Harboring Cnm-Expressing *Streptococcus Mutans* In The Oral Cavity Relates To Both Deep and Lobar Cerebral Microbleeds

Shuhei Ikeda
National Cerebral and Cardiovascular Center
Satoshi Saito
National Cerebral and Cardiovascular Center
Satoshi Hosoki
National Cerebral and Cardiovascular Center
Shuichi Tonomura
National Cerebral and Cardiovascular Center
Yumi Yamamoto
National Cerebral and Cardiovascular Center
Hajime Ikenouchi
National Cerebral and Cardiovascular Center
Hiroyuki Ishiyama
National Cerebral and Cardiovascular Center
Tomotaka Tanaka
National Cerebral and Cardiovascular Center
Yorito Hattori
National Cerebral and Cardiovascular Center
Robert P. Friedland
University of Louisville
Roxana O. Carare
University of Southampton
Nagato Kuriyama
Shizuoka Graduate University of Public Health
Yusuke Yakushiji
Saga University Faculty of Medicine
Hideo Hara
Saga University Faculty of Medicine
Masatoshi Koga
National Cerebral and Cardiovascular Center
Kazunori Toyoda

National Cerebral and Cardiovascular Center
Ryota Nomura
Osaka University Graduate School of Dentistry
Misa Takegami
National Cerebral and Cardiovascular Center
Kazuhiko Nakano
Osaka University Graduate School of Dentistry
Masafumi Ihara (ihara@ncvc.go.jp)
National Cerebral and Cardiovascular Center

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Abstract

Streptococcus mutans, a major cariogenic bacterium, expressing the collagen-binding protein Cnm induces cerebrovascular inflammation, resulting in the impairment of blood brain barrier integrity followed by cerebral bleeding. We here examined the association of Cnm-positive *S. mutans* with cerebral microbleeds (CMBs) in acute stroke patients selected from a single-center registry database. Of 428 patients who received oral bacterial examinations among 3154 stroke patients, 326 patients who harbored *S. mutans* were identified. After excluding four patients without imaging data, we compared 72 patients with Cnm-positive *S. mutans* and 250 with Cnm-negative *S. mutans*. Deep, lobar and infratentorial CMBs were observed in 46 (63.9%), 36 (50.0%), 25 (34.7%) patients with Cnm-positive *S. mutans* and 144 (57.6%), 114 (45.6%), 101 (40.4%) with Cnm-negative *S. mutans*. Possession of Cnm-positive *S. mutans* was related to higher numbers of both deep and lobar, but not infratentorial, CMBs (risk ratios 1.57 [1.07–2.30], deep; 5.44 [2.50–11.85], lobar). Statistical significance persisted after adjusting for age, sex, hypertension, stroke type, National Institutes of Health Stroke Scale score, and cerebral amyloid angiopathy (risk ratios 1.61 [1.14–2.27], deep; 5.14 [2.78–9.51], lobar). Our study indicated that reduction of Cnm-positive *S. mutans* may serve as a therapeutic approach for improving the prognosis of stroke patients.

Introduction

Cerebral microbleeds (CMBs), an independent predictor of dementia and stroke[1–3], are radiologically defined constructs found on magnetic resonance imaging (MRI)[4,5]. The underlying pathology of most CMBs consists of iron-positive focal or dispersed accumulations of siderophages corresponding to previous hemorrhages from small cerebral vessels[6,7]. However, CMBs are also accompanied by various pathological lesions such as inflammatory vasculitis and fibrinoid necrosis[6]; therefore, clarifying the pathogenesis of CMBs remains challenging[4].

Hypertensive arteriopathy and cerebral amyloid angiopathy (CAA) are the two most common causes of CMBs. Previous studies have suggested that deep CMBs are mostly associated with hypertensive arteriopathy, whereas lobar CMBs reflect CAA[8]. However, growing evidence has shown that hypertensive arteriopathy induces both deep and lobar CMBs[8]. One pathological investigation showed that lobar CMBs were related to hypertensive arteriopathy in the absence of CAA[7].

We previously reported that oral carriage of *Streptococcus mutans*, a major cariogenic bacterium, expressing the Cnm protein is related to an increased risk of deep CMBs[9–11]. Cnm, encoded by the *cnm* gene, is a cell-surface 120-kDa collagen-binding protein of *S. mutans*[12]. Intravenous administration of Cnm-expressing *S. mutans* (Cnm-positive *S. mutans*) aggravates cerebral bleeding in both the cortical and deep gray matter in stroke-prone spontaneously hypertensive rats (SHRs)[13]. Nevertheless, the contribution of Cnm-positive *S. mutans* to lobar CMBs in humans remains unclear[9,14,15]. Infective endocarditis is a critical consequence of dental bacteremia including that caused by Cnm-positive *S.*

mutans[16], and lobar CMBs precede intracerebral hemorrhage (ICH) in infective endocarditis[17]. We therefore hypothesized that Cnm-positive *S. mutans* is associated with the development of lobar and deep CMBs. This cross-sectional study investigated the involvement of Cnm-positive *S. mutans* in deep and lobar CMBs in stroke patients.

Results

Patient Selection

Among 3154 stroke patients, 428 patients (13.6%) underwent an oral bacterial examination (Figure 1). The characteristics of these 428 patients are described in the Supplementary Materials (see Supplementary Table 1). Patients who underwent oral bacterial examinations had younger ages and lower National Institutes of Health Stroke Scale (NIHSS) scores compared to those who did not undergo examinations (age: 73.0 [63.0–81.0] years versus 76.0 [67.0–83.0] years, P<0.001; NIHSS score: 3.0 [1.0–6.0] versus 4.0 [1.0–14.0], P<0.001). In addition, higher frequencies of ICH and hypertension and a lower prevalence of atrial fibrillation were observed in patients who underwent a bacterial examination (ICH: 36.9% versus 20.1%, P<0.001; hypertension: 89.3% versus 82.1%, P<0.001: atrial fibrillation: 17.3% versus 24.0%, P=0.002).

In the oral bacterial examination, *S. mutans* was detected in 326 patients (76.2%) and was absent in the remaining 102 patients (23.8%). The clinical profiles were similar between patients with and without *S. mutans* in the oral cavity (see Supplementary Table 2). We identified 72 patients with the Cnm-positive *S. mutans* (Cnm [+] group) and 254 with the Cnm-negative *S. mutans* (Cnm [−] group). Four patients in the Cnm (−) group were excluded from the analyses because no MRI data were available.

Demographics and Clinical Characteristics

The clinical profiles of the Cnm (+) and Cnm (−) groups are described in Table 1. The age, sex, blood pressure, vascular risk factors, and medication histories were similar between the two groups. The Cnm (+) group showed slightly lower NIHSS scores than the Cnm (−) group (2.0 [1.0–5.0] versus 3.0 [1.0–7.3], P=0.125). The prevalence of CAA was comparable between the two groups.

Table 1
Clinical profiles of Cnm-positive and negative groups

	Cnm (+) group (n=72)	Cnm (−) group (n=250)	*P* value
Age, y	72.5 (61.0–79.0)	72.0 (62.0–81.0)	0.554
Males, n (%)	48 (66.7)	161 (64.4)	0.723
Systolic blood pressure, mmHg	124.0 (117.3–134.0)	120.0 (113.0–132.3)	0.394
Diastolic blood pressure, mmHg	72.0 (65.0–79.8)	70.0 (61.0–78.3)	0.338
Vascular risk factors			
Hypertension, n (%)	67 (93.1)	222 (88.8)	0.294
Dyslipidemia, n (%)	41 (56.9)	122 (48.8)	0.223
Diabetes, n (%)	19 (26.4)	51 (20.4)	0.278
Atrial fibrillation, n (%)	11 (15.3)	43 (17.2)	0.700
Smoking, n (%)	14 (19.4)	46 (18.4)	0.841
Medication			
Antihypertensive agents, n (%)	40 (55.6)	140 (56.0)	0.947
ATAs, n (%)	31 (43.1)	97 (38.8)	0.516
Antiplatelet agents, n (%)	20 (27.8)	71 (28.4)	0.918
Anticoagulants, n (%)	13 (18.1)	35 (14.0)	0.395
Use of ≥2 ATAs, n (%)	4 (5.6)	15 (6.0)	1.000
CAA			0.316
Probable CAA, n (%)	6 (8.3)	10 (4.0)	
Possible CAA, n (%)	1 (1.4)	7 (2.8)	
No CAA, n (%)	65 (90.3)	233 (93.2)	
CMBs, n (%)			0.141
Strictly lobar CMBs, n (%)	5 (6.9)	8 (3.2)	
D/I or mixed CMBs, n (%)	50 (69.4)	158 (63.2)	

Data represent the median (interquartile range) or number (percent). Cnm (+), Cnm-positive group; Cnm (−), Cnm-negative group; ATAs, anti-thrombotic agents; CAA, cerebral amyloid angiopathy; CMBs, cerebral microbleeds; D/I, deep and/or infratentorial; ICH, intracerebral hemorrhage; NIHSS, National Institutes of Health Stroke Scale.

	Cnm (+) group (n=72)	Cnm (−) group (n=250)	*P* value
No CMBs, n (%)	17 (23.6)	84 (33.6)	
Stroke type			0.843
Ischemic stroke, n (%)	44 (61.1)	156 (62.4)	
ICH, n (%)	28 (38.9)	94 (37.6)	
NIHSS score	2.0 (1.0–5.0)	3.0 (1.0–7.3)	0.125

Data represent the median (interquartile range) or number (percent). Cnm (+), Cnm-positive group; Cnm (−), Cnm-negative group; ATAs, anti-thrombotic agents; CAA, cerebral amyloid angiopathy; CMBs, cerebral microbleeds; D/I, deep and/or infratentorial; ICH, intracerebral hemorrhage; NIHSS, National Institutes of Health Stroke Scale.

Cerebral Microbleeds

CMBs were observed in 55 (76.4%) patients in the Cnm (+) group and 166 (66.4%) in the Cnm (−) group (*P*=0.107). Strictly lobar CMBs were found in 5 patients (6.9%) in the Cnm (+) group and 8 (3.2%) in the Cnm (−) group (*P*=0.175). The median (interquartile range) numbers of CMBs in the Cnm (+) group were 4.0 (1.0–11.0) for all CMBs, 1.0 (0.0–6.5) for deep CMBs, 0.5 (0.0–4.8) for lobar CMBs, and 0.0 (0.0–1.0) for infratentorial CMBs, while the numbers of CMBs in the Cnm (−) group were 2.0 (0.0–7.0) for all, 1.0 (0.0–3.3) for deep, 0.0 (0.0–2.0) for lobar, and 0.0 (0.0–1.3) for infratentorial CMBs. When the number of CMBs was stratified into six categories, the Cnm (+) group was significantly distributed in the “higher CMB” categories for all and lobar CMBs but not for deep or infratentorial CMBs (all CMBs, *P*=0.030; deep, *P*=0.178; lobar, *P*=0.009; infratentorial, *P*=0.721, Table 2).

Table 2
Number of CMBs in the Cnm-positive and negative groups

	Number of CMBs						*P* value
	0	1	2–4	5–10	11–20	≥21	
All CMBs							
Cnm (+)	17 (23.6)	8 (11.1)	15 (20.8)	11 (15.3)	9 (12.5)	12 (16.7)	0.030
Cnm (−)	84 (33.6)	25 (10.0)	46 (18.4)	53 (21.2)	27 (10.8)	15 (6.0)	
Deep CMBs							
Cnm (+)	26 (36.1)	13 (18.1)	13 (18.1)	13 (18.1)	5 (6.9)	2 (2.8)	0.178
Cnm (−)	106 (42.4)	39 (15.6)	55 (22.0)	40 (16.0)	6 (2.4)	4 (1.6)	
Lobar CMBs							
Cnm (+)	36 (50.0)	6 (8.3)	12 (16.7)	8 (11.1)	4 (5.6)	6 (8.3)	0.009
Cnm (−)	136 (54.4)	40 (16.0)	43 (17.2)	25 (10.0)	2 (0.8)	4 (1.6)	
Infratentorial CMBs							
Cnm (+)	47 (65.3)	10 (13.9)	8 (11.1)	5 (6.9)	0 (0)	2 (2.8)	0.721
Cnm (−)	149 (59.6)	39 (15.6)	38 (15.2)	21 (8.4)	1 (0.4)	2 (0.8)	

Data represent the number (percent) of patients in the six cerebral microbleed (CMB) categories (0, 1, 2–4, 5–10, 11–20, and ≥21) in the Cnm (+) (n=72) and Cnm (−) groups (n=250). Cochran–Armitage test for trend. Cnm (+), Cnm-positive group; Cnm (−), Cnm-negative group.

Table 3 shows the unadjusted and adjusted risk ratios between the presence of Cnm-positive *S. mutans* and the number of CMBs. Harboring Cnm-positive *S. mutans* was significantly associated with a greater number of all, deep, and lobar CMBs, but not infratentorial CMBs, in the unadjusted model (risk ratio of all CMBs, 2.84 [95% confidence interval, 1.66–4.87], $P<0.001$; deep CMBs, 1.57 [1.07–2.30], $P=0.021$; lobar CMBs, 5.44 [2.50–11.85], $P<0.001$; infratentorial CMBs, 1.55 [0.72–3.33], $P<0.263$). Statistical significance persisted after adjusting for age, sex, hypertension, stroke type, NIHSS score, and CAA (adjusted risk ratio of all CMBs, 2.73 [1.72–4.33], $P<0.001$; deep CMBs, 1.61 [1.14–2.27], $P<0.001$; lobar CMBs, 5.14 [2.78–9.51], $P<0.001$).

Table 3
Risk ratios and 95% confidence intervals for associations between Cnm-positive *S. mutans* and the number of CMBs

	Unadjusted		Model 1*		Model 2†		Model 3‡	
	RR (95% CI)	*P* value	RR (95% CI)	*P* value	RR (95% CI)	*P* value	RR (95% CI)	*P* value
All CMBs	2.84 (1.66–4.87)	<0.001	2.78 (1.64–4.7)	<0.001	2.86 (1.69–4.86)	<0.001	2.73 (1.72–4.33)	<0.001
Deep CMBs	1.57 (1.07–2.30)	0.021	1.53 (1.04–2.23)	0.029	1.56 (1.09–2.24)	0.016	1.61 (1.14–2.27)	<0.001
Lobar CMBs	5.44 (2.50–11.85)	<0.001	5.39 (2.57–11.30)	<0.001	5.63 (2.75–11.53)	<0.001	5.14 (2.78–9.51)	<0.001
Infra-tentorial CMBs	1.55 (0.72–3.33)	0.263	1.50 (0.73–3.07)	0.272	1.50 (0.72–3.10)	0.277	1.46 (0.72–2.96)	0.298

The unadjusted risk ratio (RR) and adjusted RR were estimated by quasi-Poisson regression models. *Adjusted for age, sex, and hypertension. †Adjusted for model 1 plus stroke type (ischemic stroke or intracerebral hemorrhage) and National Institutes of Health Stroke Scale score. ‡Adjusted for model 2 plus cerebral amyloid angiopathy. CMBs, cerebral microbleeds; CI, confidence interval.

The inter-rater correlation coefficients for deep, lobar, and infratentorial CMBs were 0.87, 0.88, and 0.95, respectively. Representative images showing a patient with a substantial number of deep and lobar CMBs are illustrated in Figure 2.

Discussion

The current study demonstrated that oral carriage of Cnm-positive *S. mutans* was independently associated with a greater number of all, deep, and lobar, but not infratentorial, CMBs. The similar frequencies of CAA and strictly lobar CMBs between patients with Cnm-positive and those with Cnm-negative *S. mutans* suggests that Cnm-positive *S. mutans* does not accelerate the pathophysiology of CAA.

S. mutans is an anaerobic Gram-positive coccus that is detected in the oral cavity of approximately 90% of the general population[15]. Bacteremia caused by *S. mutans* is almost inevitable in daily life because of toothbrushing, flossing, or tooth extraction[10]. The major sources of *S. mutans* are mothers or

caregivers[18]. *S. mutans* is vertically transmitted and colonizes the mouths of infants[18] and is rarely implanted during adulthood[19]. However, it frequently disappears from the oral cavity of edentulous people because *S. mutans* resides on the tooth surface[20]. We therefore excluded patients from whom no *S. mutans* was detected and compared those with Cnm-positive and Cnm-negative *S. mutans* in this study.

Cnm-positive *S. mutans* is characterized by its binding to components of the vascular basement membrane, such as collagen-IV and laminin[12,16,21], while Cnm-negative *S. mutans* cannot attach to soft tissues[16,21]. Aging and vascular risk factors including hypertension induce endothelial injury and increase the thickness of the basement membranes, resulting in collagen-IV and laminin exposure in small cerebral arteries[22,23]. Once Cnm-positive *S. mutans* adheres to the basement membrane[21], infiltration of neutrophils may aggravate local inflammation, resulting in increased permeability of the blood-brain barrier and increased production of enzymes, such as matrix metalloproteinase-9[13], that accelerate endothelial damage, leading to CMBs (Figure 3)[11].

Our previous cross-sectional[9] and retrospective longitudinal studies[11] showed a strong association of Cnm-positive *S. mutans* with deep, but not lobar, CMBs. The seemingly different results regarding lobar CMBs may stem from the different sample sizes between the previous and current studies. Twenty-one or fewer stroke patients with Cnm-positive *S. mutans* were registered in the previous studies[9,11]. Hachinski described the “vascular centrencephalon” as the phylogenetically ancient part of the brain that is perfused by short straight arteries with few branches, transmitting pressure directly from the large arteries to small arterioles[24,25]. However, the cortex is supplied by long arteries with many branches, resulting in a large blood pressure decrement in the brain[26]. In a computational hemodynamics model of hypertension patients with a blood pressure of 192/113 mmHg in the brachial artery, the small arterial pressure was 169/101 mmHg in the lenticulostriate bed but only 117/68 mmHg in the posterior parietal artery bed[26]. This marked difference in the arterial pressure between the deep and cortical regions could explain why lacunar infarcts related to vasculopathies induced by high blood pressure preferentially occur in the vascular centrencephalon rather than in the cortex[25].

However, stroke-prone SHRs, a rat model of systemic hypertension, exhibit endothelial damage not only in the deep arteries but also in the cortical arteries[23]. Furthermore, cerebrovascular integrity is more severely damaged in stroke-prone SHRs than in SHRs, even though stroke-prone SHRs and SHRs show similar degrees of hypertension[27]. Hypertensive patients who have achieved target blood pressure levels still display autonomic dysfunction[28] and a high residual cardiovascular risk[29]. These findings suggest that factors other than high blood pressure also contribute to endothelial injury in patients with systemic hypertension[10], which may explain the increased numbers of lobar and deep CMBs in patients with Cnm-positive *S. mutans*. Furthermore, patients with a high-salt diet exhibit impaired endothelium-dependent vasodilation even without any blood pressure changes[30]. The age of onset of hypertension is associated with end organ damage, independent of the measured blood pressure[31]. Hence, several known and

unknown factors other than hypertension may contribute to endothelial injury in small arteries, enabling Cnm-positive *S. mutans* to attach to the basement membranes and induce both deep and lobar CMBs.

There are some limitations to this study. First, this study was retrospectively performed, posing a potential risk of selection bias. Second, only 428 patients (13.6%) of the total 3154 stroke patients underwent an oral bacterial evaluation. We attempted to widely recruit stroke patients, but older and severe stroke patients tended not to participate in the study, largely because of difficulties in explaining the research to those with impaired consciousness or various disabilities including dementia and advanced frailty. This resulted in a younger age and lower NIHSS scores in patients who underwent bacterial assessments. Third, the proportion of patients with ICH and the frequencies of patients with a history of hypertension or atrial fibrillation were different between those who did and did not undergo oral bacterial examination. We previously reported that Cnm-positive *S. mutans* is more closely associated with hypertensive ICH than ischemic stroke[9], which might have influenced participation in the research or affected the success rate of informed consent acquisition. Fourth, while cognitive function was not assessed in this study, we are now performing a prospective observational study to evaluate the effects of Cnm-positive *S. mutans* on cognition.

In conclusion, we found that Cnm-positive *S. mutans* was associated with a higher number of both lobar and deep CMBs. The close association between Cnm-positive *S. mutans* and CMBs suggests that reduction of Cnm-positive *S. mutans* in the oral cavity may serve as a novel therapeutic approach for improving the long-term prognosis of stroke patients.

Methods

Data Availability Statement

Raw data were generated and stored at the National Cerebral and Cardiovascular Center. Derived data supporting the findings of this study are available from the corresponding authors on request.

Study Design

The significance of oral carriage of Cnm-positive *S. mutans* with regard to CMBs was evaluated in a cross-sectional study in accordance with the Declaration of Helsinki standards and the Japanese Ethical Guidelines for Medical and Health Research Involving Human Subjects after approval was obtained from the Ethical Committee of the National Cerebral and Cardiovascular Center (M23-073-8, M27-015-5).

Acute stroke patients were selected from the National Cerebral and Cardiovascular Center Stroke Registry database (https://www.clinicaltrials.gov; Unique identifier: NCT02251665) who fully satisfied the following criteria: (1) patients who underwent oral bacterial assessments from February 2014 to May 2016 or from May 2017 to October 2018; (2) patients who developed ischemic stroke or ICH during the above period; (3) patients older than 40 years old; and (4) patients or their legal representative provided written informed consent for the current study. We did not perform oral bacterial assessments for any

patients between June 2016 and April 2017. The sample size and study period were determined based on feasibility. Patients without MRI data were excluded from the analyses. Patients in Cnm (+) group were compared with those in Cnm (−) group. We also compared acute stroke patients receiving oral bacterial examination to those who did not receive the examination in order to uncover potential sources of bias.

Clinical Characteristics

Clinical information, except for MRI findings, was obtained from the National Cerebral and Cardiovascular Center Stroke Registry database. Hypertension was defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or a history of antihypertensive medication use. Diabetes mellitus was considered present when a patient used antidiabetic drugs or insulin, the fasting plasma glucose level was ≥126 mg/dL, or the glycated hemoglobin A1c level was ≥6.5%. The definition of dyslipidemia was a low density lipoprotein cholesterol level ≥140 mg/dL, a high density lipoprotein cholesterol level ≤40 mg/dL, a triglyceride level ≥150 mg/dL, or use of lipid-lowering drugs. NIHSS scores were recorded at admission. The diagnosis of possible or probable CAA was based on the modified Boston criteria[32].

Detection of Cnm-Positive S. mutans

Dental plaque specimens were collected and inoculated in Mitis-Salivarius medium with bacitracin (Sigma-Aldrich, St. Louis, MO, USA) and on 15% sucrose agar plates and anaerobically incubated at 37°C for 48 hours. *S. mutans* strains were identified and isolated on the basis of rough morphological features on the agar plates. The strains were then cultured in brain-heart infusion broth (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) at 37°C for 24 hours. Bacterial genomic DNA was extracted from each strain. A polymerase chain reaction assay was performed using an *S. mutans*-specific primer set (forward, 5'-GGCACCACAACATTGGGAAGCTCAGTT-3'; reverse, 5'- GGAATGCCGATCAGTCAACAGGAT-3') according to a method described previously[18]. The presence or absence of the *cnm* gene was determined using primers designed to amplify the entire length of the *cnm* gene (forward, 5'-GACAAAGAAATGAAAGATGT-3'; reverse, 5'-GCAAAGACTCTTGTCCCTGC-3')[18]. Experiments were conducted by researchers who were blinded to the clinical information.

Evaluation of CMBs

In this study, CMBs were illustrated on T2*-weighted MRI. We evaluated the ‘certain’ brain microbleeds as CMBs according to the Brain Observer MicroBleed Scale[5]. The MRI parameters are summarized in the Supplementary Materials (see Supplemetary Table 3). We defined deep CMBs as hypointensities on T2*-weighted MRI located in the deep gray matter of the basal ganglia or thalamus or the white matter of the corpus callosum or internal, external, or extreme capsule. Lobar CMBs were defined as those in the cortical gray or subcortical white matter. Infratentorial CMBs were defined as those in the brainstem or cerebellum. Strictly lobar CMBs were defined as CMBs restricted to a lobar region. We also classified deep and/or infratentorial and mixed CMBs[33]. Mixed CMBs included those located in both lobar and deep and/or infratentorial regions[33]. The term “all CMBs” encompasses CMBs in any brain region. The number

of CMBs was independently determined by two trained neurologists blinded to the clinical data. In the case of disagreement, the opinion of a third neurologist was sought.

Statistical Analyses

Variables are presented as medians and interquartile ranges or numbers and percentages. The Mann-Whitney U test was used to analyze continuous data, and the χ^2 or Fisher exact test was used for categorical data. The numbers of all, deep, lobar, and infratentorial CMBs were stratified into six categories: 0, 1, 2–4, 5–10, 11–20, and ≥21[1,2]. We examined the linear trend between Cnm-positive *S. mutans* and each CMB category using the Cochran-Armitage test. In addition, the association of Cnm-positive *S. mutans* with the number of CMBs was analyzed using quasi-Poisson regression models. The adjusted risk ratios and their 95% confidence intervals were estimated after adjustment for age, sex, systolic blood pressure, stroke type (ischemic stroke or ICH) and CAA (possible or probable CAA)[34]. A *P* value <0.05 (two-tailed) was considered statistically significant. Statistical analyses were conducted using SPSS version 27 (IBM Corp., Armonk, NY, USA) and SAS version 9.4 (SAS Institute, Cary, NC, USA).

Declarations

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Author contributions statement

Study conception: SS, RN, KN and MI. Data acquisition: SI, SS, SH, ST, Hajime I, and Hiroyuki I. Analysis and interpretation of data: SI, SS, Yumi Y, RN, MT and KN. Drafting the manuscript: SI, SS and MI. Revising the manuscript critically for intellectual content: TT, YH, RPF, ROC, NK, Yusuke Y, HH, MK, and KT. Supervision of the study: SS, HH and MI.

Additional information

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Figures

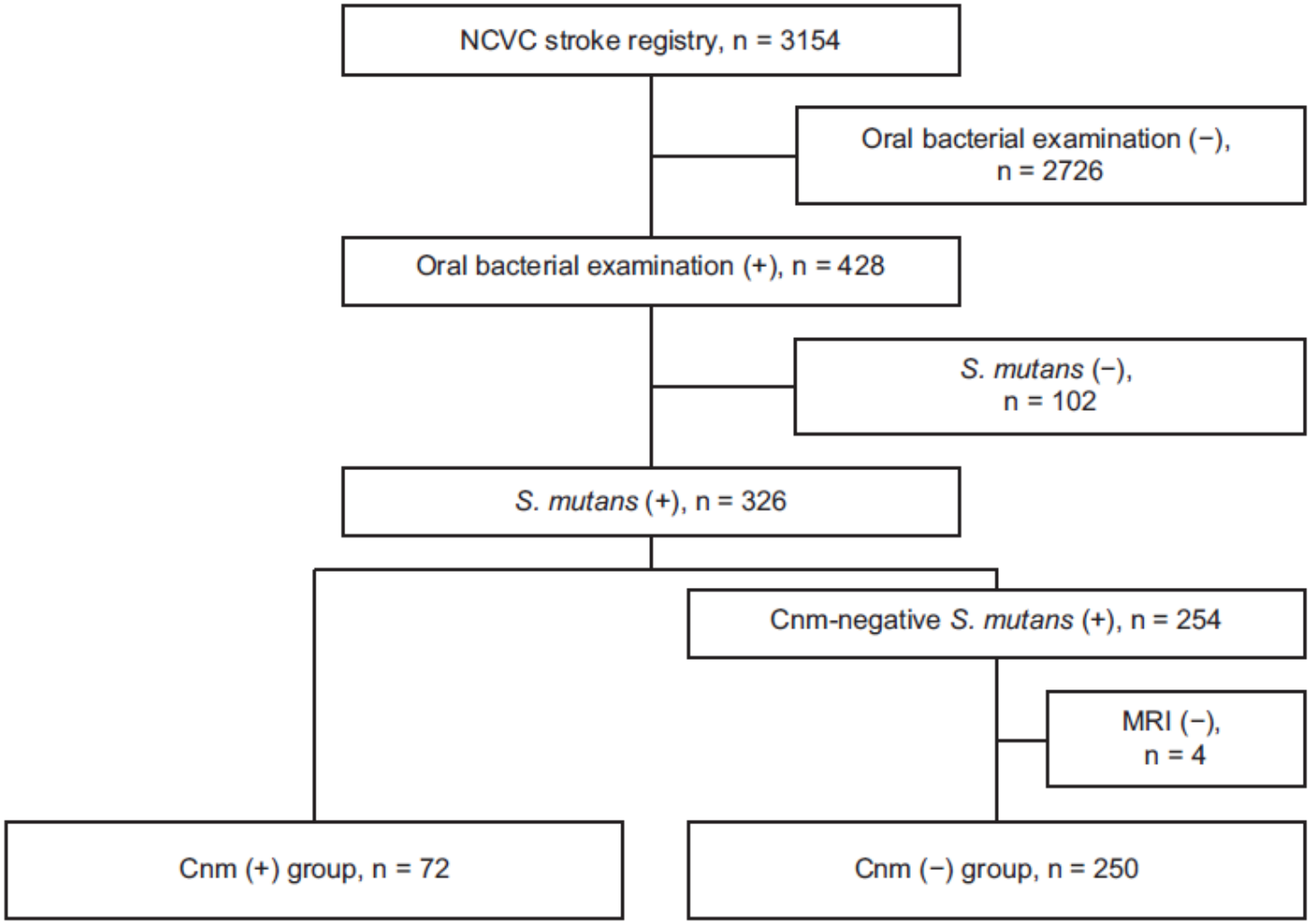

Figure 1

Flow diagram of patient selection

Patients with Cnm-positive *S. mutans* (Cnm [+] group) were compared with those with Cnm-negative *S. mutans* (Cnm [−] group).

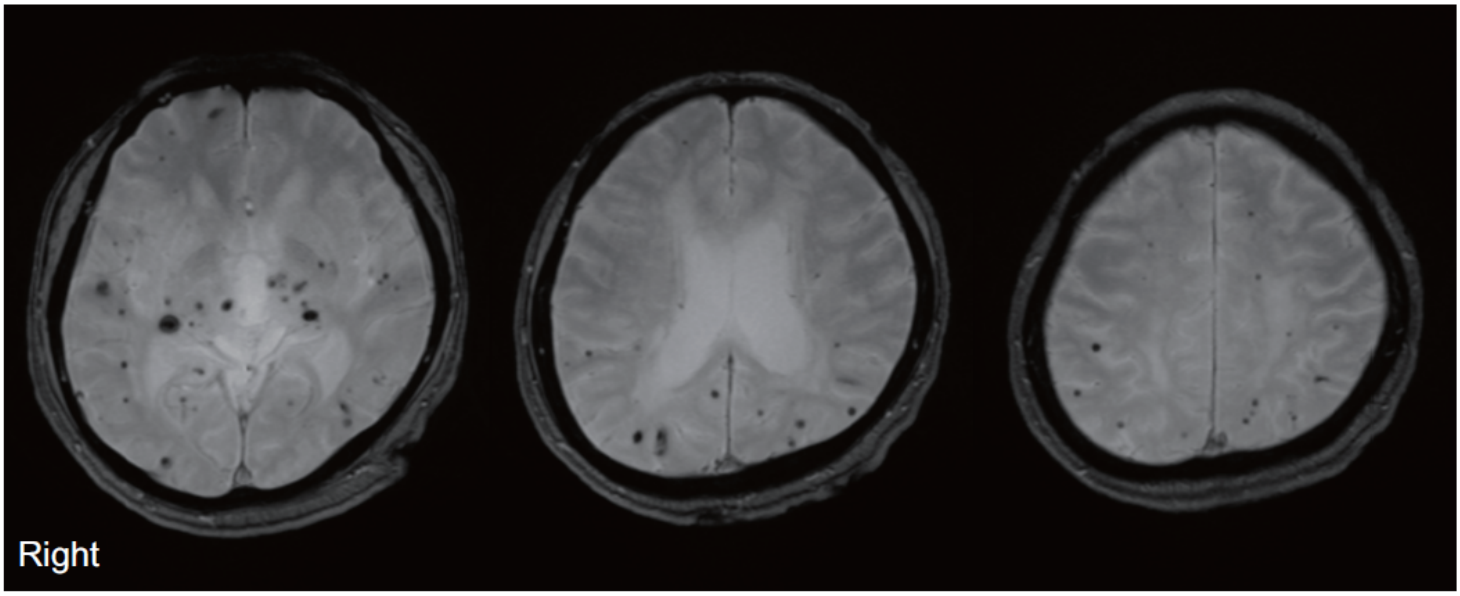

Figure 2

Representative images of cerebral microbleeds in a patient with Cnm-positive *S. mutans*

T2*-weighted magnetic resonance images of 59-year-old man with Cnm-positive *S. mutans* in his oral cavity showing multiple deep and lobar cerebral microbleeds.

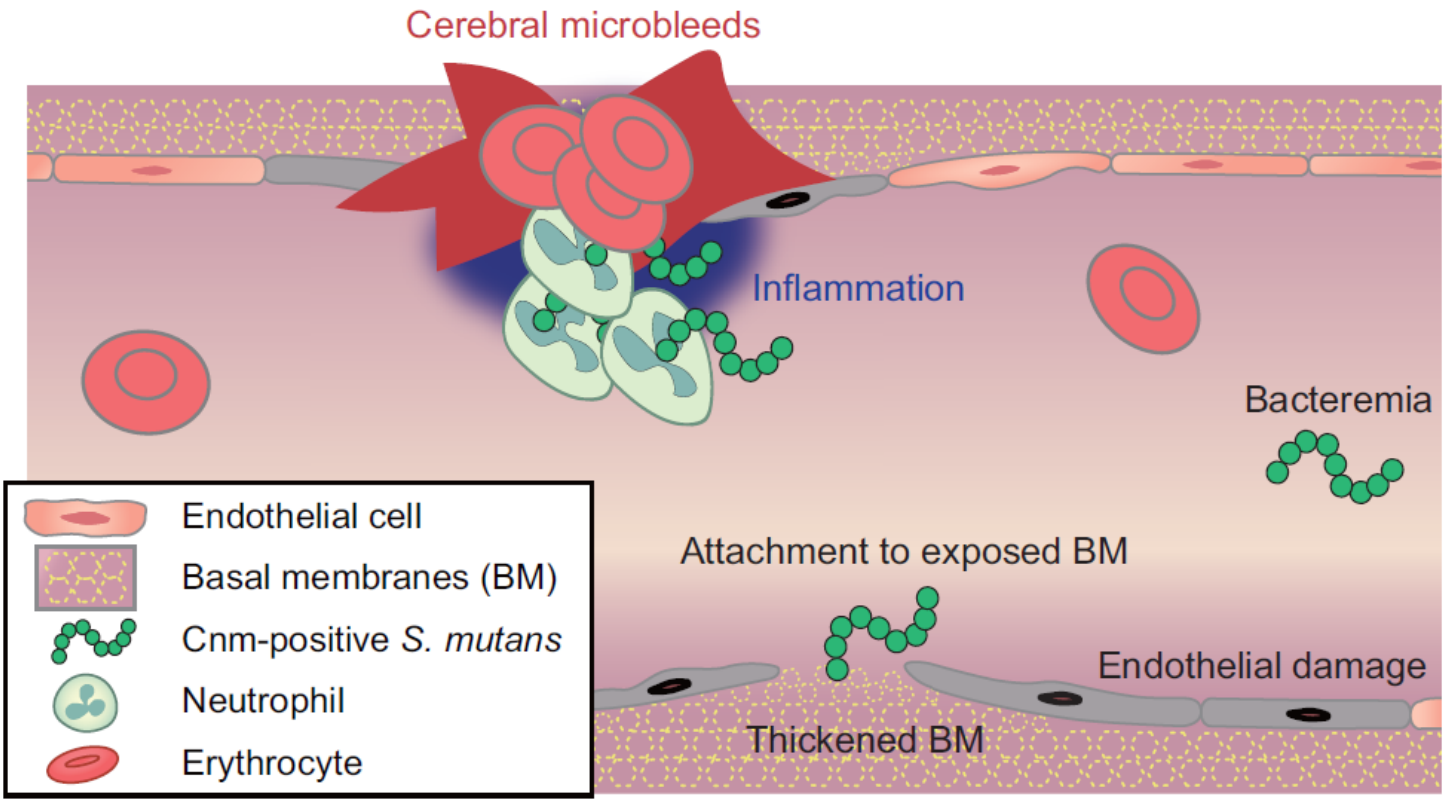

Figure 3

Hypothetical model of Cnm-induced development of cerebral microbleeds

Cerebral bleeding may occur at the level of arterioles and capillaries. Several factors including aging and hypertension result in endothelial damage and thickening of the basement membrane (BM). Cnm-positive *S. mutans* that enters the bloodstream after toothbrushing, flossing, or tooth extraction can attach to the exposed BM, where infiltration of neutrophils results in local inflammation, leading to cerebral microbleeds.

Supplementary Files

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- supple12222SI.pdf