Seropositivity to Chlamydia pneumoniae Is Associated With Risk of First Ischemic Stroke

Mitchell S.V. Elkind, MD, MS; Maria Lucia C. Tondella, PhD; Daniel R. Feikin, MD, MSPH; Barry S. Fields, PhD; Shunichi Homma, MD; Marco R. Di Tullio, MD

Background and Purpose—Serologic evidence of infection with Chlamydia pneumoniae has been associated with cardiovascular disease, but its relationship with stroke risk remains uncertain. The objective of this study is to determine whether serological evidence of C pneumoniae infection is associated with risk of ischemic stroke.

Methods—A population-based case-control study was performed in an urban, multiethnic population. Cases (n=246) had first ischemic stroke, and controls (n=474) matched for age, sex, and race–ethnicity were derived through random-digit dialing. Titers of C pneumoniae–specific IgG and IgA antibodies were measured using microimmunofluorescence, and positive titers were prospectively defined. Conditional logistic regression was used to calculate odds ratios (ORs) and 95% CIs adjusting for medical, behavioral, and socioeconomic factors.

Results—Mean age among cases was 72.3±9.7 years; 50.8% were women. Elevated C pneumoniae IgA titers were associated with increased risk of ischemic stroke after adjusting for hypertension, diabetes mellitus, current cigarette use, atrial fibrillation, and levels of high-density lipoprotein and low-density lipoprotein (adjusted OR, 1.5; 95% CI, 1.0 to 2.2). Elevated IgG titers were not associated with stroke risk (adjusted OR, 1.2; 95% CI, 0.8 to 1.8). There was a trend toward an association of elevated IgA titers with atherosclerotic and lacunar stroke but less so cardioembolic or cryptogenic subtypes.

Conclusions—Serologic evidence of C pneumoniae infection is associated with ischemic stroke risk. IgA titers may be a better marker of risk than IgG. This association is independent of other stroke risk factors and is present for atherosclerotic, lacunar, and cardioembolic subtypes. Further studies of the effect of C pneumoniae on stroke risk are warranted. (Stroke. 2006;37:790-795.)

Key Words: cerebrovascular disorders ■ Chlamydophila pneumoniae ■ risk factors ■ stroke, ischemic

Chronic infection with common organisms has been proposed as a potential risk factor for atherosclerosis and heart disease. Serological evidence of past infection with Chlamydia pneumoniae, a common respiratory pathogen, has been found in epidemiological studies to be associated with risk for atherosclerosis and cardiac disease, although prospective cohort studies have not confirmed this association. Relatively few studies have been conducted in patients with ischemic stroke, with conflicting results, but most have not measured IgA antibody subtypes or assessed ischemic stroke subtypes. Many studies have used post hoc criteria for positive antibody titer cutoffs. Because risk factor profiles likely differ according to ischemic stroke subtype, it is essential to classify ischemic stroke patients by subtype.

In a previous post hoc pilot case-control study in northern Manhattan, C pneumoniae IgA antibodies were associated with risk for first ischemic stroke. Because of small numbers of patients (n=89 cases), we were unable to examine individual stroke subtypes. In this larger population-based study, we hypothesized a priori that IgA antibody titers to C pneumoniae would be associated with first ischemic stroke. We also hypothesized that the association between C pneumoniae antibody titers and stroke would be more specific for atherosclerotic stroke than other subtypes.

Subjects and Methods

Selection of Cases and Controls

The present study shared patient recruitment with the previously described Aortic Plaque and Risk of Ischemic Stroke study and control recruitment with the Northern Manhattan Study. Eligible cases were prospectively enrolled if they were: (1) diagnosed with first cerebral infarction, (2) >55 years of age at onset of stroke, and (3) a resident in northern Manhattan in a household with a telephone. Patients with intracerebral or subarachnoid hemorrhage or transient ischemic attack (TIA), defined as neurological deficits lasting <24 hours and no ischemic infarct found on brain imaging, were excluded. Fatal and nonfatal infarcts were enrolled. The methods of
case detection were similar to those described previously for the Northern Manhattan Stroke Study.10

The methods of control recruitment and enrollment have been described previously.8,10,11 Briefly, control participants were identified by random-digit dialing. Community controls for this study were enrolled if they: (1) had never been diagnosed with stroke, (2) were >55 years of age, and (3) resided in northern Manhattan for ≥3 months in a household with a telephone. In-person evaluations were performed at the hospital or at home for those who could not come in person. Telephone response rate was 91% and 75% of those respondents participated in in-person evaluations (overall response rate 68%). The study was approved by the institutional review boards of Columbia University Medical Center and the Centers for Disease Control and Prevention (CDC). All stroke cases and stroke-free controls gave consent directly or through a surrogate where appropriate. Cases were matched 1:2 to controls by age (within 5-year increments), sex, and race–ethnicity. Where necessary, 1:1 matching was used.

Index Evaluation of Cases and Controls

Data were collected through interviews of cases and controls, medical record review, physical and neurological examination, and measurements in fasting blood specimens, as described previously.11 When possible, data were obtained directly from subjects. When the subject was unable to provide answers, a knowledgeable proxy was interviewed. Stroke-free controls were interviewed in person and evaluated in the same manner as cases. Direct subject data were obtained from 70% of cases and 99% of controls. Participants self-identified ethnicity as Hispanic or non-Hispanic and race as white, black, or other specific categories. Standardized questions were adapted from the Behavioral Risk Factor Surveillance System13 regarding common medical conditions. Standard techniques were used to measure blood pressure, height, weight, glucose, and lipids as described previously.10,13 Hypertension was defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg based on the average of the 2 blood pressure measurements, a physician diagnosis of hypertension, or a patient’s self-report of a history of hypertension or antihypertensive use, and diabetes mellitus was defined by a fasting blood glucose level ≥126 mg/dL, the subject’s self-report of such a history, or insulin or hypoglycemic use.

Assessment of Stroke Subtype

All patients underwent brain imaging (computed tomography and, as clinically appropriate, MRI), transthoracic echocardiography and transesophageal echocardiography, noninvasive vascular imaging (duplex Doppler, transcranial Doppler, or magnetic resonance angiography). Additional tests were performed as clinically appropriate. Two neurologists blinded to C pneumoniae status classified the strokes independently after review of all of the data. Final ischemic stroke subtype was decided by consensus of the 2 neurologists, and disagreements were adjudicated by a third neurologist evaluator. Strokes were classified as extracranial atherosclerotic, intracranial atherosclerotic, lacunar (small vessel), cardioembolic, or cryptogenic after full evaluation, and 2.4% either other mechanism (eg, dissection, vasculitis) or multiple conflicting mechanisms.

Characteristics of cases and controls (n=474) are shown in Table 1. Cases were significantly more likely to have diabetes mellitus and hypertension, although a high proportion of both cases and controls had hypertension (86.8% versus 76.2%). Cases had lower mean total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL). There were no differences in the proportion of cases and controls with current or past smoking, coronary artery disease, or a high school education.

Blood samples were drawn within 48 hours of admission in 85.4% of cases. Antibody levels could not be performed in 7 cases and 46 controls for technical reasons, leaving a sample of 239 cases and 428 controls with antibody titer results. The prevalence of elevated C pneumoniae IgG and IgA titers in the control population was high: 60% for IgG and 40% for IgA titers (Table 2). No participants had elevated IgM titers. Among controls, men had a higher proportion of elevated titers: 71% of men versus 50% of women had elevated IgG titers, and 49% of men versus 32% of women had elevated IgA titers. The distribution of titers was similar across race–ethnic groups, although white non-Hispanics had a slightly lower proportion of elevated IgG titers than non-Hispanics blacks and Hispanics (P=0.02).
Elevated IgG and IgA titers were more commonly found in cases than controls (Table 2). In conditional logistic regression analysis matched for age, sex, and race–ethnicity using the prespecified titer cutoff of 1:16, elevated IgA titers were associated with stroke risk after adjusting for hypertension, diabetes mellitus, current cigarette use, atrial fibrillation, and levels of HDL and LDL (adjusted OR, 1.5; 95% CI, 1.0 to 2.2; Table 3). Risk estimates were similar when using other titer cutoffs (Table 3). The risk estimates for IgA were slightly higher when analyses were adjusted using systolic blood pressure instead of a categorical definition of hypertension (adjusted OR, 1.7; 95% CI 1.1 to 2.5).

Elevated IgG titers were not associated with stroke risk, either at prespecified levels (adjusted OR, 1.2; 95% CI, 0.8 to 2.8) or at a higher cutoff of 1:64 (Table 3). Only 2 control participants, and no cases, had oropharyngeal swabs positive for C. pneumoniae by culture. No participants had oropharyngeal swabs positive for C. pneumoniae by PCR.

In subgroup analyses (Table 4), there was some evidence for greater risk associated with elevated IgA antibodies in women than men, although there was no statistically significant interaction. The risk associated with elevated IgA ≥1:16 was independently significant in women, and this risk appeared greater in women (adjusted OR, 2.0; 95% CI, 1.2 to 3.6) than in men (adjusted OR, 1.1; 95% CI, 0.7 to 1.9). Subgroup analyses by race–ethnicity suggested the possibility of a greater effect in non-Hispanic blacks than whites, although there was no significant interaction. However, these results must be interpreted cautiously given the small numbers involved in these subgroup analyses. Tests for interactions between C. pneumoniae IgA and the other risk factors similarly revealed no interactions.

The association between IgA titers and risk of ischemic stroke was also stratified according to ischemic stroke subtype (Table 4). There was a trend toward an association of IgA titers with large vessel atherosclerotic and lacunar stroke (adjusted OR for the combined category, 1.7; 95% CI, 0.9 to 3.1). Results for atherosclerotic and lacunar stroke considered independently were similar, although the numbers were smaller. There was less consistent evidence of an association for cardioembolic (adjusted OR, 2.5; 95% CI, 0.1 to 80.2) and cryptogenic stroke (adjusted OR, 1.2; 95% CI, 0.6 to 2.3).

### TABLE 2. Prevalence of Elevated C. pneumoniae Antibody Titers*

<table>
<thead>
<tr>
<th>Case/control</th>
<th>Overall</th>
<th>Age &lt;70 years</th>
<th>Age ≥70 years</th>
<th>Men</th>
<th>Women</th>
<th>Non-Hispanic white</th>
<th>Non-Hispanic black</th>
<th>Hispanic</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>239/428</td>
<td>156 (65.3)</td>
<td>73 (70.2)</td>
<td>84 (73.0)</td>
<td>84 (58.1)</td>
<td>24 (57.1)</td>
<td>43 (72.9)</td>
<td>89 (65.0)</td>
</tr>
<tr>
<td>IgG ≥1:32</td>
<td>156 (65.3)</td>
<td>73 (70.2)</td>
<td>84 (73.0)</td>
<td>84 (58.1)</td>
<td>24 (57.1)</td>
<td>43 (72.9)</td>
<td>89 (65.0)</td>
<td></td>
</tr>
<tr>
<td>n (%)</td>
<td>239/428</td>
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<td>84 (73.0)</td>
<td>84 (58.1)</td>
<td>24 (57.1)</td>
<td>43 (72.9)</td>
<td>89 (65.0)</td>
</tr>
<tr>
<td>IgA ≥1:16</td>
<td>112 (46.9)</td>
<td>52 (50.0)</td>
<td>60 (44.4)</td>
<td>62 (53.9)</td>
<td>50 (40.3)</td>
<td>17 (40.5)</td>
<td>33 (55.9)</td>
<td>62 (45.3)</td>
</tr>
<tr>
<td>n (%)</td>
<td>239/428</td>
<td>112 (46.9)</td>
<td>52 (50.0)</td>
<td>60 (44.4)</td>
<td>62 (53.9)</td>
<td>50 (40.3)</td>
<td>33 (55.9)</td>
<td>62 (45.3)</td>
</tr>
</tbody>
</table>

*Antibody titers could not be performed in 7 cases for technical reasons. One case and 1 control subject were characterized as “other” race–ethnicity.

### TABLE 3. Association of C. pneumoniae Titers With Risk of Ischemic Stroke (Conditional Logistic Regression Analysis)

<table>
<thead>
<tr>
<th>Prespecified titer thresholds</th>
<th>IgG ≥1:32</th>
<th>IgA ≥1:16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted OR (95% CI)*</td>
<td>1.3 (0.9–1.8)</td>
<td>1.3 (0.9–1.8)</td>
</tr>
<tr>
<td>Adjusted OR (95% CI)**</td>
<td>1.2 (0.8–1.8)</td>
<td>1.5 (1.0–2.2)</td>
</tr>
</tbody>
</table>

*Matched for age, gender, and race–ethnicity; ** matched for age, gender, and race–ethnicity and adjusted for diabetes mellitus, current cigarette use, atrial fibrillation, hypertension, and levels of HDL and LDL.
TABLE 4. Subgroup Analyses of Elevated C pneumoniae IgA Titers (≥1:16) and Ischemic Stroke Risk

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>n (Cases)</th>
<th>Adjusted OR* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total population</td>
<td>239</td>
<td>1.5 (1.0–2.2)</td>
</tr>
<tr>
<td>&lt;70 years of age</td>
<td>104</td>
<td>1.3 (0.7–2.4)</td>
</tr>
<tr>
<td>≥70 years of age</td>
<td>135</td>
<td>1.7 (1.0–2.8)</td>
</tr>
<tr>
<td>Men</td>
<td>115</td>
<td>1.1 (0.7–1.9)</td>
</tr>
<tr>
<td>Women</td>
<td>124</td>
<td>2.0 (1.2–3.6)</td>
</tr>
<tr>
<td>White</td>
<td>42</td>
<td>1.7 (0.6–4.7)</td>
</tr>
<tr>
<td>Black</td>
<td>59</td>
<td>2.9 (1.2–7.0)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>137</td>
<td>1.2 (0.7–1.9)</td>
</tr>
<tr>
<td>Stroke subtypes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large vessel atherosclerotic</td>
<td>50</td>
<td>2.2 (0.7–7.4)</td>
</tr>
<tr>
<td>Small vessel</td>
<td>61</td>
<td>1.7 (0.8–4.0)</td>
</tr>
<tr>
<td>Large vessel atherosclerotic and lacunar</td>
<td>111</td>
<td>1.7 (0.9–3.1)</td>
</tr>
<tr>
<td>Cardioembolic</td>
<td>40</td>
<td>2.5 (0.1–80.2)</td>
</tr>
<tr>
<td>Cryptogenic</td>
<td>88</td>
<td>1.2 (0.6–2.3)</td>
</tr>
</tbody>
</table>

*Adjusted for diabetes mellitus, current cigarette use, atrial fibrillation, hypertension, and levels of HDL and LDL.

Discussion

This population-based case-control study in which antibody titer thresholds were chosen before assay performance provides evidence for an association between antibodies against C pneumoniae and risk of ischemic stroke. It further suggests that the association of C pneumoniae IgA antibodies may be stronger than for IgG antibodies,4,7,18 and that risk may be more prominent in women and for ischemic stroke of non-cryptogenic origin.

Previous studies examined the role of C pneumoniae in cerebrovascular disease. In a hospital-based case-control study of 58 consecutive ischemic stroke or TIA patients <50 years of age and 52 hospitalized controls,4 47% of cases and 23% of controls had elevated IgA antibody titers (≥1:16) to C pneumoniae (adjusted OR, 1.7; 95% CI, 1.1 to 2.7). Elevated IgG levels were highly prevalent in both cases (74.1%) and controls (77.0%) and were not associated with stroke or TIA. Another case-control study5 of 176 stroke/TIA patients 35 to 86 years of age and 1518 hospitalized controls found that serologic evidence of previous infection was associated with ≥4× the risk of cerebrovascular disease. A prospective study19 that examined a combined exposure of elevated IgG or IgA antibody titers in patients with hypertension also found an elevated relative risk for stroke. These studies examined the relationship of C pneumoniae to stroke in nonelderly populations, used both stroke and TIA patients, and included recurrent as well as incident strokes. Our study found similar results in a multiethnic, elderly population limited to incident ischemic stroke, and not TIA, patients. Other prospective studies found no association of IgG or IgA titers and stroke risk.20,21 However, these latter studies may have been influenced by selection bias, use of populations with pre-existent coronary artery disease, suboptimal assay techniques, and a recent epidemic of C pneumoniae.20

In our study, IgA titers, but not IgG titers, were associated with risk of stroke. Changing the criterion for a positive IgG titer to 1:64 did not materially affect our results. The stronger association for IgA titers reflects the possibility that IgA antibodies, which are produced for only 3 to 5 days after exposure, are a marker of persistent, chronic infection, whereas IgG antibodies, which remain elevated for several years after infection, are a marker of remote, completed infection.18 Evidence from studies of IgA in other chlamydial diseases, including chronic bronchitis associated with C pneumoniae22,23 and pelvic inflammatory disease associated with C trachomatis,24 support this hypothesis. In addition, IgA is associated with persistent infection in other chronic bacterial diseases.25–27 However, according to a recent consensus statement16 and other reviews,28,29 there is not yet agreement that IgA titers are indicative of chronic, persistent infection. Measurement of IgA antibodies may be complicated by cross-reactivity with antibodies to other chlamydial species and potentially other microorganisms.28 They may also be less frequently detected in the presence of high titers of C pneumoniae IgG and rheumatoid factor.28 There is also significant interlaboratory, and even intra-laboratory variability, in measurement of C pneumoniae IgA.30 However, our laboratory was blinded to case-control status, and thus variability in the assay would be expected to bias our results toward the null value, potentially underestimating the size of the effect.30 Another potential reason for the discrepancy between associations for IgG and IgA in this population is the high prevalence of IgG.

Most studies of the association of C pneumoniae and vascular clinical events have been conducted in patients with atherosclerotic heart disease. Ischemic stroke is more heterogeneous than coronary artery disease and is caused by atherosclerosis in only 10% to 20% of cases.14 Risk factors for ischemic stroke likely differ according to underlying stroke subtype, and distinguishing among these in epidemiological analyses of potential novel risk factors is therefore important. Our study provides evidence that C pneumoniae is associated with large vessel atherosclerotic and small vessel (“lacunar”) stroke, consistent with the hypothesis that C pneumoniae contributes to atherosclerosis. Lipohyalinosis, the underlying pathophysiology in the small vessels that leads to lacunar stroke, has been considered to be an early form of atherosclerosis.31 We found evidence that atherosclerosis is involved in lacunar stroke in previous analyses of patients in northern Manhattan as well.14 Several studies identified C pneumoniae in the endothelium, smooth muscle cells, and macrophages within the vascular wall.6 Although studies have reported the presence of C pneumoniae in middle cerebral32,33 and other large cerebral vessels, no published reports have identified C pneumoniae in the small penetrating vessels of the brain. Although the point estimate for the association between C pneumoniae and cardioembolic stroke in our population was elevated (2.5), the CI was wide, and it is difficult to be certain about the effect in cardioembolic stroke. Because cardioembolic stroke in many cases results from coronary atherosclerosis, it is plausible that these strokes would also be associated with C pneumoniae. We found less evidence for association with cryptogenic stroke,
but again numbers were small. Most other studies of C pneumoniae did not distinguish between different ischemic stroke subtypes. We found some evidence for a differential effect of C pneumoniae titers in men and women. Other investigators suggested a differential effect on vascular function of infectious and inflammatory measures among women and men. Moreover, in cross-sectional analyses of C pneumoniae antibody titers in our population, we found a decrease in brachial artery endothelial reactivity associated with elevated C pneumoniae IgA titers among women but not men. There also may be differences in prevalence of antibody titers according to sex, race, and socioeconomic status. In our population, men were more likely to have positive titers, possibly obscuring the effect on stroke risk among men. Our population, although racially and ethnically diverse, shares a common environment that may minimize differences in infectious disease history. Our results should be generalizable to most urban, multiethnic populations in the United States.

Our study has strengths. Cases included only incident ischemic stroke cases, and controls were drawn from a population-based sample identified through random-digit dialing. Serological testing with MIF, used in our study, remains the “gold standard” for clinical diagnosis of C pneumoniae infection. Other postulated measures, such as PCR and flow cytometry, remain under investigation.

Our study has limitations as well. Because of its retrospective design, we cannot exclude the possibility that stroke caused antibody titers to rise in the cases. Patients with stroke could be more susceptible to C pneumoniae infection, or C pneumoniae antibody levels could rise because of nonspecific immunologic activation or from an immune response to common epitopes in C pneumoniae and infarcted brain tissue (ie, “molecular mimicry”). However, the majority (85.4%) of samples were drawn within 48 hours of admission, which should minimize poststroke changes in serology. We did not measure serum antibody titers at intervals or antibody titers to other organisms. Serologies are also known to correlate poorly with presence of C pneumoniae in vascular specimens using immunohistochemistry and other pathological techniques. In addition, although we initially performed oropharyngeal swabs in participants to determine the presence of microorganisms by culture and PCR, the yield of these techniques was low and we discontinued this practice. We were thus unable to determine the source of microorganisms that may have contributed to the elevated IgA antibody titers, as well as the specificity of these IgA titers for C pneumoniae. We did not collect data on the presence of acute or chronic respiratory tract infections. We cannot exclude the possibility that lower respiratory tract or even vascular tissue is the source of persistent exposure, but we do not have data to address this issue. In addition, although we adjusted for major stroke risk factors, there could be other unmeasured risk factors. Our subgroup analyses are also limited by small sample sizes.

Additional well-designed prospective studies of the relationship between C pneumoniae and ischemic stroke, as well as between reliable markers of other chronic infections and stroke, are needed. Although recent prospective studies have not confirmed that serologic evidence of C pneumoniae infection is associated with heart disease, most studies have not measured IgA titers. Studies of the relationship between C pneumoniae and atherosclerotic heart disease also may not reflect the relationship between C pneumoniae and stroke. Other risk factors have differential effects on heart disease and stroke. Dyslipidemia is a more important risk factor for heart disease than stroke, whereas another common infection, periodontal disease, may be more important in predicting stroke than myocardial infarction. Similarly, although animal studies and early clinical trials of anti-chlamydial agents in patients with coronary artery disease provided evidence that the risk of atherosclerotic disease associated with C pneumoniae may be modifiable, subsequent well-designed randomized controlled trials have not confirmed this benefit in patients with heart disease. Nonetheless, corroboration from large prospective studies of the role of C pneumoniae or other infections in stroke would indicate the potential for clinical trials of anti-chlamydial therapy to prevent incident or recurrent stroke, independent of effects on heart disease.

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References


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