

The Relationship Between Seropositivity Against *Chlamydia pneumoniae* and Stroke and its Subtypes in a Latvian Population

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Key words: cerebrovascular disease; stroke; *Chlamydia pneumoniae*; risk factor.

Summary. Background and Objective. Serological evidence of infection with *Chlamydia pneumoniae* has been associated with cardiovascular diseases, but the relationship with stroke and its risk factors remains not completely understood. The aim of this study was to determine whether serological evidence of infection with *Chlamydia pneumoniae* was associated with the risk of ischemic stroke and any of investigated stroke subtypes.

Material and Methods. Confirmed stroke cases ($n=102$) were compared with gender- and age-matched control patients ($n=48$). The patients with stroke were divided into 3 groups according to the TOAST criteria: atherothrombotic ($n=36$), cardioembolic ($n=47$), and of undetermined etiology ($n=19$). Plasma levels of IgG antibodies to *Chlamydia pneumoniae* were measured by enzyme-linked immunosorbent assay.

Results. There was a significant association between seropositivity to *Chlamydia pneumoniae* and stroke. Anti-*Chlamydia pneumoniae* IgG antibodies were detected in 64 case patients (62.7%) and 17 control patients (35.4%) ($\chi^2=9.8$; $df=1$; $P=0.002$). IgG seropositivity to *Chlamydia pneumoniae* was linked to all the analyzed etiological subtypes of stroke.

Conclusion. This study showed that IgG seropositivity to *Chlamydia pneumoniae* was associated with stroke and all the analyzed etiological subtypes of stroke.

Introduction

Vascular diseases, undoubtedly, are one of the major causes of mortality worldwide, whereas stroke remains a leading cause of long-term disability. Mortality from stroke in Latvia is rather high; moreover, it is one of the highest in the region comparing with the neighboring countries (1).

Atherosclerosis is one of the main risk factors for cerebrovascular diseases, not only directly affecting intra- and extracranial blood vessels, but also affecting other organs and systems thus increasing disease burden and costs and lowering quality of life.

Chronic inflammatory reaction is hypothesized to play a role in the development of atherosclerotic process; furthermore, it might be considered as an independent risk factor for stroke (2).

C. pneumoniae has been linked to atherosclerosis (3) and vascular accidents. The relationship between *C. pneumoniae* and stroke has not been confirmed by all trials; therefore, the data are controversial. Several serologic studies have established a link between seropositivity and acute cerebrovascular accident (4–8), while others have not demonstrated any association (9–11).

The aim of this study was to determine whether serologic evidence of infection with *C. pneumoniae* was associated with the risk of ischemic stroke and any of investigated stroke subtypes.

Material and Methods

A retrospective study was carried out. It was approved by the hospital's Ethics Commission. All the case patients ($n=102$) were patients with acute ischemic stroke admitted to the Paula Stradins Clinical University Hospital from October 2007 to March 2009. Stroke was defined as a focal (or at the time global) neurological impairment of sudden onset, and lasting more than 24 hours (or leading to death), and of presumed vascular origin according to the WHO definition.

Eligible cases were enrolled if they had a first-ever stroke or recurrent cerebral infarction. Patients with cerebral infarction secondary to other diseases, lacunar stroke, past medical history of oncologic or chronic inflammatory diseases, and severe impairment of renal function were excluded from the study. All the patients were categorized into 3 groups using the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) criteria (12): atherothrombotic, cardioembolic, and of undetermined etiology. In addition, the study questionnaire included the evaluation of neurological status on admission, modified Rankin scale, stroke localization (middle cerebral artery [MCA]) anterior cerebral artery [ACA] and vertebrobasilar [VB] territories), and stroke risk factors. Computed tomography was used as a routine neuroimaging method (100% of study subjects), while magnetic resonance imaging was performed if required. Control patients were matched to case patients by age and gender ($n=48$; 26 men and 22

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women; mean age, 64.3 years [SD, 11.8]; age range, 42 to 82 years) and were patients with nonvascular diseases, predominantly noninflammatory lower back pain, admitted to the P. Stradins Clinical University Hospital. The ratio of control patients to case patients was about 1:2. Stenosis of brachiocephalic vessels exceeding 60% detected by Doppler ultrasound using flow velocity analysis was considered significant. Overweight was defined as body mass index greater than 25 kg/m² according to the WHO criteria.

To detect IgG to *C. pneumoniae*, Novagnost™ (Germany) ELISA system was used. A value of 8 IU/mL was considered as indicative of positive result. Both semiquantitative and qualitative tests were employed for the present study.

Statistical Analysis. SPSS 16.0 for Windows was used for data processing and analysis. To compare the mean values, both the analysis of variance (ANOVA) and the *t* test were used. Frequency expressed in % was obtained by applying multifactorial (r×c) frequency tables. Frequency distribution was detected by the Pearson χ^2 and Fisher tests using “Statcalc,” but covariance was calculated by applying Pearson correlation coefficient. Differences were considered significant at $P \leq 0.05$.

Results

Demographic characteristics of the recruited subjects are shown in Table 1.

There were no significant age ($t=0.806$; $P=0.422$)

Table 1. Demographic Characteristics of the Study Population

Characteristic	Stroke Group (n=102)	Control Group (n=48)
Men, n	61	26
Women, n	41	22
Age, mean (SD) [range], years	65.8 (10.9) [42–89]	64.3 (11.8) [42–82]

Table 2. Demographic Characteristics of Case Patients by Stroke Subtype

Characteristic	Atherothrombotic (n=36)	Cardioembolic (n=47)	Undefined Etiology (n=19)	<i>P</i>
Men, n (%)	28.0 (77.8)	21.0 (44.6)	12.0 (63.2)	<0.005
Women, n (%)	8.0 (22.2)	26.0 (45.4)	7.0 (36.8)	<0.005
Age, mean (SD), years	63.19 (11.3)	69.9 (8.8)	60.7 (11.9)	<0.005

Table 3. Risk Factors by Study Groups

Risk factor	Case Group	Control Group	<i>P</i>
Arterial hypertension	86 (84.3)	18 (37.5)	<0.001
Dyslipidemia	47 (46.0)	6 (12.5)	<0.001
Coronary heart disease	46 (45.1)	2 (4)	<0.001
Diabetes mellitus	42 (41.2)	3 (6)	<0.001
Atrial fibrillation	42 (41.2)	5 (10)	<0.001
Significant stenosis of brachiocephalic blood vessels	36 (35.3)	4 (8)	<0.001
Increased body mass index	51 (50.0)	5 (10)	<0.001
Smoking	59 (57.8)	7 (14.6)	<0.001

Values are number (percentage).

and gender ($\chi^2=0.426$; $df=1$; $P=0.514$) differences comparing the case and control groups (Table 1).

The mean age of patients with atherothrombotic stroke was 63.19 years (SD, 11.3); with cardioembolic stroke, 69.9 years (SD, 8.8); and with stroke of undetermined etiology, 60.7 years (SD, 11.9). The difference in the mean age of stroke patients according to the subtypes was significant ($F=4.631$; $P=0.004$) (Table 2).

Of all the patients, 72 (70.6%) had stroke in the MCA territory, 28 patients (27.5%) in the VB, 1 patient (1.0%) in the ACA, and 1 patient (1%) in the watershed zone.

Table 3 illustrates data on the main risk factors for stroke. Arterial hypertension was found to be the most common risk factor detected in 83.4% of case patients ($n=86$).

Seropositivity against *C. pneumoniae* was documented in 64 (62.7%) of the 102 patients and only in 17 (35.4%) of the 48 controls ($\chi^2=9.8$; $df=1$; $P=0.002$). The mean serum antibody level in the patient group was slightly higher compared with the control group, but the difference was not significant (9.89 ± 4 U vs. 8.8 ± 4.4 U; $t=1.465$, $P=0.15$). Analysis of each group with different stroke subtype revealed IgG seropositivity against *C. pneumoniae* in 58.33% of atherothrombotic patients, and this percentage was significantly greater than that of seropositive patients in the control group (35.42%) ($\chi^2=4.36$; $P=0.037$). In the groups of cardioembolic stroke and stroke of undefined etiology, 65.96% and 63.16% of patients, respectively, were found to be seropositive, and these percentages were significantly greater as compared to the control group as well ($\chi^2=8.86$, $P=0.003$; and $\chi^2=4.27$, $P=0.039$; respectively).

Lipid profile and its association with *C. pneumoniae* IgG status were retrospectively analyzed. For this purpose, patients with stroke were divided into two

Table 4. *C. Pneumoniae* Serum Antibody IgG Level by Different Groups

	Atherothrombotic stroke, n=36	Stroke of undefined etiology, n=19	Cardioembolic stroke, n=47	Controls, n=48	Total, n=150
No. of seropositive patients	21	12	31	17	81
<i>C. pneumoniae</i> Ig G, mean (SD) [95% CI], U	9.57 (5.48) [7.72–11.42]	10.27 (2.94) [8.85–11.68]	10.20 (3.84) [9.07–11.33]	8.80 (4.38) [7.53–10.07]	9.61 (4.37) [8.91–10.32]

groups: seropositive (n=64) and seronegative patients (n=38). Among seropositive and seronegative patients, 51.56% and 34.21% respectively had an elevated serum total cholesterol level, ($\chi^2=2.90$; $P=0.089$). Elevated serum low-density lipoprotein (LDL) level was found in 35 seropositive as compared with 12 seronegative patients ($\chi^2=5.12$; $P=0.024$), while elevated serum triglyceride level was documented in 9 seropositive and 6 seronegative patients ($\chi^2=0.06$; $P=0.8$).

Discussion

The link between *C. pneumoniae* and an acute stroke has not been established in all studies, which have looked at this issue. Furthermore, various studies employed different methods. Some trials that employed serologic methods have demonstrated a link between seropositivity to *C. pneumoniae* and an acute cerebrovascular accident (4–8), whereas others have not (9–11). Moreover, some studies have confirmed the presence of *C. pneumoniae* in the atherosclerotic plaque by molecular genetic methods (13–17), but a considerable number of studies have not reported any association (18–21). Likewise, the relationship between bacteria and atherosclerotic changes in intracerebral blood vessels is not straightforward. A study from Netherlands using polymerase chain reaction (PCR) was unable to detect *C. pneumoniae* in large intracerebral vessels (22); whereas the study that examined the atherosclerotic middle cerebral artery was able to detect it by nested PCR in 5 of the 15 atherosclerotic arterial samples and none of the control tissues (23). Despite a plausible link between bacteria and atherosclerosis described in a review by Watson and Alp (24), the situation with stroke might be more complicated due to a possibly weaker link between an acute ischemic cerebrovascular event and atherosclerosis if compared to coronary artery disease and myocardial infarction. Consequently, it may be that no certain bacteria are associated with stroke, but perhaps infectious burden of common pathogens actually might be linked to stroke (25) or carotid atherosclerosis (26).

Stroke is a heterogenous disease that is classified into several subtypes of different etiology, and associations between subtypes and *C. pneumoniae* has not been thoroughly investigated yet. Moreover, results of the studies appear to be controversial, not only due to the difficulties in the detection of *C. pneumoniae*, but also due to differences in the definition for stroke subtypes among researchers.

In the present study, an association between seropositivity to *C. pneumoniae* and a stroke was observed. Unfortunately, the present study has some limitations: certain stroke subtypes, such as lacunar and a stroke associated with other pathologies, were excluded, and the sample size was small. However, our study showed evidence for an association between *C. pneumoniae* and investigated subtypes of stroke.

Arterial hypertension, coronary artery disease, peripheral artery disease, etc. are well-known and well-studied classic risk factors for stroke (27). Dyslipidemia is one of the classic risk factors, which have been demonstrated to have some link with *C. pneumoniae* seropositivity in some studies (28–29). Currently, there is room for more studies to clarify an interaction between infectious agents and dyslipidemia, whether *C. pneumoniae* is just an innocent bystander or an active player. This study showed a significant link between *C. pneumoniae* seropositivity and elevated serum LDL level. It might be worth to investigate further a role of microorganisms in lipid profile of atherogenesis.

Population health, efficient use of state budget, and quality of life are relevant questions requiring a continuing search for effective solutions of the stroke problem. However, there are many problems to be solved to confirm the infection theory and its role in stroke etiology. Every new research is a next step in assembling this multi-layer jigsaw puzzle and finding a solution to the complicated problem.

Conclusions

Our serological study showed an association between the presence of *C. pneumoniae* antibodies and stroke in the Latvian population. In addition, it demonstrated the significance of *C. pneumoniae* seropositivity for several major stroke subtypes and correlation with elevated serum low-density lipoproteins level. To support atherogenic characteristics of *Chlamydia pneumoniae*, we need large prospective cohort studies and randomized controlled interventional trials, which would combine serologic and nonserologic methods for confirmation of chronic infection in selected population.

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Statement of Conflict of Interest

The authors state no conflict of interest.

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