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Chronic *Chlamydia pneumoniae* Infection and Stroke in Cameroon

A Case-Control Study

Alfred K. Njamnshi, Kathleen Ngu Blackett, Josephine N. Mbuagbaw, Freedom Gumedze, Sandeep Gupta, Charles S. Wiysonge

Background and Purpose—To determine the relationship between chronic *Chlamydia pneumoniae* infection and stroke in Cameroon.

Methods—Sixty-four consecutive stroke patients 26 to 80 years of age were enrolled at 2 tertiary hospitals in Yaoundé, Cameroon, between March 2000 and December 2001 and matched for age and sex to 64 controls. We measured IgG (1/64) and IgA (1/16) titers against *C pneumoniae* in both patients and controls using a validated microimmunofluorescence technique.

Results—There was no significant difference between cases and controls with respect to hypertension ($P=0.2$), smoking ($P=0.53$), alcohol intake ($P=0.8$), body mass index ($P=0.49$), waist-to-hip ratio ($P=0.14$), and diabetes ($P=0.76$). IgA antibodies were detected in 50 (78.1%) patients and 27 (42.2%) controls (odds ratio [OR] 4.29; 95% CI, 1.84 to 11.56; $P=0.0002$), and IgG antibodies in 41 (64.1%) patients and 35 (54.7%) controls (OR, 1.46; 95% CI, 0.68 to 3.22; $P=0.29$). For confirmed thrombotic stroke, the association with IgA antibodies became stronger (OR, 21.0; 95% CI, 3.38 to 868.45; $P<0.0001$), but there was still no association with IgG antibodies (OR, 1.86; 95% CI, 0.69 to 5.50; $P=0.18$).

Conclusions—Our study shows a strong statistical association between (IgA, and not IgG, as a serological marker of) chronic *C pneumoniae* infection and stroke for the first time in a resident indigenous African population. These findings, if confirmed, may have important policy implications (in terms of antibiotic use in stroke prevention) in sub-Saharan Africa. (*Stroke*. 2006;37:796-799.)

Key Words: atherosclerosis ■ infection ■ stroke

Atherosclerosis is a leading cause of mortality and morbidity worldwide.¹ Conventional stroke risk factors including hypertension, cigarette smoking, diabetes mellitus, and hyperlipidemia do not fully explain the incidence of atherosclerotic vascular disease. Inflammation has been postulated to play an important role in the initiation and development of atherosclerosis.² Recently, increasing interest has focused on the putative causal role of chronic infections such as *Helicobacter pylori*³ and *Chlamydia pneumoniae*.⁴⁻⁶ *C pneumoniae* is a Gram-negative intracellular bacterium that commonly causes upper respiratory tract infection, bronchitis, pharyngitis, sinusitis, and pneumonia.⁷ The link between *C pneumoniae* and cerebrovascular disease has been investigated in a number of seroepidemiological⁸⁻¹³ and antibiotic intervention¹⁴ studies. However, the causal role of *C pneumoniae* infection is still controversial.¹⁵ In addition, to the best of our knowledge, no such study has been performed in

any resident indigenous African population. The present study was designed to investigate whether serological markers of *C pneumoniae* infection were associated with acute stroke or transient ischemic attack (TIA) in an indigenous population of Cameroon in sub-Saharan Africa. The prevalence of stroke in patients attending the university teaching hospital in Yaoundé, Cameroon varies from 2% in the internal medicine unit to 9% in the medical intensive care unit.¹⁶ In the Yaoundé Central Hospital, stroke prevalence is 3% in the internal medicine unit, with 52% hemorrhagic and 48% ischemic in patients with brain scan confirmation.¹⁷

Patients and Methods

This case-control study was undertaken in the university teaching hospital and central hospital in Yaounde, Cameroon. Consecutive patients presenting with stroke or TIA in these hospitals were recruited into the study from March 2000 to December 2001. Patients were eligible for inclusion if they were natives of Cameroon,

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The results of this research have been published in abstract form only in the *Cameroon Journal of Medicine* and the abstract book of the World Stroke Conference in Vancouver, Canada (June 2004).

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TABLE 1. Characteristics of Study Population

Risk Factor	Cases	Controls	P Value
Hypertension*	53/64 (82.8%)	48/64 (75.0%)	0.20
Current smokers	12/62 (19.7%)	8/31 (25.8%)	0.53
Current alcohol consumers	34/63 (54.8%)	20/31 (64.5%)	0.80
Diabetes	8/59 (13.6%)	7/44 (15.9%)	0.76
Height, meters (m)	1.64 (0.08)	1.67 (0.08)	0.16
Weight, kilograms (kg)	74 (14)	72 (13)	0.54
Body mass index, kg/m ²	27.4 (4.5)	26.6 (4.5)	0.49
Waist circumference, centimeters (cm)	89 (14)	97 (11)	0.06
Hip circumference, cm	95 (16)	99 (11)	0.40
Waist-to-hip ratio	0.94 (0.09)	0.98 (0.08)	0.14

Values are count/total (proportion) for categorical and mean (SD) for continuous variables. Denominators differ because of missing values; this information was not completed on the data collection forms. *Hypertension indicates blood pressure ≥140/90 mm Hg or current use of blood pressure-lowering drugs.

admitted to either hospital with stroke or TIA, and had no signs of acute infection. Stroke was defined according to the recommended standard World Health Organization definition as a focal (or at times global) neurological impairment of sudden onset, lasting >24 hours or leading to death, and of presumed vascular origin.¹⁸ An abrupt-onset neurological deficit lasting <24 hours was considered a TIA. Sixty-four consecutive patients were recruited prospectively. Controls, matched for age, sex, and (as much as possible) hypertensive status (blood pressure ≥140/90 mm Hg or use of blood pressure-lowering agents), were recruited consecutively from the university teaching hospital during the same study period, from patient visitors, hospital staff, and noncardiac outpatient attendees. The exclusion criteria for the controls were a history of stroke or TIA or coronary heart disease and acute or active cardiopulmonary or infective conditions.

Age and sex were recorded, as well as a medical history of hypertension, smoking, diabetes mellitus, hypercholesterolemia, ischemic heart disease, stroke, or TIA. Weight and height were measured using standard procedures. Hip and waist circumferences were measured in a standardized way using a plastic inelastic tape measure. Hip circumference was measured at the widest part (lower buttock level). Waist circumference was measured at expiration (where possible), with the tape passing midway between the costal margin and the iliac crest. The body mass index was computed as the weight in kilograms divided by the height in meters squared. The waist-to-hip ratio was calculated as the waist divided by hips. Forty-seven stroke patients had computerized tomographic brain scan, 47 had an ECG, and 40 had lipid profiles undertaken.

At least 2 mL of serum obtained from each patient was stored at -20°C before laboratory analyses. Detection of IgA and IgG antibodies to *C pneumoniae* was done with a validated microimmunofluorescence technique (MRL Diagnostics).¹⁹ The cutoff point for

seropositivity was 1/16 for IgA and 1/64 for IgG. Testing procedures followed the recommendations of the manufacturer. Control tests were performed using positive serum preparations as well as negative patient serum. For every batch of slides tested, 2 control preparations were applied to 2 slides. The laboratory technician who performed all the analyses was unaware of the study hypothesis.

Verbal informed consent before participation was obtained from the patient or attending family member when not possible from patient. The ethics committee of the faculty of medicine and biomedical sciences at the University of Yaoundé I approved the study.

Statistical analyses were performed using STATA version 9.0 (STATA Corporation). Probabilities of significant differences between cases and controls were compared by the paired *t* test, McNemar test, and conditional logistic regression. All significance tests were 2-tailed, and significance was defined at the 5% α level.

Results

There were 64 cases and 64 controls with a median age of 60 years (range 26 to 80 years) and 64 (50%) women. Table 1 shows the general characteristics of the study population. There was no significant difference between the cases and controls with respect to hypertension ($P=0.2$), current smoking status ($P=0.53$), alcohol intake ($P=0.80$), body mass index ($P=0.49$), waist-to-hip ratio ($P=0.14$), and diabetes ($P=0.76$). There was a tendency toward a higher waist circumference among the controls (mean 97 [SD 14] cm versus 89 [SD 14] cm; $P=0.06$), but this trend was not significant ($P=0.06$).

Table 2 shows that positive *C pneumoniae* serology was detected in 50 (78.1%) patients versus 27 (42.2%) controls (odds ratio [OR], 4.29; 95% CI, 1.84 to 11.56; $P=0.0002$) for IgA and in 41 (64.1%) patients compared with 35 (54.7%) controls (OR, 1.46; 95% CI, 0.68 to 3.22; $P=0.29$) for IgG. The relationship between thrombotic stroke and *C pneumoniae* seropositivity (35 case-control pairs) is also shown in Table 2. Thirty-three (94.3%) thrombotic patients had positive *C pneumoniae* IgA titers compared with 13 (37.1%) controls (OR, 21.0; 95% CI, 3.38 to 868.45; $P<0.0001$). For IgG, 22 (62.9%) patients had a positive serology compared with 16 (45.7%) controls (OR, 1.86; 95% CI, 0.69 to 5.50; $P=0.18$). We did not use multiple logistic regression analysis to control for nonmatched patient characteristics, which are known or suspected to affect stroke risk (hypertension, obesity, smoking, diabetes, and alcohol intake) because none of these factors were significantly different between cases and controls.

We explored whether *C pneumoniae* seropositivity increased with age among the cases. The prevalence of antibodies in the 54 stroke patients ≥50 years of age was not

TABLE 2. Seroprevalence of *C pneumoniae* Antibodies in Cases and Controls

Antibody Type	Stroke Cases	Controls	OR, 95% CI
All cases of stroke (64 case-control pairs)			
IgA	50 (78.1%)	27 (42.2%)	4.29, 1.84 to 11.56; $P=0.0002$
IgG	41 (64.1%)	35 (54.7%)	1.46, 0.68 to 3.22; $P=0.29$
Thrombotic stroke cases (35 case-control pairs)			
IgA	33 (94.3%)	13 (37.1%)	21.00, 3.38 to 868.45; $P<0.0001$
IgG	22 (62.9%)	16 (45.7%)	1.86, 0.69 to 5.50; $P=0.18$

Values are absolute count (percentage).

TABLE 3. Frequency of Each Stroke Subtype in the Sample Population

Subtype	Frequency (%)
Cardioembolic	2 (3.1)
Thrombotic	35 (54.7)
Hemorrhagic	10 (15.6)
Unknown*	17 (26.6)
Total	64 (100)

*Seventeen patients did not do the computerized tomographic scan, and we do not know the nature of their lesions.

different from that of the 10 patients <50 years of age (77.8% versus 80.0%; OR, 0.88; 95% CI, 0.16 to 4.68; $P=0.62$ for IgA; 64.8% versus 60.0%, OR, 1.23; 95% CI, 0.31 to 4.90; $P=0.52$ for IgG).

Table 3 shows that 47 patients had computerized tomographic brain scans done: 35 (74.5%) had ischemic strokes presumed to be thrombotic, and 2 ischemic strokes (4.2%) were considered cardioembolic given that ECG findings in these patients showed atrial fibrillation and 10 (21.3%) strokes were hemorrhagic.

Discussion

This case-control study was performed in 2 tertiary hospitals in Yaoundé, Cameroon, aimed at examining the relationship between chronic *C pneumoniae* infection and stroke or TIA. To the best of our knowledge, this is the first study of its kind on indigenous patients of an African country. Using microimmunofluorescence assay, we found that 94.3% of thrombotic stroke patients tested positive for IgA antibodies against *C pneumoniae* and 62.9% for IgG, similar to the high prevalence reported in studies from Japan,²⁰ Taiwan,²¹ Israel,²² United Kingdom,²³ and Turkey.²⁴ The seroprevalence of chronic *C pneumoniae* infection (IgA) was significantly higher in stroke patients than in the controls (OR, 4.29; 95% CI, 1.84 to 11.56; $P=0.0002$ for all cases of stroke and OR, 21.0; 95% CI, 3.38 to 868.45; $P<0.0001$ for ischemic stroke). A possible limitation of our study is that not all cases had brain scans to distinguish the stroke type, and cardiac workup for cardioembolism was incomplete in the cases. The latter could explain the low frequency of embolic stroke. In addition (one could argue that), some controls could have had silent infarcts, but this would have led to an underestimation (rather than an overestimation) of the association between chronic *C pneumoniae* infection and stroke.

Many case-control studies^{8,9,12,13,25,26} have demonstrated an association between antibodies against *C pneumoniae* (mainly IgA) and stroke in other populations. Some seroepidemiological studies have also reported an association between past *C pneumoniae* infection and asymptomatic carotid atherosclerosis,^{27–30} although this finding has not been consistent.^{11,23} The reasons for this inconsistency are not yet determined. It is possible that the inconsistency may be explained by different criteria for controls, different serological methods with different cutoff titers for seropositivity, different subtypes of stroke, and variable degree of statistical adjustment for confounding factors. In 100 whites >65 years of age admitted with acute stroke or TIA and 87 controls

admitted with acute noncardiopulmonary and noninfective disorders, Ngeh et al, using an ELISA method for detecting *C pneumoniae* infection, did not find a difference in prevalence between patients and controls.²³ In a subgroup analysis of 43 cases and 44 controls without a known history of ischemic heart disease, there was a trend toward a significant association between *C pneumoniae* IgG seropositivity and stroke. However, other studies assessing only IgG antibodies^{31,32} have not reported an association.

Most reports on the relationship between *C pneumoniae* infection and stroke have been from case-control studies. As such, the temporal sequence of the events, infection-atherosclerosis-stroke, needs to be ascertained through cohort studies or randomized controlled trials. *C pneumoniae* infection may increase systemic inflammation and immune-mediated vascular damage and cause altered lipid metabolism.^{15,34–36} These changes may adversely modify conventional risk factors of atherosclerosis and consequently lead to stroke.

Some prospective studies have shown that *C pneumoniae* seropositivity predicted the risk of future stroke,^{10,32,33} but a study in women only failed to show this risk.³¹ In our study, 50% of both cases and controls were women. Preliminary evidence is becoming available from intervention studies to support the link between chronic *C pneumoniae* infection and stroke. Thirty-day antibiotic treatment of *C pneumoniae*-infected patients was shown in a randomized controlled trial to sustain a significant reduction of atherosclerosis progression in the common carotid artery for up to 2 years.¹⁴ However, 2 recent large randomized controlled trials did not observe a beneficial effect of antichlamydial antibiotic therapy on the secondary prevention of cardiovascular heart disease.^{37,38} Neither trial used the serological marker of chronic *C pneumoniae* infection during patient selection. *C pneumoniae* IgA antibody was assessed in only 32.4% of the participants of one trial and no participant of the other trial. In the absence of chronic *C pneumoniae* infection among the trial participants, there is little reason to expect a clinical benefit with antichlamydial antibiotic treatment. In light of the foregoing evidence, the association between *C pneumoniae* and stroke is emerging. However, because of paucity of data from prospective cohort studies and randomized controlled trials, causality³⁹ cannot be firmly established at present.

Conclusion

Our data confirm a strong association between (IgA, and not IgG, as a serological marker of) chronic *C pneumoniae* infection and stroke for the first time in an indigenous population of the African continent. These findings, if confirmed by larger studies, may have important policy implications in sub-Saharan Africa, where stroke is a major public health problem, because antibiotic treatment of chronic *C pneumoniae* infection would be a simple and easily available intervention for primary prevention of thrombotic stroke. Given the inherent difficulty of defining the direction of causality in case-control studies, there is need for large prospective cohort studies and randomized controlled interventional trials to examine this association.

Acknowledgments

A.K.N., K.N.B., S.G., and C.S.W. conceived and designed the study. A.K.N., K.N.B., J.N.M., and C.S.W. collected the data. F.G. and C.S.W. conducted the statistical analyses. A.K.N., K.N.B., and C.S.W. drafted the article, and all authors contributed to revision and the approved final version.

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