Chlamydia pneumoniae and the Risk of First Ischemic Stroke
The Northern Manhattan Stroke Study

Mitchell S.V. Elkind, MD; I-Feng Lin, MS; J.T. Grayston, MD; Ralph L. Sacco, MD

Background and Purpose—Serological evidence of infection with Chlamydia pneumoniae has been associated with cardiovascular disease in multiple epidemiological studies. The data on its association with ischemic stroke are limited. We sought to determine whether chronic C pneumoniae infection is associated with ischemic stroke in a multi-ethnic population.

Methods—The Northern Manhattan Stroke Study contains a population-based, case-control study component. Cases had first ischemic stroke and matched control subjects were derived through random digit dialing. Titers of IgG, IgA, and IgM antibodies specific for C pneumoniae were measured with the use of microimmunofluorescence, and titers $\geq 1:16$ were considered positive. Conditional logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (95% CIs) after adjustment for medical, behavioral, and socioeconomic factors.

Results—Eighty-nine cases and 89 control subjects were selected. Mean age among cases was 68.5±12.8 years; 53% were women and 15% of the subjects were white, 28% were black, and 54% were Hispanic. Elevated C pneumoniae IgA titers were significantly associated with risk of ischemic stroke after adjusting for other stroke risk factors (adjusted OR 4.51, 95% CI 1.44 to 14.06). IgG titers were less strongly associated with stroke risk (adjusted OR 2.59, 95% CI 0.87 to 7.75). The association of IgA with stroke risk was detected in both younger and older groups, in men and women, and in whites, blacks, and Hispanics. There was also a significant continuous increase in risk associated with the log-transformation of the titer for IgA (adjusted OR 1.32, 95% CI 1.05 to 1.66) but not IgG.

Conclusions—Serological evidence of chronic infection with C pneumoniae is associated with risk of ischemic stroke in an urban, multi-ethnic population. IgA titers may be a better marker of this risk than are IgG titers. This association is independent of other vascular disease risk factors. Further prospective epidemiological studies of the effect of this infection on stroke risk are warranted. (Stroke, 2000;31:1521-1525.)

Key Words: atherosclerosis ■ cerebrovascular disorders ■ epidemiology ■ infection ■ risk factors

Knowm risk factors for ischemic stroke fail to account for all cases. Chronic infection with Chlamydia pneumoniae, a common respiratory pathogen capable of infecting endothelium, arterial smooth muscle, and monocytes, is a recently proposed risk factor for atherosclerosis and coronary artery disease. Recent studies have also suggested a role for C pneumoniae in carotid atherosclerosis and cerebrovascular disease. We sought to determine whether serological evidence of infection with C pneumoniae is associated with first ischemic stroke in a case-control study in an elderly, multi-ethnic urban population.

Subjects and Methods
The Northern Manhattan Stroke Study (NOMASS) is a population-based study designed to determine stroke incidence, risk factors, and prognosis in a multi-ethnic urban population. Northern Manhattan consists of the area north of 145th Street, south of 218th Street, bordered on the west by the Hudson River and on the east by the Harlem River. In 1990, ~260,000 people lived in the community, with 40% aged $>39$ years and a race-ethnic mixture consisting of 20% black, 63% Hispanic, and 15% white residents.

Selection of NOMASS Cases and Control Subjects
Cases eligible for NOMASS were prospectively enrolled if they met the following criteria: (1) diagnosed with first cerebral infarction after July 1, 1993, (2) aged $>39$ years at onset of stroke, and (3) resident in northern Manhattan in a household with a telephone. Patients with intracerebral or subarachnoid hemorrhage or transient ischemic attack, 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 in NOMASS have been described previously. For this analysis, 89

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noncryptogenous cases were selected at random from among the 711 stroke cases enrolled in NOMASS and matched by age, sex, and race-ethnicity to 89 stroke-free community control subjects. Cryptogenous stroke cases were excluded to allow study of a sample of cases with well-defined causes of stroke.

The methods of control recruitment and enrollment have been described in a previous publication. Random digit dialing of ~16,000 households was performed by Audits and Surveys, Inc. Community control subjects were enrolled if they (1) had never been diagnosed with stroke, (2) were aged >39 years, 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. The telephone response rate was 94%, and 70% of those respondents participated in an in-person evaluation. The study was approved by the institutional review boards at Columbia-Presbyterian Medical Center and other primary hospitals. All stroke cases and stroke-free control subjects gave consent directly or through a surrogate when appropriate.

Index Evaluation of Cases and Control Subjects
Data were collected through interviews of cases and control subjects by trained research assistants, medical record review, physical and neurological examination by the study physicians, in-person measurements, and fasting blood specimens for lipid and glucose measurements, as described elsewhere. When possible, data were obtained directly from subjects by use of the standardized data collection instruments. When the subject was unable to provide answers, a proxy knowledgeable about the subject’s history 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.

Standardized questions were adapted from the Behavioral Risk Factor Surveillance System by the Centers for Disease Control and Prevention regarding the following conditions: hypertension, diabetes, hypercholesterolemia, peripheral vascular disease, transient ischemic attack, cigarette smoking, and cardiac conditions such as myocardial infarction, coronary artery disease, anagia, congestive heart failure, atrial fibrillation, other arrhythmias, and valvular heart disease. Standard techniques were used to measure blood pressure, height, weight, and fasting glucose as described in prior publications. Fasting lipid panels (including total cholesterol, LDL, HDL, and triglyceride) were measured with the use of a Hitachi 705 analyzer (Boehringer). Hypertension was defined as in prior publications, and diabetes mellitus was defined by a fasting blood glucose level ≥127 mg/dL. The subject’s self-report of such a history, or insulin or hypoglycemic use. The definitions are noted in the legends. Because of concern that season in which stroke occurred might influence the association with an infection such as C pneumoniae, the date at which stroke occurred was also classified as to season, and this was included as a covariate in adjusted models.

Strokes were classified as extracranial atherosclerosis, intracranial atherosclerosis, lacunar (small vessel), or cardioembolic by a consensus of vascular neurologists, using the results of the diagnostic evaluation.

Assessment of C pneumoniae Status
At the time of enrollment of cases and control subjects, blood samples were obtained, centrifuged, and aliquoted into 1-mL specimens. These were frozen at −70°C until the time of analysis for C pneumoniae serology. Frozen sera were analyzed for IgG, IgA, and IgM antibody titers to C pneumoniae with the use of microimmunofluorescence, the gold-standard serological test for C pneumoniae, with the use of techniques described elsewhere. Titers of IgG and IgA were reported as 0, 2, 4, 8, 16, 32, 64, 128, 256, 512, and 1024, based on serial dilutions. Titers ≥1:16 were considered positive.

Statistical Analyses
Statistical analyses were conducted with the use of SAS computer software (SAS Institute). Means were calculated for continuous variables and proportions for dichotomous variables, and z tests were performed for comparisons of means and χ² tests for comparisons of proportions. Conditional logistic regression was used to estimate the odds ratio for matched case-control pairs before and after adjustment for potential confounders. Subgroup analyses were performed in strata defined by age, sex, and race-ethnicity. To determine whether a possible dose-response effect of titer was present, log transformation of IgG and IgA titers was performed, and a negative titer (0) was considered to be equal to 1. Univariate and multivariate regression analysis with the log-transformed titers was then used to estimate odds ratios for continuously increasing levels of titer for matched case-control pairs. Subgroup analyses were performed in strata defined by age, sex, and race-ethnicity. Statistical significance was determined at the α=0.05 level with use of 2-sided tests.

Results
The mean age of the 89 ischemic stroke cases was 68.5±12.8 years. Cases were 54% Hispanic, 28% black, and 15% white; 53% were women. Cases were significantly more likely to be current smokers and to have hypertension and diabetes. Cases had a significantly lower mean total cholesterol level, but this may be due to their concurrent significantly lower mean HDL level. There were no significant differences in LDL levels.

Controls were more likely to have a high school education (Table 1). The stroke subtypes were extracranial atherosclerosis (12%), intracranial atherosclerosis (21%), lacunar (49%), and cardioembolic (17%). Cryptogenous stroke cases were excluded by design.

The prevalence of elevated C pneumoniae titers in the control population was high: IgG 83.2% and IgA 30.3% (Table 2). No subject had elevated IgM titers. Among control subjects, a higher proportion of elevated titers was found among those ≥65 years old and among men. The distribution of titers was similar across race-ethnic groups (Table 2).

### Table 1. Characteristics of Cases and Control Subjects Matched for Age, Sex, and Race/Ethnicity

<table>
<thead>
<tr>
<th>Cases</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (%) or Mean ± SD</td>
<td>n (%) or Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>Completed high school</td>
<td>26 (29.6)</td>
<td>42 (47.2)</td>
</tr>
<tr>
<td>Diabetes mellitus*</td>
<td>32 (36.0)</td>
<td>17 (19.1)</td>
</tr>
<tr>
<td>Hypertension*</td>
<td>67 (75.3)</td>
<td>44 (49.4)</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>25 (28.1)</td>
<td>19 (21.4)</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>10 (11.2)</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>11 (12.4)</td>
<td>4 (4.5)</td>
</tr>
<tr>
<td>Current smoking</td>
<td>17 (19.3)</td>
<td>6 (6.7)</td>
</tr>
<tr>
<td>Ever smoked</td>
<td>53 (59.6)</td>
<td>51 (57.3)</td>
</tr>
<tr>
<td>Enrolled in winter</td>
<td>29 (32.6)</td>
<td>19 (21.4)</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>192.1±37.6</td>
<td>205.6±40.2</td>
</tr>
<tr>
<td>HDL, mg/dL</td>
<td>40.1±12.7</td>
<td>46.8±15.4</td>
</tr>
<tr>
<td>LDL, mg/dL</td>
<td>120.5±33.3</td>
<td>129.3±38.0</td>
</tr>
<tr>
<td>WBC, ×10^9/mL</td>
<td>8.7±3.1</td>
<td>6.4±1.9</td>
</tr>
</tbody>
</table>

*Hypertension was defined as systolic blood pressure recording ≥160 mm Hg or diastolic blood pressure recording ≥95 mm Hg or the patient’s self-report of a history of hypertension or antihypertensive use. Diabetes mellitus was defined by a fasting blood glucose level >126 mg/dL, the patient’s self-report of such a history, or insulin or hypoglycemic use.
Elevated IgG and IgA titers (≥1:16) were present in 81% and 46% of cases, respectively. In conditional logistic regression analysis (Table 3), elevated IgG titers were not associated with overall odds of first ischemic stroke in an unadjusted analysis (unadjusted OR 0.86, 95% CI 0.40 to 1.85). After adjustment for hypertension, diabetes, current smoking, HDL, education, and season of enrollment, there was a trend toward an increased odds of association (OR 2.59, 95% CI 0.87 to 7.75). Elevated IgA titers were significantly associated with risk of ischemic stroke (unadjusted OR 2.27, 95% CI 1.12 to 4.62). After adjustment for the above risk factors, the risk was increased (adjusted OR 4.51, 95% CI 1.44 to 14.06). The association was independent of cardiac disease, total cholesterol, LDL, and leukocyte count. In an analysis performed with log-transformed titers, there was a continuous increase in risk for each level of antibody titer for IgA (adjusted OR 1.32, 95% CI 1.05 to 1.66) but not IgG (Table 3). In a further analysis that used IgG ≥1:32 as the cutoff for an elevated IgG titer, the result was virtually identical to that for the cutoff of 1:16 (Table 3).

Subgroup analyses by age, sex, and race-ethnic group supported the consistency of the association of IgA and stroke risk in each subgroup, although numbers were not large enough to achieve statistical significance in each subgroup (Table 4). Odds ratios were on the order of 2.0 to 3.0. Tests for interactions between C pneumoniae IgA and the other risk factors revealed no significant interactions with any of the other risk factors examined.

### Discussion

Our case-control study supports the previously described association between chronic C pneumoniae infection and risk of ischemic stroke and extends the observation to an elderly, multiethnic population, in whom the burden of stroke is greatest. This suggests that chronic C pneumoniae infection may be a risk factor for stroke in the elderly as well as the young. Because our study was restricted to incident stroke cases, it provides a more reliable estimate of the association between infection and stroke than earlier studies. Patients with prior stroke might be more susceptible to infection with C pneumoniae, and the inclusion of such patients could artificially elevate the prevalence of antibody titers among cases. Our data also suggest that there may be a continuous relationship between level of IgA but not IgG antibody titer and stroke risk.

Five studies besides our own have examined the role of C pneumoniae in cerebrovascular disease. Wimmer et al4 reported on a hospital-based case-control study of 58 consecutive stroke or transient ischemic attack (TIA) patients aged <50 years and 52 hospitalized control subjects. Among controls and cases, 23.1% and 46.6% had elevated IgA antibody titers (≥1:16) to C pneumoniae, respectively (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. Cook et al5 found in a case-control study of 176 stroke/TIA and 1518 hospitalized controls that serological evidence of previous infection...
was associated with risk of cerebrovascular disease (OR 4.4). Patients ranged in age from 35 to 86 years. These studies examined the relation 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 multi-ethnic, elderly population limited to incident stroke (not TIA) patients.

The Atherosclerosis Risk in Communities (ARIC) Study Investigators reported a similar magnitude of association of *C pneumoniae* IgG antibodies with asymptomatic carotid atherosclerosis assessed by ultrasound imaging (adjusted OR 2.0, 95% CI 1.2 to 3.4). Patients were aged 45 to 64 years, but IgA levels were not reported. A prospective study that examined a combined exposure of elevated IgG and/or IgA antibody titers in patients with hypertension and another cardiovascular risk factor also found an elevated relative risk for stroke (8.58). Another prospective study, a nested case-control study from Sweden, found no association of IgG or IgA titers and stroke risk. This latter study may have been influenced, however, by selection bias and a recent epidemic of *C pneumoniae*.

In our study, IgA titers were more strongly associated with risk of stroke than were IgG titers. This may reflect the possibility that IgA antibodies, which last only 3 to 5 days in the circulation, are a marker of persistent, chronic infection, whereas IgG antibodies, which are produced for 3 to 5 years, are a marker of remote, completed infection. Evidence from studies of IgA in other chlamydial diseases, including chronic bronchitis associated with *C pneumoniae* and pelvic inflammatory disease associated with *Chlamydia trachomatis*, support this hypothesis. In addition, IgA is associated with persistent infection in other chronic bacterial diseases, including yersinial reactive arthritis and *Pseudomonas aeruginosa* in cystic fibrosis. Changing the criterion for a positive IgG titer to 1:32 did not materially affect our results. Another potential reason for the discrepancy between the association of IgG and IgA as stroke risk markers could be due to the high prevalence of IgG in our elderly population.

We found consistency in the association between ischemic stroke and *C pneumoniae* serology in both young and old, men and women, and in each of the 3 race-ethnic groups. Because our sample size was small, there was insufficient power to detect statistically significant associations after adjustment for other risk factors, and any subgroup analyses of this data must be viewed with caution. We did find odds ratios ranging between 1.7 and 3.0 in the different subgroups, and these were statistically significant among those aged ≥65 years and among women in unadjusted models. We did not find a difference in the prevalence of *C pneumoniae* among the different race-ethnic groups in northern Manhattan. Differences in prevalence according to race and socioeconomic status have been reported by others. Our population, though racially and ethnically diverse, shares a common environment that may minimize differences in infectious disease history. Our results, moreover, should be generalizable to most urban, multi-ethnic populations in the United States.

Our study is limited by its inclusion of patients only with defined stroke subtypes (extracranial atherosclerosis, intracranial atherosclerosis, cardioembolic, and lacunar). We chose not to include cryptogenic strokes to enrich our sample for strokes with atherosclerosis as the underlying mechanism. Almost 50% of our cases were thought to have small-vessel disease as the mechanism of stroke, and an additional 33% had either intracranial or extracranial atherosclerosis. Because small-vessel disease may share pathophysiological mechanisms with large-artery atherosclerotic disease, it is likely that our sample is weighted toward atherosclerotic mechanisms. Our numbers were too small to draw conclusions about the association of *C pneumoniae* titers with individual stroke subtypes and in particular in nonatherosclerotic subtypes, but an ongoing study in the northern Manhattan population is investigating this issue.

Because of the retrospective design of this case-control study, we cannot be certain that stroke itself did not cause the antibody titers to rise in the case population. Patients with stroke could be more susceptible to *C pneumoniae* infection, but this would not be expected to occur immediately. Alternatively, *C pneumoniae* antibody levels could rise after stroke because of immune response to common epitopes in *C pneumoniae* and infarcted brain tissue (ie, “molecular mimicry”) or because of nonspecific immunological activation. The majority (71%) of the samples in NOMASS were drawn within 48 hours of admission, however, which should minimize the possibility of poststroke changes in serology. We did not measure serial antibody titers at intervals after stroke, which might help to resolve this issue, nor did we measure antibody titers to other organisms. In addition, although we attempted to adjust in our analysis for the major stroke risk factors, there still could be residual confounding in this case-control study by other risk factors that were not measured.

At the present time, serology with the microimmunofluorescence technology used in our study remains the gold standard for the clinical diagnosis of *C pneumoniae* infection. Serologies are known to correlate poorly with presence of *C pneumoniae* in vascular specimens with the use of immunohistochemistry and other pathological techniques. Other postulated markers of infection, such as polymerase chain reaction and flow cytometry, are currently under investigation but are not yet standardized for general use. Whether these techniques will provide a more reliable marker of *C pneumoniae* infection remains under active investigation.

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. Recent prospective studies have not confirmed that serological evidence of *C pneumoniae* infection is associated with heart disease. These studies did not measure IgA antibody titers, however. Studies of the relationship between infection and atherosclerotic heart disease, moreover, may not reflect the relationship between infection and stroke. In northern Manhattan, atherosclerosis accounts for only 10% to 20% of ischemic stroke. The role of chlamydial infection as a risk factor for stroke caused by nonatherosclerotic mechanisms, such as small-vessel disease caused by lipoysis interstitial granuloma, remains unknown. Future studies will therefore need to evaluate associations within different sub-
types of stroke as well as to carefully measure and control for other potential risk factors that may confound the association.


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