to be noted that the incidence of coronary spasm also has a circadian variation with a peak occurring from midnight to early morning corresponding to that of inflammatory cytokines.
There are recent reports that CRP suppresses endothelial NO activity and activates RhoA signaling. Suppression of CRP with statins was shown to be independently associated with less adverse outcomes in patients with coronary heart disease and a specific inhibitor of human CRP was recently reported to reduce myocardial infarction size in rats. These findings may support the hypothesis that CRP is a modifiable risk factor in coronary artery disease, including coronary spasm. However, conflicting evidence still remains and much more work is needed before CRP can become a target for treatment.

**Study Limitations**

Serum hs-CRP levels were not measured repeatedly in all the patients to examine whether the levels varied over time for each patient in this study. Coronary spasm tends to be cyclic and hs-CRP levels might be higher at times when spas tic episodes are frequent. However, there were no differences in the hs-CRP levels between those who had spontaneous chest pain within 1 week of the study and those who were free of chest pain for more than 1 week, although it must be recognized that spontaneous coronary spasm is more often silent than symptomatic and the absence of chest pain does not necessarily mean the absence of coronary spasm. This is in line with the findings of previous workers who reported that CRP levels were unrelated to the number and duration of ischemic events in patients with variant angina. A number of study shows that baseline serum CRP levels are stable, independent on food intake, and have no diurnal or seasonal variation for each individual subject.

**Conclusions**

The present study shows that minor elevations of serum CRP levels are significantly and independently associated with coronary spasm in patients with normal coronary angiograms, suggesting that chronic low-grade inflammation may be involved in the pathogenesis of coronary spasm. Minor elevation of hs-CRP levels may thus be a useful risk marker for coronary spasm in patients with normal coronary angiograms.

**Acknowledgments**

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**References**

29. Somlyo P, Somlyo AV. Calcium sensitivity of smooth muscle and nonmuscle myosin II: Modulated by G proteins, kinases, and myosin


41. Scirica BM, Morrow DA. Is C-reactive protein an innocent bystander or proatherogenic culprit?: The verdict is still out. Circulation 2006; 113: 2128–2134.