

Chlamydia pneumoniae, *Mycoplasma pneumoniae*, and *Legionella pneumophila* in Elderly Patients With Stroke (C-PEPS, M-PEPS, L-PEPS)

A Case-Control Study on the Infectious Burden of Atypical Respiratory Pathogens in Elderly Patients With Acute Cerebrovascular Disease

Joseph Ngeh, MSc, MRCP; Colin Goodbourn, MSc, MRCPATH

Background and Purpose—Multiple studies have suggested an association between *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* infection and cardiovascular disease. We investigated whether the risk of cerebrovascular disease is associated with *Legionella pneumophila* infection and the aggregate number/infectious burden of these atypical respiratory pathogens.

Methods—One hundred patients aged >65 years admitted with acute stroke or transient ischemic attack (TIA) and 87 control patients admitted concurrently with acute noncardiopulmonary, noninfective conditions were recruited prospectively. Using enzyme-linked immunosorbent assay (ELISA) kits, we previously reported the seroprevalences of *C pneumoniae* and *M pneumoniae* in these patients. We have now determined the seroprevalences of *L pneumophila* IgG and IgM in this cohort of patients using ELISA.

Results—The seroprevalences of *L pneumophila* IgG and IgM were 29% (n=91) and 12% (n=81) in the stroke/TIA group and 22% (n=86) and 10% (n=72) in the controls, respectively. Using logistic regression to adjust for age, sex, hypertension, smoking, diabetes, ischemic heart disease, and ischemic ECG, the odds ratios for stroke/TIA in relation to *L pneumophila* IgG and IgM were 1.52 (95% CI, 0.70 to 3.28; $P=0.29$) and 1.49 (95% CI, 0.45 to 4.90; $P=0.51$), respectively. The odds ratios in relation to IgG seropositivity for 1, 2, or 3 atypical respiratory pathogens after adjustment were 3.89 (95% CI, 1.13 to 13.33), 2.00 (95% CI, 0.64 to 6.21), and 6.67 (95% CI, 1.22 to 37.04), respectively ($P=0.06$).

Conclusions—*L pneumophila* seropositivity is not significantly associated with stroke/TIA. However, the risk of stroke/TIA appears to be associated with the aggregate number of chronic infectious burden of atypical respiratory pathogens such as *C pneumoniae*, *M pneumoniae*, and *L pneumophila*. (*Stroke*. 2005;36:259-263.)

Key words: *Chlamydia pneumoniae* ■ elderly ■ epidemiology ■ infection ■ ischemic attack, transient ■ *Legionella pneumophila* ■ *Mycoplasma pneumoniae* ■ stroke

Multiple studies have suggested acute and chronic respiratory infections as potential risk factors for stroke.¹⁻³ The link between atypical respiratory pathogens such as *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* and cerebrovascular disease has been investigated in seroepidemiological, pathological, and clinical case studies.^{4,5} Recent studies have reported an association between exposure to a number of infectious pathogens, ie, infectious burden, and the occurrences and prognoses of atherosclerotic vascular diseases.⁶⁻¹⁶ These studies mainly focused on ischemic heart disease (IHD) and were performed in younger patients.

Legionella pneumophila, like *C pneumoniae* and *M pneumoniae*, is an atypical respiratory pathogen.^{17,18} The aim of the present study was to investigate whether serological

markers or immunoglobulins of *L pneumophila* infection and the aggregate number (infectious burden) of *C pneumoniae*, *M pneumoniae*, and *L pneumophila* seropositivity were associated with acute stroke/transient ischemic attack (TIA) in subjects aged ≥ 65 years.

Subjects and Methods

We have reported the *Chlamydia pneumoniae* in Elderly Patients With Stroke (C-PEPS)⁴ and *Mycoplasma pneumoniae* in Elderly Patients With Stroke (M-PEPS)⁵ case-control studies. The C-PEPS study⁴ had 100 cases and 87 controls, whereas the M-PEPS study⁵ had 95 cases and 82 controls. The subjects of the current *Legionella pneumophila* in Elderly Patients With Stroke (L-PEPS) case-control study originated from the C-PEPS study.⁴ The L-PEPS study had excluded 7 cases of hemorrhagic stroke without acute infarction as

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From the Department of Geriatric and Stroke Medicine (J.N.) Warwick Hospital, Warwick; and the Department of Microbiology, Whipps Cross University Hospital (C.G.), London, UK.

Correspondence to Joseph Ngeh, Department of Geriatric and Stroke Medicine, Warwick Hospital, Lakin Rd, Warwick CV34 5BW, UK. E-mail Joseph.Ngeh@swh.nhs.uk

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detected on CT head scan. Additionally, the L-PEPS study had excluded 2 cases and 1 control because of a shortage of enzyme-linked immunosorbent assay (ELISA) kits. Thus, the L-PEPS study consisted of the remaining 91 cases and 86 controls. The seroprevalence of *L pneumophila* IgG was measured for all of them, but that of *L pneumophila* IgM was measured for only 81 cases and 72 controls because of a shortage of IgM ELISA kits. Of the 91 cases and 86 controls of the L-PEPS study, the seroprevalences of *C pneumoniae*, *M pneumoniae*, and *L pneumophila* were measured for only 88 cases and 82 controls for IgG and 81 cases and 72 controls for IgM because of a limited supply of ELISA kits. All studies were approved by the hospital's local research ethics and research and development committees.

The study population was recruited at a 750-bed acute district general hospital in northeast London from December 20, 1999, to March 31, 2000.⁴ Patients were eligible for inclusion if they were white, aged ≥ 65 years, and admitted acutely to the hospital under the care of general physicians. Whereas cases were admitted patients with a primary diagnosis of acute stroke/TIA, controls subjects were patients with no past history of stroke/TIA who were admitted concurrently with acute noncardiopulmonary, noninfective conditions. All patients' demographics and history of vascular risk factors such as hypertension, smoking, diabetes mellitus, hypercholesterolemia, IHD, and stroke/TIA were obtained.⁴ Investigations such as CT brain scan, ECG, and routine blood tests were ordered/performed by clinicians not directly involved in the recruitment of patients.

The exclusion criteria were immunodeficiency, hypergammaglobulinemia, connective tissue disease, and other autoimmune disease because these may interfere with immunoglobulin production. For controls, those who had a history of stroke/TIA, acute/active cardiopulmonary disorder, or infective conditions on admission were also excluded.

Serological Analysis

Up to 2 mL of each patient's serum obtained on admission was stored at -20°C for subsequent analysis at the end of the study period. A commercial ELISA kit (Vircell SL) was used for the analysis of *L pneumophila* serogroup 1-specific IgG and IgM antibodies in serum. The antigens used are derived from inactivated LPS antigens of *L pneumophila* serogroup 1. Sera intended for *L pneumophila* IgM testing were pretreated with manufacturer's anti-IgG sorbent to remove rheumatoid factor and reduce IgG interference. The results of the ELISA were interpreted as negative, equivocal, or positive on the basis of a single dilution of a serum sample and optical density measured by a spectrophotometer at a wavelength of 450/620 nm. Sera were analyzed independently by 2 investigators blinded to the case or control status of the patients, in accordance with the manufacturer's protocol.

Statistical Methods

The sample size calculation was based on the C-PEPS study,⁴ which had 90% power to detect an odds ratio (OR) of ≥ 3.0 . Raw data were initially computed with the use of the Microsoft Excel program. With the use of the SAS statistical package, a logistic regression model adjusting for confounders such as age, sex, hypertension, smoking, diabetes mellitus, hypercholesterolemia, IHD, and ischemic ECG was constructed to analyze the association of *L pneumophila* serogroup 1 antibody and stroke/TIA. We identified cases and controls who tested IgG or IgM seropositive for either 0, 1, 2, or all 3 pathogens. Fisher exact tests were used for crude analysis, and logistic regression was used to adjust for confounders. The ORs for stroke/TIA in relation to infectious burden were determined. We also investigated whether specific combinations of *C pneumoniae*-*M pneumoniae*, *M pneumoniae*-*L pneumophila*, or *L pneumophila*-*C pneumoniae* IgG seropositivity would increase the risk of stroke/TIA.

Results

In this L-PEPS study, the median age was 80 years (range, 65 to 98 years) in the 91 cases and 82 years (range, 65 to 95

TABLE 1. Distribution of Risk Factors in 91 Stroke/TIA Cases and 86 Controls*

Vascular Risk Factor	Cases (%)	Controls (%)
Hypertension		
No	47 (52)	51 (59)
Yes	43 (48)	35 (41)
No.	90	86
Smoking		
Never	34 (38)	28 (32)
Former	41 (45)	47 (55)
Current	15 (17)	11 (13)
No.	90	86
Diabetes mellitus		
No	83 (91)	76 (88)
Yes	8 (9)	10 (12)
No.	91	86
Hypercholesterolemia		
No	5 (50)	8 (67)
Yes	5 (50)	4 (33)
No.	10	12
Missing	81	74
IHD		
No	74 (81)	62 (72)
Yes	17 (19)	24 (28)
No.	91	86
ECG ischemia		
No	38 (44)	38 (54)
Yes	49 (56)	32 (46)
No.	87	70

*Because of missing values, the numbers of cases and controls do not always add to 91 and 86, respectively.

years) in the 86 controls. Fifty-three cases (58%) and 59 controls (69%) were women. CT head scan results were available for 71 of the 91 cases: 49 had an infarct and 2 had a hemorrhagic infarct, and in 20 no lesion was demonstrated.

Table 1 shows the distribution of vascular risk factors in 91 stroke/TIA cases and 86 controls. The seroprevalences of *L*

TABLE 2. Seroprevalence of *L pneumophila* Antibody in Cases and Controls

<i>L pneumophila</i> Antibody	Cases, No. (%)	Controls, No. (%)	P*
IgG			
Negative	52 (57)	57 (66)	0.50
Equivocal	13 (14)	10 (12)	
Positive	26 (29)	19 (22)	
Total	91	86	
IgM			
Negative	67 (83)	62 (86)	0.84
Equivocal	4 (5)	3 (4)	
Positive	10 (12)	7 (10)	
Total	81	72	

*Fisher exact test.

TABLE 3. ORs for Stroke/TIA in Relation to Serological Markers of *L pneumophila* Infection

<i>L pneumophila</i> Antibody	OR*	95% CI	P
Unadjusted for confounders			
IgG	1.41	0.71–2.79	0.32
IgM	1.31	0.47–3.64	0.61
Adjusted for hypertension, smoking, diabetes, IHD, age, sex, and ischemic ECG			
IgG	1.52	0.70–3.28	0.29
IgM	1.49	0.45–4.90	0.51

*OR of being antibody positive compared with antibody negative/equivocal by logistic regression analysis.

pneumophila IgG and IgM in the stroke/TIA group were 29% and 12%, respectively, and were similar to those in the control group (22% and 10%) (Table 2). The ORs for stroke/TIA in relation to *L pneumophila* seropositivity were 1.52 (95% CI, 0.70 to 3.28; $P=0.29$) for IgG and 1.49 (95% CI, 0.45 to 4.90; $P=0.51$) for IgM after statistical adjustment for potential confounders (Table 3).

The ORs for stroke/TIA in relation to the number of pathogens tested seropositive for IgG or IgM are presented in Table 4. The ORs in relation to IgG seropositivity for 1, 2, or 3 atypical respiratory pathogens after adjustment were 3.89

TABLE 4. ORs for Stroke/TIA in Relation to IgG or IgM Seropositivity for 0, 1, 2 or 3 of *C pneumoniae*, *M pneumoniae*, and *L pneumophila*

No. of Pathogens	No. of Cases	No. of Controls	OR (Crude)	OR (Adjusted)*
IgG				
0	8	16	1	1
1	30	21	2.86 (1.03–7.89)	3.89 (1.13–13.33)
2	39	42	1.86 (0.72–4.82)	2.00 (0.64–6.21)
3	11	3	7.33 (1.58–33.97)	6.67 (1.22–37.04)
All	88	82	$P=0.04\ddagger$	$P=0.06\ddagger$
IgM				
0	60	55	1	1
1	17	10	1.56 (0.66–3.69)	1.44 (0.53–3.91)
2	4	6	0.61 (0.16–2.28)	0.77 (0.17–3.50)
3	0	1	Not estimable	Not estimable
All	81	72	$P=0.87\ddagger$	$P=0.91\ddagger$
IgG				
0	5†	16	1	1
1	19†	21	2.90 (0.89–9.43)	3.80 (0.89–16.39)
2	26†	42	1.98 (0.65–6.05)	2.25 (0.59–8.55)
3	7†	3	7.47 (1.39–40.25)	7.09 (1.05–47.62)
All	57†	82	$P=0.09\ddagger$	$P=0.15\ddagger$

Numbers in parentheses are 95% CIs. P value for test of trend across the ORs was 0.10 (crude) and 0.23 (adjusted).

*Adjusted for age, sex, hypertension, diabetes mellitus, smoking, IHD, and ischemic ECG.

†No. of first-ever cases. P value for test of trend across the ORs was 0.12 (crude) and 0.20 (adjusted).

‡Logistic regression analysis (test of association between No. of pathogens and risk of stroke/TIA).

TABLE 5. ORs for Stroke/TIA in Relation to Specific Combinations of *C pneumoniae*–*M pneumoniae*, *M pneumoniae*–*L pneumophila*, and *L pneumophila*–*C pneumoniae* IgG Seropositivity

Combination of Pathogens (IgG)	Seropositivity	OR*	P†
Cp-Mp	No pathogens‡	1.00	0.20
	Either Cp or Mp	2.84 (0.90–8.93)	
	Both Cp and Mp	2.50 (0.79–7.87)	
Mp-Lp	No pathogens‡	1.00	0.47
	Either Mp or Lp	1.29 (0.56–2.95)	
	Both Mp and Lp	2.09 (0.65–6.71)	
Lp-Cp	No pathogens‡	1.00	0.18
	Either Lp or Cp	2.38 (0.80–7.09)	
	Both Lp and Cp	3.22 (0.91–11.36)	

Cp indicates *C pneumoniae*; Mp, *M pneumoniae*; and Lp, *L pneumophila*. Numbers in parentheses are 95% CIs.

*Adjusted for age, sex, hypertension, diabetes mellitus, smoking, IHD, and ischemic ECG.

†Logistic regression analysis.

‡Not positive for any of Cp, Mp, or Lp.

(95% CI, 1.13 to 13.33), 2.00 (95% CI, 0.64 to 6.21), and 6.67 (95% CI, 1.22 to 37.04), respectively ($P=0.06$). Table 5 shows the ORs for stroke/TIA in relation to specific combinations of *C pneumoniae*–*M pneumoniae*, *M pneumoniae*–*L pneumophila*, and *L pneumophila*–*C pneumoniae* IgG seropositivity.

The acute medical conditions of the 87 controls in the C-PEPS study⁴ were grouped as follows: (1) gastrointestinal (40 subjects); (2) musculoskeletal pain/immobility/falls (20 subjects); (3) hematological (12 subjects); (4) neurological/psychiatric (11 subjects); and (5) renal/metabolic disturbances (4 subjects). The seroprevalences of *C pneumoniae*, *M pneumoniae*, and *L pneumophila* were similar among the medical condition groups of the controls (except *M pneumoniae* and *L pneumophila* IgM; Table I, available online only at <http://www.strokeaha.org>).

Among the 81 stroke and 10 TIA cases in the L-PEPS study, 58 subjects were admitted with first-ever stroke/TIA. Thirty-two subjects had ≥ 1 episodes of stroke/TIA in the past. Table II (available online only at <http://www.strokeaha.org>) compared the IgG and IgM seroprevalences of *C pneumoniae*, *M pneumoniae*, and *L pneumophila*, as well as their aggregate number in first-ever (including 1 case without data for previous stroke/TIA) and recurrent stroke/TIA cases, showing no significant differences in IgG and IgM status between the first-ever and recurrent cases.

Discussion

In the L-PEPS study, the seroprevalences of *L pneumophila* serogroup 1 were 12% to 29% for the stroke/TIA cases and 10% to 22% for the controls, both within the range of 0.4% to 32% reported worldwide.¹⁷ The seroprevalence of *L pneumophila* in stroke/TIA patients did not differ significantly from that of controls (Table 2). With the use of logistic regression to adjust for hypertension, smoking, diabetes, IHD, age, sex, and ischemic ECG, the ORs for stroke/TIA in

relation to IgG and IgM seropositivity were 1.52 ($P=0.29$) and 1.49 ($P=0.51$), respectively (Table 3). These results suggested that serological markers of *L pneumophila* infection were not significantly associated with acute stroke/TIA.

Recent studies have suggested that an increasing number of microorganisms, ie, infectious burden, as evidenced by serological markers of chronic infection such as IgG, was associated with an incremental risk of cardiovascular and cerebrovascular diseases.^{6–16} The completion of the L-PEPS study provides a unique opportunity to investigate for the first time, in a case-control fashion, the infectious burden of the 3 most common atypical respiratory pathogens in elderly stroke/TIA patients. Although the data are suggestive of an association between stroke/TIA and chronic infectious burden of atypical respiratory pathogens as evidenced by IgG, but not IgM, seropositivity, a small sample size is suggested by the wide CIs (Table 4). The association was statistically significant ($P=0.04$) before adjustment for confounders and close to significance after adjustment ($P=0.06$). When recurrent stroke/TIA cases were excluded from analysis, the overall association became statistically less significant, with $P=0.09$ before adjustment and $P=0.15$ after adjustment. With only 3 estimates of ORs, the probability value for a test of trend across the ORs was not significant. However, the OR for stroke/TIA and infectious burden after adjustment appeared strongest in subjects infected with at least 3 pathogens: OR=6.67, 95% CI, 1.22 to 37.04, with OR=7.09, 95% CI, 1.05 to 47.62 for first-ever cases of stroke/TIA. Table 5 showed that specific combinations of *C pneumoniae*–*M pneumoniae*, *M pneumoniae*–*L pneumophila*, or *L pneumophila*–*C pneumoniae* did not increase the risk of stroke/TIA significantly.

A number of bacteria and viruses may contribute to atherogenesis and atherothrombosis through inflammatory/immunological mechanisms.³ Respiratory infections like influenza virus,¹ *C pneumoniae*,^{3,4} and *M pneumoniae*⁵ have been associated with cerebrovascular disease. *C pneumoniae* is the microorganism most investigated in the “infectious hypotheses” of atherosclerosis. *L pneumophila*, like *C pneumoniae*, is a gram-negative, intracellular bacterium that may exert a chronic immunological response.^{3,10,18–21} Together, *L pneumophila*, *C pneumoniae*, and *M pneumoniae* share similar microbiological and clinical features, including neurological manifestations.^{18,19,22–24} They are important atypical respiratory pathogens that cause 8% to 50% of cases of community-acquired pneumonia.¹⁸

Serological methods such as indirect fluorescence antibody (IFA) and ELISA have test sensitivity of 40% to 80% and specificity of 95% to 99%.^{18,19,25} They have been recommended for epidemiological studies of *Legionella* infection.^{18,19,25} The ELISA test is less subjective than the IFA standard reference test.^{25,26} According to the manufacturer, Vircell ELISA IgM had sensitivity of 83% and specificity of 97% compared with an immunofluorescence test. The sensitivity and specificity for Vircell ELISA IgG were reported as 95% and 97%, respectively.

The C-PEPS,⁴ M-PEPS,⁵ and L-PEPS studies had several limitations. The number of controls was small and less than that of cases. The controls were selected from admitted

patients and could be biased. We have analyzed the seroprevalences of *C pneumoniae*, *M pneumoniae*, and *L pneumophila* among the medical condition groups of the controls (Table I, available online at <http://stroke.ahajournals.org>). There was no association between atypical respiratory infection and medical conditions in the controls. Although the result was statistically significant for *M pneumoniae* IgM ($P=0.02$), the number of positive controls was so small ($n=9$) that this could be due to chance. Silent or subclinical cerebrovascular disease in the controls could not be excluded, although CT would have been helpful to detect asymptomatic stroke in this group. Subclinical atypical respiratory infection or even epidemic during the study period might induce an antibody response in the controls, thereby obscuring any genuine serological association between infection and stroke/TIA.

Patients with stroke could be more susceptible to respiratory tract infection. It could be argued that infection may not be a cause of the present stroke event but a result of past/recurrent stroke. However, the results in Table II (available online at <http://stroke.ahajournals.org>) showed that the seroprevalences of the pathogens did not differ between cases with first-ever or recurrent stroke/TIA.

Although a significant association between individual serological markers of *C pneumoniae*,⁴ *M pneumoniae*,⁵ *L pneumophila*, and acute cerebrovascular events was not apparent, this preliminary case-control study nevertheless suggested that a collective burden of chronic atypical respiratory infection (IgG seropositivity) may be associated with an increased risk of acute stroke/TIA. Whether seroprevalences of atypical respiratory pathogens in elderly patients could reflect a lifelong recurrent infection or an infective burden related to an etiological link with atherosclerosis will need to be confirmed in larger, prospective outcome studies.

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