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# Association of Serum Anti-Periodontal Pathogen Antibody with Ischemic Stroke

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**Key Words**
Periodontitis · Cerebral infarction · Atherosclerosis · Infectious disease · Atrial fibrillation

**Abstract**
***Background:*** Periodontitis increases the risk of atherosclerotic cardiovascular disease and ischemic stroke. In this study, we evaluated whether serum antibody levels against individual periodontal pathogens are significantly associated with ischemic stroke subtypes and their risk factors. ***Methods:*** Patients with acute ischemic stroke (n = 132; 74 male and 58 female, 71.3 ± 10.7 years) and patients with no previous stroke (n = 77; 38 male and 39 female, 70.7 ± 9.5 years) were consecutively enrolled in this study. Stroke subtype was evaluated based on the Trial of Org 10172 in Acute Stroke Treatment classification. Serum was obtained from each patient after obtaining their consent to participate in the study. The levels of serum antibodies against *Aggregatibacter actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg) and *Prevotella intermedia* (Pi) were evaluated by ELISA. Serum high-sensitivity C-reactive protein (hs-CRP) levels were measured by nephelometry. ***Results:*** Serum hs-CRP levels were significantly associated with acute ischemic stroke even after controlling for acute ischemic stroke, hypertension, diabetes mellitus and bulb/ internal carotid artery (ICA) atherosclerosis which were statistically selected (coefficient 0.245, 95% CI 0.142–0.347, $p < 0.0001$). The serum-antibody level of Pi was significantly higher in atherothrombotic-stroke patients than in patients with no previous stroke ($p = 0.0035$). Detectable serum anti-Pg antibody was significantly associated with atrial fibrillation (overall $\chi^2 = 35.5$, $R^2 = 0.18$, $n = 209$, $p < 0.0001$; anti-Pg antibody: OR 4.36, 95% CI 1.71–12.10, $p = 0.0017$), and detectable serum anti-Pi antibody was significantly associated with bulb/ICA atherosclerosis after controlling for the statistically selected associ-

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ated factors (overall $\chi^2 = 46.1$, $R^2 = 0.18$, n = 209, $p < 0.0001$; anti-Pg antibody: OR 16.58, 95% CI 3.96–78.93, $p < 0.0001$). The levels of serum anti-Pi antibody were significantly associated with atherothrombotic stroke with the statistically selected associated factors excluding bulb/ICA atherosclerosis (overall $\chi^2 = 77.0$, $R^2 = 0.44$, n = 129, $p < 0.0001$; anti-Pi antibody: OR 23.6, 95% CI 2.65–298.2, p = 0.008). However, when we included bulb/ICA atherosclerosis in this model, the levels of serum anti-Pi antibody were no longer significantly associated with atherothrombotic stroke (overall $\chi^2 = 98.0$, $R^2 = 0.56$, n = 129, $p < 0.0001$; anti-Pi antibody: p = 0.107). ***Conclusions:*** Our results suggest that anti-Pg antibody is associated with atrial fibrillation and that anti-Pi antibody is associated with carotid artery atherosclerosis. In addition, anti-Pi antibody may be associated with atherothrombotic stroke through its association with carotid artery atherosclerosis. Thus, periodontitis may lead to serious systemic diseases.



## Introduction

Stroke is largely explained by several generally accepted risk factors; these do not, however, fully account for stroke epidemiology. Increasing evidence indicates that recent acute infection and various chronic infectious diseases are important risk factors for stroke. In a combined analysis of 2 prospective studies, periodontitis was found to increase the risk of stroke nearly 3-fold [1]. More recently, a larger case-control study confirmed the graded association between the severity of periodontitis and the risk of stroke [2]. Prospective case-control studies have identified patients who have infections caused by major periodontal pathogens as being at risk for future stroke [3, 4]. On the other hand, periodontal pathogens have been detected with other microbial antigens in carotid plaques. In addition, herpes simplex virus infection was recently reported to be associated with an increased risk of future atrial fibrillation [5]. Atrial fibrillation is also reported to be associated with inflammation [6]; however, its associations with other infectious diseases such as periodontitis are not well defined.

In this study, we evaluated whether serum antibody levels against individual periodontal pathogens are significantly associated with ischemic stroke subtypes and their risk factors. *Aggregatibacter actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg) and *Prevotella intermedia* (Pi), three major periodontitis pathogens, were selected because of their reported associations with systemic diseases.

## Methods

*Subjects*

A total of 132 consecutive patients with acute ischemic stroke were admitted to the Kagawa University Hospital within 24 h after stroke onset between January 1, 2005 and December 31, 2008. The control patients were consecutively enrolled when they were over 45 years old and they were subjected to brain magnetic resonance imaging (MRI) to detect any relevant non-stroke-related issues. They were excluded when they had any neurological deficit or had had a stroke previously (detected as signal loss in fluid-attenuated inversion recovery with MRI); 77 were ultimately used as control patients in the study. They attended our outpatient clinic and exhibited atypical symptoms. Informed consent was obtained from all patients. This study was approved by the investigational review board of the Kagawa University Hospital (H16–26).

Stroke specialists diagnosed ischemic stroke patients with cardioembolic, atherothrombotic or lacunar stroke, or any other type of stroke ('other') using echocardiography, brain computed tomography, MRI, magnetic resonance angiography and carotid ultrasonography. The final diagnosis of the stroke subtype was made before discharge, based on the Trial of Org 10172 in Acute Stroke Treatment classification [7].

Detailed data were collected from all patients by physicians, including baseline characteristics (age, sex, blood pressure and drinking and smoking habits) and vascular risk factors (hypertension, diabetes mellitus and dyslipidemia).

Hypertension, diabetes mellitus and dyslipidemia were diagnosed by physicians. Patients were designated as hypertensive if they were taking antihypertensive agents and had a systolic blood pressure ≥140 mm Hg and/or a diastolic blood pressure ≥90 mm Hg. Patients were diagnosed with diabetes mellitus if they were treated with oral hypoglycemic agents or insulin and/or their serum fasting blood glucose level was ≥7 mmol/l. Patients were diagnosed with dyslipidemia if they were taking antidyslipidemia medication or presented the following levels: serum LDL-cholesterol ≥3.62 mmol/l, triglycerides ≥1.69 mmol/l and/or HDL-cholesterol <1.03 mmol/l.

In this study, a patient was classified as 'drinker' if they had at least one alcoholic drink per day. Occasional drinkers and nondrinkers were both classified as 'nondrinkers'. A patient was classified as a 'smoker' if they smoked at the time of the study or had quit smoking less than 1 year previously. Those patients who had quit smoking more than 1 year previously and those who had never smoked were classified as 'nonsmokers'. These categories were assigned by a physician who interviewed the patient and/or the patient's family.

*Carotid Ultrasonography*

Carotid ultrasound examinations were performed by one trained physician (H.O.) with a 5- to 10-MHz annular array transducer connected to an ultrasound imaging system (LOGIQ 500; General Electric Yokogawa Medical Systems, Tokyo, Japan) with a monitor that displayed the electrocardiogram, as previously reported [8, 9]. Bulb/internal carotid artery (ICA) atherosclerosis was diagnosed when a plaque [intima-media thickness (IMT) ≥1.1 mm] was detected [10].

**Table 1.** Baseline characteristics

| | No previous stroke (n = 77) | Ischemic stroke (n = 132) | p value |
|---|---|---|---|
| Mean age ± SD, years | 70.7 ± 9.5 | 71.3 ± 10.7 | 0.6782 |
| Female sex, n (%) | 39 (50.7) | 58 (43.9) | 0.3895 |
| Hypertension, n (%) | 20 (26.0) | 83 (62.9) | <0.0001 |
| Diabetes mellitus, n (%) | 34 (44.2) | 39 (29.6) | 0.0362 |
| Dyslipidemia, n (%) | 28 (36.4) | 41 (31.1) | 0.4491 |
| Drinking, n (%) | 16 (20.8) | 40 (30.3) | 0.1478 |
| Smoking, n (%) | 18 (23.4) | 37 (28.0) | 0.5169 |
| Atrial fibrillation, n (%) | 8 (10.4) | 31 (23.5) | 0.0262 |
| Bulb/ICA atherosclerosis, n (%) | 15 (19.5) | 49 (37.1) | 0.0082 |
| Subtypes of ischemic stroke | | | |
| Lacunar stroke, n (%) | 0 | 42 (31.8) | N.D. |
| Atherothrombotic stroke, n (%) | 0 | 52 (39.4) | N.D. |
| Cardioembolic stroke, n (%) | 0 | 38 (28.8) | N.D. |
| hs-CRP, log, ng/ml | 2.75 ± 0.69 | 3.23 ± 0.67 | <0.0001 |

N.D. = Not determined.

*Quantification of Serum Anti-Periodontal Pathogen Antibodies*

Serum samples were obtained from patients with acute ischemic stroke upon hospital admission and from those without previous stroke at an outpatient clinic. The samples were kept at –78°C until testing. The level of serum antibody against Aa, Pg or Pi was quantified using the enzyme-linked immunosorbent assay (ELISA), as reported previously [11]. The strains of periodontitis pathogens were ATCC33384, ATCC33277 and ATCC25611 for Aa, Pg and Pi, respectively. Each suspension was incubated at 4°C overnight in 96-well microtiter plates for coating. The reference IgG was purified from serum with a high IgG level to the periodontal pathogens of interest. The diluted reference IgG and vehicle (phosphate-buffered saline) were used to construct standard curves. The patient serum or the reference IgG was applied in duplicate to the wells, and the plates were incubated for 1 h. After washing, the wells were incubated for 1 h with anti-human IgG labelled with peroxidase (DakoCytomation, Carpinteria, Calif., USA). The plates were washed and incubated with the peroxidase substrate (3,3′,5,5′-tetramethylbenzidine) for 30 min. The optical density was read at a wavelength of 450 nm and a sub-wavelength of 650 nm on a plate reader. The serum IgG antibody levels of each patient were calculated using the IgG reference curves and were expressed in relative arbitrary ELISA units. The coefficients of variation were 7.63, 7.19, and 8.12 (original values) and 1.34, 1.21, and 1.42% (logarithmic values) for anti-Aa antibody, anti-Pg antibody and anti-Pi antibody levels, respectively (n = 6). We blinded the physicians who diagnosed the ischemic stroke subtypes to the serum anti-periodontal-pathogen antibody values.

*Quantification of Serum High-Sensitivity C-Reactive Protein*

Serum high-sensitivity C-reactive protein (hs-CRP) levels were measured by nephelometry (NA Latex CRP kit; Dade Behring) in SRL, Inc. (Tokyo, Japan). When the hs-CRP value was below the limit of detection (50 ng/ml), it was recorded as 50 ng/ml.

*Statistical Analysis*

The levels of serum anti-periodontal-pathogen antibodies and serum hs-CRP were analyzed in logarithmic form because these values exhibit a Gaussian distribution. The data were expressed as the means ± standard deviations (SD) for the continuous variables and as frequencies and percentages for discrete variables. Univariate analyses were performed to evaluate the differences between the groups regarding the baseline characteristics, risk factors and levels of serum hs-CRP and serum anti-periodontal-pathogen antibodies. The groups were compared using a one-way analysis of variance (ANOVA; for continuous variables) or the Fisher exact test (for discrete variables). As there are many independent variables, two-step regression strategies were pursued. First, a forward stepwise regression was used with no forced independent variables unless otherwise mentioned and p values of 0.25 to enter and remove. The linear regression model or the logistic regression model reported in the next section with selected factors that were determined from the forward stepwise regression.

Statistical analysis was performed using JMP software version 9.0 for Macintosh. All analyses were two-tailed and a value of $p < 0.05$ was considered statistically significant.

## Results

We evaluated 132 acute ischemic-stroke patients and 77 patients with no previous stroke. Baseline characteristics and the levels of serum hs-CRP are shown in table 1. In the patients with the acute ischemic stroke, prevalence of hypertension, atrial fibrillation and bulb/ICA atherosclerosis was significantly higher than that of the patients with no previous stroke. In contrast, the prevalence of

**Table 2.** Serum anti-periodontal-pathogen antibody levels

| | Aa, logU/ml | Pg, logU/ml | Pi, logU/ml |
|---|---|---|---|
| Age (years) (n = 209) | $R^2 = 0.0058$ | $R^2 = 0.0004$ | $R^2 = 0.0002$ |
| Gender | | | |
| Female (n = 97) | 1.68 ± 0.47 | 1.82 ± 0.49 | 2.42 ± 0.30 |
| Male (n = 112) | 1.66 ± 0.50 | 1.96 ± 0.44* | 2.52 ± 0.28* |
| Hypertension | | | |
| No (n = 106) | 1.71 ± 0.51 | 1.91 ± 0.48 | 2.44 ± 0.29 |
| Yes (n = 103) | 1.63 ± 0.47 | 1.88 ± 0.45 | 2.50 ± 0.30 |
| Diabetes mellitus | | | |
| No (n = 136) | 1.67 ± 0.46 | 1.87 ± 0.48 | 2.45 ± 0.32 |
| Yes (n = 73) | 1.67 ± 0.54 | 1.94 ± 0.45 | 2.51 ± 0.24 |
| Dyslipidemia | | | |
| No (n = 140) | 1.61 ± 0.45 | 1.88 ± 0.46 | 2.41 ± 0.28 |
| Yes (n = 69) | 1.79 ± 0.53* | 1.93 ± 0.48 | 2.61 ± 0.27*** |
| Drinking | | | |
| No (n = 153) | 1.66 ± 0.47 | 1.84 ± 0.49 | 2.47 ± 0.29 |
| Yes (n = 56) | 1.69 ± 0.53 | 2.03 ± 0.39** | 2.49 ± 0.29 |
| Smoking | | | |
| No (n = 154) | 1.70 ± 0.47 | 1.91 ± 0.46 | 2.46 ± 0.29 |
| Yes (n = 55) | 1.60 ± 0.53 | 1.86 ± 0.50 | 2.50 ± 0.30 |
| Atrial fibrillation | | | |
| No (n = 170) | 1.65 ± 0.48 | 1.83 ± 0.46 | 2.45 ± 0.29 |
| Yes (n = 39) | 1.74 ± 0.51 | 2.15 ± 0.41*** | 2.58 ± 0.28** |
| Bulb/ICA atherosclerosis | | | |
| No (n = 145) | 1.64 ± 0.47 | 1.88 ± 0.46 | 2.41 ± 0.29 |
| Yes (n = 64) | 1.74 ± 0.53 | 1.93 ± 0.49 | 2.61 ± 0.25*** |
| Stroke | | | |
| No previous (n = 77) | 1.74 ± 0.54 | 1.86 ± 0.51 | 2.44 ± 0.28 |
| Acute ischemic (n = 132) | 1.63 ± 0.45 | 1.91 ± 0.44 | 2.49 ± 0.30 |
| hs-CRP, log, ng/ml (n = 209) | $R^2 = 0.0085$ | $R^2 = 0.0002$ | $R^2 = 0.0008$ |

All values are presented as the mean ± SD in logarithmic form. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.005$.

diabetes mellitus was higher in the patients with no previous stroke. In the acute ischemic stroke group, serum hs-CRP levels were significantly elevated compared to the levels in patients with no previous stroke ($p < 0.0001$). With a forward stepwise regression, acute ischemic stroke, hypertension, diabetes mellitus and bulb/ICA atherosclerosis were selected as factors that associated with serum hs-CRP levels. Serum hs-CRP levels were significantly associated with acute ischemic stroke even after controlling for those statistically selected associated factors (coefficient 0.245, 95% CI 0.142–0.347, $p < 0.0001$).

The association between the baseline characteristics and the levels of serum anti-periodontal-pathogen antibodies were evaluated (table 2). The levels of serum anti-Aa antibody were higher in patients with dyslipidemia than in patients without. The levels of serum anti-Pg antibody were higher in male patients and patients with drinking habit and atrial fibrillation than in patients without these conditions. The levels of serum anti-Pi antibody were higher in male patients and patients with dyslipidemia, atrial fibrillation and bulb/ICA atherosclerosis than in patients without these conditions. In this study, no significant differences were identified between the groups in terms of serum anti-periodontal-pathogen antibodies and smoking habits.

To assess the association of the levels of serum anti-periodontal-pathogen antibodies with stroke risk factors, the association of the levels of serum anti-periodontal-pathogen antibodies with atrial fibrillation and the bulb/ICA atherosclerosis was evaluated. There were 39 patients (18.7%) with atrial fibrillation in total. The levels of serum antibodies of Pg and Pi, but not of Aa, were significantly

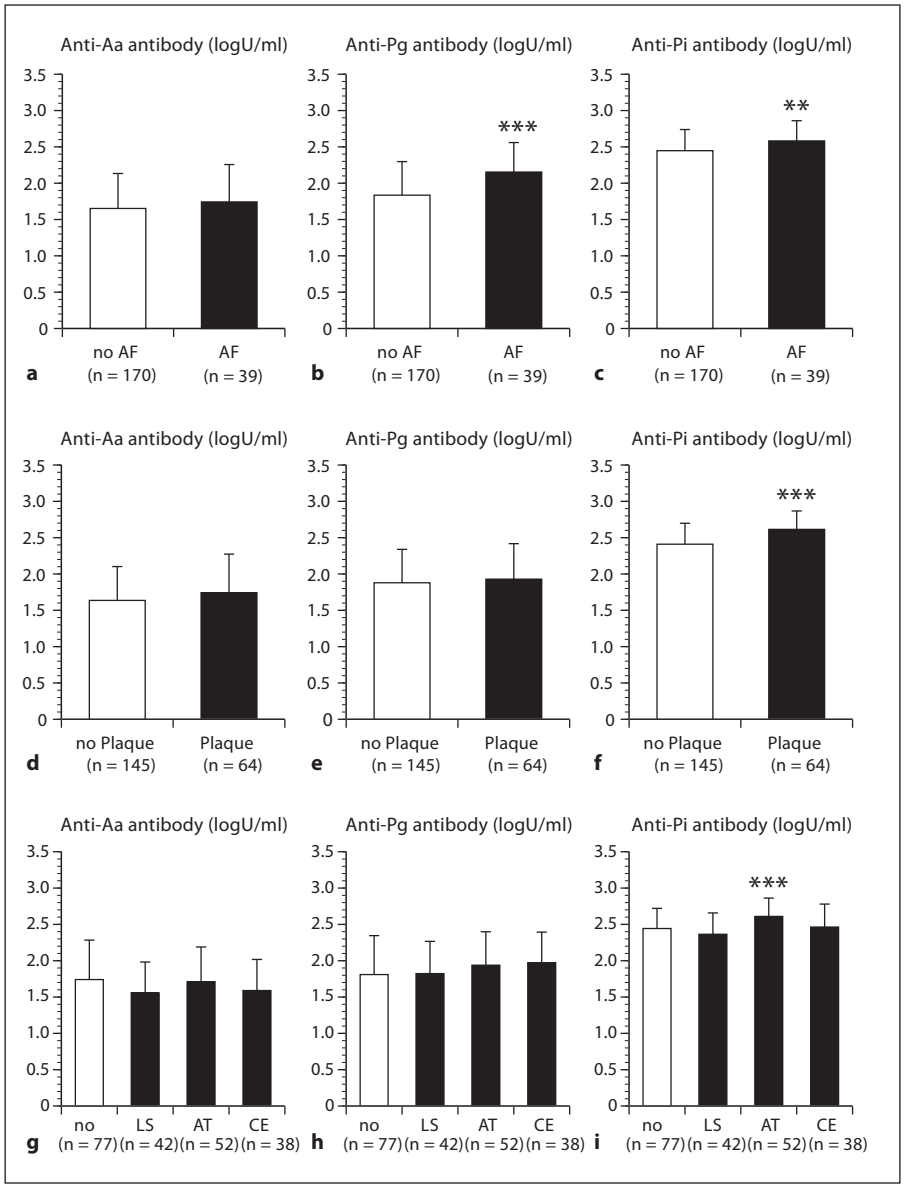


**Fig. 1.** Differences in the levels of serum anti-periodontal-pathogen antibodies between with/without atrial fibrillation, with/without bulb/ICA atherosclerosis and the subtypes of ischemic stroke. Levels of serum antibody against Aa (**a**), Pg (**b**) and Pi (**c**) in patients with atrial fibrillation (AF) versus the patients without it (no AF). Levels of serum antibody against Aa (**d**), Pg (**e**) and Pi (**f**) in patients with bulb/ICA atherosclerosis (plaque) versus the patients without it (no plaque). Levels of serum antibody against Aa (**g**), Pg (**h**) and Pi (**i**) in the patients with lacunar stroke (LS), atherothrombotic stroke (AT) or cardioembolic stroke (CE) were compared to those in patients with no previous stroke (no). ** $p < 0.05$ and *** $p < 0.005$ compared to the patients without these conditions.

higher in the patients with atrial fibrillation compared to that in patients without it ($p < 0.0001$ and $p = 0.0093$; respectively, fig. 1a–c; table 2). With a forward stepwise regression forced to include the levels of serum antibodies against Aa, Pg and Pi, age and hypertension were selected as factors that associated with atrial fibrillation. The level of serum anti-Pg antibody was significantly associated with atrial fibrillation even after controlling for those statistically selected associated factors (overall $\chi^2 = 35.5$, $R^2 = 0.18$, $n = 209$, $p < 0.0001$. Anti-Pg antibody: OR 4.36, 95% CI 1.71–12.10, $p = 0.0017$; fig. 2a), but not with the levels of serum antibodies against Aa and Pi.

There were 64 patients (30.6%) with bulb/ICA atherosclerosis in total. The levels of serum anti-Pi antibody were significantly higher in the patients with bulb/ICA atherosclerosis than that in patients without it ($p < 0.0001$), but not when compared to the levels of serum antibodies against Aa and Pg (fig. 1d–f; table 2). With a forward stepwise regression forced to include the levels of serum antibodies against Aa, Pg and Pi, sex, hypertension, diabetes mellitus, dyslipidemia, drinking and serum hs-CRP levels were selected as factors that associated with bulb/ICA atherosclerosis. Bulb/ICA atherosclerosis was significantly associated with the level of

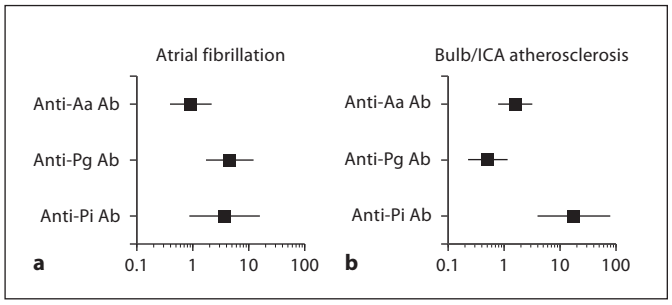


**Fig. 2.** Association of serum anti-periodontal pathogen antibodies. OR of serum antibody (Ab) levels against Aa, Pg and Pi in association with atrial fibrillation (**a**) including analyses that controlled for age and hypertension, and with bulb/ICA atherosclerosis (**b**) controlled for sex, hypertension, diabetes mellitus, dyslipidemia, drinking and serum hs-CRP levels.

serum anti-Pi antibody, even after controlling for the statistically selected associated factors (overall $\chi^2$ = 46.1, $R^2$ = 0.18, n = 209, $p < 0.0001$. Anti-Pg antibody: OR 16.58, 95% CI 3.96–78.93, $p < 0.0001$; fig. 2b), but not with the levels of serum antibodies against Aa and Pg.

The association between the levels of serum anti-periodontal-pathogen antibodies and acute ischemic stroke was evaluated. There was no significant difference in the levels of serum antibodies against Aa, Pg and Pi between the patients with acute ischemic stroke and those with no previous stroke (p = 0.104, 0.486 and 0.273, respectively). Considering the subtypes of acute ischemic stroke, there were significant differences in the levels of serum anti-Pi antibody between the patients with atherothrombotic stroke and those with no previous stroke (p = 0.0035, fig. 1i). With a forward stepwise regression forced to include the levels of serum anti-Pi antibody, age, gender, bulb/ICA atherosclerosis, hypertension, diabetes mellitus, drinking, atrial fibrillation and serum hs-CRP levels were selected as factors that associated with atherothrombotic stroke. When we performed a logistic regression analysis with statistically selected associated factors excluding bulb/ICA atherosclerosis, the levels of serum anti-Pi antibody were significantly associated with atherothrombotic stroke (overall $\chi^2 = 77.0$, $R^2 = 0.44$, n = 129, $p < 0.0001$. Anti-Pi antibody: OR 23.6, 95% CI 2.65–298.2, p = 0.008). However, when we included bulb/ICA atherosclerosis into this model, the levels of serum anti-Pi antibody were no longer significantly associated with atherothrombotic stroke (overall $\chi^2 = 98.0$, $R^2 = 0.56$, n = 129, $p < 0.0001$. Anti-Pi antibody: p = 0.107).

## Discussion

This study demonstrated that serum hs-CRP levels were independently associated with acute ischemic stroke. The levels of serum antibodies against select periodontal pathogens were significantly higher in the patients with dyslipidemia, drinking habit, atrial fibrillation and bulb/ICA atherosclerosis than in patients without these conditions. No significant association between smoking habits and serum antibody levels against any periodontal pathogen was observed. The levels of serum anti-Pg antibody were significantly associated with atrial fibrillation and the levels of serum anti-Pi antibody were significantly associated with bulb/ICA atherosclerosis, independent of the statistically selected associated factors. The levels of serum anti-Pi antibody were significantly higher in the patients with atherothrombotic stroke and were associated with atherosclerotic stroke through bulb/ICA atherosclerosis.

Periodontitis, which is caused by local infections with periodontal pathogens, leads to systemic reactions, such as inflammation, and immunological reactions. In periodontitis, gingival inflammation accompanied by microulceration of the periodontal pocket epithelium and increasing subgingival space for bacterial deposits provide bacteria and their constituents with access to the bloodstream. Local infection in the periodontal pockets triggers a systemic inflammatory response and the release of inflammatory mediators, e.g. CRP. The presence of major periodontal pathogens in subgingival samples was positively associated with elevated CRP levels [12]. The elevation of CRP is directly associated with atherogenesis [13]. Inflammatory markers such as hs-CRP predict myocardial infarction and cerebral infarction, which occur later in life. Systemic or local infection with periodontal pathogens may partially account for the increased serum CRP levels and may be associated with stroke pathogenesis. Our results suggest that serum hs-CRP levels were associated with bulb/ICA atherosclerosis and acute ischemic stroke independent of classical vascular risk factors. These results are supported by many previous reports. Elevated serum hs-CRP levels in acute ischemic stroke are largely caused by inflammation resulting from ischemic insults. No significant associations were observed between the levels of serum antibodies against periodontal pathogens and the serum hs-CRP levels. Thus, inflammation and immunological reactions caused by periodontitis may be independently associated with systemic reactions, e.g. atherosclerosis. Our results support the hypothesis that serum anti-periodontal-

pathogen antibodies levels are associated with atrial fibrillation and bulb/ICA atherosclerosis, independent of serum hs-CRP levels.

In the Framingham Heart Study, the risk factors for atrial fibrillation were identified as age, sex, body mass index, systolic blood pressure, treatment for hypertension, PR interval, clinically significant cardiac murmurs and heart failure [14]. In addition, atrial fibrillation is associated with inflammation, e.g. CRP [6]. The degeneration of pulmonary veins is an important cause of atrial fibrillation [15]. In this study, we evaluated the possible association of serum anti-periodontal-pathogen antibodies with atrial fibrillation. Our results indicate that the levels of serum anti-Pg antibody were significantly associated with the prevalence of atrial fibrillation, independent of serum hs-CRP levels and the other classical vascular risk factors. To our knowledge, this study is the first to show a possible association between the levels of serum anti-periodontal-pathogen antibodies and atrial fibrillation. Inflammation or immunological responses resulting from periodontitis may lead to the degeneration of pulmonary veins, causing atrial fibrillation.

Chronic infection with *Chlamydia pneumoniae*, *Helicobacter pylori* and cytomegalovirus is associated with cardiovascular disease and atherosclerosis [16–18]. A recent study detected the bacterial DNA of major periodontal pathogens in large-artery atheromatous plaques, showing that 30% of the specimens are positive for *Tannerella forsythus*, 26% are positive for Pg, 18% are positive for Aa and 14% are positive for Pi [19]. In the Oral Infections and Vascular Disease Epidemiology Study (INVEST), a significant association was observed between the levels of tooth loss and the prevalence of carotid artery plaque [20]. Among patients with 0–9 missing teeth, 46% had carotid artery plaque, whereas among those with more than 10 missing teeth, approximately 60% had carotid artery plaque. This cross-sectional study reveals that carotid IMT is regressed on tertiles of periodontal pathogens in the subgingival plaque [21]. Furthermore, the levels of serum antibodies (IgA and IgG) against Aa and Pg were positively correlated with mean IMT [22]. On the other hand, the immunoreactivity to Aa leukotoxin correlates negatively with a future stroke in women, but not in men [23]. Our results show that the levels of serum anti-periodontal-pathogen antibodies, especially against Pi, independently predict large-artery diseases, e.g. carotid artery atherosclerosis.

The risk profiles differ significantly among ischemic stroke subtypes. It is reported that a total of 9.7% patients in the stroke patients had suffered a previous infection within the month before the stroke [24], and previous infection was more frequent in ischemic stroke cases than in intracerebral hemorrhage. A limited number of reports have evaluated the influence of systemic periodontal pathogen infections on stroke. Pussinen et al. [3, 4] demonstrated that IgA seropositivity against Pg is associated with stroke incidence in prospective case-control studies. A recent case-control study revealed that high levels of periodontal clinical attachment loss, gingivitis and radiographic bone loss are independently associated with stroke after adjusting for age, gender, tooth loss and the established cardiovascular risk factors [25]; however, no studies have evaluated the association of the levels of serum anti-periodontal-pathogens antibodies with the subtypes of ischemic stroke. In this study, the levels of serum anti-Pi antibody were significantly higher in the patients with atherothrombotic stroke.

It is important to determine whether different periodontal pathogens or their serum antibodies have different associations with ischemic stroke. The different periodontal pathogens show different levels of association with periodontitis; however, to our knowledge, there are still no data about the different degrees to which periodontal pathogens or their antibodies are associated with cardiovascular disease. This study showed the different degrees to which anti-periodontal-pathogen antibodies are associated with ischemic-stroke subtypes and their risk factors: the levels of serum anti-Pi antibody were higher in the patients with atherothrombotic stroke, the levels of anti-Pi antibody were associated with bulb/ICA atherosclerosis and the levels of anti-Pg antibody were associated with atrial fibrillation. Therefore, the different periodontal pathogens or their antibodies may have different associations with cardiovascular disease.

This study has some limitations. The control group was composed of patients from neurological and neurosurgical clinics with no previous stroke symptomatic or detectable by MRI, in order to avoid possible confounding from anamnestic stroke with a silent infarct. Thus, there was a selection bias favoring nonstroke patients from the general population. In addition, the basic characteristics of the patients with no previous stroke differed significantly from those of the acute ischemic stroke patients. Periodontal disease status was not evaluated in the study; therefore, the relationship between this status and the levels of serum anti-periodontal-pathogen antibodies was not assessed.

In conclusion, our results suggest that anti-Pg antibody may be associated with atrial fibrillation and that anti-Pi antibody may be associated with carotid artery

atherosclerosis. In addition, anti-Pi antibody may be associated with atherothrombotic stroke. Thus, periodontitis may lead to serious systemic diseases. Further studies will be needed to clarify whether intervention against periodontitis could prevent systemic diseases.


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