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Original Article

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Chlamydia pneumoniae accompanied by inflammation is associated with the progression of atherosclerosis in CAPD patients: a prospective study for 3 years*

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Abstract

Background. The causes of accelerated atherosclerosis in end-stage renal disease (ESRD) patients are unknown, although recent studies have suggested that *Chlamydia pneumoniae* (Cp) infection and inflammation might be contributing factors. We aimed to evaluate the association of carotid atherosclerosis progression with Cp infection and inflammation in patients undergoing peritoneal dialysis (PD).

Methods. This is a prospective observational study. A total of 52 non-diabetic prevalent PD patients were included. The intima-media thickness of a common carotid artery (CCA-IMT) was measured at baseline and after 36 months by B-mode ultrasonography. Serum antibodies to Cp and inflammatory markers were obtained at the time of initial measurement of the CCA-IMT.

Results. CCA-IMT progressors (ΔCCA-IMT $\geq$ 0.015 mm/year) had a higher prevalence of seropositivity for Cp IgA antibody, a higher level of Cp IgA antibodies indices, log IL-(interleukin-)6, and intercellular adhesion molecule-1 (ICAM-1) compared to the non-progressors (ΔCCA-IMT $<$ 0.015 mm/year). On multivariate analysis, Cp IgA index and log IL-6 were independent risk a factors for CCA-IMT progression. Also, Cp IgA index had independent positive correlation with the magnitude of annual ΔCCA-IMT. Cp IgA antibody seropositive patients showed significantly higher mean annual ΔCCA-IMT than seronegative patients. Moreover, patients with both positive Cp IgA antibodies and elevated IL-6 above the median level showed higher ΔCCA-IMT than those with either factor alone.

Conclusions. Our data showed that Cp and inflammation were significant risk factors of CCA-IMT change in PD patients. This study strengthens evidence that Cp is involved in the pathogenesis of atherosclerosis and also suggests that the effect of Cp infection under high inflammatory status might be a risk factor for progression of atherosclerosis.

Keywords: atherosclerosis; *Chlamydia pneumoniae*; inflammation; intima-media thickness

Introduction

Although continuous advances in dialysis and renal transplantation have significantly decreased the mortality rate from end-stage renal disease (ESRD) patients undergoing dialysis still have a higher mortality rate than the general population, and cardiovascular disease is the main contributor to this higher mortality. This phenomenon cannot be simply explained by the traditional cardiovascular risk factors such as hypertension, diabetes mellitus, cigarette smoking and dyslipidaemia. Therefore, much attention has recently been paid to on the non-traditional risk factors, such as chronic inflammation and infection.

Chlamydia pneumoniae (Cp) has been the most implicated micro-organism associated with atherosclerosis. It has been proposed that the organism infects the vessel wall, induces inflammatory mediators and promotes vascular smooth muscle proliferation [1]. A previous study has reported an increased prevalence of Cp seropositivity in ESRD patients compared with the general population [2]. Several reports have described a relationship between Cp and atherosclerosis in patients on haemodialysis [2–4]. While it is still under debate, Cp infection may be a risk factor for cardiovascular mortality in patients on continuous ambulatory peritoneal dialysis (CAPD) [5,6]. However, there has been no prospective study to evaluate the effect of Cp on the progression of atherosclerosis or morphological change of artery in CAPD patients.

Inflammatory processes play an important role in the pathogenesis of atherosclerosis, and elevated serum markers of vascular wall inflammation, such as IL-(interleukin-)6, intercellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1), C-reactive

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protein (CRP) and high sensitivity CRP (hsCRP) have been shown to predict cardiovascular events.

The measurement of the intima-media thickness of common carotid artery (CCA-IMT) assessed by high-resolution duplex ultrasonography has been used as a marker for subclinical atherosclerosis and predicting future cardiovascular disease [7].

We undertook this prospective study to investigate a possible association of Cp infection and markers of inflammation with changes in CCA-IMT.

Subjects and methods

Study population

We initially performed baseline carotid duplex ultrasonography and biochemical analyses in 64 non-diabetic CAPD patients at a single dialysis unit of Severance hospital. We followed the patients for 36 months thereafter. During this period, 12 patients were excluded from the study, 5 were expired, 3 received kidney transplantation and 3 were switched to haemodialysis. Therefore, 52 (26 male, mean age 51.8 ± 10.8 years, range 32–74) out of 64 patients undertook follow-up carotid duplex ultrasonography after 36.0 ± 0.3 months of baseline ultrasonography. Causes of ESRD were chronic glomerulonephritis in 17 patients (32.7%), hypertension in 12 (23.1%), polycystic kidney disease in 2 (3.8%), and other (or unknown) causes in 21 (40.4%). Patients with diabetes mellitus, malignancy, autoimmune disease, general infection sign (fever, leukocytosis) and CAPD related infection (exit site infection, peritonitis) in the recent 6 months were excluded to avoid the potential confounding effects of these comorbid conditions. In addition, patients who had a history of coronary artery disease, cerebrovascular disease and/or peripheral vascular disease, and CAPD duration less than 6 months were not included. All patients received PD using a glucose-based lactate buffered solution. Informed consents were obtained from all study subjects.

Carotid ultrasonography

In all patients, both carotid arteries were examined with a duplex scanner (Toshiba, Tokyo, Japan) using a 7.5 MHz linear array transducer. All scans, both baseline and follow-up, were performed by the same trained sonographer.

Each subject was examined in the supine position in a semi-dark room. The far wall of the CCA-IMT, 0.5–1.0 cm proximal to the beginning of the carotid bulb, was used for measurements of CCA-IMT and lumen diameter. The CCA-IMT was defined as the distance between the leading edge of the lumen-intima echo and the leading edge of the media-adventitia echo. The lumen diameter was defined as the distance between the leading edge of the intima-lumen echo of the near wall and that of the lumen-intima echo of the far wall. The mean values of the CCA-IMT and lumen diameter were calculated from at least two measurements for each artery. A carotid plaque was defined as a localized intima-media thickening of greater than 1 mm with a minimum 100% increase in thickness compared with adjacent wall segments. The cross-sectional intima-media area (cIM area) of the artery was calculated using the following formula: $3.14 \times [(\text{lumen diameter}/2 + \text{CCA-IMT})^2 - (\text{lumen diameter}/2)^2]$ [8].

We measured the mean CCA-IMT at the onset and end of the study and evaluated the difference in the CCA-IMT over 36 months. For the assessment of factors that affect changes in CCA-IMT, the patients were divided into two groups, the progressors and non-progressors, based on the mean annual increment of CCA-IMT (ΔCCA-IMT, mm/year). Progressors were defined as those with a mean annual ΔCCA-IMT ≥ 0.015 mm/year, whereas non-progressors as those with a ΔCCA-IMT < 0.015 mm/year according to the result of the meta-analysis which estimated the mean annual change in CCA-IMT among control groups from 13 published randomized placebo-controlled studies [7].

Biochemical analyses

A blood sample was drawn after a 12-h fast on the same day of the baseline carotid ultrasonography examination. Haemoglobin (g/dL), haematocrit (%), creatinine (mg/dL), albumin (g/dL), total cholesterol (mg/dL), triglyceride (mg/dL), high-density lipoprotein (HDL)-cholesterol (mg/dL), calcium (mg/dL) and phosphate (mg/dL) were measured. The enzymatic method was used to measure total cholesterol and triglyceride. The results were analyzed using a Hitachi 736–40 automated analyzer. HDL-cholesterol was measured by selective inhibition and the direct enzymatic method (Daichii, Tokyo, Japan) and low-density lipoprotein (LDL)-cholesterol was estimated by the Friedewald formula: LDL-cholesterol (mg/dL) = total cholesterol (mg/dL) − HDL-cholesterol (mg/dL) − triglyceride (mg/dL) / 5. CRP, hsCRP and IL-6 were measured as indicators of chronic inflammation. hsCRP was measured by a BNII nephelometer analyzer (Dade Behring Inc., Newark, DE, USA). Serum IL-6 (Pierce endogen, Rockford, Italy), ICAM-1 and VCAM-1 (R&D system Europe Ltd, Abingdon, UK) levels were measured by enzyme-linked immunosorbent assay (ELISA).

IgA and IgG antibodies to Cp were measured by ELISA (Bioclone, Sydney, Australia). The index value for distinguishing between positive and negative samples was calculated from the formula

$$\text{Cut-off value (COV)} = (\text{mean negative control OD X K}) + 0.20$$

$$\text{Index value} = (\text{mean sample OD X K})/\text{COV}$$

where 'mean negative control OD' is the mean value of negative control serum absorbance at 405 nm in duplicate and *K* is the ratio of the positive serum sample value to the mean values of positive control serum absorbance at 405 nm.

Seropositivity of IgG and IgA to Cp was defined as an index value > 1.10. The sensitivity and specificity of Cp IgG and IgA by ELISA compared to microimmunofluorescence (MIF) assay were as follows: The sensitivity was 90.4% for detecting IgG and 84.6% for IgA, and the specificity was 89.9% for detecting IgG and 86.7% for IgA [9].

Statistical analysis

All the statistics were performed with SPSS (Windows release 12.0) for personal computers. *P*-values less than 0.05 were considered statistically significant. Data were expressed as mean ± standard deviation. Differences between the two groups were analyzed by the Student's *t*-test and the Chi-square test and among three or more groups by ANOVA. A multiple regression analysis was used to examine the relationship between atherosclerotic change and select variables.

Results

Demographic characteristics and biochemical data

Clinical and biochemical data from the study populations are shown in Table 1.

Changes in carotid duplex ultrasonography parameters

A significant increase in the mean values of CCA-IMT (0.74 ± 0.11 versus 0.79 ± 0.12 mm, $P < 0.001$), and the cIM area (19.99 ± 4.57 versus 22.66 ± 5.12 mm^2, $P < 0.001$) were noted between baseline and follow-up measurements. The mean annual ΔCCA-IMT was 0.015 ± 0.018 mm/year. The percentage of patients with carotid plaques did not change significantly (Table 2). To analyze factors that affect changes in CCA-IMT, patients were divided into two groups according to the change in CCA-IMT. In the progressors (annual ΔCCA-IMT ≥ 0.015 mm/year, $n = 31$), the mean CCA-IMT significantly increased from 0.74 ± 0.12 mm to 0.82 ± 0.12 mm ($P < 0.001$) and the mean cIM area significantly increased from 20.00 ± 4.84 mm^2 to 23.68 ± 5.06 mm^2 ($P < 0.001$). The proportion of patients with carotid plaque increased, but was not statistically significant (Table 3). The mean annual ΔCCA-IMT was significantly higher in the progressors than in the non-progressors (0.028 ± 0.010 versus −0.005 ± 0.001, $P < 0.01$).

Table 1. The initial clinical and biochemical characteristics of patients

Age (years)	51.8 ± 10.8 (32–74)
Sex (M:F)	26:26
CAPD duration (months)	58.6 ± 40.8 (7–151)
Body mass index (kg/m^2)	23.5 ± 3.2
Mean arterial pressure (mmHg)	103.7 ± 16.0
Hemoglobin (g/dL)	10.3 ± 1.4
Hematocrit (%)	30.5 ± 4.3
Creatinine (mg/dL)	11.3 ± 2.4
Albumin (g/dL)	3.7 ± 0.4
Calcium (mg/dL)	9.5 ± 0.8
Phosphorus (mg/dL)	5.1 ± 1.3
Calcium-Phosphorus product (mg^2/dL^2)	48.39 ± 12.85
Intact parathyroid hormone (pg/mL)	323.42 ± 301.34
Total cholesterol (mg/dL)	177.9 ± 29.2
Triglyceride (mg/dL)	145.7 ± 106.4
HDL-cholesterol (mg/dL)	50.0 ± 14.5
LDL-cholesterol (mg/dL)	103.8 ± 31.4
C-reactive protein (mg/dL)	0.35 ± 0.44
High sensitivity C-reactive protein (mg/L)	2.70 ± 4.93 (0.17–31.70)
Interleukin-6 (pg/mL)	27.46 ± 87.37 (0.3–428.5)
Log interleukin-6	0.65 ± 0.66 (−0.52–2.63)
Cp IgA antibody index	1.21 ± 0.57 (0.43–3.71)
Cp IgG antibody index	1.38 ± 0.89 (0.54–4.84)
Positive IgA to Cp, *n* (%)	24 (46.2%)
Positive IgG to Cp, *n* (%)	26 (50.0%)
Intercellular adhesion molecule-1 (ng/mL)	273.00 ± 92.99 (89.92–567.54)
Vascular cell adhesion molecule-1 (ng/mL)	1795.51 ± 311.78 (1292.12–2519.21)

Data are mean ± SD or mean ± SD (range). CAPD: continuous ambulatory peritoneal dialysis; HDL-cholesterol: high-density lipoprotein cholesterol; LDL-cholesterol: low-density lipoprotein cholesterol; Cp: *Chlamydia pneumoniae*.

Table 2. The carotid ultrasonographic parameters of patients over 3 years ($n = 52$)

	Baseline	Follow-up	*P*-value
CCA-IMT (mm)	0.74 ± 0.11	0.79 ± 0.12	<0.001
cIM area (mm^2)	19.99 ± 4.57	22.66 ± 5.12	<0.001
Presence of plaque			
Unilateral, *n* (%)	10 (19.2)	12 (23.1)	NS
Bilateral, *n* (%)	5 (9.6)	6 (11.5)	NS
Total, *n* (%)	15 (28.8)	18 (34.6)	NS

Data are mean ± SD. NS: not significant; IMT: intima-media thickness of common carotid artery; cIM area: calculated intima-media area.

Clinical and biochemical parameters in the CCA-IMT progressors and non-progressors

There were no significant differences in age, sex, smoking, body mass index, mean arterial pressure, haemoglobin, haematocrit, albumin, calcium, phosphorus, intact parathyroid hormone, total cholesterol and triglyceride level between the progressors and the non-progressors. However, the progressors had lower HDL-cholesterol levels than the non-progressors (41.5 ± 11.8 versus 50.1 ± 16.7 mg/dL, $P < 0.05$). LDL-cholesterol was higher in the progressors, but was not statistically significant ($P = 0.07$). Among indicators of Cp infection and inflammation, CRP, hsCRP, IgG antibody index to Cp and VCAM-1 were higher in the progressors but not with statistical significance. However, log IL-6 (0.90 ± 0.69 versus 0.32 ± 0.43, $P < 0.001$), IgA antibody index to Cp (1.37 ± 0.64 versus 0.97 ± 0.33, $P < 0.01$) and ICAM-1 level (294.29 ± 96.81 versus 241.58 ± 79.09 ng/mL, $P < 0.05$) were significantly higher in the progressors than the non-progressors (Table 4). Also prevalence of seropositivity of IgA to Cp was significantly higher in the progressors (58.1 versus 28.6%, $P < 0.05$), whereas seropositivity of IgG to Cp was not different.

On multiple logistic regression analysis adjusted for age, sex, log IL-6, Cp IgA antibody index, HDL-cholesterol and ICAM-1, the Cp IgA index [HR = 1.54 per 0.1 increase (1.07–2.22), $P = 0.019$] and log IL-6 [HR = 1.24 per 0.1 increase (1.04–1.54), $P = 0.046$] were independent risk factors of CCA-IMT progression, while ICAM-1,

Table 3. Comparison of carotid ultrasonographic parameters between baseline and follow-up study

	Non-progressors ($n = 21$)		Progressors ($n = 31$)	
	Baseline	Follow-up	Baseline	Follow-up
CCA-IMT (mm)	0.75 ± 0.10	0.74 ± 0.10	0.74 ± 0.12	0.82 ± 0.12*
cIM area (mm^2)	19.97 ± 4.26	21.16 ± 4.38	20.00 ± 4.84	23.68 ± 5.06*
Presence of plaque, *n* (%)	5 (25.0)	6 (28.6)	10 (31.3)	12 (37.5)

Data are mean ± SD. Progressors: annual ΔIMT ≥ 0.015 mm/year; non-progressors: annual ΔIMT < 0.015 mm/year. CCA-IMT: intima-media thickness of common carotid artery; cIM area: calculated intima-media area.
*$P < 0.001$ versus baseline.

Table 4. Comparison of clinical and biochemical characteristics between progressors and non-progressors

	Non-progressors ($n = 21$)	Progressors ($n = 31$)	*P*-value
Age (years)	51.3 ± 10.3	52.1 ± 11.3	NS
Sex (M:F)	10:11	16:15	NS
Smoking, *n* (%)	5 (25.0)	9 (28.1)	NS
CAPD duration (months)	57.2 ± 42.6	59.6 ± 40.2	NS
Body mass index (kg/m^2)	22.8 ± 3.4	24.1 ± 3.1	NS
Mean arterial pressure (mmHg)	103.3 ± 13.6	103.9 ± 17.6	NS
Haemoglobin (g/dL)	10.1 ± 1.5	10.4 ± 1.3	NS
Haematocrit (%)	30.5 ± 5.0	30.4 ± 3.8	NS
Albumin (g/dL)	3.6 ± 0.4	3.7 ± 0.4	NS
Calcium (mg/dL)	9.5 ± 0.9	9.5 ± 0.7	NS
Phosphorus (mg/dL)	5.0 ± 1.4	5.1 ± 1.2	NS
Calcium–phosphorus product (mg^2/dL^2)	47.87 ± 14.65	48.74 ± 11.71	NS
Intact parathyroid hormone (pg/mL)	345.63 ± 349.86	308.37 ± 268.67	NS
Total cholesterol (mg/dL)	173.1 ± 26.9	181.2 ± 30.7	NS
Triglyceride (mg/dL)	143.9 ± 149.7	146.9 ± 65.5	NS
HDL-cholesterol (mg/dL)	50.1 ± 16.7	41.5 ± 11.8	<0.05
LDL-cholesterol (mg/dL)	94.2 ± 31.5	110.3 ± 30.1	NS
C-reactive protein (mg/dL)	0.31 ± 0.50	0.38 ± 0.41	NS
High sensitive C-reactive protein (mg/L)	2.61 ± 3.12	2.83 ± 6.87	NS
Log interleukin-6 (pg/mL)	0.32 ± 0.43	0.90 ± 0.69	<0.01
Cp IgA antibody index	0.97 ± 0.33	1.37 ± 0.64	<0.01
Cp IgG antibody index	1.25 ± 0.85	1.46 ± 0.92	NS
Positive IgA to Cp, *n* (%)	6 (28.6)	18 (58.1)	<0.05
Positive IgG to Cp, *n* (%)	9 (42.6)	17 (54.8)	NS
Intercellular adhesion molecule-1 (ng/mL)	241.58 ± 79.09	294.29 ± 96.81	<0.05
Vascular cell adhesion molecule-1 (ng/mL)	1764.88 ± 320.48	1816.26 ± 309.32	NS

Data are mean ± SD. Progressors: annual ΔIMT ≥ 0.015 mm/year, non-progressors: annual ΔIMT < 0.015 mm/year. NS: not significant; CAPD: continuous ambulatory peritoneal dialysis; HDL-cholesterol: high-density lipoprotein cholesterol; LDL-cholesterol: low-density lipoprotein cholesterol; Cp: *Chlamydia pneumoniae*.

HDL-cholesterol and body mass index were not. When the Cp IgA antibody index was replaced by Cp IgA seropositivity, Cp IgA seropositivity [HR = 8.32 (1.28–54.14), $P = 0.027$] was the only independent risk factor of CCA-IMT progression (Table 5).

Magnitude of CCA-IMT progression and traditional risk factors

The mean annual ΔCCA-IMT (m/year) showed a significant positive correlation with the initial body mass index ($r = 0.320$, $P < 0.05$) and LDL-cholesterol ($r = 0.335$, $P < 0.05$), and a negative correlation with HDL-cholesterol ($r = -0.392$, $P < 0.01$) (Figure 1). In contrast, there was no significant association between mean annual ΔCCA-IMT and age, mean arterial pressure, haemoglobin, albumin, total cholesterol and triglyceride (data not shown).

Table 5. Predictors of progression of intima-media thickness (multiple logistic regression analysis)

Variables[a]	HR	95% CI	*P*-value
Cp IgA antibody index per 0.1	1.54	1.07–2.22	0.019
Log interleukin-6 per 0.1	1.24	1.04–1.54	0.046
HDL-cholesterol (mg/dL)	0.93	0.87–1.00	0.056
Intercellular adhesion molecule-1 (ng/mL)	1.01	1.00–1.02	0.141
Variables[b]	**HR**	**95% CI**	***P*-value**
Cp IgA seropositivity	8.32	1.28–54.14	0.027
Log interleukin-6 per 0.1	1.20	0.98–1.49	0.080
HDL-cholesterol (mg/dL)	0.94	0.88–1.01	0.086
Intercellular adhesion molecule-1 (ng/mL)	1.01	1.00–1.02	0.089

[a]Adjusted for age, sex, log IL-6, Cp IgA antibody index, HDL-cholesterol and ICAM-1.
[b]Adjusted for age, sex, log IL-6, Cp IgA seropositivity, HDL-cholesterol and ICAM-1.
HR: hazard ratio; CI: confidence interval; Cp: *Chlamydia pneumoniae*; HDL-cholesterol: high-density lipoprotein cholesterol.

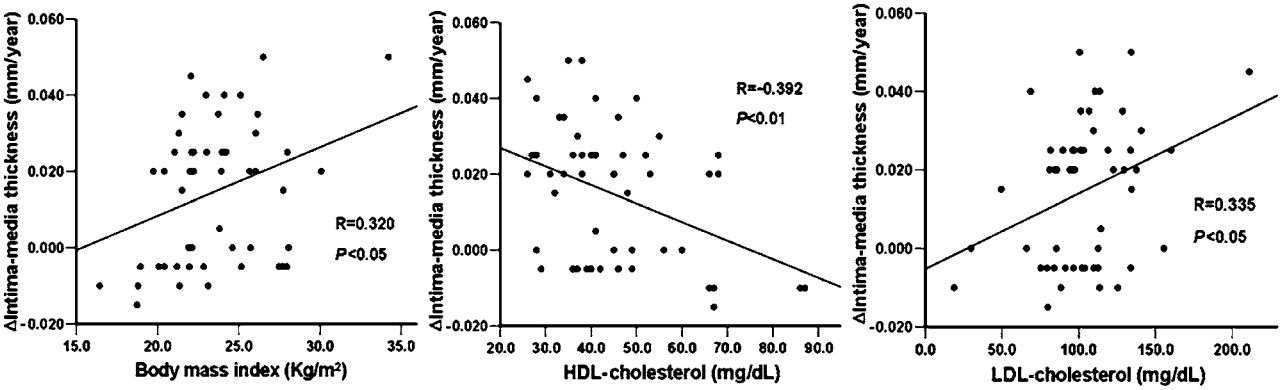

Fig. 1. Mean annual Δintima-media thickness of common carotid artery showed positive correlation with initial body mass index, LDL-cholesterol and negative correlation with HDL-cholesterol. LDL: low density lipoprotein; HDL: high density lipoprotein.

Table 6. Results of multiple linear regression analysis for the determinants of the independent factors associated with progression of intima-media thickness

Multiple $R = 0.647$	β	P-value
Cp IgA index	0.326	<0.01
HDL-cholesterol	−0.272	<0.05

Cp: *Chlamydia pneumoniae*; HDL-cholesterol: high-density lipoprotein cholesterol.

Magnitude of CCA-IMT progression and Cp infection, inflammatory markers

The annual ΔCCA-IMT was significantly correlated with the IgA antibody index to Cp ($r = 0.392$, $P < 0.01$), ICAM-1 ($r = 0.347$, $P < 0.05$) and log IL-6 ($r = 0.400$, $P < 0.01$) (Figure 2). However, no significant correlations were found between the mean annual ΔCCA-IMT and CRP, hsCRP, IgG antibody index to Cp and VCAM-1 (data not shown). A stepwise multiple linear regression analysis showed that index of Cp IgA antibody and HDL-cholesterol were independently associated with the mean annual ΔCCA-IMT ($P < 0.05$) after adjustment for age, body mass index, log IL-6, ICAM-1 and LDL-cholesterol (Table 6).

Changes in carotid duplex ultrasonographic findings and differences of baseline risk factors between Cp IgA seropositive and seronegative patients

Cp IgA seropositive patients ($n = 24$) showed significantly higher mean annual ΔCCA-IMT (0.020 ± 0.019 versus 0.010 ± 0.016 mm/year, $P < 0.05$) and log IL-6 levels (0.80 ± 0.67 versus 0.49 ± 0.45, $P < 0.05$) than seronegative patients (Figure 3). The mean annual ΔcIM area was higher in seropositive patients, but did not reach statistical significance. Among traditional and non-traditional risk factors, only log IL-6 was significantly higher in IgA seropositive patients (0.80 ± 0.69 versus 0.49 ± 0.45, $P < 0.05$). Moreover, when patients were stratified into four groups according to Cp IgA serology status and IL-6 levels (high log IL-6 $\geq$ 0.455: median value of log IL-6; low log IL-6 < 0.455), the magnitude of the annual ΔCCA-IMT was highest in the Cp IgA seropositive and high log IL-6 group (0.030 ± 0.009 mm/year, $n = 14$) (versus IgA seronegative and low log IL-6 group, $n = 16$, 0.008 ± 0.017 mm/year, $P < 0.01$; versus IgA seronegative and high log IL-6 group, $n = 12$, 0.013 ± 0.015 mm/year, $P < 0.05$; versus IgA seropositive and low log IL-6 group, $n = 10$, 0.006 ± 0.020 mm/year, $P < 0.01$) (Figure 4).

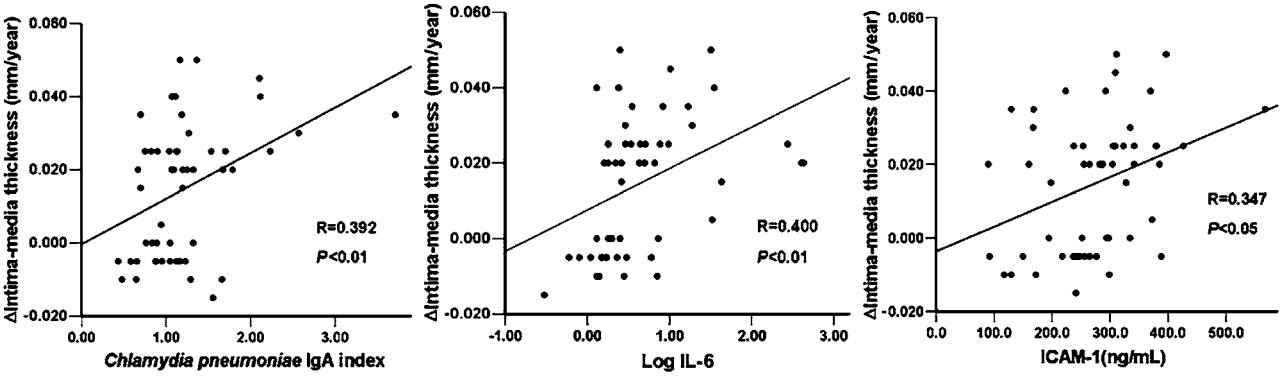

Fig. 2. Mean annual Δintima-media thickness showed positive correlation with *Chlamydia pneumoniae* IgA index, ICAM-1 and log IL-6. ICAM-1: intercellular adhesion molecule-1; IL-6: interleukin-6.

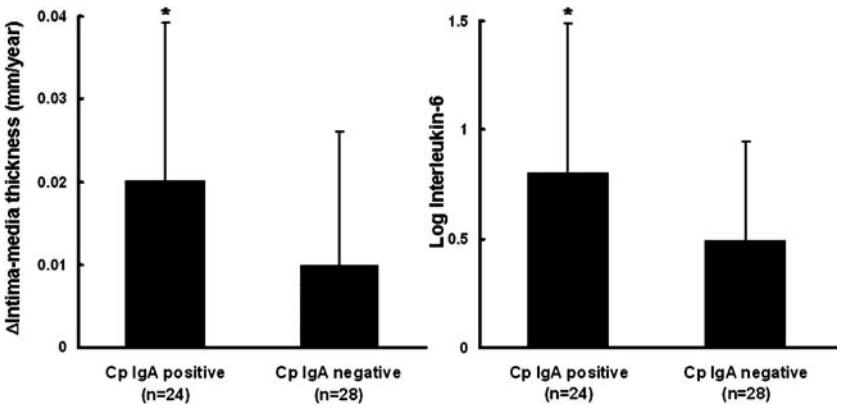

Fig. 3. *Chlamydia pneumoniae* IgA antibody seropositive patients ($n = 24$) showed significantly higher mean annual Δintima-media thickness and log interleukin-6 level compared with seronegative patients. $^{*}P < 0.05$ versus seronegative; Cp: *Chlamydia pneumoniae*.

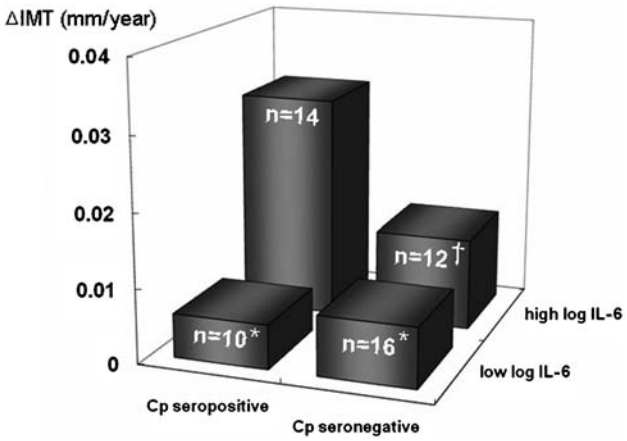

Fig. 4. Group of patients with *Chlamydia pneumoniae* IgA antibody seropositive and high log IL-6 (≥ 0.455: median of log IL-6) showed a higher mean annual Δintima-media thickness of common carotid artery than other groups of patients. $^{*}P < 0.01$ versus IgA seropositive and high log IL-6; $^{\dagger}P < 0.05$ versus IgA seropositive and high log IL-6. IMT: intima-media thickness; Cp: *Chlamydia pneumoniae*; IL-6: interleukin-6.

Discussion

Chronic persistent infection in dialysis patients, a non-traditional cardiovascular risk factor, could have rationale accounting for the atherogenic property of chronic inflammation. Many reports have suggested that infectious agents can evoke cellular and molecular changes that might cause or accelerate the atherosclerotic process. Among these pathogens, Cp, a Gram-negative obligate intracellular bacterium, has the strongest epidemiological evidence for an association with cardiovascular and cerebrovascular disease [1]. Cp is a common respiratory pathogen responsible for a wide range of chronic inflammatory conditions. It could be disseminated inside monocytes and macrophages from the respiratory tract to susceptible tissues including arteries. Indeed, local infection of Cp, as determined by the presence of chlamydial DNA, antigens or elementary bodies was found in 52% of atheromatous lesions but in only 5% of control samples [1]. In addition, there are several experimental evidences about initiation or progression of atherosclerosis by Cp infection. Cp infection in human monocytic cells induces various cytokines, such as tumor necrosis factor-α, IL-1 and IL-6 that are associated with the development of inflammation and atherosclerosis in an *in vitro* study [10]. Also, Kalayoglu *et al*. demonstrated that Cp lipopolysaccharide induced the foam-cell formation of macrophages that is one of the important processes of early atherosclerosis [11]. Moreover, Cp secretes a protease-like activity factor for degrading host cell transcription factors, which is required for major histocompatibility complex antigen expression. This mechanism may help the organism evade host immunity resulting in chronic persistent infection [12]. In addition to these *in vitro* studies, Cp infection was demonstrated to accelerate the complex atherosclerotic lesions in an *in vivo* study with Apo E3-leiden mice [13]. In spite of these quite strong experimental evidences, there have been controversies over seroepidemiological studies on ESRD patients and the general population regarding the atherogenic properties of Cp and its significance as a risk factor for cardiovascular morbidity and mortality. In ESRD patients, several prospective trials demonstrated higher cardiovascular morbidity and mortality rates in Cp seropositive patients compared to seronegative patients [5,6]. Moreover, Kato *et al*. have reported in a cross-sectional study that IgA antibody titer to Cp in patients on haemodialysis has a positive correlation with the maximal diameter of carotid artery plaques [3]. They later suggested IgA seropositivity to Cp as an independent risk factor for IMT progression in haemodialysis patients in a prospective study over 4 years [4]. The important or considerable role of Cp in atherosclerosis was further supported by the prospective study by Nishimura *et al*. They showed that Cp IgA seropositivity by the ELISA method is an independent risk factor for coronary artery stenosis on angiography in haemodialysis patients [14]. In accordance with these reports, our data clearly indicated that Cp IgA seropositivity is an independent risk factor for CCA-IMT progression, and the level of IgA antibody index to Cp has a significant positive correlation with the severity of CCA-IMT progression after adjustment for traditional risk factors and the levels of inflammatory markers. Cp IgA antibodies

have a very short half-life; thus, IgA seropositivity is considered as a better marker for chronic infection or recent re-infection. A report of meta-analysis indicated that IgA seropositivity has a stronger association with coronary heart disease than IgG seropositivity [15]. On the other hand, a recent prospective observational study for patients on haemodialysis showed that Cp seropositivity does not increase all-cause and cardiovascular mortality [16]. In addition, in a univariate analysis Zoccali *et al*. reported that Cp IgA seropositivity is associated with higher cardiovascular mortality and morbidity in patients on haemodialysis, but this effect lost its statistical significance after adjustment for conventional risk factors, such as age, smoking, diabetes and hypercholesterolaemia [17]. These two studies implied that serologic assessment by the detection of Cp antibody might not have clinical availability for the risk factor of atherosclerosis in patients maintaining haemodialysis. However, these results cannot refute the capability of Cp to induce atherosclerosis as proven by *in vitro* and *in vivo* experimental studies [10–13]. This might be due to the methodological limitation of the serologic assay alone to detect vascular Cp infection. Because this organism is difficult to culture and neither pathologic diagnosis from arterial tissue nor Cp DNA detection by polymerase chain reaction (PCR) in cultured peripheral blood mononuclear cells is possible for clinical application, antibody detection by serologic assay is currently the only clinically available method and routine approach to identify Cp infection. However, several reports suggested that this method has a higher prevalence of positive results than other methods of Cp identification, such as DNA detection by PCR and culture in the general population as well as in ESRD patients. Also, there is a lack of evidence for an association between local vascular infection and positive serology [18]. Although serologic assay of Cp alone could be considered less valid based on these conflicting reports, Cp seropositivity limited to high inflammatory status could obtain clinical significance with the hypothesis that Cp infection leads to the pathogenesis of atherosclerosis by causing chronic inflammation. In addition, seroepidemiological studies for ESRD patients found a positive correlation between Cp IgA titer and IL-6 level, which is the most potent atherogenic pro-inflammatory cytokine and a growth factor for vascular smooth muscle cell [3]. Higher IL-6 levels were also observed in IgA seropositive patients compared with negative patients [19]. Our results showed that IL-6 is significantly higher in Cp IgA seropositive patients in accordance with the previous reports. CCA-IMT progression was highly enhanced in Cp IgA seropositive patients, especially in high inflammatory status assessed by IL-6 but not in patients with low inflammatory status. These findings suggest that Cp may trigger the production of this pro-inflammatory, atherogenic cytokine resulting in the aggravation of atherosclerosis or at least it can be speculated that this organism accelerates the process of atherosclerosis only in the case of high inflammatory status. Thus, it is possible that the serologic assay of Cp combined with the measurement of inflammatory markers especially IL-6 could be of help in predicting the progression of atherosclerosis in patients undergoing CAPD.

The limitations of this study should be considered. First, we used an ELISA method to detect antibodies of Cp, not a MIF assay. Although the MIF method is recommended for serologic assays because of its high sensitivity and specificity, the interpretation of the results can be time consuming, subjective and have poor reproducibility [20]. On the other hand, the ELISA method is relatively simple, more objective, reproducible and clinically applicable due to the photometrical interpretation method used to detect Cp. Second, we relied on a single blood sample from each patient and thus cannot account for a possible variation in IgA seropositivity and IgA antibody index to Cp over time. However, we excluded the patients who had symptoms and signs of acute infection. Therefore, initial IgA antibody seropositivity and index were not related to acute infection of Cp, but reflect a chronic persistent infection. Third, only 52 patients were studied and our data need to be confirmed in a larger group.

In conclusion, the present study showed that Cp IgA seropositivity in high inflammation status might be an important clinical risk factor for atherosclerosis in patients undergoing CAPD, and the Cp serologic test by the ELISA method in combination with a test for IL-6 level could be a useful predictor of future atherosclerosis.

Conflict of interest statement. None declared.

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