Neurology Asia 2012; 17(3) : 183 – 192

ORIGINAL ARTICLES

Chlamydophila (Chlamydia) pneumoniae but not *Helicobacter pylori* infection, is associated with cerebral infarction in Japanese community-dwelling populations: The Jichi Medical School Cohort Study

[1]Reiko Yamamoto, [1]Shizukiyo Ishikawa, [2]Masafumi Mizooka, [1]Eiji Kajii, and the Jichi Medical School Cohort Study

[1]Division of Community and Family Medicine, Center for Community Medicine, Jichi Medical University, Simotsuke, Tochigi; [2]Department of General Medicine, Hiroshima University Hospital, Hiroshima, Japan

Abstract

Background: Chronic infections, such as *Helicobacter pylori (H. pylori)* and *Chlamydophila pneumoniae (C. pneumoniae)*, are known to contribute to atherosclerosis. However, the relationship of the infections to cerebral infarction is still controversial. *Methods:* The Jichi Medical School (JMS) Cohort Study, a prospective population-based study, investigated the risk factors of cardiovascular disease in Japanese community-dwelling populations. In 1999, we measured serum *H. pylori* IgG, *C. pneumoniae* IgG and IgA levels in 2,632 subjects. Logistic regressions were used to analyze associations between *H. pylori* and *C. pneumoniae* seropositivities and cerebral infarction. *Results:* A total of 2,243 subjects were followed up and, during 10.7-years, 64 developed cerebral infarctions, whose prevalence of *H. pylori* IgG, *C. pneumoniae* IgG and IgA seropositivities were 51.6%, 71.9%, and 67.2%, respectively. Among seropositive subjects, adjusted odds ratios (ORs) for cerebral infarctions were 1.04 (95% confidence interval (CI), 0.58-1.87, P=0.89), 2.02 (1.03-3.95, P=0.04), and 1.35 (0.73-2.49, P=0.34) respectively, after adjusting for sex, age, body mass index, total cholesterol, high-density lipoprotein cholesterol, fasting blood sugar, smoking, alcohol, and fibrinogen. *C. pneumoniae* IgG seropositivities in subjects aged ≥ 65 years were associated with cerebral infarctions, whereas those in subjects aged < 65 years, were not.
Conclusions: C. pneumoniae IgG was associated with cerebral infarction, *C. pneumoniae* IgA and *H. pylori* IgG were not.

INTRODUCTION

Many risk factors have been suggested to play roles in the development of atherosclerosis and cardiovascular disease, including age, hypertension, smoking, diabetes mellitus and hyperlipidemia, but understanding of the pathogenesis of atherosclerosis remains incomplete. Therefore, it is important to account for potential confounders. Chronic infections such as *Helicobacter pylori (H. pylori)*[1] and *Chlamydophila pneumoniae (C. pneumoniae)*[2] have been epidemiologically linked to atherosclerosis and cardiovascular disease, and also pathophysiologically detected directly in atherosclerotic plaques.[3-6] However, the contributions of *H. pylori* [7,8] and *C. pneumoniae*[9-12] to cerebral infarctions remain controversial.

In the Jichi Medical School (JMS) Cohort Study[13], we determined the *H. pylori* IgG, *C. pneumoniae* IgG and IgA antibodies and examined the role of chronic infection in the pathogenesis of cardiovascular disease. Previously, our colleagues reported that *C. pneumoniae* seropositivities were associated with smoking and physical activity among Japanese men living in rural areas.[14] In this study, we investigated the association between these seropositivities and the incidence of cerebral infarction.

METHODS

Briefly, baseline data were collected from mass screening examinations as described below in 1999 as part of the JMS cohort study.

Subjects

The JMS Cohort Study is a prospective population-based study investigating risk factors for cardiovascular disease in Japanese community-dwelling populations. Details of the JMS Cohort Study design and some descriptive data have been published previously.[13] Baseline data were collected between 1992 and 1995 in 12 rural communities. A total of 12,490 subjects (4,911 men and 7,579 women) living in 12 districts participated in the study. These subjects were participants in mass screening examinations for cardiovascular disease in accordance with the Health and Medical Service Law for the Aged in Japan.

Ethical issues

The study design and procedure were approved by each community government and by the Ethical Committee of Epidemiologic Research at Jichi Medical University. Written informed consent for the study was obtained individually from those who responded to the mass screening examinations at their health check-up. At visits, participants were informed that data would be obtained from questionnaires and blood samples, and that their health status would be followed up through a review of their hospital medical records if a stroke or MI was suspected to have occurred.

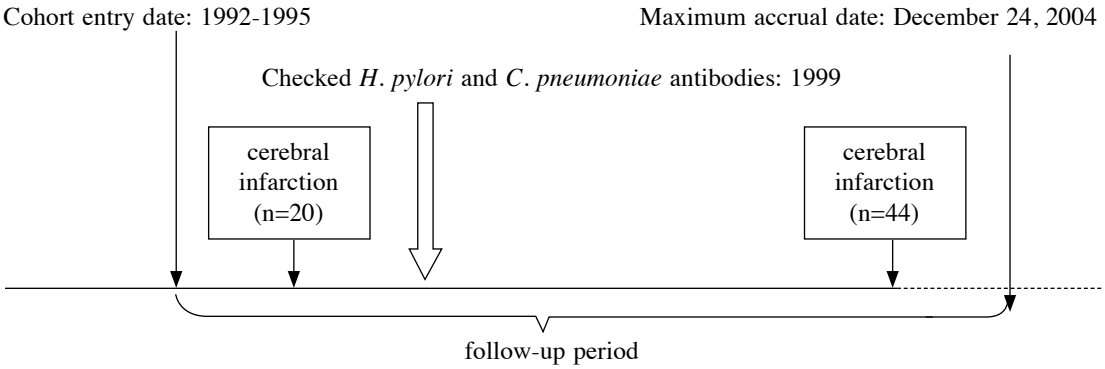
Study population

In 1999, we measured serum *H. pylori* IgG, *C. pneumoniae* IgG and IgA levels and cardiovascular risk factors in 2,632 subjects in 6 of these 12 districts (Takasu, Kuse, Wara, Sakugi, Akaike, and Ainoshima); antibodies to *H. pylori* and *C. pneumoniae* were not measured at the baseline in 1992-1995. (Figure 1). The 6 districts that agreed to participate in the intermediate survey were selected from the 12 districts where baseline data were collected in the JMS cohort study. Also, 2,632 subjects whose data were collected in the JMS cohort study agreed to participate in this study. They were volunteers but were not selected at random.

Measurement of baseline variables

To synchronize data collection methods, we established a central committee composed of the chief medical officers of each participating district. This committee developed a detailed manual for data collection. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured with a fully-automated sphygmomanometer, the BP203RV-II (Nippon Colin), placed on the right arm of a seated participant who had rested in a sitting position for 5 minutes before measurement. Body weight was recorded with the participant clothed, and 0.5 kg in summer or 1 kg during other seasons was subtracted from the recorded weight. Body mass index (BMI) was calculated as weight (kg)/height^2 (m^2).

Information about medical history and lifestyle was obtained using a written questionnaire. Trained interviewers assessed the histories of smoking and alcohol intake. Smoking status was categorized into active smoker or not. A drinker was defined as a person who regularly consumed



Antibodies were checked in study subjects during the course of the JMS cohort follow-up period.
H. pylori: *Helicobacter pylori*

Figure 1: Description of the study design.

alcohol on more than 3 days a week, and drinking status was categorized into drinker or not.

All blood samples were collected after fasting for at least 8 hours by standard methods.[13] Total cholesterol (TC), fasting blood sugar (FBS), and fibrinogen were measured using some commercial kits detailed elsewhere.[13,15] High-density lipoprotein cholesterol (HDL-C) was measured using a homogeneous assay kits (Cholestest HDL, Daiichi Pure Chemical, Co., Ltd, Tokyo, Japan). The serum immunoglobulin G (IgG) antibody to *H. pylori* was measured by an enzyme linked immunosorbent assay (ELISA) (HM-CAP, Enteric Products, INC. (EPI), USA). IgG to *H. pylori* ≥ 2.3 was considered positive, 1.8 to 2.2 as pseudopositive, and <1.8 as negative; therefore, we designated a result of ≥ 2.3 as positive and <2.3 as negative. The titers for IgG and IgA antibodies to *C. pneumoniae* were measured by a commercial ELISA kit (Hitazyme *C. pneumoniae* kits, Hitachi Chemical Co., Ltd., Tokyo) and recorded as the index value calculated according to the manufacturer's instructions. IgG and IgA to *C. pneumoniae* ≥ 1.10 was considered positive, 0.90-1.09 as pseudopositive, and <0.90 as negative; therefore, we designated a result of ≥ 1.10 as positive and <1.10 as negative.

Blood variables were measured at the central laboratory of SRL (Tokyo, Japan), a commercial hematology laboratory. The present study was approved by the institutional review board of the Central Committee of the JMS Cohort Study. All procedures were performed in accordance with the guidelines of the institutional committee.

Follow-up

A detailed description of the follow-up systems has been provided elsewhere.[16] After enrollment in the study, subjects were asked whether they had a history of stroke. Those with such a history were asked when and which hospitals they visited. Subjects who did not attend the screening examination were contacted by mail or phone. If they were hospitalized for any reason, their medical records, including duplicate computed tomography scans and magnetic resonance imaging, were checked for evidence of stroke. Public health nurses also visited the homes of the subjects to obtain further information. Each municipal government annually obtained information about subjects who had relocated out of the area. We followed up them until the end of 2005.

The criteria for stroke were sudden onset of a focal and nonconvulsive neurological deficit that persisted for longer than 24 hours; stroke subtypes, i.e, cerebral infarction, cerebral hemorrhage, or subarachnoid hemorrhage, were determined according the criteria of the National Institute of Neurological Disorders and Stroke.[17] All probable cases of cerebral infarction in this study were evaluated independently by a diagnosis committee composed of a radiologist and a neurologist, with the aid of computed tomography and magnetic resonance imaging.[16]

Statistical analysis

Continuous variables were compared between *H. pylori* IgG-, *C. pneumoniae* (IgG and IgA)-positive and negative subjects using an unpaired *t* test, respectively. Categorical variables were compared using a Chi-squared test or Fisher exact test. Logistic regression models were used to calculate ORs for the incidence of cerebral infarction, after adjusting for sex, age, BMI, TC, HDL-C, FBS, smoking status, and alcohol status, which were known as conventional cardiovascular risk factors. Logistic regression models were also used to calculate ORs for cerebral infarctions, after further adjustment for fibrinogen. A P value <0.05 was considered significant. Statistical analyses were carried out using SPSS for Windows, version 19.0J (SPSS inc., Japan).

RESULTS

A total of 2,243 subjects were followed up and cerebral infarction occurred in 64 participants (38 men and 26 women) during a 10.7 year follow-up. Twenty cases (14 men and 6 women) had cerebral infarctions before antibodies were checked (Figure 1).

Characteristics of the study subjects were shown in Table 1. *H. pylori* IgG, *C. pneumoniae* IgG and IgA seropositivity were 54.6%, 55.9%, and 53.9%, respectively (Table 1-3). The prevalences of seropositivity to *H. pylori* IgG, *C. pneumoniae* IgG and IgA among men were higher than those of among women. *H. pylori* IgG seropositive subjects were older than seronegative subjects. *H. pylori* IgG seropositivity was associated with decreased HDL-C levels. Both *C. pneumoniae* IgG seropositive subjects and IgA seropositive subjects were older than seronegative subjects. These serpositivities were associated with increased fibrinogen levels and smoking status.

The prevalence of *H.pylori* IgG, *C. pneumoniae* IgG and IgA seropositivity with cerebral infarctions were 51.6%, 71.9%, and 67.2%, respectively (Table 4). There were differences

Table 1: Characteristics of subjects with seroposive or seronegative to *H. pylor*i IgG

	number	Seropositive mean ± SD	Seronegative mean ± SD	P value*
H. pylori IgG**	2243	1225 (54.6 %)	1018 (45.4 %)	
sex (men/women)	888/1355	528/697	360/658	<0.001
age (years)	2243	63.4 ± 10.3	61.8 ± 11.7	<0.01
SBP (mmHg)	2018	131.1 ± 21.0	129.4 ± 20.3	0.06
DBP (mmHg)	2018	76.1 ± 12.8	75.3 ± 12.7	0.15
BMI	2017	22.8 ± 2.9	22.9 ± 3.1	0.44
TC (mg/dL)	2243	206.3 ± 33.9	207.0 ± 35.4	0.64
HDL-C (mg/dL)	2243	55.8 ± 14.6	58.1 ± 15.1	<0.001
FBS (mg/dL)	2243	97.3 ± 20.1	96.4 ± 18.1	0.30
fibrinogen (mg/dL)	2243	265.1 ± 53.6	260.8 ± 52.5	0.05
smoking**	2188	230/1193 (19.3 %)	170/995 (17.1 %)	0.19
alcohol**	1196	589/1075 (54.8 %)	607/1121 (54.1 %)	0.76

*Variables in two groups with seropositive or seronegative were compared by unpaired t-test for consective variables and the chi-square test or Fisher exact test for categorical data.

** number (percentage).

H. pylori: Helicobacter pylori, SD: standard deviation. SBP: systolic blood pressure. DBP: diastolic blood pressure. BMI: body mass index. TC: total cholesterol. HDL-C: high-density lipoprotein cholesterol. FBS: fasting blood sugar.

between subjects with or without cerebral infarction in the ratio of men, the values of SBP, DBP, HDL-C and fibrinogen, and the prevalence of *C. pneumoniae* IgG and IgA seropositivities.

The results of logistic regression models for cerebral infarctions were shown in Table 5. Among subjects with *H. pylori* IgG, *C. pneumoniae* IgG and IgA seropositivities, after adjusting for sex, age, BMI, TC, HDL-C, FBS, smoking status, and alcohol status, the adjusted ORs for cerebral

Table 2: Characteristics of subjects with seroposive or seronegative to *C. pneumoniae* IgG

	number	Seropositive mean ± SD	Seronegative mean ± SD	P value*
C. pneumoniae IgG**	2243	1254 (55.9 %)	989 (44.1 %)	
sex (men/women)	888/1355	538/716	350/639	<0.001
age (years)	2243	63.8 ± 11.0	61.1 ± 10.8	<0.001
SBP (mmHg)	2018	131.2 ± 20.7	129.3 ± 20.7	0.03
DBP (mmHg)	2018	76.0 ± 12.9	75.4 ± 12.6	0.27
BMI	2017	22.8 ± 3.0	22.9 ± 3.0	0.52
TC (mg/dL)	2243	205.2 ± 35.2	208.4 ± 33.7	0.03
HDL-C (mg/dL)	2243	56.5 ± 15.1	57.2 ± 14.7	0.28
FBS (mg/dL)	2243	97.4 ± 21.0	96.3 ± 16.7	0.15
fibrinogen (mg/dL)	2243	267.1 ± 54.9	258.2 ± 50.4	<0.001
smoking**	2188	244/1226 (19.9 %)	156/962 (16.2 %)	0.03
alcohol**	2196	591/1075 (55.0 %)	639/1121 (57.0 %)	0.34

*Variables in two groups with seropositive or seronegative were compared by unpaired t-test for consective variables and the chi-square test or Fisher exact test for categorical data.

** number (percentage).

C. pneumoniae: Chlamydophila pneumoniae, SD: standard deviation. SBP: systolic blood pressure. DBP: diastolic blood pressure. BMI: body mass index. TC: total cholesterol. HDL-C: high-density lipoprotein cholesterol. FBS: fasting blood sugar.

Table 3: Characteristics of subjects with seroposive or seronegative to *C. pneumoniae* IgA

	number	Seropositive mean ± SD	Seronegative mean ± SD	P value*
C. pneumoniae IgA**	2236	1205 (53.9 %)	1031 (46.1 %)	
sex (men/women)	883/1353	531/674	352/679	<0.001
age (years)	2236	64.1 ± 10.8	60.9 ± 10.9	<0.001
SBP (mmHg)	2018	130.8 ± 20.9	129.8 ± 20.5	0.26
DBP (mmHg)	2018	75.6 ± 12.7	75.8 ± 12.8	0.81
BMI	2017	22.8 ± 2.9	22.9 ± 3.1	0.54
TC (mg/dL)	2236	205.3 ± 35.7	208.2 ± 33.2	0.04
HDL-C (mg/dL)	2236	56.3 ± 15.3	57.5 ± 14.5	0.06
FBS (mg/dL)	2236	97.4 ± 21.0	96.4 ± 17.1	0.24
fibrinogen (mg/dL)	2236	265.9 ± 55.9	260.0 ± 49.7	<0.01
smoking**	2182	257/1183 (21.7 %)	140/999 (14.0 %)	<0.001
alcohol**	2189	576/1068 (53.9 %)	609/1121 (54.3 %)	0.85

*Variables in two groups with seropositive or seronegative were compared by unpaired t-test for consective variables and the chi-square test or Fisher exact test for categorical data.

** number (percentage).

C. pneumoniae: Chlamydophila pneumoniae, SD: standard deviation. SBP: systolic blood pressure. DBP: diastolic blood pressure. BMI: body mass index. TC: total cholesterol. HDL-C: high-density lipoprotein cholesterol. FBS: fasting blood sugar.

Table 4: Characteristics of subjects with or without cerebral infarction

	cerebral infarction (+) mean ± SD	cerebral infarction (-) mean ± SD	P value*
men**	38/64 (59.4 %)	851/2179 (39.1 %)	<0.01
age (years)	72.2 ± 7.5	62.4 ± 10.9	<0.001
SBP (mmHg)	145.6 ± 21.0	130.0 ± 20.6	<0.001
DBP (mmHg)	80.2 ± 12.0	75.6 ± 12.7	0.01
BMI	22.8 ± 2.6	22.9 ± 3.0	0.86
TC (mg/dL)	200.1 ± 35.2	206.8 ± 34.6	0.16
HDL-C (mg/dL)	50.0 ± 11.3	57.0 ± 15.0	<0.001
FBS(mg/dL)	96.8 ± 16.6	96.9 ± 19.3	0.97
fibrinogen (mg/dL)	276.5 ± 57.1	262.8 ± 53.0	0.04
smoking**	9/63 (14.3 %)	391/2125 (18.4 %)	0.41
alcohol**	30/63 (47.6 %)	1045/2133 (49.0 %)	0.83
H. pylori IgG†	2.5 (1.0-5.2)	2.6 (0.9-5.2)	0.69
H. pylori IgG seropositivity**	33/64 (51.6 %)	1192/2179 (54.7 %)	0.62
C. pneumoniae IgG†	1.5 (1.0-2.5)	1.2 (0.7-1.9)	<0.01
C. pneumoniae IgG seropositivity**	46/64 (71.9 %)	1208/2179 (55.4 %)	<0.01
C. pneumoniae IgA†	1.5 (0.9-1.9)	1.2 (0.6-1.8)	0.08
C. pneumoniae IgA seropositivity**	43/64 (67.2 %)	1162/2172 (53.5 %)	0.03

*Variables in two groups with or without cerebral infarction were compared by unpaired t-test for consective variables and the chi-square test or Fisher exact test for categorical data.

**number (percentage).
†median (interquartile range).

H. pylori: *Helicobacter pylori*, *C. pneumoniae*: *Chlamydophila pneumoniae*, SD: standard deviation. SBP: systolic blood pressure. DBP: diastolic blood pressure. BMI: body mass index. TC: total cholesterol. HDL-C: high-density lipoprotein cholesterol. FBS: fasting blood sugar.

Table 5: Crude and adjusted ORs for cerebral infarction with *H. pylori* IgG, *C. pneumoniae* IgG and IgA seropositivity

	Adjusted OR (95% CI)	P value**	Adjusted OR (95% CI)	P value†
H. pylori IgG seropositivity	1.03 (0.57-1.87)	0.92	1.04 (0.57-1.89)	0.90
sex	0.47 (0.23-0.96)	0.04	0.47 (0.23-0.96)	0.04
age (years)	1.11 (1.06-1.16)	<0.001	1.12 (1.06-1.16)	<0.001
SBP (mmHg)	1.03 (1.02-1.05)	<0.001	1.03 (1.02-0.05)	<0.001
BMI	0.92 (0.82-1.03)	0.15	0.91 (0.81-1.03)	0.13
TC (mg/dL)	1.01 (1.00-1.02)	0.23	1.01 (1.00-1.02)	0.20
HDL-C (mg/dL)	0.95 (0.93-0.98)	<0.001	0.95 (0.93-0.98)	<0.001
FBS(mg/dL)	0.99 (0.98-1.01)	0.50	0.99 (9.98-1.01)	0.49
smoking**	0.63 (0.26-1.54)	0.31	0.67 (0.27-1.66)	0.39
alcohol**	0.95 (0.48-1.85)	0.87	0.93 (0.48-1.82)	0.83
fibrinogen (mg/dL)	-	-	0.36 (1.00-1.00)	0.36
C. pneumoniae IgG seropositivity	2.13 (1.06-4.27)	0.03	2.22 (1.10-4.48)	0.03
sex	0.49 (0.24-1.01)	0.05	0.49 (0.24-1.01)	0.05
age (years)	1.10 (1.05-1.15)	<0.001	1.10 (1.06-1.15)	<0.001
SBP (mmHg)	1.03 (1.02-1.05)	<0.001	1.03 (1.02-1.05)	<0.001
BMI	0.92 (0.82-1.03)	0.15	0.91 (0.82-1.03)	0.12
TC (mg/dL)	1.01 (1.00-1.02)	0.19	1.01 (1.00-0.12)	0.15
HDL-C (mg/dL)	0.95 (0.93-0.98)	<0.001	0.95 (0.93-0.98)	<0.001
FBS(mg/dL)	0.99 (0.98-1.01)	0.49	0.99 (0.97-1.01)	0.47
smoking**	0.59 (0.24-1.46)	0.26	0.64 (0.26-1.57)	0.33
alcohol**	0.95 (0.49-1.86)	0.88	0.93 (0.47-1.83)	0.84
fibrinogen (mg/dL)	-	-	1.00 (0.99-1.00)	0.25
C. pneumoniae IgA seropositivity	1.26 (0.67-2.36)	0.47	1.27 (0.68-2.37)	0.46
sex	0.47 (0.23-0.97)	0.04	0.48 (0.23-0.98)	0.04
age (years)	1.10 (1.06-1.15)	<0.001	1.11 (1.06-1.16)	<0.001
SBP (mmHg)	1.03 (1.02-1.05)	<0.001	1.03 (1.02-1.05)	<0.001
BMI	0.92 (0.82-1.03)	0.15	0.91 (0.81-1.03)	0.13
TC (mg/dL)	1.01 (1.00-1.02)	0.21	1.01 (1.00-1.02)	0.18
HDL-C (mg/dL)	0.95 (0.93-0.98)	<0.001	0.95 (0.93-0.98)	0.18
FBS(mg/dL)	0.99 (0.98-1.01)	0.50	0.99 (0.98-1.01)	0.49
smoking**	0.61 (0.25-1.49)	0.27	0.65 (0.26-1.60)	0.35
alcohol**	0.96 (0.49-1.88)	0.47	0.95 (0.48-1.86)	0.88
fibrinogen (mg/dL)	-	-	1.00 (0.99-1.00)	0.37

H. pylori: *Helicobacter pylori*. *C. pneumoniae: Chlamydophila pneumoniae*.
OR: odds ratio. CI: confidence interval.
ORs were calculated with multiple logistc regression.
*logistic regression for cerebral infarction
**logistic regression for cerebral infarction adjusting for sex, age, systolic blood pressure, body mass index, total cholesterol, high-density lipoprotein cholesterol, fasting blood sugar, smoking status, and alchohol status.
†logistic regression for cerebral infarction adjusting for ** plus fibrinogen.

infarction among subjects with *H. pylori* IgG, *C. pneumoniae* IgG and IgA seropositives were 1.03 (0.57-1.87, P=0.92), 2.13 (1.06-4.27, P=0.03), and 1.26 (0.67-2.36, P=0.47), respectively. Moreover, after adjusting for these factors plus fibrinogen, adjusted ORs for cerebral infarctions among the subjects with them were 1.04 (0.57-1.89, P=0.90), 2.22 (1.10-4.48, P=0.03), and 1.27 (0.68-2.37, P=0.46), respectively.

Table 6 showed subgroup analysis according

to age. Among subjects aged ≥ 65 years, *C. pneumoniae* IgG seropositivity was associated with cerebral infarction after adjusting for sex, BMI, TC, HDL-C, FBS, smoking status, alcohol status, and fibrinogen (OR 1.57, 95% CI, 0.87-2.84, P=0.13, adjusted OR 2.29, 1.06-4.95, P=0.04). Among subjects aged <65 years, it was not associated with cerebral infarction.

DISCUSSION

In our cohort study, *C. pneumoniae* IgG seropositives, especially in subjects aged ≥ 65 years, were associated with cerebral infarction in Japanese community-dwelling populations. *H. pylori* IgG and *C. pneumoniae* IgA seropositivities were not associated with cerebral infarction.

Many risk factors for the development of cerebral infarctions have been identified. Atherosclerosis is the major pathological process underlying cerebral infarction. Because inflammation plays a central role in the pathogenesis of atherosclerosis, infectious agents may be involved with atherosclerosis. Among the organisms that have been implicated are *H. pylori* and *C. pneumonia*.

H. pylori infection

We could not show that *H. pylori* IgG seropositivity was associated with the incidence of cerebral infarction. Whincup *et al*. reported the association between *H. pylori* infection and stroke in middle aged men.[7] Epidemiological studies have shown the association between *H. pylori* and stroke, and pathogenic studies[4] also reported the role of *H. pylori* infection in stroke. However, the association between *H. pylori* and stroke remains controversial.

Recent studies suggested an association between *H. pylori* infection and ischemic stroke subtypes, but not all stroke subtypes.[18] Grau *et al*. showed the *H. pylori* seropositivity increased the odds for the atherothrombotic origin univariate (OR 3.63; 95% CI 1.37-9.65) and multivariate analysis (Adjusted OR 3.53; 1.09-11.4).[19] Sawayama *et al*. also reported that chronic *H. pylori* infection was associated with a higher risk of stroke due to small artery occlusion and a lower risk of cardioembolic stroke in Japan.[10] It is possible that because we did not differentiate the cerebral infarction subtypes, we could have missed the association.

C. pneumoniae infection

In our cohort study, *C. pneumoniae* IgG, but not IgA seropositivity was associated with the incidence of cerebral infarction in Japanese community-dwelling populations. Many risk factors have been suggested to play roles in the incidence of cardiovascular disease, including age, hypertension, smoking, diabetes mellitus,

Table 6: Crude and adjusted ORs for cerebral infarction with *H. pylori* IgG, *C. pneumoniae* IgG and IgA seropositivity by age

	Crude OR (95 % CI)	P value*	Adjusted OR (95 % CI)	P value**
age ≥ 65				
H. pylori IgG	0.63 (0.37-1.07)	0.09	0.75 (0.40-1.41)	0.37
C. pneumoniae IgG	1.57 (0.87-2.84)	0.13	2.29 (1.06-4.95)	0.04
C. pneumoniae IgA	1.28 (0.73-2.23)	0.39	1.16 (0.60-2.23)	0.66
age < 65				
H. pylori IgG	-	0.99	-	0.99
C. pneumoniae IgG	2.66 (0.51-13.69)	0.25	4.02 (0.69-23.5)	0.12
C. pneumoniae IgA	6.61 (0.79-55.07)	0.08	8.10 (0.88-75.3)	0.07

H. pylori: Helicobacter pylori.
C. pneumoniae: Chlamydophila pneumoniae.
OR: odds ratio. CI: confidence interval.
ORs were calculated with multiple logistc regression.
*logistic regression for cerebral infarction
**logistic regression for cerebral infarction adjusting for sex, systolic blood pressure, body mass index, total cholesterol, high-density lipoprotein cholesterol, fasting blood sugar, smoking status, alchohol status, and fibrinogen.

and hyperlipidemia. However, these risk factors could not predict all cardiovascular diseases. Other risk factors for cardiovascular disease, such as chronic infection with *C. pneumoniae*[2], have been proposed. Melnick *et al*. reported the association between *C. pneumoniae* and carotid atherosclerosis.[20] Some pathological studies have also showed the presence of *C. pneumoniae* DNA to be present in the symptomatic carotid atherosclerotic disease.[21] Several cross-sectional and case-control studies showed *C. pneumoniae* IgA, but not IgG was associated with cerebral infarction.[22-24] Our results seemed not to be compatible with them. Because *C. pneumoniae* IgA is thought to be the better marker for recent exposure to infection[25], study design differences would lead to different results.

Our colleagues[14] showed the association between *C. pneumoniae* seropositivity and smoking status. Smieja *et al*. hypothesized a synergy between *C. pneumoniae* infection and smoking to atherosclerosis disease.[26] The association between *C. pneumoniae* infection and increased fibrinogen levels [27], indicating inflammation [28] and a procoagulant state[29], were also shown. These were interpreted as one of underlying pathological physiological mechanisms as to how *C. pneumoniae* infection had contributed to the development of atherosclerotic processes.[30] In our study, the association between *C. pneumoniae* IgG seropositivity and cerebral infarction remained after adjusting for conventional cardiovascular risk factors plus fibrinogen. Even though smoking, fibrinogen, and *C. pneumoniae* IgG seropositivity may be part of the same causal pathway, other excess risks of *C. pneumoniae* infection may exist (Table 5). Moreover, in subgroup analysis, our study showed that the association between subjects with *C. pneumoniae* seropositivities aged $\geq$ 65 years and cerebral infarctions remained. Watson *et al*. pointed out that *C. pneumoniae* can play a role in all of the stages of atherosclerosis and increase the impact of the classical risk factors for atherosclerosis.[31] Our results showed the possibility that *C. pneumoniae* infection increased the impact of aging for atherosclerosis.

There were some limitations to this study. First, evaluation of *H. pylori* infection status in our study was based on detection of the *H. pyori*-specific antibody alone. Although the specificity and sensitivity of ELISA used in this study were more than 95.0%, a minority of false-positive or false-negative subjects may have been included among the study subjects.[32] Evaluation of *C. pneumoniae* status in our study was also based on the detection of *C. pneumoniae*-specific IgG and IgM by ELISA. Most previous studies use the MIF technique, which is recommended with regard to serological testing. In comparison with the antibody titers demonstrated by the MIF in serum specimens from *C. pneumoniae* antigen positive (throat swab: PCR positive), ELISA showed good correlation coefficients of 0.950 for IgG and 0.852 for IgA. The two assay methods showed high agreement rates: 90.2% for IgG an 84.3% for IgA. Specimens which did not yield the same result with ELISA and MIF were subjected to analysis by Western blot method, and the rates of agreement with the ELISA results were 80.0% for IgG and 87.5% for IgA. Moreover, the cross reactivity of ELISA with the other *Chlamydia* species was weaker than that of MIF. We thought that our results from ELISA were appropriate.[33] Second, we also did not check *H. pylori* CagA status, which was more strongly associated with the risk of stroke compared to other strains.[34] CagA positive *H. pylori* infections are common in Japan and our *H. pylori* seropositive subjects may have a CagA positive status.[35] Third, *H. pylori* IgG was checked in the course of the follow-up period (Figure 1). *H. pylori* infection is usually acquired early in life, and the serological status was unlikely changed during the JMS Cohort Study follow-up period.[36,37] The evaluation of *C. pneumoniae* infection status was also based on the detection of *C. pneumoniae* specific IgG and IgA antibodies checked only once in the course of the study. *C. pneumoniae* antibody titers change according to infectious states; it may fall substantially within a few years of seroconversion, and may reincrease substantially if re-infection occurs.[38] Fagerberg *et al*. showed that high titers to *C. pneumoniae* IgG or IgA were associated with an increased risk for future stroke in prospective studies during 6.5 years follow-up (relative risk, 8.58; 95% CI, 1.07-68.82, P=0.043).[39] They evaluated antibodies two times and confirmed that in those with high titers at entry, 97% remained high at the 3.5 year reexamination. This result seemed to support our study results. However, we had the possibility of under or over estimating *C. pneumoniae* infection because of the difficulty in obtaining relevant information regarding the persistence of *C. pneumoniae* infection using serological tests including such temporal variations. Fourth, we did not know whether subjects had previously undergone eradication therapy for *H. pylori* infection. Eradication therapy decreases the *H. pylori* IgG titer.[38] Our data were obtained in 1999, before eradication therapy for

H. pylori had become widely available in Japan. As such it would not seem necessary for us to take into consideration the effect of eradication therapy. Finally, we did not check past medical histories and medications, but, obtained these data from questionnaires as baseline data. The proportions of subjects treated for hypertensions, hyperlipidemia, or diabetes mellitus were low. We thought our study subjects were healthy.[16] Further well-designed, large scale prospective studies whose outcome measures the incidence of cerebral infarction subtypes are needed to clarify these associations.

In conclusion, *C. pneumoniae* IgG seropositivities, especially in subjects aged ≥65 years, were associated with cerebral infarction in Japanese community-dwelling populations. However, *H. pylori* IgG and *C. pneumoniae* IgA seropositivities were not associated with cerebral infarction. Further studies are needed to clarify the epidemical role of *H. pylori* and *C. pneumoniae*.

ACKNOWLEDGEMENTS

This study was supported by a Scientific Research Grant from the Ministry of Education, Culture, Sports, Science, and Technology, Japan, and by grants from the Foundation for the Development of the Community, Tochigi, Japan.

REFERENCES

1. Mendall MA, Goggin PM, Molineaux N, *et al*. Relation of Helicobacter pylori infection and coronary heart disease. *Br Heart J* 1994; 71:437-9.
2. Saikku P, Leinonen M, Mattila K, *et al*. Serological evidence of an association of a novel Chlamydia, TWAR, with chronic coronary heart disease and acute myocardial infarction. *Lancet* 1988; 2:983-6.
3. Kowalski M. Helicobacter pylori (H. pylori) infection in coronary artery disease: influence of H. pylori eradication on coronary artery lumen after percutaneous transluminal coronary angioplasty. The detection of H. pylori specific DNA in human coronary atherosclerotic plaque. *J Physiol Pharmacol* 2001; 52:3-31.
4. Rassu M, Cazzavillan S, Scagnelli M, *et al*. Demonstration of Chlamydia pneumoniae in atherosclerotic arteries from various vascular regions. *Atherosclerosis* 2001; 158:73-9.
5. Shor A, Kuo CC, Patton DL. Detection of Chlamydia pneumoniae in coronary arterial fatty streaks and atheromatous plaques. *S Afr Med J* 1992; 82:158-61.
6. Grayston JT, Kuo CC, Campbell LA, *et al*. Chlamydia pneumoniae, strain TWAR and atherosclerosis. *Eur Heart J* 1993; 14 (Suppl K):66-71.
7. Whincup PH, Mendall MA, Perry IJ, *et al*. Prospective relations between Helicobacter pylori infection, coronary heart disease, and stroke in middle aged men. *Heart* 1996; 75:568-72.
8. Markus HS, Mendall MA. Helicobacter pylori infection: a risk factor for ischaemic cerebrovascular disease and carotid atheroma. *J Neurol Neurosurg Psychiatry* 1998; 64:104-7.
9. Wimmer ML, Sandmann-Strupp R, Saikku P, *et al*. Association of chlamydial infection with cerebrovascular disease. *Stroke* 1996; 27:2207-10.
10. Sawayama Y, Ariyama I, Hamada M, *et al*. Association between chronic Helicobacter pylori infection and acute ischemic stroke: Fukuoka Harasanshin Atherosclerosis Trial (FHAT). *Atherosclerosis* 2005; 178:303-9.
11. Coles KA, Knuiman MW, Plant AJ, *et al*. A prospective study of infection and cardiovascular diseases: the Busselton Health Study. *Eur J Cardiovasc Prev Rehabil* 2003; 10:278-82.
12. Tanne D, Haim M, Boyko V, *et al*. Prospective study of Chlamydia pneumoniae IgG and IgA seropositivity and risk of incident ischemic stroke. *Cerebrovasc Dis* 2003; 16:166-70.
13. Ishikawa S, Gotoh T, Nago N, *et al*. The Jichi Medical School (JMS) Cohort Study: design, baseline data and standardized mortality ratios. *J Epidemiol* 2002; 12:408-17.
14. Mizooka M, Ishikawa S. Prevalence of chlamydia pneumoniae in Japanese rural districts; association of smoking and physical activity with Chlamydia pneumoniae seropositivity. *Intern Med* 2003; 42:960-6.
15. Ishikawa S, Kayaba K, Gotoh T, *et al*. Metabolic syndrome and C-reactive protein in the general population: JMS Cohort Study. *Circ J* 2007; 71:26-31.
16. Ishikawa S, Kayaba K, Gotoh T, *et al*. Incidence of total stroke, stroke subtypes, and myocardial infarction in the Japanese population: the JMS Cohort Study. *J Epidemiol* 2008; 18:144-50.
17. Adams HP, Jr., Bendixen BH, Kappelle LJ, *et al*. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. *Stroke* 1993; 24:35-41.
18. Heuschmann PU, Neureiter D, Gesslein M, *et al*. Association between infection with Helicobacter pylori and Chlamydia pneumoniae and risk of ischemic stroke subtypes: Results from a population-based case-control study. *Stroke* 2001; 32:2253-8.
19. Grau AJ, Buggle F, Lichy C, *et al*. Helicobacter pylori infection as an independent risk factor for cerebral ischemia of atherothrombotic origin. *J Neurol Sci* 2001; 186:1-5.
20. Melnick SL, Shahar E, Folsom AR, *et al*. Past infection by Chlamydia pneumoniae strain TWAR and asymptomatic carotid atherosclerosis. Atherosclerosis Risk in Communities (ARIC) Study Investigators. *Am J Med* 1993; 95:499-504.
21. Sessa R, Di Pietro M, Schiavoni G, *et al*. Chlamydia pneumoniae DNA in patients with symptomatic carotid atherosclerotic disease. *J Vasc Surg* 2003; 37:1027-31.

22. Elkind MS, Lin IF, Grayston JT, *et al*. Chlamydia pneumoniae and the risk of first ischemic stroke : The Northern Manhattan Stroke Study. *Stroke* 2000; 31:1521-5.
23. Elkind MS, Tondella ML, Feikin DR, *et al*. Seropositivity to Chlamydia pneumoniae is associated with risk of first ischemic stroke. *Stroke* 2006; 37:790-5.
24. Voorend M, Faber CG, van der Ven AJ, *et al*. Chlamydia pneumoniae is a likely risk factor for ischemic stroke in young patients. *J Stroke Cerebrovasc Dis* 2004; 13:85-91.
25. Dowell SF, Peeling RW, Boman J, *et al*. Standardizing Chlamydia pneumoniae assays: recommendations from the Centers for Disease Control and Prevention (USA) and the Laboratory Centre for Disease Control (Canada). *Clin Infect Dis* 2001; 33:492-503.
26. Smieja M, Gnarpe J, Lonn E, *et al*. Multiple infections and subsequent cardiovascular events in the Heart Outcomes Prevention Evaluation (HOPE) Study. *Circulation* 2003; 107:251-7.
27. Patel P, Mendall MA, Carrington D, *et al*. Association of Helicobacter pylori and Chlamydia pneumoniae infections with coronary heart disease and cardiovascular risk factors. *BMJ* 1995; 311:711-4.
28. Gurfinkel E. Inflammation, infection, or both in atherosclerosis: the ROXIS trial in perspective. *J Infect Dis* 2000; 181 (Suppl 3):S566-8.
29. Dechend R, Maass M, Gieffers J, *et al*. Chlamydia pneumoniae infection of vascular smooth muscle and endothelial cells activates NF-kappaB and induces tissue factor and PAI-1 expression: a potential link to accelerated arteriosclerosis. *Circulation* 1999; 100:1369-73.
30. Bachmaier K, Neu N, de la Maza LM, *et al*. Chlamydia infections and heart disease linked through antigenic mimicry. *Science* 1999; 283:1335-9.
31. Watson C, Alp NJ. Role of Chlamydia pneumoniae in atherosclerosis. *Clin Sci (Lond)* 2008; 114:509-31.
32. Obata Y, Kikuchi S, Miwa H, *et al*. Diagnostic accuracy of serological kits for Helicobacter pylori infection with the same assay system but different antigens in a Japanese patient population. *J Med Microbiol* 2003; 52:889-92.
33. Kishimoto T, Kubota Y, Matsushima T, *et al*. Assay of specific anti-Chlamydia pneumoniae antibodies by ELISA method. 2. studies on clinical usefulness and serological diagnostic standards. *Kansenshogaku Zasshi* 1996; 70:830-9.
34. Preusch MR, Grau AJ, Buggle F, *et al*. Association between cerebral ischemia and cytotoxin-associated gene-A-bearing strains of Helicobacter pylori. *Stroke* 2004; 35:1800-4.
35. Maeda S, Ogura K, Yoshida H, *et al*. Major virulence factors, VacA and CagA, are commonly positive in Helicobacter pylori isolates in Japan. *Gut* 1998; 42:338-43.
36. Asaka M, Kimura T, Kudo M, *et al*. Relationship of Helicobacter pylori to serum pepsinogens in an asymptomatic Japanese population. *Gastroenterology* 1992; 102:760-6.
37. Fujisawa T, Kumagai T, Akamatsu T, *et al*. Changes in seroepidemiological pattern of Helicobacter pylori and hepatitis A virus over the last 20 years in Japan. *Am J Gastroenterol* 1999; 94:2094-9.
38. Marchildon P, Balaban DH, Sue M, *et al*. Usefulness of serological IgG antibody determinations for confirming eradication of Helicobacter pylori infection. *Am J Gastroenterol* 1999; 94:2105-8.
39. Fagerberg B, Gnarpe J, Gnarpe H, *et al*. Chlamydia pneumoniae but not cytomegalovirus antibodies are associated with future risk of stroke and cardiovascular disease: a prospective study in middle-aged to elderly men with treated hypertension. *Stroke* 1999; 30:299-305.