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JOURNAL OF THE AMERICAN HEART ASSOCIATION

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## **Chlamydia pneumoniae Antibody Titers Are Significantly Associated With Acute Stroke and Transient Cerebral Ischemia : The West Birmingham Stroke Project**

Peter J. Cook, David Honeybourne, Gregory Y. H. Lip, D. Gareth Beevers, Richard Wise and Paul Davies

*Stroke* 1998;29;404-410

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ISSN: 1524-4628

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# *Chlamydia pneumoniae* Antibody Titers Are Significantly Associated With Acute Stroke and Transient Cerebral Ischemia

## The West Birmingham Stroke Project

Peter J. Cook, MSc; David Honeybourne, MD; Gregory Y.H. Lip, MD; D. Gareth Beevers, MD; Richard Wise, MD; Paul Davies, PhD

**Background and Purpose**—Several studies have implied an association between *Chlamydia pneumoniae* and atherosclerosis. Our research was designed to investigate the association of this organism with strokes and transient cerebral ischemia.

**Methods**—Antibodies to *C pneumoniae* were measured in 176 patients with stroke or transient cerebral ischemia and 1518 control subjects with noncardiovascular, nonpulmonary disorders. Acute infection or reinfection was defined by IgG  $\geq$ 512 or IgM  $\geq$ 8 or fourfold rise in IgG, and previous infection was defined by IgG 64 to 256 or IgA  $\geq$ 8. Logistic regression was used to examine the influences of ethnic origin, age, sex, smoking habit, diabetes mellitus, steroid medication, and social deprivation on antibody levels. Some patients underwent CT and carotid ultrasound examinations and cholesterol, triglyceride, fibrinogen, and von Willebrand factor estimations.

**Results**—We found that 13.6% of stroke/transient ischemic attack (TIA) patients and 5.7% of control subjects had antibody titers suggesting acute *C pneumoniae* (re)infection, while 32.4% of stroke/TIA patients and 12.7% of control subjects had titers suggesting previous infection ( $P < .05$ ). Stroke/TIA patients differed from control subjects in their levels of acute and previous infection, with adjusted odds ratios of 4.2 (95% CI, 2.5 to 7.1) and 4.4 (95% CI, 3.0 to 6.5), respectively. These did not differ notably between strokes resulting from major nonhemorrhagic infarcts, small-vessel infarcts, or hemorrhage. Cholesterol, triglyceride, fibrinogen, and von Willebrand factor concentrations showed no apparent association with titers.

**Conclusions**—These data support the association of cerebral vascular disease with previous *C pneumoniae* infection and the association of stroke and transient cerebral ischemia with recrudescence of infection. (*Stroke*. 1998;29:404-410.)

**Key Words:** cerebral ischemia, transient ■ infection ■ stroke, ischemic

*Chlamydia pneumoniae* was first described in 1986.<sup>1</sup> Serological studies indicate that it is one of the most prevalent infectious agents worldwide,<sup>2-4</sup> with a wide range of clinical manifestations, chiefly affecting the respiratory tract.<sup>5</sup> Serological associations have also been demonstrated with coronary artery disease<sup>6-8</sup> and asymptomatic carotid atherosclerosis.<sup>9</sup>

Our study was designed to test the association of *C pneumoniae* with stroke syndromes in the multiracial inner-city population of west Birmingham, England, after adjustments for several potential confounding variables. We subjected a proportion of stroke patients to cerebral CT and carotid arterial ultrasound examinations and measured blood concentrations of cholesterol, triglyceride, fibrinogen, and von Willebrand factor to elucidate the mechanism of any association between *C pneumoniae* and cerebrovascular disease.

### Subjects and Methods

#### Study Subjects and Investigations

Approval for this project was obtained from the hospital ethical committee at the City Hospital, Birmingham. Subjects were older

than 16 years, all were admitted to this hospital through the emergency department, and all gave informed consent to participate. All adult patients were initially considered to be eligible; recruitment was prospective and continued at a steady rate, so that subjects were admitted consecutively throughout a 24-month period (March 1993 through March 1995). Exclusion criteria were known immunodeficiency, hypergammaglobulinemia, connective tissue disease, and other autoimmune disease.

The World Health Organization definition of stroke was applied in this study, ie, rapidly developing signs of focal or global disturbance of cerebral function, with no apparent cause other than cerebrovascular disease. In this setting, fully evolved strokes were diagnosed when clinical neurological deficiency persisted for more than 24 hours or led to death, and TIAs were diagnosed when it resolved within 24 hours. Cases in which the diagnosis was uncertain, including those in which neurological deficit had resolved by the time of admission, were excluded. We also excluded all cases that were complicated by clinical or electrocardiographic evidence of acute myocardial ischemia or by clinical or radiological evidence of active pulmonary disease. Because cardiac disease so commonly accompanies cerebrovascular ischemia, we did not attempt to exclude patients with chronic cardiac disease.

Control subjects were selected randomly from all admissions to the emergency department with acute nonpulmonary, noncardiovascular

Received August 12, 1997; final revision received November 18, 1997; accepted November 18, 1997.

From the Departments of Thoracic Medicine (P.J.C., D.H.), Medicine (G.Y.H.L., D.G.B.), and Microbiology (R.W.), City Hospital, Birmingham, and School of Mathematics and Statistics, University of Birmingham (P.D.) (UK).

Correspondence to Dr P.J. Cook, Department of Medicine, Queen Elizabeth Medical Centre, Edgbaston, Birmingham B15, UK.

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### Selected Abbreviations and Acronyms

CI = confidence interval
MIF = microimmunofluorescence
OR = odds ratio
TIA = transient ischemic attack

disorders, provided that there was no evidence of active cardiac, vascular, or pulmonary disease; thus, there is no reason to suspect that they were predisposed to acquire *C pneumoniae* infection.

Blood was taken on admission (or within 12 to 24 hours, when immediate venipuncture was impossible). All patients were asked to return 2 to 3 months after discharge for convalescent blood sampling. Fasting blood samples were taken within 24 hours for serum cholesterol and triglyceride measurements, and cerebral CT and Doppler ultrasound examinations of the carotid arteries were performed within 10 days of admission. These investigations, which were ordered by the admitting physician, were not confined to any part of the study period or to any discernible subgroup of patients. We also selected patients who presented within 12 hours of the onset of neurological symptoms (to avoid a significant acute-phase response) for measurement of plasma fibrinogen and von Willebrand factor.

Demographic characteristics, medical history, smoking habit, drug therapy, and physical signs were recorded by the admitting medical staff; ethnic origin, smoking habit, and use of steroid medication were later confirmed by postal questionnaires. Townsend scores of socioeconomic deprivation were assigned to those subjects who lived in the West Midlands conurbation by linking postal code sectors to census enumeration districts. These scores, which include items that reflect predominantly personal and family income, may be considered to reflect the socioeconomic status of people living within a particular locality.<sup>10</sup> The higher the score (ie, the more strongly positive), the greater was the degree of social deprivation.

### Serological Testing

In every case, 2 to 5 mL of serum was obtained by centrifugation within 6 hours and stored at  $-20^{\circ}\text{C}$  until analysis; testing of admission and convalescent sera was deferred until both specimens were available. Each blood sample was labeled only with a serial number; thus, the investigator was blind to all patient data at the time of testing and remained so until statistical analysis of the results.

Sera were tested by one investigator using Maxiscreen *Chlamydia* MIF slides (IO International Ltd) and fluorescein-conjugated anti-human immunoglobulins. Only an even pattern of elementary body fluorescence was regarded as positive. In every batch of slides tested, two control serum preparations known to be positive for this organism and two negative control subjects were each applied to two slides. All sera were screened at a dilution of 1:8; thereafter, positive sera were tested at dilutions of 1:8 to 1:1024.

Acute infection or reinfection just before entry into the study was presumed to be indicated by titers of  $\text{IgG} \geq 512$ ,  $\text{IgM} \geq 8$ , or—in patients who provided convalescent samples—IgG rising fourfold between initial and convalescent sampling. Titers indicating previous infection without recrudescence were presumed to be IgG 64 to 256 or  $\text{IgA} \geq 8$ , provided that IgM could not be detected and there was no significant rise in IgG.

Rheumatoid factor was assayed in patients in whom connective tissue diseases were suspected on clinical grounds, despite insufficient evidence to exclude them from the study. *C pneumoniae* IgM antibodies were discounted for serological classification when rheumatoid factor was present because of nonspecific IgM reactivity.

### Analysis of Data

We compared raw frequencies of acute (re)infection and previous infection between the stroke/TIA and control groups by simple univariate analyses, using  $\chi^2$  tests. We used a logistic regression modeling method, as implemented in the EGRET statistical package, to explore possible influences of ethnic origin (Caucasian, Asian, or Afro-Caribbean), age, sex, smoking habit (current or previous versus

never), diabetes mellitus, steroid use, and Townsend score on any association of antibody levels with stroke/TIA. Therefore, we derived ORs expressing the associations of stroke/TIA with acute *C pneumoniae* (re)infection and previous infection, adjusted for potential confounding factors.

Finally, in the stroke/TIA patients only, we performed general linear model analyses of variance on cholesterol, triglyceride, fibrinogen, and von Willebrand factor concentrations, stratifying data by all potentially associated factors. Because cholesterol, triglyceride, and von Willebrand factor levels had very skewed distributions, we used their logarithmic ( $\log_e$ ) transforms for these analyses to stabilize variance.

## Results

### Study Subjects and Investigations

Twenty-six stroke/TIA patients and 312 potential control subjects were excluded by the criteria stated above, and 53 control subjects refused to participate. We recruited 1694 patients. One hundred seventy-six (10.4%) had been admitted with acute neurological dysfunction; fully evolved stroke syndromes were eventually diagnosed in 164 patients and TIAs in 12.

The remaining 1518 patients (89.6%) were assigned to the control group. In these patients, the primary indications for hospital admission were abdominal pain in 460, for which causes were established in 403 cases; chest pain, not suggestive of myocardial ischemia and for which no cause was found, in 91; malignant disease in 85; fractures of various bones in 77; urinary obstruction in 64; complications of diabetes mellitus in 44; and other miscellaneous medical and surgical diagnoses in 697.

The demographic characteristics of both groups are shown in Table 1. They were broadly similar with respect to ethnic origin and sex. The stroke/TIA group had a higher mean age, a higher proportion of diabetics, more current and previous smokers, and more steroid users (although data on smoking habit and steroid use were incomplete). These potential confounding variables were taken into account by the logistic regression analysis.

Thirty-one stroke/TIA and 282 control patients (17.6% and 18.6%, respectively) returned for convalescent blood sampling within 160 days, at a mean of 98.6 (SD 36.7) days after admission. After 160 days, we judged that sera could no longer be regarded as convalescent, and we discarded them. The demographic characteristics of these 313 patients were broadly similar to those of the study groups that they represented (Table 2).

After patients with known connective tissue or other autoimmune disease were excluded, there remained in the study 21 stroke/TIA patients and 133 control subjects in whom we suspected connective tissue diseases on clinical grounds and whom we therefore tested for the presence of rheumatoid factor. When this test was positive, in 4 stroke/TIA patients and 24 control subjects, the finding of IgM antibodies was discounted for the diagnosis of acute *C pneumoniae* infection.

Acute infection was diagnosed solely from IgM titers in 14 stroke/TIA patients and 30 control subjects, of whom 14 stroke/TIA patients and 22 control subjects would otherwise have been defined by their IgG titers as previously infected. Nine stroke/TIA patients and 11 control subjects were older than 60 years and therefore were within the age group that has been associated with unsuspected rheumatoid factor production.<sup>11</sup>

**TABLE 1. Demographic Characteristics of Stroke/TIA and Control Groups**

Patients	Stroke/TIA Group (n=176)	Control Group (n=1518)
Ethnic origin		
Caucasian	129 (73.3)	1061 (69.9)
Afro-Caribbean	30 (17.0)	290 (19.1)
Asian	17 (9.7)	167 (11.0)
Age, y		
Range	35-86	16-88
Mean	67.9	56.5
SD	10.6	18.0
Sex		
Male	103 (58.5)	844 (55.6)
Female	73 (41.5)	674 (44.4)
Smoking		
Current	58 (33.0)	448 (29.5)
Previous (>3 mo)	35 (19.9)	264 (17.4)
Never	67 (38.1)	689 (45.4)
Unknown	16 (9.1)	117 (7.7)
Diabetes mellitus		
Insulin-dependent	4 (2.3)	54 (3.6)
Non-insulin-dependent	12 (6.8)	86 (5.7)
Diabetes, type unknown	19 (10.8)	58 (3.8)
Not diabetic	141 (80.1)	1320 (87.0)
Steroid use		
Inhaled only	1 (0.6)	61 (4.0)
Systemic	1 (0.6)	52 (3.4)
None	158 (89.8)	1393 (91.8)
Unknown	16 (9.1)	12 (0.8)
Townsend scores		
Range	4.2-9.8	-7.0-10.2
Mean	5.1	4.5
SD	3.3	3.5

Values in parentheses are percentages.

Fasting serum total cholesterol concentrations were measured in 30 stroke and 421 control patients and fasting serum triglyceride levels in 28 stroke and 419 control patients. Plasma fibrinogen was measured in 71 stroke patients, and plasma von Willebrand factor was measured in 69 stroke patients. Seventy-five stroke patients underwent CT brain scanning, and 47 underwent carotid artery ultrasound examination.

Among the stroke/TIA patients, cerebral CT scans showed major nonhemorrhagic infarcts in 26, small-vessel infarcts in 23, intracerebral hemorrhage in 13, and no abnormality in 13 (12 of whom had TIAs). Carotid artery ultrasound examination showed arterial plaques in 35 stroke/TIA patients (29 of whom had clinical evidence of cerebral infarction) and was normal in 12.

### ***C pneumoniae* and Cerebral Ischemia**

Of the 176 patients in the stroke/TIA group, 24 (13.6%) had serological evidence of acute (re)infection, 57 (32.4%) previous

**TABLE 2. Demographic Characteristics of Patients Who Gave Convalescent Sera**

Patients	Stroke/TIA Group (n=31)	Control Group (n=282)
Ethnic origin		
Caucasian	24 (77.4)	200 (70.9)
Afro-Caribbean	1 (3.2)	33 (11.7)
Asian	6 (19.4)	49 (17.4)
Age, y		
Range	43-82	20-84
Mean	63.6	58.6
SD	8.6	15.5
Sex		
Male	18 (58.1)	185 (65.6)
Female	13 (41.9)	97 (34.4)
Smoking		
Current	12 (38.7)	69 (24.5)
Previous (>3 mo)	8 (25.8)	84 (29.8)
Never	10 (32.3)	121 (42.9)
Unknown	1 (3.2)	8 (2.8)
Diabetes mellitus		
Insulin-dependent	0	10 (3.5)
Non-insulin-dependent	2 (6.5)	19 (6.7)
Diabetes, type unknown	4 (12.9)	13 (4.6)
Not diabetic	25 (80.6)	240 (85.1)
Steroid use		
Inhaled only	0	19 (6.7)
Systemic	0	14 (5.0)
None	31 (100)	249 (88.3)
Unknown	0	0
Townsend scores		
Range	4.2-9.8	-5.2-9.8
Mean	5.5	4.4
SD	4.8	3.6

Values in parentheses are percentages.

infection, and 95 (54.0%) no infection. In the control group of 1518 subjects, 87 (5.7%) had evidence of acute (re)infection, 193 (12.7%) previous infection, and 1238 (81.6%) no infection. On  $\chi^2$  analysis, there was a statistically significant difference ( $P < .05$ ) between the stroke/TIA and control groups in the distribution of the three possible serological classifications—acute (re)infection, previous infection, and no infection. The crude ORs for stroke/TIA were 3.6 (95% CI, 2.2 to 5.9) for acute (re)infection and 3.9 (95% CI, 2.7 to 5.5) for previous infection. These analyses would change very little if we ignored IgM antibody titers from the 9 stroke/TIA patients and 11 control subjects older than 60 years who were not tested for rheumatoid factor and in whom we detected acute *C pneumoniae* infection solely on the basis of IgM.

Logistic regression analysis suggested statistically significant associations of stroke/TIA with both acute and previous *C pneumoniae* infection. After adjustment for ethnic origin, age, sex, diabetes mellitus, and smoking habit, the ORs for stroke/

TIA were 4.2 (95% CI, 2.5 to 7.1) for acute (re)infection and 4.4 (95% CI, 3.0 to 6.5) for previous infection. The variables that made the largest contribution to the increase in ORs after this adjustment were age and smoking habit; among young smokers, previous infection was more common in the stroke/TIA group than in the control group.

With regard to CT scan findings (in 75 stroke/TIA patients), serological results indicated acute (re)infection, previous infection, and no infection in 26.9%, 26.9%, and 46.2% of patients with major nonhemorrhagic infarcts; 17.4%, 43.4%, and 39.1% of those with small-vessel infarcts; 23.1%, 46.2%, and 30.8% of those with intracerebral hemorrhage; and 15.4%, 46.2%, and 38.5% of those with no abnormality. There was no significant variation among the four categories, individually (by  $\chi^2$  analysis) or in pairs, in their distributions of acute (re)infection, previous infection, and no infection. In this comparison, however, the small number of cases limits the power of such tests to detect variation.

With regard to carotid artery ultrasound examination (in 47 stroke/TIA patients), acute (re)infection, previous infection, and no infection were found in 17.1%, 34.3%, and 48.6% of patients with arterial plaques compared with 16.7%, 41.7%, and 41.7%, respectively, of those without plaques. Again, there was no significant difference between the two groups in this respect, but the small number of cases limits the power to detect such a difference.

In stroke/TIA patients with serological evidence of acute (re)infection, previous infection, and no infection, mean (95% CI) fasting serum concentrations of cholesterol were 5.8 (5.0 to 6.6), 5.8 (5.2 to 6.4), and 5.5 (5.2 to 6.0) mmol/L, and mean (95% CI) fasting serum concentrations of triglyceride were 2.2 (1.7 to 2.9), 1.9 (1.6 to 2.3), and 1.9 (1.7 to 2.2) mmol/L. Mean (95% CI) plasma concentrations of fibrinogen were 11.2 (9.7 to 12.3), 11.5 (10.3 to 12.6), and 12.1 (10.9 to 13.2)  $\mu\text{mol/L}$ , and mean (95% CI) plasma concentrations of plasma von Willebrand factor were 147 (127 to 167), 156 (139 to 172), and 150 (136 to 164) IU/dL, respectively. After adjustment by regression analysis for variations in ethnic origin, age, sex, and smoking habit, there were no significant differences in serum lipids, plasma fibrinogen, or plasma von Willebrand factor between stroke/TIA patients with acute *C pneumoniae* (re)infection, previous infection, and no infection.

We found no noteworthy statistical interactions (ie, effect modification) by ethnic origin, age, sex, smoking habit, diabetes mellitus, or steroid use with the *C pneumoniae*–stroke/TIA associations.

## Discussion

### Case Definition

We decided to include both fully evolved stroke syndromes and TIAs in this study, recognizing the fact that their differentiation (depending on whether clinical neurological deficit persisted for >24 hours) is somewhat arbitrary and that there are no a priori reasons to assume that different pathological processes apply in the pathogenesis of these two conditions. The World Health Organization definitions, used in this study, are widely accepted.

### *C pneumoniae* and Cerebral Ischemia

The notion that infections may be partly responsible for vascular occlusive disease is not new.<sup>12</sup> Many workers have proposed a role in atherosclerosis for Coxsackie viruses,<sup>13,14</sup> and cytomegalovirus infection is strongly associated with rapidly progressive coronary atherosclerosis in cardiac transplant recipients.<sup>15,16</sup> Other putative associations of coronary artery disease include *Helicobacter pylori*<sup>17</sup> and dental sepsis.<sup>18</sup> However, the strongest infectious contender for a role in the pathogenesis of atheroma is *C pneumoniae*. Both in chronic coronary artery disease and in acute myocardial infarction, several workers have demonstrated high levels of antibodies to *C pneumoniae*.<sup>6,19</sup> Inclusions reacting with *C pneumoniae*–specific antibodies have been reported in coronary arterial fatty streaks and fibrolipid atheromatous plaques,<sup>20,21</sup> and *C pneumoniae* DNA has been demonstrated in plaques by the polymerase chain reaction.<sup>22,23</sup>

It is noteworthy that we found substantial levels of acute and previous infection in control subjects as well as stroke/TIA patients, reflecting the high incidence of *C pneumoniae* infection in our community. Nevertheless, the results of our study support an association between cerebrovascular disease and *C pneumoniae* infection and between acute cerebral ischemia and acute recrudescence of infection. They therefore agree with the results of Melnick et al,<sup>9</sup> who reported a significant cross-sectional association between previous *C pneumoniae* infection and atherosclerosis in carotid arteries.

Since it is possible that any correlation between infection and strokes might result from a common link to atherosclerosis, our failure to distinguish ischemic from hemorrhagic strokes in some patients might weaken the apparent association with *C pneumoniae*. However, among those patients who underwent CT and carotid ultrasound examinations, we could demonstrate no significant differences in levels of *C pneumoniae* antibodies between patients with major nonhemorrhagic infarcts, small-vessel infarcts, intracerebral hemorrhage, and no abnormality; nor could we demonstrate any significant differences between patients with and without arterial plaques. There were too few patients to allow differentiation between TIAs and fully evolved stroke syndromes.

Strategies to take account of potential confounding variables are essential in studies of *C pneumoniae* antibodies, which have been associated with increasing age,<sup>24</sup> male sex,<sup>24,25</sup> and smoking.<sup>24,26</sup> In this study we found no noteworthy interactions of these factors—or of ethnic origin, use of steroid medication, diabetes mellitus, or Townsend score—with the association between *C pneumoniae* and cerebral ischemia.

Several possible mechanisms have been proposed for a role of infection in the pathogenesis of atheroma.<sup>12</sup> Lipid metabolism is clearly important<sup>27</sup>; for example, animal models have shown that circulating monocytes adhere to and penetrate the endothelium at an early stage in atherogenesis<sup>28</sup> and become engorged with oxidized LDL, thereby producing the “foam cells” that are so characteristic of atheroma.<sup>29</sup> LDL, whose concentration is increased in sepsis, becomes oxidized when free superoxide radicals are released from endothelial cells or monocyte-macrophages.<sup>30</sup> Oxidized LDL is a strong chemoattractant for monocytes<sup>31</sup> and may form a number of cytotoxic molecules important in vascular damage and plaque evolu-

tion.<sup>32</sup> In our study there was no significant association of cholesterol or triglyceride levels with *C pneumoniae* antibodies, implying that a global dysregulation of lipid metabolism plays no part in the association with cerebral ischemia.

Thrombogenesis is intimately related to atherogenesis, and it is widely believed that prothrombotic states predispose to atherosclerotic vascular disease. Many factors may influence the development of these conditions, among which fibrinogen is probably the most important.<sup>33,34</sup> High levels of this protein are significantly associated with peripheral vascular disease, in which they confer an increased risk of cardiac events and mortality.<sup>35</sup> Infection is known to increase blood concentrations of plasma fibrinogen, and in a recent study an increased plasma fibrinogen level was independently associated with seropositivity for *C pneumoniae*.<sup>36</sup> Fibrinogen and von Willebrand factor may be regarded as possible markers for a prothrombotic state and for endothelial damage, respectively. Neither was significantly associated with *C pneumoniae* seropositivity in our study, but since they were not measured in all patients, this observation demands some caution.

As discussed above, other infectious agents have been also implicated in atherosclerosis. We believe that the rise in specific antibody levels that we have observed is disproportionate to the nonspecific immunoglobulin increase that is associated with "acute-phase reactions"; but even so, this rise does not prove a causal role for *C pneumoniae*. The possibility that some of our subjects had polymicrobial infections and that *C pneumoniae* antibodies merely confound a more important association has not been addressed. In this respect, the interactions of diverse organisms may prove to be crucial and deserve to be investigated in future research.

### Serological Testing

Various techniques are available to detect *C pneumoniae* antibodies.<sup>37</sup> The best and most widely used is the MIF assay,<sup>38</sup> which is time-consuming and subject to some operator variation but is sensitive and species-specific and reliably detects IgG, IgM, and IgA.<sup>39</sup> The kit that we used has been employed in several studies<sup>2,40</sup> and performs similarly to other MIF assays.

The principle that a fourfold rise in specific IgG antibody titer should constitute evidence of acute primary *C pneumoniae* infection is generally accepted.<sup>41</sup> However, the rise may not be seen for 8 to 10 weeks<sup>42</sup> and may persist for several months<sup>43</sup>; we therefore decided to set a time limit of 160 days on the interval between initial and convalescent sera. In a single serum specimen, an IgG titer  $\geq 512$  can be interpreted as evidence of acute primary infection<sup>44</sup> or—particularly in older people—reinfection or recrudescence of chronic or latent infection.<sup>45</sup> IgG titers  $\leq 256$  may persist for many months<sup>46</sup> and have generally been accepted (in a single serum specimen) as evidence of previous infection,<sup>47</sup> provided that there is no rise in IgM antibodies. In our study patients who had IgG titers  $\geq 512$  in the first serum and who gave convalescent samples invariably showed a fall in titers between the two sera, but some who had initial titers of 64 to 256 maintained them.

Many authors have employed these serological criteria,<sup>48–52</sup> and a large study combining serology with an examination of pharyngeal swabs by polymerase chain reaction has provided evidence in support of them.<sup>53</sup> We chose to reject IgG titers  $< 64$  to minimize the probability of false-positive results.<sup>54</sup> Our

decision to include IgA is justified by experimental evidence that it persists for more than 3 weeks in chronic infection.<sup>55,56</sup>

IgM is generally considered to signify acute primary infection.<sup>38</sup> A threshold titer of  $\geq 16$  has been proposed, with titers of 8 indicating "probable acute infection,"<sup>57</sup> but we found no difference in the proportions of patients with IgM antibodies at these two titers in a pilot study and therefore saw no merit in drawing a distinction between such low levels of antibody production. It has been suggested<sup>58</sup> that rheumatoid factor may make the measurement of IgM antibodies unreliable, particularly in elderly patients.<sup>11</sup> For this reason, we excluded from the study all patients with known connective tissue or other autoimmune disease and measured rheumatoid factor in other cases in which such diseases were considered possible, discounting IgM antibodies when it was present. (The presence of connective tissue disease per se was not considered to be an important confounding variable. We therefore did not measure other markers of such diseases, such as antinuclear antibodies, which are not reported to interfere significantly with IgM assays.) As demonstrated above, ignoring IgM titers from the remaining patients older than 60 years would not have significantly altered the relationships of stroke/TIA to acute and chronic *C pneumoniae* infections.

### Limitations of This Study

Not all stroke patients underwent cerebral CT and carotid artery ultrasound examinations and estimations of plasma fibrinogen and plasma von Willebrand factor, and no control subjects did so. Only limited conclusions can be drawn from these measurements.

Fasting serum total cholesterol and triglyceride were measured in only a third of all patients. We did not attempt to differentiate between HDL and LDL cholesterol, but we suggest that such a differentiation might be informative. Furthermore, our approach does not address the possibility of altered lipid metabolism or accumulation of cholesteryl esters within cells.

We believe that our strategy for preventing interference by rheumatoid factor in IgM measurement has allowed us to make a valid interpretation of the results. However, in the future we would recommend the routine absorption of sera with anti-human IgG, as advocated by Verkooyen et al.<sup>11</sup>

### Conclusions

These data support the association of cerebrovascular disease with previous *C pneumoniae* infection and the association of acute stroke and TIAs with acute recrudescence of infection. We believe that more research to elucidate the mechanism(s) of these associations is warranted and that particular attention should be paid to dysregulation of thrombogenesis and lipid metabolism.

### Acknowledgments

This study was supported by the Peel Medical Research Trust, the British Heart Foundation, and the Stroke Association (P.J.C.). We wish to acknowledge the advice of Richard Matthews (Virology Laboratory, City Hospital, Birmingham). We thank Dr Andrew Blann for assistance with plasma fibrinogen and von Willebrand factor measurements. The advice and support of Dr John Treharne (Senior Lecturer in Virology, University of London) are also gratefully

acknowledged. We wish to thank IO International Ltd, London, for their generous help. We also thank the referees of an earlier draft of this paper for their helpful comments and suggestions.

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