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Effect of *Chlamydia pneumoniae* Infection on Coronary Flow Reserve and Intimal Hyperplasia After Stent Implantation in Patients With Angina Pectoris

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Abstract

Objectives. *Chlamydia pneumoniae*(*C. pneumoniae*)has been detected in tissue from coronary atherosclerotic vascular lesions and may be involved in the pathogenesis of atherosclerosis. However, the effect of prior *C. pneumoniae* infection on coronary intimal hyperplasia after stent implantation and on coronary microvascular function is unknown.

Methods. Seventy-three patients with stable angina pectoris and a single *de novo* coronary lesion were studied prospectively. All patients underwent successful coronary angioplasty and stent implantation for the stenotic lesion. Blood samples were tested for prior *C. pneumoniae* infection before the procedure, and patients were divided into two groups: Seropositive and seronegative. Coronary flow reserve was measured in the non-stenotic coronary vessel before angioplasty, and quantitative coronary arteriography was performed at the stent implantation site before angioplasty and 6 months later in all patients.

Results. Coronary flow reserve in the non-stenotic vessel was significantly lower in the seropositive group than in the seronegative group(2.51 ± 0.35 vs 2.76 ± 0.43, $p < 0.05$). The minimum luminal diameter was smaller and late loss was greater in the seropositive group than in the seronegative group(minimum luminal diameter: 1.52 ± 0.59 vs 1.91 ± 0.79 mm, $p < 0.05$, late loss: 1.17 ± 0.55 vs 0.76 ± 0.67, $p < 0.05$). However, there was no significant difference in the restenosis rate or target lesion revascularization rate between the two groups.

Conclusions. Prior *C. pneumoniae* infection may accelerate intimal hyperplasia after stent implantation and impair coronary microvascular function in the non-stenotic coronary vessels.

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Key Words

■**Atherosclerosis** ■**Intravascular Doppler** ■**Restenosis** ■**Stent**
■**Microcirculation**(**coronary flow reserve**) ■**Infectious disease**(***Chlamydia pneumoniae***)

INTRODUCTION

Percutaneous transluminal coronary angioplasty (PTCA) is an important method for the treatment of coronary artery disease. However, stent implantation restenosis develops in approximately 30% of patients within 6 months of PTCA, mainly because of coronary intimal hyperplasia[1–3]. Several complex interactions between cellular and extracellular factors that could contribute to restenosis have been identified.

Previous studies have demonstrated the potential

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involvement of infectious agents in the pathogenesis of atherosclerosis and coronary artery disease. *Chlamydia pneumoniae* (*C. pneumoniae*), a human respiratory pathogen, has been detected in tissue specimens obtained from coronary atherosclerotic vascular lesions and may be a contributing pathogenic factor[4,5]. Recently, preliminary studies have examined the possible relationship between *C. pneumoniae* infection and restenosis after PTCA, although the results are controversial[6,7].

In addition to the large coronary conduit vessels, the coronary microvasculature is very important in the regulation of coronary artery blood flow. Coronary flow reserve, which reflects coronary microvascular function, is determined by measuring coronary flow velocity before and after the administration of coronary vasodilators. Coronary flow reserve provides important diagnostic information in a variety of cardiac diseases[8,9]. Recent clinical reports have demonstrated that coronary flow reserve is reduced in several diseases, including diabetes[10], hypertension[11] and hyperlipidemia[12], which are also risk factors for coronary atherosclerosis. However, there are no reports on the effects of prior *C. pneumoniae* infection on coronary microvascular function.

The present study investigated the effects of *C. pneumoniae* infection on coronary microvascular function and examined whether prior *C. pneumoniae* infection affects intimal hyperplasia after stent implantation in patients with angina pectoris.

SUBJECTS AND METHODS

Study population

Seventy-three patients (60 men and 13 women, mean age 65 ± 10 years) with stable angina pectoris and a single *de novo* coronary lesion were studied prospectively. All patients had single vessel disease with a stenotic lesion $\geq 75\%$ and no other significant stenoses. Patients with chronic total occlusion, acute myocardial infarction, old myocardial infarction, dilated or hypertrophic cardiomyopathy, or marked valvular disease were excluded. All patients undergoing PTCA and stent implantation were scheduled for follow-up angiography 6 months after the procedures. All patients gave written informed consent to a protocol approved by the Ethics Committee of the Showa General Hospital.

Coronary angioplasty and angiography

Coronary angioplasty and stent implantation were performed using a standard method. A balloon catheter of appropriate size was advanced over a guide wire through a 6F or 7F guiding catheter and positioned at the site of stenosis. After sufficient predilation, coronary stent implantation was performed in all patients. After stent implantation, 100 mg of aspirin and 200 mg of ticlopidine a day were given to all patients as antiplatelet therapy until follow-up angiography. Quantitative coronary angiography (QCA) data were analyzed using a computerized QCA system (QCA-CMS, Medical Imaging System Co., Ltd.) before and immediately after PTCA and stent implantation, and after a mean follow-up of 6 months. The minimum luminal diameter, reference diameter, and lesion length were measured, and the late loss was calculated as the minimum luminal diameter at follow-up minus the minimum luminal diameter immediately after stent implantation. The rate of restenosis ($>50\%$ stenosis) at the target lesion and the target lesion revascularization rate were also analyzed.

Coronary flow reserve measurement

Left and right coronary angiography were performed using a standard method after intracoronary injection of 150 to 200 μg of nitroglycerin. Before coronary angioplasty, a 0.014-inch Doppler flow wire (FloWire, Cardiometrics, Mountain View) was advanced into the coronary artery without the stenotic lesion through a guiding catheter. The flow velocity pattern was monitored on a video display. The coronary flow velocities were determined from single-frame images (Flomap, Cardiometrics, Mountain View). Doppler velocities were recorded under steady state conditions and coronary flow velocity measurements were obtained at baseline and at peak hyperemia after bolus intracoronary injection of adenosine 25 to 50 μg[13]. The coronary flow reserve was calculated as the ratio of hyperemic to baseline averaged peak velocity. The blood pressure, heart rate, and surface electrocardiography were continuously monitored.

Blood samples and laboratory analysis

Blood was taken under standardized conditions, and all laboratory determinations were performed in a blinded fashion. Specific antibodies against *C. pneumoniae* were identified by a microimmunofluorescence method. Blood samples were used to determine the *C. pneumoniae* IgG titer. Patients were divided into two groups, seropositive and

Table 1 Patient characteristics

	Seropositive group□ (*n*=47) □	Seronegative group□ (*n*=26) □	*p* value
Age (yr) □	63±10□	67±10□	NS□
Gender (male/female) □	39/8 □	21/5□	NS□
Hemodynamics□	□□	□□	□□
Heart rate (beats/min) □	77±12□	72±10□	NS□
Systolic blood pressure (mmHg) □	136±25 □	140±23 □	NS□
Diastolic blood pressure (mmHg) □	79±15□	82±19□	NS□
Mean blood pressure (mmHg) □	99±18□	104±18 □	NS□
Coronary risk factors□	□□	□□	□□
Hyperlipidemia□	18 (38) □	11 (42) □	NS□
Hypertension□	21 (44) □	11 (42) □	NS□
Diabetes mellitus□	17 (36) □	10 (38) □	NS□
Smoking□	15 (32) □	9 (35) □	NS□
Drug treatment□	□□	□□	□□
Nitrates□	44 (94) □	23 (88) □	NS□
ACE-inhibitor□	18 (38) □	8 (30) □	NS□
Calcium antagonist□	21 (44) □	9 (35) □	NS□
Lipid lowering agent	17 (36) □	9 (35) □	NS

Continuous values are mean±SD. ( ): %.□
Seropositive=positive *Chlamydia pneumoniae* titer; Seronegative=negative *Chlamydia pneumoniae* titer; ACE =angiotensin converting enzyme.

seronegative patients, based on the titer. Patients were considered seropositive when the IgG titer was ≥ 0.9.

Statistical analysis

Values in the two groups were compared by the unpaired *t*-test or the χ^2 test for categorical variables. All measurements are expressed as the mean $\pm$ SD, and a p value < 0.05 was considered statistically significant.

RESULTS

C. pneumoniae serostatus and patient characteristics

C. pneumoniae IgG serum antibody titer was positive in 47 patients (64%) and negative in 26 patients (36%). In the study population, seropositive and seronegative individuals showed similar basic characteristics, hemodynamic parameters, risk factors for coronary artery disease, and drug treatment (**Table 1**).

PTCA and QCA data

The angiographic findings, the calculated parameters at baseline, immediately after PTCA, and at the 6-month follow-up, the target vessel, lesion type, and stents used are listed in **Table 2**. There were no differences between the two groups with respect to the reference diameter, minimal luminal diameter, percentage diameter stenosis, and lesion length before angioplasty. There were no differences in the minimum luminal diameter and percentage diameter stenosis just after the stent implantation between the two groups. No differences between the two groups were found with respect to the target vessel, lesion type, or type of stent used. Although the left anterior descending artery was treated more frequently than the other two vessels and multi-link stents were used in most patients, there were no significant differences between the two groups.

Based on the follow-up QCA data, the minimum luminal diameter was smaller in the seropositive group than in the seronegative group (1.52 ± 0.59 vs 1.91 ± 0.79 mm, $p < 0.05$), and the late loss was greater in the seropositive group than in seronegative group (1.17 ± 0.55 vs 0.76 ± 0.67 mm, $p < 0.05$). However, there were no significant differences between the two groups with respect to the restenosis rate or the target lesion revascularize-

Table 2 PTCA and QCA findings

	Seropositive group (*n*=47)	Seronegative group (*n*=26)	*p* value
Before angioplasty			
Reference diameter (mm)	2.84±0.31	2.88±0.28	NS
MLD (mm)	0.72±0.22	0.70±0.29	NS
Diameter stenosis (%)	74.1±12.7	75.0±13.3	NS
Lesion length (mm)	6.9±4.4	8.0±5.1	NS
Post stent implantation			
MLD (mm)	2.79±0.25	2.81±0.40	NS
Diameter stenosis (%)	4.1±0.23	3.3±0.39	NS
Follow-up			
MLD (mm)	1.52±0.59	1.91±0.79	<0.05
Diameter stenosis (%)	41.5±18.0	34.0±20.6	NS
Late loss (mm)	1.17±0.55	0.76±0.67	<0.05
Restenosis rate (%)	12 (26%)	7 (27%)	NS
TLR (%)	9 (19%)	6 (23%)	NS
Target vessel			NS
LAD	29	18	
Cx	9	5	
RCA	9	3	
Lesion type (ACC/AHA)			NS
Type A	15	9	
Type B	27	14	
Type C	5	3	
Stent used			NS
GFX	1	2	
Multi-link	39	22	
NIR	7	2	

PTCA=percutaneous transluminal coronary angioplasty; QCA=quantitative coronary angiography; MLD=minimum luminal diameter; TLR=target lesion revascularization; LAD=left anterior descending artery; Cx=left circumflex artery; RCA=right coronary artery; ACC/AHA=American College of Cardiology/American Heart Association. Other abbreviations as in Table 1.

tion rate. No correlation was recognized between the *C. pneumoniae* IgG serum antibody titer and minimum luminal diameter or late loss.

Coronary flow data

The basal averaged peak velocity did not differ between the two groups, but coronary flow reserve was lower in the seropositive group (2.51±0.35 vs 2.76±0.43, $p<0.05$; **Table 3**).

DISCUSSION

The results of the present study indicate that *C. pneumoniae* infection might accelerate intimal hyperplasia after coronary stent implantation. We also found that *C. pneumoniae* infection might alter coronary microvascular function, as reflected by impaired coronary flow reserve.

Restenosis after coronary stent implantation is a serious complication due to its effect on secondary coronary morbidity. Despite considerable efforts, the results of various pharmacologic and interventional approaches to prevent restenosis after stent implantation have been unsatisfactory[1,2]. In the present study, *C. pneumoniae* infection was found not to be a risk factor for restenosis or indicator for target lesion revascularization, although the incidence of intimal hyperplasia represented by late loss was significantly greater in seropositive than in seronegative patients. The most likely reason for

Table 3 Coronary flow data

	Seropositive group (*n*=47)	Seronegative group (*n*=26)	*p* value
Baseline APV (cm/sec)	16.8±18.7	14.9±16.6	NS
Coronary flow reserve	2.51±0.35	2.76±0.43	<0.05

Values are mean±SD.
APV=averaged peak velocity. Other abbreviations as in Table 1.

this discrepancy is that the angiographic differences in the minimum luminal diameter and late loss between the two groups were so small. As a result, the restenosis rate and target lesion revascularization rate were not reflected by the minor luminal changes.

Preliminary studies have examined the relationship between *C. pneumoniae* infection and restenosis after coronary intervention, but the results are controversial[6,7]. *C. pneumoniae* was been detected in tissue specimens obtained from coronary atherosclerotic vascular lesions by coronary atherectomy and was implicated as a contributing pathogenic factor[4]. *C. pneumoniae* was detected in a larger number of specimens obtained from restenotic tissue than from primary lesions[14]. However, the difference did not reach statistical significance. Recent *in vitro* studies have indicated that *C. pneumoniae* can infect and reproduce in human endothelial cells, smooth muscle cells, and macrophages[15]. Based on these studies, *C. pneumoniae* infection may affect the intimal hyperplasia that occurs after stent implantation.

The present study indicated that *C. pneumoniae* infection might impair coronary microvascular function. Coronary flow reserve was significantly lower in seropositive patients than in seronegative patients. The baseline coronary flow velocity was equivalent in the two groups, so the mechanism responsible for the impairment of coronary flow reserve in *C. pneumoniae* seropositive patients might involve restricted diastolic function at the coronary microvascular level. Serologic evidence for *C. pneumoniae* infection precedes both the development of early and advanced atherosclerotic lesions[16], suggesting that *C. pneumoniae* infection might have an atherogenic effect on both the coronary conduit vessels and on the microvascular vessels. Based on these factors, *C. pneumoniae* seropositive patients might have a lower ischemic threshold than seronegative patients with coronary artery disease. Ongoing trials of pharmacologic therapy will determine whether anti-chlamydial antibiotics such as azithromycin can prevent the acceleration of atherosclerosis associated with *C. pneumoniae* infection[17]. In the near future, these antibiotics may reduce intimal hyperplasia after stent implantation and coronary microvascular dysfunction.

There are a few limitations to the present study. First, the study population was so small that the restenosis rate and target lesion revascularization rate could not be shown to be statistically different. Second, this study did not include a control group, so our results only permit comparisons between patients with or without demonstrated *C. pneumoniae* infection and with coronary artery disease. We could not determine the exact time when patients were infected with *C. pneumoniae*, so it is impossible to demonstrate that *C. pneumoniae* infection induced coronary atherosclerosis. To solve these problems, we must include patients with *C. pneumoniae* seropositive or seronegative but without coronary artery disease. Third, only *C. pneumoniae* IgG titers were measured in the present study, but we must examine other parameters, including *C. pneumoniae* IgA, IgM, and *C. pneumoniae* DNA in the future[18–20]. Fourth, intravascular ultrasonography is a more specific diagnostic method to evaluate intimal hyperplasia than coronary angiography. However, intravascular ultrasonography was not used in the follow-up examination of coronary vessels, so a further study is necessary to acquire more accurate data on the intimal hyperplasia after stent implantation.

CONCLUSIONS

Our data indicate that *C. pneumoniae* infection affects coronary artery intimal hyperplasia and impairs coronary microvascular function. These results may support the beneficial effect of antimicrobial therapy for the treatment of coronary artery disease.

要　　約

狭心症患者における冠動脈ステント留置後の冠血流予備能と内膜増殖に及ぼす*Chlamydia pneumoniae*感染の影響

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目　的: 冠動脈の動脈硬化病変組織に*Chlamydia pneumoniae*（*C. pneumoniae*）が病理学的に証明されて以来，*C. pneumoniae*感染が冠動脈硬化の一因との説がある．しかしながら，*C. pneumoniae*先行感染が冠動脈ステント留置後の内膜増殖反応，再狭窄率，さらに冠末梢動脈における微小循環に与える影響についての報告はない．そこで，*C. pneumoniae*感染の冠動脈ステント留置後の内膜増殖および冠血流予備能からみた冠末梢循環に与える影響について検討した．

方　法: 待期的に経皮的冠動脈形成術を施行し，ステントを留置した1枝病変の労作性狭心症73例を対象とし，採血上*C. pneumoniae*抗体陽性症例および抗体陰性の2群に分類した．両群においてDoppler flow wireを用い，非狭窄冠動脈枝の冠血流予備能を，また6ヵ月後のステント留置部位の冠動脈造影所見をそれぞれ比較検討した．

結　果: 非狭窄冠動脈枝の冠血流予備能は抗体陰性群に比べて，抗体陽性群で有意に低値であった（2.51±0.35 vs 2.76±0.43，$p<0.05$）．抗体陰性群に比べて，抗体陽性群では治療6ヵ月後のステント留置部の最小血管径は有意に小さく（1.52±0.59 vs 1.91±0.79 mm, $p<0.05$），晩期損失径は有意に大きかった（1.17±0.55 vs 0.76±0.67 mm，$p<0.05$）．しかしながら，両群間で再狭窄率，再血行再建術施行率に差は認められなかった．

結　語: *C. pneumoniae*の先行感染は冠末梢動脈の拡張機能障害作用を惹起し，ステント留置後の内膜増殖反応を促進させる．

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