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ORIGINAL ARTICLE

Periodontitis in ischemic stroke: impact of *Porphyromonas gingivalis* on thrombus composition and ischemic stroke outcomes

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Abstract

Background: Periodontitis is associated with an increased risk of ischemic stroke, but the mechanisms underlying this association remain unclear.

Objectives: Our objective was to determine whether *Porphyromonas gingivalis* (Pg), a periodontal bacterium, could be detected within thrombus aspirates, modify thrombus composition, and endovascular therapy responses.

Methods: The presence of Pg gingipain in 175 consecutive thrombi from patients with large vessel occlusion stroke enrolled in the multicenter research cohort compoCLOT was investigated by immunostaining. Thrombus blood cell composition according to gingipain status was analyzed in a subset of 63 patients.

Results: Pg gingipain immunostaining was positive in 33.7% of thrombi (95% CI, 26.7%-40.8%). The percentage of near to complete reperfusion (modified Thrombolysis in Cerebral Infarction Score 2c/3) at the end of the procedure was lower in the Pg^{pos} group than the Pg^{neg} group (39.0% vs 57.8% respectively; adjusted odds ratio, 0.38;

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95% CI, 0.19-0.77). At 3 months, 35.7% of patients in the Pg^{pos} group had a favorable neurological outcome vs 49.5% in the Pg^{neg} group (odds ratio, 0.65; 95% CI, 0.30-1.40). Quantitative analysis of a subset of 63 thrombi showed that neutrophil elastase content was significantly ($P < .05$) higher in Pg^{pos} thrombi than in Pg^{neg} thrombi.

Conclusion: Our results indicate that intrathrombus Pg gingipain is associated with increased neutrophil content and resistance to endovascular therapy.

KEYWORDS
endovascular therapy, ischemic stroke, neutrophils, periodontitis, *Porphyromonas gingivalis*, thrombus

Essentials

- Thrombus composition influences the success of endovascular therapy in ischemic stroke.
- The impact of periodontitis on endovascular therapy and stroke outcomes is unknown.
- This study demonstrated that *Porphyromonas gingivalis* increases neutrophil content in thrombi.
- *Porphyromonas gingivalis* makes ischemic stroke thrombi more resistant to endovascular therapy.

1 | INTRODUCTION

Despite current prevention care and recent breakthroughs in acute management, the worldwide incidence and morbidity of ischemic stroke remain on the rise [1]. This evolution is partially explained by the aging of the population but also suggests the role of neglected vascular risk factors such as chronic infections and inflammatory diseases [2]. Among them, periodontitis has been found to be associated with an increased risk of vascular events [3]. Periodontitis is a common oral infection with a high prevalence of 45% to 50% overall, with the most severe form affecting 11.2% of the world's population [4]. Several epidemiologic studies have demonstrated a link between ischemic stroke and periodontitis. It was first reported that periodontitis was independently associated with a 4.34-fold increase in the risk of ischemic stroke [5]. A recent meta-analysis found a 2.72 increased relative risk of stroke in 4 case-control studies [6]. It was shown more recently that regular dental care was associated with a relative reduction in the risk of ischemic stroke by 23%, reinforcing the strength of this association [7]. *Porphyromonas gingivalis* (Pg) is an anaerobic gram-negative bacterium of the oral cavity and is one of the main bacteria involved in the pathogenesis of periodontitis [8]. Gingival ulceration of periodontal pockets during dental activities induces bleeding [9] and provokes low-noise, transient, and repetitive bacteremia [10]. Once in circulation, periodontal bacteria can disseminate to distant sites in the body and nest within injured tissue, as observed in atherothrombotic lesions [11], endarterectomy specimens [12], thrombus aspirates from patients with myocardial infarction [13] and in human or experimental abdominal aortic aneurysm where Pg accelerated the growth of abdominal aortic aneurysm [14] due to intraluminal thrombus neutrophil content and activation. Moreover, possible physical interactions between Pg and Toll-like receptors at the surface of circulation leukocytes and platelets [15] can trigger procoagulant platelets through the activation of gingipain-activated receptors [16], and Pg has a prothrombotic effect on smooth muscle cells [17].

The aim of our study was to determine whether Pg could harm ischemic stroke outcomes by modifying the thrombus composition and endovascular therapy (EVT) responses. We report here that Pg antigen was present in 33% of thrombi and was responsible for large vessel occlusion stroke, modifying thrombus composition and affecting EVT-induced recanalization and clinical outcome.

2 | METHODS

2.1 | Clinical and biological data

Thrombi were collected at the end of EVT. The EVT procedure was chosen at the discretion of the interventional neuroradiologist, using a stent-retriever and/or a contact aspiration technique. Patient data were collected prospectively using a standardized questionnaire (Endovascular Treatment in Ischemic Stroke registry NCT03776877).

2.2 | Ischemic stroke thrombi collection and processing

We used 175 consecutive thrombi that were recovered at the end of EVT from August 2016 to November 2018 at the Rothschild Foundation Hospital and fixed in paraformaldehyde for immunohistology. Sixty-three thrombi provided sufficient material to be both fixed in paraformaldehyde and immediately frozen at −80 °C for quantitative biochemical assays (Supplementary Figure S1).

2.3 | Demographic, clinical, and radiological data

For each patient, demographic data, history (hypertension, dyslipidemia, diabetes, smoking, stroke, and coronary artery disease), antithrombotic treatment (antiplatelet agent and/or direct oral anticoagulant and/or antivitamin K), clinical data (National Institutes of Health Stroke Scale at H0 and H24, baseline modified Rankin Scale [mRS]), and presence of prior intravenous thrombolysis were recorded. The mRS at 3 months was determined by a standardized questionnaire by calling the patient according to a validated procedure [18]. Ischemic stroke etiology was determined after a complete workup including at least 48 hours of telemetry, transthoracic ultrasound, and imaging of the extracranial and aortic vessels (computed tomography [CT] angio or Doppler ultrasound) by a vascular neurologist, according to the Trial of Org 10172 in Acute Stroke Treatment classification [19]. Ischemic strokes of undetermined etiology were defined as Embolic Strokes of Undetermined Source because they fulfilled the criteria [20].

2.4 | Imaging analysis

Each patient had a baseline brain magnetic resonance imaging with diffusion-weighted imaging (DWI), fluid-attenuated inversion recovery, susceptibility weighted imaging or T2* sequences and time of flight or CT scan and CT angiography. A 24-hour follow-up imaging with a brain CT scan was used to assess the intracranial hemorrhagic complications.

The baseline Alberta Stroke Programme Early CT (ASPECT) score was calculated on the DWI sequence (DWI-ASPECTS), if available, or on the brain CT scan by a neuroradiologist blinded to the study.

The level of occlusion, the final modified Thrombolysis in Cerebral Infarction (mTICI) Score, the number of passes, and the presence of intracranial hemorrhage at H24 were determined by the neuroradiologist in charge of the patient and confirmed by an experienced neuroradiologist blinded to the study.

For each patient with a CT scan through the maxillary arch, the number of remaining teeth was collected retrospectively blinded to the Pg patient's status. Patients who had only a brain magnetic resonance imaging and patients with a complete absence of teeth (considered as denture wearers) were excluded from the analysis.

2.5 | Characterization of thrombus composition and detection of *Porphyromonas gingivalis*

The presence of Pg in thrombi aspirates was determined by immunostaining with a commercial antibody that recognizes specifically gingipain/hemagglutinin of Pg (clone 61BG1.3, Developmental Studies Hybridoma Bank). After blocking in 5% bovine serum albumin/0.1% fish gelatin/100 mM glycine buffer for 30 minutes, sections were incubated with mice anti-gingipains (4 μg/mL) and rabbit anti-myeloperoxidase (MPO, 10 μg/mL; Dako) antibodies overnight at 4 °C. Then, sections were incubated for 1 hour with goat anti-mouse A488 and goat anti-rabbit Cy5 (1:400 dilution, Thermo Fisher Scientific). Nuclei were labeled with Hoechst solution (1:1000 dilution). The acquisition was performed on an Axio Observer microscope, and pictures were analyzed with Zen software (Zeiss). Specificity controls were also performed (Supplementary Figure S2).

Thrombi were homogenized in cold phosphate-buffered saline (30 μL/mg thrombus) containing 1% protease inhibitor (Sigma-Aldrich) with stainless steel beads (5 mm, Qiagen, 69989) using a TissueLyser *II* system (25 Hz, 4 minutes, Qiagen). Incompletely ground thrombi were subjected to a second round through the TissueLyser. The supernatant of thrombus homogenates was then recovered after ultracentrifugation (14,000 g × 20 minutes, 4 °C) to remove nonsoluble debris.

Red blood cell content was assessed by hemoglobin quantification with an ELISA kit (Abcam). Neutrophils were assessed by the determination of MPO (Hycult Biotech), elastase by ELISA (Hycult Biotech), and DNA by immunofluorescence (Quant-iT PicoGreen dsDNA Assay Kit, Invitrogen). Platelet content was assessed by quantification of glycoprotein VI (GPVI) by sandwich ELISA (Human GPVI Antibody, Bio-Techne; Recombinant Human GPVI Protein, Bio-Techne; and Streptavidin SULFO-TAG labeled, Meso Scale Discovery) and P-selectin/CD62P by ELISA (Quantikine, R&D Systems). Results are expressed as the amount/mg of initial thrombus weight.

2.6 | Statistical analysis

Quantitative variables are expressed as mean (SD) or median (IQR) for nonnormal distribution. The normality of distributions was assessed graphically and using the Shapiro–Wilk test. Categorical variables were expressed as frequencies and percentages. The prevalence of Pg in thrombus was reported with binomial 95% CI. Patients were further divided into 2 study groups according to the presence or absence of Pg in the thrombus. Patient and treatment characteristics were described according to study groups, and the magnitude of the differences was assessed by calculating the standardized differences; an absolute standardized difference >20% was interpreted as meaningful differences. Patient and treatment characteristics were also described according to the availability of histological analysis to evaluate the selection bias.

Among the full study sample (N = 175), we compared the 2 study groups, angiographic outcomes (number of passes, first-pass effect, perfect recanalization, and recanalization time from groin puncture), and clinical outcomes (90-day favorable outcome) before and after adjustment for symptom onset to groin puncture delay, occlusion site, and current smoking; for clinical outcome, additional adjustment for age and ASPECTS was made. Multivariable analyses were performed using logistic regression models for binary outcomes and nonparametric covariance analyses (ANCOVA on rank-transformed values) for the 2 quantitative outcomes (number of passes and recanalization time). Effect sizes

were expressed as odds ratio (OR) for binary outcomes and standardized differences (calculated on rank-transformed data) for quantitative outcomes.

Among the 63 patients with biochemical analysis, the thrombus cell marker concentrations (hemoglobin, DNA, elastase, MPO, P-selectin, and GPVI) were compared between the 2 study groups using Mann–Whitney U-tests, and the standardized differences (calculated on rank-transformed values) were calculated as effect sizes (Pg^{pos} vs Pg^{neg}) with their 95% CIs; absolute values of 0.2, 0.5, and 0.8 were interpreted as small, medium, and large effect sizes, respectively.

Statistical testing was conducted at the 2-tailed α-level of .05. No corrections for multiple testing were performed regarding the exploratory nature of the present study, and results should be interpreted with caution. Data were analyzed using the SAS software version 9.4 (SAS Institute).

3 | RESULTS

3.1 | *Porphyromonas gingivalis* is frequent in ischemic stroke thrombi irrespective of stroke etiology

Of the 175 consecutive thrombi, 33.6% (*n* = 59; 95% CI, 26.7%-40.8 %) contained Pg antigen. Patient and treatment characteristics of the patients with ischemic stroke according to the presence or absence of Pg in retrieved thrombi are reported in Table 1. We found several between-group differences (absolute standardized difference >20%) in the main characteristics with a lower rate of hypercholesterolemia, internal carotid artery occlusion, and intravenous thrombolysis use in patients with Pg^{pos} thrombus compared with those with Pg^{neg} thrombus. Pg^{pos} thrombus was associated with a higher number of missing teeth, with a median of 5 (IQR, 3-9) compared with 3 (IQR, 2-6) in patients with Pg^{neg} thrombus. There was no difference concerning prestroke mRS, the National Institutes of Health Stroke Scale at baseline, and the ischemic stroke etiology prevalence.

3.2 | *Porphyromonas gingivalis*-positive thrombi are associated with a reduced rate of complete reperfusion

The distribution of reperfusion grade and 90-day mRS according to the presence of Pg in thrombi were analyzed. As reported in Figure 1A, patients with Pg^{pos} thrombus often had less complete reperfusion at the end of EVT (mTICI 2c or 3, 39.0% vs 57.8%, *P* = .018) and had a nonstatistically significant trend toward less often favorable outcomes (Figure 1B) at 90 days (35.7% vs 49.5%, *P* = .095). There was no difference in number of EVT passes, first-pass effect rate, or time from groin puncture to reperfusion between the 2 groups of patients (Table 2). After prespecified adjustment for confounding factors, the difference in post-EVT complete recanalization rate remained significant (adjusted OR, 0.38; 95% CI, 0.19-0.77) but not for a favorable outcome at 3 months (adjusted OR, 0.62; 95% CI, 0.29-1.34). Sensitivity analysis excluding patients with history of stroke or transient ischemic attack showed that the difference in post-EVT complete recanalization remained significant (adjusted OR, 0.44 [0.20-0.97]) (Supplementary Table S2).

3.3 | *Porphyromonas gingivalis*-positive thrombi were richer in neutrophil biomarkers

We observed that Pg^{pos} thrombi were characterized by intense MPO immunostaining (Figure 2) compared with Pg^{neg} thrombi, suggesting a higher neutrophil content in Pg^{pos} thrombi. To confirm this result, we analyzed the thrombus composition in a subgroup of 63 patients to quantitatively assess their red blood cell (RBC), platelet, and neutrophil content.

In this subset of patients, Pg was detected in 20 thrombi, corresponding to a prevalence of 31.8% (95% CI, 20.2%-43.3%), which was similar to that of the global study analysis. However, as shown in Supplementary Table S1, the global study group (*N* = 175) and the subgroup (*n* = 63) were not well balanced regarding the patient and treatment characteristics, with a difference in prestroke mRS (≥1, 12.7% vs 33.6%), the occlusion site (internal carotid artery, 55.6% vs 21.1%), and the use of intravenous thrombolysis (61.9% vs 42.0%).

The concentrations of cell markers of retrieved thrombi according to the presence or absence of Pg are reported in Table 3. Pg^{pos} thrombi contained a significantly higher amount of neutrophil elastase compared with Pg^{neg} thrombi (Pg^{pos}: median, 180; IQR, 124-304 vs Pg^{neg}: median, 129; IQR, 86-195) with a medium effect size (standardized mean difference, 0.54; 95% CI, 0.001-1.08). The concentration of MPO was also increased in Pg^{pos} thrombi, but the difference was not significant. The concentration of GPVI tended to be increased in Pg^{pos} thrombi, whereas P-selectin concentration was similar between the 2 groups. RBC content, assessed by measurement of hemoglobin, was not affected by the presence of Pg within the thrombus.

4 | DISCUSSION

In this study, we explored the prevalence of Pg within ischemic stroke thrombi and its relationships with response to endovascular treatment and ischemic stroke outcome. To our knowledge, this is the first study assessing Pg by specific immunostaining in the composition of thrombi and its consequence on post-EVT recanalization rate and 3-month clinical outcome of patients with ischemic stroke.

We show that 33% of thrombi causing ischemic stroke were positive for Pg gingipains detected by immunostaining analysis, independently of the ischemic stroke etiology. Our results are in contrast to those of Patrakka and al. [21], who showed that thrombus aspirates from patients with ischemic stroke were negative for Pg and *Aggregatibacter actinomycetemcomitans*, another periodontal bacterium but positive for *Streptococcus* species. Nevertheless, our data are in agreement with previous studies performed on tissues from other

TABLE 1 Patient characteristics according to *Porphyromonas gingivalis* status.

Characteristics	Pg status		Standardized
	Pg^{neg} (n = 116)	Pg^{pos} (n = 59)	difference (95% CI)
Demographics			
Age, years	69 (55-81)	75 (55-83)	0.12 (−0.20 to 0.44)
Men	58/116 (50.0)	33/59 (55.9)	0.12 (−0.20 to 0.44)
Medical history			
Hypertension	63/114 (55.3)	38/59 (64.4)	0.19 (−0.14 to 0.51)
Diabetes mellitus	24/114 (21.1)	10/59 (16.9)	−0.10 (−0.42 to 0.21)
Hypercholesterolemia	35/114 (30.7)	11/59 (18.6)	−0.28 (−0.59 to 0.02)
Current smoking	15/114 (13.2)	12/59 (20.3)	0.19 (−0.13 to 0.52)
Coronary artery disease	17/112 (15.2)	10/59 (16.9)	0.05 (−0.27 to 0.37)
Previous stroke or TIA	20/113 (17.7)	10/59 (16.9)	−0.02 (−0.34 to 0.30)
Antithrombotic medications	46/113 (40.7)	29/58 (50.0)	0.19 (−0.13 to 0.51)
Number of missing teeth[a]	3 (2-6)	5 (3-9)	0.38 (−0.002 to 0.77)
Prestroke mRS > 1	18/114 (15.8)	6/59 (10.2)	0.19 (−0.13 to 0.51)
Current stroke event			
NIHSS score	17 (12-21)	19 (11-22)	0.14 (−0.18 to 0.46)
Baseline magnetic resonance imaging	98/116 (84.5)	45/58 (77.6)	0.18 (−0.15 to 0.50)
ASPECT score[b]	7 (5-8)	7 (5-9)	0.13 (−0.20 to 0.46)
Occlusion site			
Isolated MCA	64/116 (55.2)	37/59 (62.7)	0.45 (0.13-0.77)
Intracranial and/or extracranial ICA	48/116 (41.4)	14/59 (23.7)	
Vertebrobasilar	4/116 (3.4)	8/59 (13.6)	
Thrombus weight[c], mg	27 (13-69)	35 (17-62)	0.12 (−0.41 to 0.66)
Intravenous rtPA therapy	65/116 (56.0)	21/59 (35.6)	−0.42 (−0.72 to −0.12)
Symptom onset to imaging[d], min	120 (90-159)	122 (92-180)	0.12 (−0.20 to 0.44)
Symptom onset to groin puncture[d], min	265 (201-312)	233 (190-327)	−0.07 (−0.39 to 0.25)
Etiology[e]			
Cardioembolic	62/116 (53.4)	31/59 (52.5)	−0.04 (−0.35 to 0.28)
ESUS	29/116 (25.0)	19/59 (32.2)	
Large-artery atherosclerosis	16/116 (13.8)	7/59 (11.9)	
Stroke of other determined etiology	7/116 (6.0)	2/59 (3.4)	

Values are expressed as n (%) or median (IQR).

ASPECT, Alberta Stroke Programme Early CT; ESUS, embolic strokes of undetermined source; ICA, internal carotid artery; MCA, middle cerebral artery; mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale; rtPA, recombinant tissue plasminogen activator; TIA, transient ischemic attack.

[a]Fifty-eight missing values (17 in Pg^{pos}).

[b]Nine missing values (4 in Pg^{pos}).

[c]Data available in 63 patients (20 in Pg^{pos}) with histological analysis of retrieval thrombi.

[d]One missing value (1 in Pg^{pos}).

[e]Two missing values in Pg^{neg}.

cardiovascular complications of atherosclerotic lesions. Periodontal bacterial DNA, in particular from Pg, was detected in thrombus aspirates from acute myocardial infarction [13,22]. Indeed, during periodontitis, bacteria can translocate into blood circulation to provoke recurrent silent and transient bacteremia [23] and finally colonize atherosclerosis lesions where they could participate in the plaque

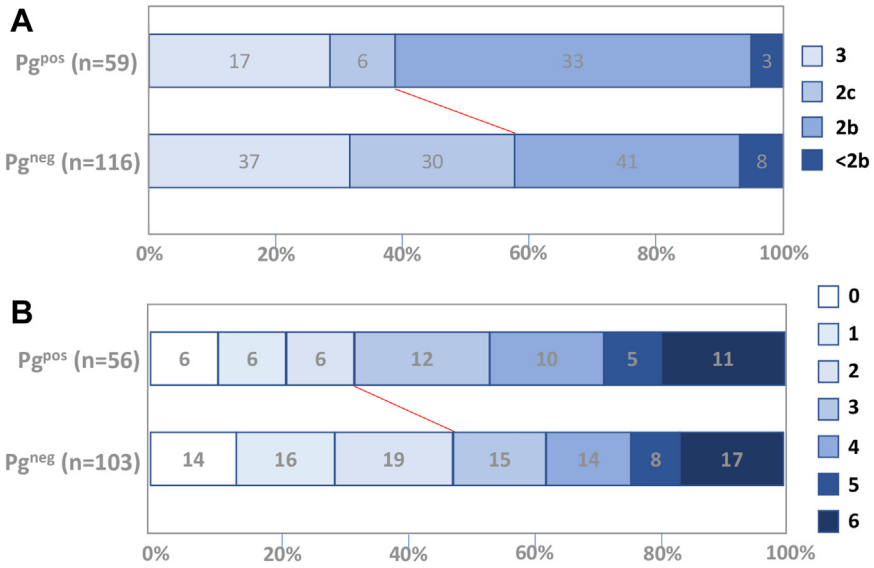

FIGURE 1 Impact of *Porphyromonas gingivalis* (Pg) status on recanalization and stroke outcome. (A) Distribution of recanalization grade in percentage. The bar indicates the separation between excellent (modified Thrombolysis in Cerebral Infarction Score 3 and 2c) and poor (modified Thrombolysis in Cerebral Infarction Score ≤ 2b) recanalization grades. (B) Distribution of 90-day modified Rankin Scale (mRS) in percentage. The bar indicates the separation between favorable (mRS 0 to 2) and poor (mRS ≥ 3) outcomes.

instability and rupture [24,25] by promoting platelet activation and thrombosis [17,26] triggering thrombus formation, which leads to artery occlusion. It is likely that differences in the methodology used for the assessment of periodontitis and the detection of Pg (ie, bacterial DNA vs antigen) have contributed to the discrepancies between studies on the association of periodontitis and stroke.

We also observed that in Pg^{pos} thrombi, gingipain staining largely colocalized with MPO, suggesting that Pg could be phagocytosed or trapped by circulating neutrophils inducing their activation. Once in circulation, Pg can induce thromboinflammation and can be phagocytosed by neutrophils or exhibit neutrophil extracellular trap formation [27], which, in turn, could promote thrombus formation. Interestingly, Pg was independently found in cardioembolic and non-cardioembolic thrombi, reinforcing the idea that Pg could be involved in thrombus formation.

Next, we evaluated whether the presence of Pg was associated with EVT recanalization success and clinical outcome. The detection of Pg was associated with less post-EVT complete reperfusion, as indicated by a significant difference in mTICI score between the 2 groups. The presence of Pg was also associated with a trend toward less often favorable outcomes at 3 months. Numerous previous works have reported a strong association between thrombus composition and per-procedural and clinical outcomes [28]. Here, we observed differences in thrombus composition according to the Pg status. Indeed, Pg^{pos} thrombi were enriched in neutrophil elastase and MPO, with no difference in platelets and RBCs. Yet, it is well shown that achieving complete recanalization was associated with better clinical outcomes than successful but incomplete recanalization [29].

Interestingly, previous studies have shown that thrombus neutrophil content negatively impacts the realization and clinical

TABLE 2 Endovascular therapy procedure outcomes and 3-month clinical outcome according to *Porphyromonas gingivalis* status.

	Pg status		95% CI	
Outcomes	**Pg^{neg} (*n* = 116)**	**Pg^{pos} (*n* = 59)**	**Unadjusted effect size**	**Adjusted effect size[b]**
Number of passes	2 (1-3)	1 (1-3)	−0.15 (−0.46 to 0.17)	−0.003 (−0.30 to 0.29)[c]
FPE	33/116 (28.5)	14/59 (23.7)	0.78 (0.38-1.61)	0.56 (0.25-1.22)[d]
mTICI 2c/3	67/116 (57.8)	23/59 (39.0)	0.47 (0.24-0.89)	0.40 (0.20-0.80)[d]
Groin puncture to rec (min)	43 (29-63)	40 (26-60)	−0.15 (−0.46 to 0.17)	−0.10 (−0.40 to 0.20)[c]
Favorable outcome[a]	51/103 (49.5)	20/56 (35.7)	0.57 (0.29-1.11)	0.65 (0.30-1.40)[d]

Values are expressed as median (IQR).

FPE, first-pass effect; mRS, modified Rankin Scale; mTICI, modified Thrombolysis in Cerebral Infarction Score; Pg, *Porphyromonas gingivalis*; Rec, recanalization.

[a]Ninety-day mRS 0-2 or equal to prestroke mRS.

[b]Adjusted for symptom onset to groin puncture, current smoking, and occlusion site for angiographic outcomes and symptom onset to groin puncture, occlusion site, age, current smoking, and ASPECT (Alberta Stroke Programme Early CT) score for favorable outcome.

[c]Standardized differences calculated on rank-transformed data.

[d]Odds ratios.

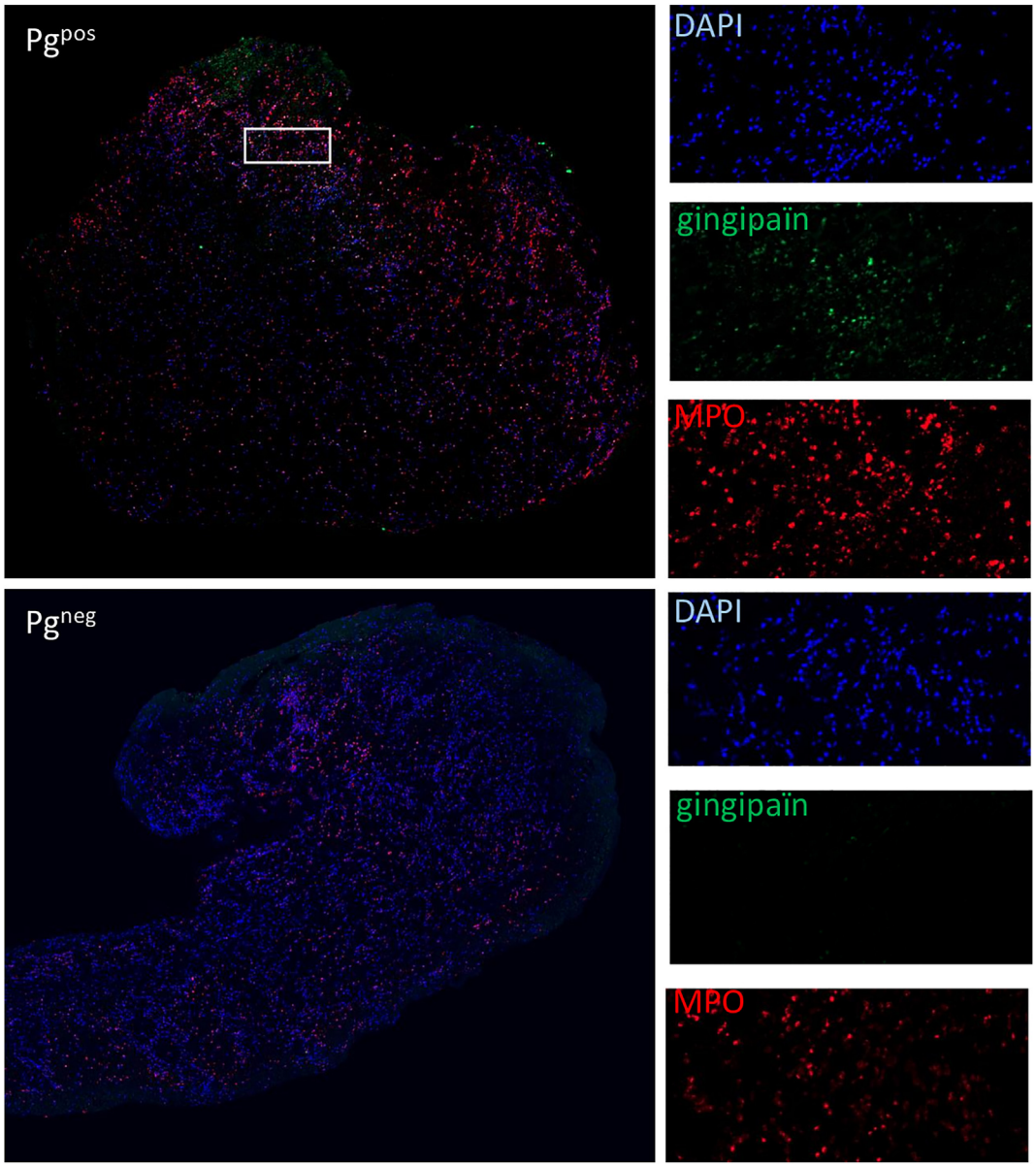

FIGURE 2 Detection of *Porphyromonas gingivalis* (Pg) within ischemic stroke thrombi. Thrombus samples obtained after mechanical thrombectomy were used for immunohistochemistry. In a representative image of Pg^{neg} and Pg^{pos} thrombi, Pg (green) was detected with a mouse anti-gingipain, and neutrophils (red) were detected using a human anti-myeloperoxidase (MPO). Nuclei (blue) were stained with DAPI. DAPI, 4',-6 ddiamino-2-phenylindole.

benefits of mechanical thrombectomy in ischemic stroke (diverse thrombus composition in patients with thrombectomy stroke with longer time to recanalization) [30,31]. Furthermore, periodontitis provokes changes in the circulation of white blood cell count with high neutrophil and low lymphocyte levels [32], suggesting an increase of the neutrophil to lymphocyte ratio, a parameter whose elevation is linked with reduced efficacy of reperfusion therapy in ischemic stroke [33,34]. Thus, in line with these studies, our results suggest that neutrophils may, at least in part, mediate the deleterious effects of periodontitis on response to recanalization therapy and stroke outcome.

Prevalence of periodontitis in the general population, independently of the causal bacteria, has been estimated at 57.3% and 70.1% of 50-64-year-olds and >65-year-olds, respectively [32]. More specifically, in a prospective cohort study, 34.3% of the subjects had positive anti-Pg immunoglobulin A antibodies at baseline [8], and in a study of consecutive patients with ischemic stroke, 37.7% had high-level periodontal disease assessed by dental examination [35]. The prevalence of Pg^{pos} thrombus found in our study is consistent with those results. Moreover, patients of the Pg^{pos} thrombus group had increased tooth loss compared with the Pg^{neg} thrombus patients, suggesting that the presence of Pg is indeed related to the presence of periodontitis.

TABLE 3 Cell marker content of retrieval thrombus according to *Porphyromonas gingivalis* status.

	Pg status (ng/mg)[a]		
	Pg^{neg} (n = 43), median (IQR)	Pg^{pos} (n = 20), median (IQR)	Standardized difference (95% CI)
Hemoglobin[b] (μg/mg)	246 (112-317)	262 (228-342)	0.31 (−0.26 to 0.88)
DNA	35 (12-113)	46 (12-124)	0.11 (−0.43 to 0.64)
Elastase	129 (86-195)	180 (124-304)	0.54 (0.001-1.08)
MPO	34 (13-66)	51 (20-94)	0.38 (−0.16 to 0.92)
Pselectin[c]	2.9 (1.9-6.0)	2.9 (2.3-3.7)	−0.13 (−0.67 to 0.40)
GPVI[d]	185 (129-248)	234 (181-260)	0.31 (−0.26 to 0.88)

Results are expressed as the amount/mg of initial thrombus weight. Standardized differences were calculated on rank-transformed values.
DNA, deoxyribonucleic acid; GPVI, glycoprotein VI; MPO, myeloperoxidase; Pg, *Porphyromonas gingivalis*.
[a]Unless otherwise specified.
[b]Seven missing values (2 in Pg^{pos}).
[c]One missing value in Pg^{neg}.
[d]Six missing values (3 in Pg^{pos}).

Ethnicity and socio-demographic conditions are major determinants of oral health [36] and stroke [37], and it has been demonstrated that individuals with low socio-cultural levels or belonging to a specific ethnic group have an increased risk of having an ischemic stroke or developing periodontitis. However, due to ethical considerations, these data were not routinely collected during the recruitment of patients in our stroke unit. The absence of these data may represent a limitation to our results, and future investigations considering ethnicity and demographic conditions should be necessary to improve the interpretation of our findings. Another limitation of our study is the number of patients enrolled and, notably, the number of thrombi for which composition was assessed. We focused our attention on Pg since it is the main pathogen involved in human periodontitis; however, we cannot exclude that other periodontal pathogens might be detected in ischemic stroke thrombus as previously demonstrated in atherosclerotic plaques [11] from carotid endarterectomy and may participate to unfavorable ischemic stroke outcome in patients with periodontitis. To our knowledge, specific antibodies that recognize antigens from other periodontal bacteria are not commercially available. To override this limitation, future research will assess the presence of periodontal bacteria by molecular biology using the nested polymerase chain reaction technique and primers for these periodontitis bacteria.

It is now recognized that periodontitis is associated with an increased risk of ischemic stroke [38]. The main mechanisms discussed are indirect damage to vascular function via the production of proinflammatory mediators and, more rarely, direct damage by infectious agents [39]. We illustrate here, through the example of Pg, the hypothesis that infectious agents might affect thrombus composition regardless of the underlying associated etiology; however, more data are needed to conclude. We demonstrated that intrathrombus Pg gingipain is associated with increased neutrophil content and resistance to EVT, which may explain the worsened outcome in Pg^{pos} patient group. In conclusion, our results illustrate a possible mechanism by which periodontitis and periodontal pathogens may be linked to ischemic stroke, both through the presence of bacteria within thrombi and through quantitative changes in thrombi. These results support and provide a new argument for considering periodontitis as an independent risk factor for ischemic stroke, which has been reported several times. Furthermore, they support the idea that targeted management of periodontitis could have an impact on the recurrence and severity of ischemic stroke, which remains to be demonstrated by other studies.

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ETHICS STATEMENT

The local Ethics Committee approved this research protocol (Comité de Protection des Personnes Nord Ouest II, ID-RCB number: 2017-A01039-44).

INFORMED PATIENT CONSENT

Informed consent was provided by all patients. Patients were provided with a written explanation of the study, and the patients or their representatives were given the opportunity to refuse participation. This trial was registered at clinicaltrials.gov as NCT03268668.

AUTHOR CONTRIBUTIONS

A.F.V.S., S.D., J.-P.D., and B.H.-T.-N. designed the study and collected and interpreted the data. A.F.V.S., S.D., J.-P.D., and B.H.-T.-N. wrote the manuscript. S.D., M.S.N., V.O., and S.D. performed all the biological manipulations. J.L. performed the statistical analyses. All authors collected data, reviewed and edited the manuscript, and approved the final version of the manuscript.

RELATIONSHIP DISCLOSURE

There are no competing interests to disclose.

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SUPPLEMENTARY MATERIAL

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