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Chronic *Chlamydia pneumoniae* infection in patients with symptomatic atherothrombosis

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Summary *Objectives:* The aim of the present study was to search for an association between chronic *Chlamydia pneumoniae* infection, indicated by elevated antibody titers against the pathogen, atherothrombosis and the occurrence of arterial ischemic events.
Methods: We studied 52 patients presenting at baseline with at least one symptomatic episode of atherothrombosis. A screening for fasting blood glucose and a lipid profile was performed on all patients who had no known history of diabetes or hypercholesterolemia.
Results: The prevalence of IgG and IgA anti-*C. pneumoniae* antibodies at baseline was 90% (95% CI: 79–97) and 81% (67–90), respectively. Forty-two of the 52 patients (81%) experienced a new arterial ischemic event after a mean follow-up of 9 years [heart: 19 (37%); brain: 12 (23%); lower limbs: 8 (15%); and other: 13 (25%)]. Occurrence of a new arterial ischemic event was related to age ($p = 0.003$), sex ($p = 0.009$), and tobacco smoking ($p = 0.06$). Prevalences of IgA and IgG anti-*C. pneumoniae* were significantly higher in patients with atherothrombosis at baseline than that in controls.
Conclusion: Our study confirmed the links between *C. pneumoniae* and atherothrombosis. However, neither IgA nor IgG antibodies for *C. pneumoniae* was a significant predictive factor for new ischemic arterial events in patients with atherothrombosis.

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Introduction

In addition to the well-established cardiovascular risk factors such as hypercholesterolemia, hypertension, and cigarette smoking, multiple additional factors have been suspected in both the development and progression of atherothrombosis.[1] *Chlamydia pneumoniae* (*C. pneumoniae*)has been the focus of research associating infection with atherothrombosis.[2,3] *C. pneumoniae* is an obligate intracellular parasite that commonly infects mononuclear phagocytes. Macrophages derived from monocytes are localized in human atherosclerotic plaques and provide a mechanism for entry of the organism into the vessel wall. *C. pneumoniae* has been identified in atherosclerotic plaques of patients with cerebrovascular and cardiovascular diseases.[4,5] The biology of *C. pneumoniae* is characterized by alternate phases of activity and latency in inflammatory lesions, followed by fibrosis and scarring, and ultimate degradation, the antigens being detectable for a considerable longer time than intact DNA.[5]

Based on seroepidemiological studies, *C. pneumoniae* has been suspected to play a role in atherosclerosis.[6,7] Association of high level of IgA anti-*C. pneumoniae* with symptomatic disease suggests that chronic *C. pneumoniae* infection anywhere in the body could play a role in atherosclerotic plaque activation and be used as a marker to target populations in future prevention trials.[5] Given the potential for enhancing pro-inflammatory mediators that could lead to plaque rupture or luminal thrombosis, we hypothesized that *C. pneumoniae* would be present in a significantly larger percentage of symptomatic vs. asymptomatic patients with atherothrombosis. The aim of the present study was to search for an association between chronic *C. pneumoniae* infection, indicated by elevated antibody titers against the pathogen, atherothrombosis and the occurrence of arterial ischemic events.

Methods

Patients

We studied 52 patients presenting at baseline with at least one symptomatic episode of atherothrombosis (i.e. stroke, angina, myocardial infarction, peripheral arterial disease, and others). Patients were followed-up during a mean period of 9 years, and occurrence of new arterial ischemic events was collected for each patient. Risk factors for atherothrombosis were recorded in all patients including hypertension (blood pressure > 140/90 mm Hg), past history of smoking (≥0.5 pack-years), diabetes (treated by oral agent or insulin for >1 year), and hypercholesterolemia (LDL cholesterol > 160 mg/dL, untreated). A screening for fasting blood glucose and a lipid profile was performed on all patients who had no known history of diabetes or hypercholesterolemia. A group of 56 healthy blood donors, mean age 49 years (range 27–58), have been selected as a control group.

Serological detection of *C. pneumoniae*

ELISA was performed on frozen (−80 °C) serum samples using the Sero *C. pneumoniae* Quant.[8] This test, based on specific *C. pneumoniae* peptides, is a semi-quantitative determination of specific IgG and IgA antibodies to *C. pneumoniae*. A result higher than 10 IU/mL was considered positive [9].

Statistical methods

For comparisons of categorical variables, Chi-square and Fisher's exact tests (2-sided *p* value) were used. Continuous variables were compared using either *t* test or Wilcoxon test. The occurrence of any new arterial ischemic event was analyzed using a logistic regression model. Factors related to both IgA antibodies and occurrence of a new arterial ischemic event in univariate analysis at a significance level of 0.20 were included in the logistic regression model. The statistical analyses were performed using the SAS® software.

Results

The baseline characteristics of the cohort according to *C. pneumoniae* serology are presented in Table 1. The prevalence of IgG and IgA anti-*C. pneumoniae* antibodies at baseline was 90% (95% CI: 79–97) and 81% (67–90), respectively. Patients with either IgA or IgG antibodies were significantly older than those without antibodies. Males were more likely to have either IgA or IgG antibodies than women. All other baseline characteristics, including ischemic area, were not significantly related to anti-*C. pneumoniae* antibodies.

Forty-two of the 52 patients (81%) experienced a new arterial ischemic event after a mean follow-up of 9 years [heart: 19 (37%); brain: 12 (23%); lower limbs: 8 (15%); and other: 13 (25%)]. Patients who experienced a new arterial ischemic event during follow-up were as much as likely to have either IgA or IgG antibodies at baseline than those without any event (91% vs. 90% for IgG, and 83% vs.

Table 1 Baseline characteristics of patients according to *Chlamydia pneumoniae* serology

	IgG and IgA negative ($n = 5$)	IgG or IgA positive ($n = 47$)	p
Mean age, years (std)	47.2 (15.2)	61.0 (12.6)	0.03
Male sex [n (%)]	1 (20)	34 (72)	0.03
Body mass index ≥ 25 [n (%)]	2 (40)	17 (36)	1.00
Mean follow-up duration, years (std)	7 (3.3)	9.4 (7.2)	0.46
Tobacco smoking [n (%)]	4 (80)	31 (66)	1.00
Hormone replacement therapy, in women [n (%)]	3 (75)	4 (31)	0.25
Diabetes [n (%)]	0	6 (13)	0.31
Hypertension [n (%)]	3 (60)	21 (45)	0.65
Hypercholesterolemia [n (%)]	2 (40)	28 (60)	0.64
Familial history of atherothrombosis [n (%)]	3 (60)	13 (28)	0.16
Arterial ischemic territory			
Heart	0	14 (30)	0.31
Brain	2 (40)	10 (21)	0.33
Legs	4 (80)	21 (45)	0.18
Other	0	4 (9)	1.00

70%, $p =$ NS). Occurrence of a new arterial ischemic event was related to age ($p = 0.003$), sex ($p = 0.009$), and tobacco smoking ($p = 0.06$). The logistic regression model showed that, after adjusting for sex, age, family history of atherosclerosis disease, and tobacco smoking at baseline, IgA anti-*C. pneumoniae* was not significantly related to the occurrence of a new arterial ischemic event (Table 2). Prevalences of IgA and IgG anti-*C. pneumoniae* were significantly higher in patients with atherothrombosis at baseline than that in controls (Table 3).

Discussion

In the present cohort study, the very high prevalence of anti-*C. pneumoniae* antibodies in patients with atherothrombosis brings further evidence for a relationship between such infection and atherothrombosis, as reported by others.[10–13] However, neither IgA nor IgG antibodies for *C. pneumoniae* was a significant predictive factor for new ischemic arterial events in patients with atherothrombosis.

C. pneumoniae antibodies are more likely to be found among male and older patients. Khairy et al.

Table 2 Factors related to new arterial ischemic event (logistic regression model, $n = 52$)

	Odds ratio	95% Confidence interval of odds ratio	p
Age	1.10	1.02–1.19	0.02
Sex (female as reference)	13.8	1.4–131.1	0.02
Smoking (non-smoking as reference)	5.84	0.83–40.7	0.07
Family history of atherothrombosis (no history as reference)	3.57	0.35–35.9	0.28
IgA anti-*C Pneumoniae* > 10 IU/mL (IgA $\leq$ 10 IU/mL as reference)	0.68	0.08–5.82	0.72

found an absence of association between infectious agents, included *C. pneumoniae* and endothelial function in healthy young men.[14] Few data on the epidemiology of *C. pneumoniae* in blood donors and general population are available.[15] Our results of 41% seropositivity for IgG contrast with the Irish study that reported a 70% seropositivity in a randomly selected adult population.[16] O'Neill et al. did not find significant relation between *C. pneumoniae* antibodies and age, gender or smoking.[16] They found, however, that *C. pneumoniae* seroprevalence was higher in patients with low socio-economic status. Further studies are needed for a better knowledge of the epidemiology of *C. pneumoniae* in general population and patients with atherothrombosis.

The obligate intracellular bacterium *C. pneumoniae* has recently been implicated in the pathogenesis of atherosclerosis.[17] In the present study, using a highly specific test, serological response to *C. pneumoniae* indicates an increased prevalence in patients suffering from symptomatic atherothrombosis disease. Two recent studies have demonstrated that the detection of a link between *C. pneumoniae* and coronary artery disease depends on the choice of serologic methods.[18,19] However, Hermann and colleagues reported that ELISAs that are fast and objective deliver seroprevalence results, sensitivities, and specificities that are very similar to those of the MIF test.[8] Our results corroborate other investigations that reported the presence of chlamydial structures and even viable *C. pneumoniae* in atherosclerotic plaques. Experimental proof, however, of an

Table 3 IgG and IgA for *Chlamydia Pneumoniae* infection in controls and patients with atherothrombosis

	Controls ($n = 56$)	Patients with atherothrombosis ($n = 52$)	p
IgG titers [mean (95% CI)]	40.8 [31.1;51.1]	69.2 [54.2;80.9]	0.001
IgG > 10 IU/mL [n (%)]	41 (73%)	47 (90%)	0.02
IgA titers [mean (95% CI)]	18.5 [12.5;24.9]	52.4 [38.1;64.3]	0.0001
IgA > 10 IU/mL [n (%)]	18 (32%)	42 (81%)	0.0001

etiological role of *C. pneumoniae* in atherothrombosis has not yet been accomplished since a well-established animal model and a system of genetic recombination are not yet available.

Elevated anti-chlamydial IgA levels are believed to occur with reinfection of *C. pneumoniae*.[20–22] IgA titers begin to decline within weeks to several months after reinfection. Persistently elevated IgA levels are believed to be associated with a chronic infection state and have been noted in both chronic and acute coronary diseases.[20] However, no association was seen between the presence of *C. pneumoniae* in atherosclerotic plaques and immunoglobulin titers. More than 70% of patients with plaque positive for *C. pneumoniae* by PCR did not show elevated IgA anti-chlamydial titers. This lack of association makes anti-chlamydial IgA titer levels a poor indicator for intraplaque presence of *C. pneumoniae* although elevated anti-chlamydial IgA levels seem associated with symptomatic disease, as previously reported.[23,24] Elevated serum anti-chlamydial IgA may represent a more chronic infection state, inducing activation of atherosclerotic plaque by circulating activated leukocytes.

Another possible explanation for association of IgA levels with *Chlamydia* and symptomatic disease is a non-specific increase in immunoglobulin response to antigens. Persons who have been previously infected with *C. pneumoniae* could display an elevated anti-chlamydial IgA after exposure to a variety of antigens. This supports the hypothesis that generalized inflammatory response can activate atherosclerotic plaque, with anti-chlamydial IgA acting only as a marker and not as an indicator of the specific antigen exposure. Further studies that focus on intraplaque differences between symptomatic and asymptomatic patients in presence of *C. pneumoniae* need to be performed to identify causative effect of infectious agents in atherosclerotic disease.

Numerous clinical trials that have studied the use of antibiotics in the secondary prevention of ischemic heart diseases have had conflicting results. A recent meta-analysis based on nine published studies, with a total of 11,015 participants, showed that in patients with known ischemic heart disease, macrolide antibiotics for *C. pneumoniae* did not result in a statistically significant reduction in recurrent cardiac events or mortality over 3 months to 3 years.[25] However, other studies using doxycycline in doses prescribed in routine clinical practice for brucellosis decreases the risk of coronary artery disease.[26] These conflicting results may be due to the obligate intracellular infectious life cycle of *C. pneumoniae*. Only the intracellular forms, i.e. the reticulate bodies, are metabolically active and can be treated with membrane-permeative antibiotics such as macrolides. The persistent state is characterized by a metabolically active period without replication. Until now, no antibiotics have proven capable of eradicating persistent chlamydial infections.[27,28]

In conclusion, our study suggests the links between *C. pneumoniae* and atherothrombosis. These results must be confirmed on larger cohort. Clinical evaluation of patients is complicated by the lack of a useful parameter to indicate the risk of endovascular infection. At the current state of scientific knowledge, an experimental anti-*Chlamydia* treatment in patients with atherothrombosis to prevent new ischemic events may only be justified in randomized controlled clinical trials.

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