

Disequilibrium of Plasma Protease/Anti-Protease Due to Severe Periodontal Disease Contributes to Human Subarachnoid Hemorrhage

Shotaro Yoshioka, MD,* Takeshi Miyamoto, MD, PhD,* Junichiro Satomi, MD, PhD,* Yoshiteru Tada, MD, PhD,* Kenji Yagi, MD, PhD,* Kenji Shimada, MD, PhD,* Koji Naruishi, DS, PhD,† Eiji Shikata, MD,* Izumi Yamaguchi, MD,* Tadashi Yamaguchi, MD,* Masaaki Korai, MD, PhD,* Yoshihiro Okayama, ME,§ Masafumi Harada, MD, PhD,¶ Keiko T. Kitazato, BS,* Yasuhisa Kanematsu, MD, PhD,* Shinji Nagahiro, MD, PhD,* Yasushi Takagi, MD, PhD*

*Department of Neurosurgery, Graduate School of Biomedical Sciences, Tokushima University, Tokushima, Japan; †Department of Periodontology and Endodontology, Graduate School of Biomedical Sciences, Tokushima University, Tokushima, Japan; §Clinical Trial Center, Tokushima University and Tokushima University Hospital, Tokushima, Japan; ¶Department of Radiology, Graduate School of Biomedical Sciences, Tokushima University, Tokushima, Japan

Correspondence: Takeshi Miyamoto, MD, PhD, Department of Neurosurgery, Graduate School of Biomedical Sciences, Tokushima University, 3-18-15, Kuramoto-cho, Tokushima, 770-8503 Japan. Email: takeshi_edit@yahoo.co.jp

Received, December 19, 2019; **Accepted,** May 31, 2020

Copyright © The Author(s) 2020. Published by Oxford University Press on behalf of the Congress of Neurological Surgeons. This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

BACKGROUND: The pathophysiology of subarachnoid hemorrhages (SAHs) due to ruptured intracranial aneurysms (IAs) remains unclear. Although a relationship between SAHs and periodontal disease (PD) has been suggested, the mechanism requires clarification.

OBJECTIVE: To evaluate the relationship between PD and SAHs and to identify periodontal pathogens associated with SAHs.

METHODS: This prospective study included consecutive patients with ruptured ($n = 11$) and unruptured ($n = 14$) IAs and healthy controls ($n = 8$). The plasma and plaque subgingival bacterial deoxyribonucleic acid (DNA) levels in PD were evaluated by a dentist using the Community Periodontal Index of Treatment Needs (CPITN). Plasma levels of matrix metalloproteinase (MMP-9), tissue inhibitors of matrix metalloproteinase (TIMP2), and procollagen I were analyzed.

RESULTS: Patients with ruptured IAs, had significantly higher CPITN scores than the controls, suggesting that ruptured IAs were associated with severe PD. Although no rupture-specific bacteria were identified, the positive rate of plaque subgingival bacterial DNA was significantly higher in patients with severe PD than in those without severe PD. Multivariate logistic regression analysis indicated that bleeding on probing (BOP) was associated with ruptured IAs (odds ratio, 1.10; 95% confidence interval 1.04–1.20; $P = .0001$). BOP was positively associated with plasma MMP-9 levels and a disequilibrium in the MMP-9/TIMP2 ratio. BOP was negatively correlated with plasma procollagen I levels ($P < .05$, for each). This suggested that local inflammation with severe PD might have systemic effects and lead to ruptured IAs.

CONCLUSION: Disequilibrium of plasma protease/anti-protease associated with a high BOP rate in severe PD may be attributable to IA rupture.

KEY WORDS: Gingival bleeding, Intracranial aneurysm, Matrix metalloproteinase 9, Periodontal disease, Procollagen type I, Subarachnoid hemorrhage, Tissue inhibitor of metalloproteinase 2

Neurosurgery Open 1:1–9, 2020

DOI: 10.1093/neuopn/okaa007

www.neurosurgeryopen-online.com

The pathophysiology of subarachnoid hemorrhages (SAHs) due to ruptured intracranial aneurysms (IAs) remains unclear.¹ As endovascular coiling or clipping involves non-negligible risks,² the pharmacological management of IAs is preferred. Therefore, a better understanding of the

detailed mechanisms of aneurysmal growth and rupture is required.

Periodontal disease (PD), including periodontitis and gingivitis, may play a role in the pathophysiology of diabetes mellitus, atherosclerosis, and ischemic stroke.^{3,4} Periodontal

gram-negative bacteria and their products could elicit inflammatory responses in periodontal tissues and cause systemic sequelae.³ Periodontitis, including tooth loss, is associated with elevated levels of C-reactive protein and baseline characteristics including stroke, diabetes, hypertension, coronary heart disease, income, and education.⁴ Periodontal pathogens have been identified in aortic aneurysms,⁵⁻⁷ carotid plaques,⁸ and IAs,^{9,10} suggesting that PD plays a role in their pathophysiology. PD severity is associated with cerebral ischemia.¹¹ A relationship between periodontitis, gingival bleeding, and IAs has been reported.¹² However, this association and the deterioration of intracranial arteries leading to IA rupture remains unclear. Furthermore, the link between periodontal bacteria and their pathological effects on organs and vessels, whether periodontal bacteria are directly or indirectly associated with IAs, and which periodontal bacteria affect intracranial arteries remain to be established.

Using the rat IA model,¹³⁻¹⁷ we previously demonstrated the relationships between inflammation and IAs and found that the inflammation-related interleukin (IL-1beta) and IL-6 were elevated with macrophage migration in the walls of intracranial vessels that are prone to rupture. Furthermore, we found that matrix metalloproteinase (MMP)-9 and an imbalance in the MMP-9/tissue inhibitor of matrix metalloproteinase (TIMP2) ratio plays a crucial role in the formation and rupture of IAs.

Hence, in this study, we focused on inflammation-related molecules. We speculated that a local inflammatory response mediated by subgingival bacteria leads to PD and that severe PD may be a risk factor for ruptured aneurysms.

We aimed to evaluate the relationship between severity of PD and IAs and to determine which periodontal pathogens and inflammation-related biomarkers are associated with these conditions.

METHODS

This prospective study included consecutive patients with ruptured ($n = 11$) and unruptured ($n = 14$) IAs and healthy controls ($n = 8$). The plasma and plaque subgingival bacterial deoxyribonucleic acid (DNA) levels in PD were evaluated by a dentist (see **Supplemental Methods**, for further details).

ABBREVIATIONS: **AUC**, area under the curve; **BOP**, bleeding on probing; **CPITN**, Community Periodontal Index of Treatment Needs; **DNA**, deoxyribonucleic acid; **IAs**, intracranial aneurysms; **MMP**, matrix metalloproteinase; **NET**, neutrophil extracellular trap; **PD**, periodontal disease; **ROC**, receiver operating characteristic; **SAH**, subarachnoid hemorrhage; **TIMP**, tissue inhibitors of matrix metalloproteinase; **TLR**, toll-like receptor

Supplemental digital content is available for this article at www.neurosurgeryopen-online.com.

RESULTS

Characteristics of Patients and Aneurysms

As shown in Table 1, no significant difference in age, sex, comorbidities, drinking and smoking habits, or diabetes mellitus was noted between the study subjects and controls. Moreover, there was no significant difference in the size, location, or number of aneurysms between the patients with ruptured or unruptured IAs. However, subjects having, both, hypertension and a smoking habit were significantly higher in the ruptured- than unruptured patients or control subjects (**Supplemental Figure 1A**).

Relationships Between Ruptured IAs, Severe PD, and Risk Factors

To assess the association between IAs and PD, the subgingival conditions were assessed by a dentist in all groups. As shown in Figure 1A, the number of missing teeth (mean \pm SD, 13.4 ± 11.4 vs 1.9 ± 2.5) and the number of patients with mobile teeth (16.1 ± 12.5 vs $1.0 \pm 1.9\%$) were significantly greater in the ruptured IA patients than in the controls, as was the rate of patients with deep periodontal pockets (>6 mm, 45.5 ± 32.1 vs $8.3 \pm 14.0\%$) and bleeding on probing (BOP) (42.6 ± 21.5 vs $19.2 \pm 13.5\%$) (all $P < .05$; Figure 1A). Moreover, patients with ruptured IAs had a higher rate of bleeding than patients with unruptured IAs (42.6 ± 21.5 vs $19.2 \pm 14.8\%$, $P < .05$).

Subsequently, we classified the severity of PD based on the Community Periodontal Index of Treatment Needs (CPITN) code (0, healthy; 1, bleeding; 2, calculus deposition; 3, shallow; 4, deep periodontal pocket) (Figure 1B). We added "edentulous" as the most severe grade of PD because advanced tooth loss is an indicator of past oral inflammation, such as severe periodontitis.¹⁸ Of the 8 controls, 4 had shallow periodontal pockets (CPITN 3); however, none had pockets that were 6 mm or deeper (CPITN 4) or an edentulous mandible. A total 3 of 14 (21%) patients with unruptured IAs and 4 of 11 (36%) with ruptured IAs had deep periodontal pockets or an edentulous mandible, suggesting a relationship between ruptured IAs and severe PD.

To further clarify the relationships between the IAs and the severity of PD, all subjects including the control group were classified into 3 groups based on the CPITN score: slight, moderate, and severe. The rate of severe PD in the ruptured patients was significantly higher than in the unruptured patients (Figure 1B, $P < .05$). The rate of severe PD was significantly higher in patients with a smoking habit and either with or without hypertension (Table 2 and **Supplemental Figure 1B**, $P < .05$), suggesting a relationship between severe PD and smoking.

Severe PD Associated With High Content of Subgingival Bacteria but no Rupture-Specific Pathogen

Analysis of the plasma IgG titer against four species of periodontal bacteria showed that at least one type of bacterial

TABLE 1. Characteristics of Control Subjects and Patients With Unruptured- and Ruptured IAs

	Control (n = 8)	Unruptured (n = 14)	Ruptured (n = 11)	P value
Age (mean ± SD, yr)	58.4 ± 7.9	66.0 ± 7.7	64.5 ± 9.2	NS
Female sex, n (%)	6 (75)	12 (86)	5 (45)	NS
Hypertension, n (%)	2 (25)	8 (57)	6 (55)	NS
Diabetes mellitus, n (%)	1 (13)	1 (7)	2 (18)	NS
Dyslipidemia, n (%)	1 (13)	4 (29)	2 (18)	NS
Drinking, n (%)	4 (50)	3 (21)	5 (45)	NS
Smoking, n (%)	1 (13)	5 (36)	5 (45)	NS
Aneurysm				
Largest dimension (mean ± SD, mm)		7.1 ± 2.6	6.0 ± 2.3	NS
Distribution, n (%)				
≥ 5 mm		8 (57)	3 (27)	NS
Location, n (%)				
Middle cerebral artery		4 (29)	3 (27)	
Anterior communicating artery		2 (14)	5 (45)	
Internal carotid-posterior communicating artery		4 (29)	2 (18)	NS
Other		4 (29)	1 (9)	
Multiple aneurysm, n (%)		4 (29)	2 (18)	NS

Abbreviation: NS, no significant difference. P value by Student's t-test for largest dimension or by Pearson's χ^2 test. "Other" indicates aneurysms at the internal carotid-ophthalmic artery, distal posterior inferior cerebellar artery, and other supratentorial locations not categorized above.

IgG was present in 62.5% of the controls, in 78.6% of patients with unruptured IAs, and in 87.5% of those with ruptured IAs (Figure 1C). Thus, the positive rate of some bacteria IgG tended to increase in ruptured IAs; however, no significant difference was observed. Although the periodontal bacteria investigated in this study are assumed to play an important role in the pathogenesis of PD,¹⁹ IgG antibodies against *P. gingivalis* were detected in almost 50% of the 3 groups, and we identified no specific bacteria associated with the pathogenesis of IAs.

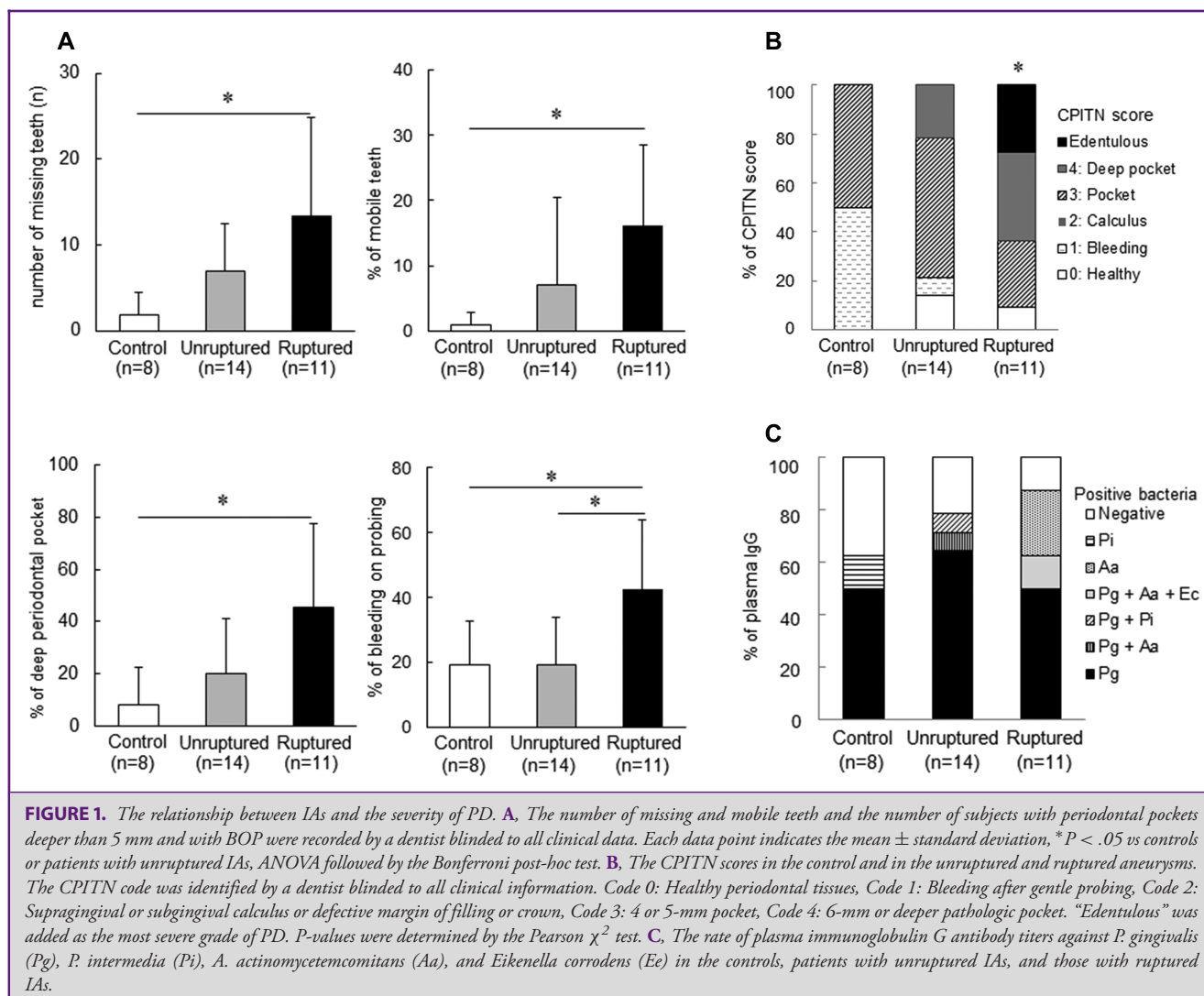
Conversely, DNA of *P. gingivalis* and *T. denticola* bacteria, which commonly cohabit in the deep sites of periodontal pockets, are considered to be associated with PD.³ We recorded the number of individuals with positive periodontal bacterial DNA in subgingival plaques. All pathogens, except *A. actinomycetem-comitans*, were detected in all groups. Although no rupture-specific pathogen in oral plaques could be identified (data not shown), the positive rates of DNA for *P. gingivalis* and *T. denticola* bacteria were significantly higher in the severe PD group than in the slight PD group (Table 2), indicating a relationship between severe PD and high content of subgingival bacteria in plaque.

Plasma Biomarkers and IA Rupture Associated with BOP in Severe PD

Based on previous findings that MMP-9 and an imbalance of the MMP/TIMP-2 ratio were elevated in vascular walls that are prone to rupture in IA model rats,¹⁵⁻¹⁷ we examined the relationships between types of IAs and plasma biomarkers in human. The elevation of plasma level of MMP-9 and an imbalance of the MMP/TIMP-2 ratio and the reduction of collagen I level were observed in the ruptured patients (Supplemental Figure 2).

In the classification of severity of PD, the rate of ruptured IAs was significantly higher in the severe PD than in other PD groups (Figure 2A). We examined the relationships between the ruptured IAs and the severe PD by multivariate logistic regression analysis with stepwise method for factors including age, sex, hypertension, diabetes, dyslipidemia, moving teeth, missing teeth, PD pocket and BOP. BOP was an independent factor associated with the rupture of IAs (odds ratio, 1.10; 95% confidence interval 1.04–1.20; $P = .0001$).

The plasma level of MMP-9 and the disequilibrium of MMP-9/TIMP-2 were positively correlated with BOP (Figure 2B). Procollagen I as targets of MMP-9²⁰ were negatively



correlated with BOP, but no correlation was found with procollagen III (data not shown). Based on the receiver operating characteristic (ROC) curve, we found the optimal cut-off point of BOP for ruptured IAs ($\leq 20\%$; area under the curve [AUC] = 0.886; sensitivity = 1.00; specificity = 0.73).

No ruptured IAs were observed in the BOP $\leq 20\%$ group, while 90% of the BOP $\geq 40\%$ group had ruptured IAs (Figure 3A). Furthermore, in the BOP $\geq 40\%$ group, the plasma MMP-9 level and the MMP-9/TIMP-2 ratios were significantly higher than that in the other groups, and the level of plasma procollagen I was significantly decreased (Figure 3B). Taken together, BOP rate was associated with ruptured IAs and higher MMP-9 levels and MMP-9/TIMP-2 ratios and lower procollagen I levels.

DISCUSSION

To our knowledge, this is the first study demonstrating that ruptured IAs were associated with severe PD, especially with a high BOP rate. Importantly, we found that a high BOP rate in severe PD was correlated with an increase in plasma MMP-9 levels, disequilibrium between MMP-9 and TIMP-2, and decreased plasma procollagen I levels. Unfortunately, we could not identify the rupture-specific periodontal bacteria in plasma. There was no significant difference in the positive rate of some gingival bacterial DNA in plasma, while positive cases of bacterial DNA in subgingival plaques were significantly elevated in correlation with the severity of PD. These findings suggest that local inflammation mediated by severe PD may affect the ruptured IAs, which were mirrored by the changes of plasma biomarkers. Severe

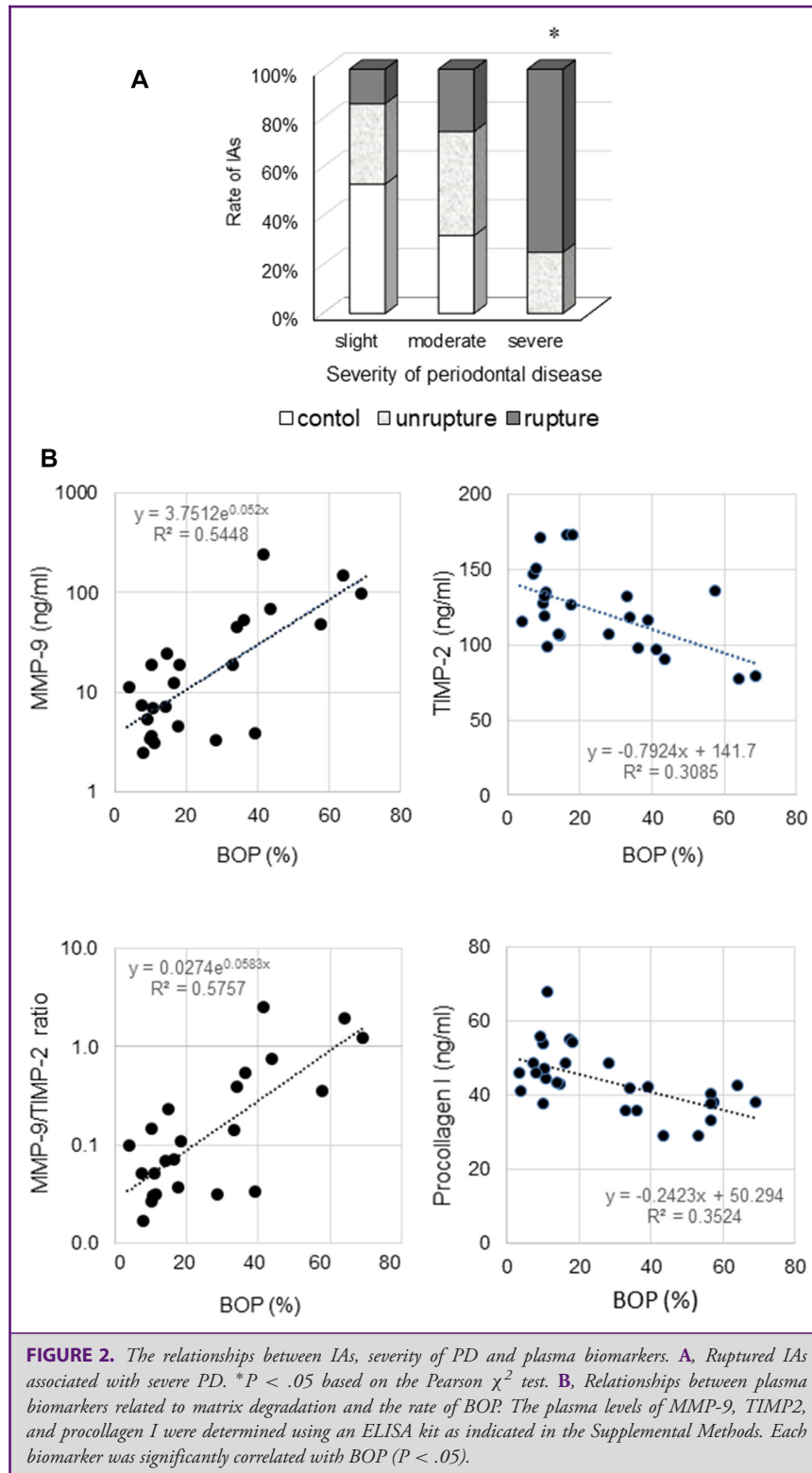


TABLE 2. Characteristics of all Subjects Based on the Severity of PD

	Slight (n = 18)	Moderate (n = 7)	Severe (n = 8)	P-value
Age (mean ± SD), yr	60.7 ± 8.9	67.4 ± 7.7	66.9 ± 6.7	NS
Female sex, n (%)	15 (83)	5 (71)	3 (38)	NS
Hypertension, n (%)	5 (28)	6 (86)	5 (63)	<.05
Diabetes mellitus, n (%)	1 (6)	2 (29)	1 (13)	NS
Dyslipidemia, n (%)	2 (11)	3 (43)	2 (25)	NS
Drinking, n (%)	5 (28)	2 (29)	5 (63)	NS
Smoking, n (%)	3 (17)	2 (29)	6 (75)	<.05
Positive cases of PD bacteria DNA in subgingival plaque				
<i>Porphyromonas gingivalis</i> ; Pg	11	57	100	< .05
<i>Treponema denticola</i> ; Td	33	29	100	< .05
<i>Prevotella intermedia</i> ; Pi	28	29	75	NS
<i>Tannerella forsythia</i> ; Tf	83	100	100	NS
<i>Fusobacterium nucleatum</i> ; Fn	94	100	100	NS
<i>Aggregatibacter actinomycetemcomitans</i> ; Aa	0	0	0	NS

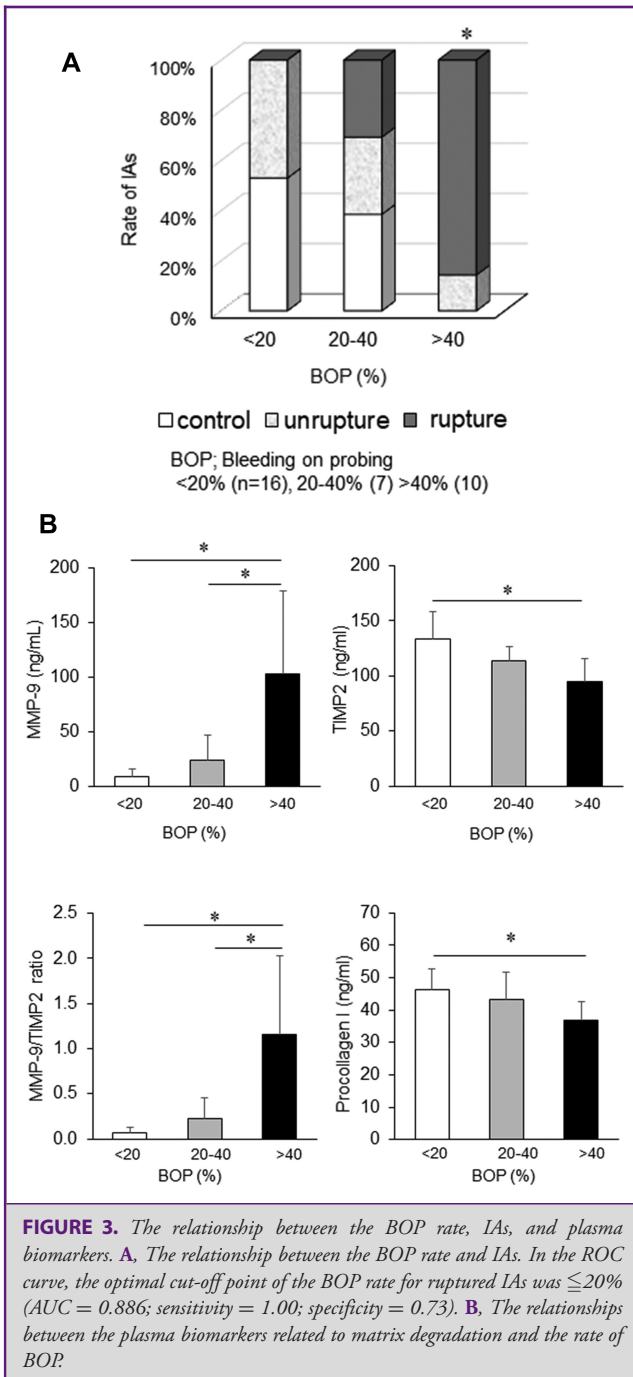
Abbreviation: NS, no significant difference. *p* value calculated by ANOVA for mean by Fisher's exact test for categorical variables.

PD may be at least partly associated with the pathophysiology of IAs, especially ruptured IAs.

PD is associated with increased systemic inflammation, and periodontal inflammation may play a role in the initiation or progression of atherosclerosis-induced diseases, such as coronary artery disease and ischemic stroke.²¹⁻²³ Grau et al¹¹ reported that severe gingivitis is strongly and independently associated with cerebral ischemia and that gingivitis is an indicator of the actual status of periodontal inflammation. The incidence of severe PD and anodontia was significantly higher in patients with ruptured IAs than in those with unruptured IAs or the controls, suggesting a relationship between severe PD and the pathophysiology of IAs.

Pyysalo et al^{9,10} detected bacterial DNA in several oral specimens from tissue samples of ruptured and unruptured aneurysms. The specimens contained significantly more bacterial DNA than the samples from the control vessels. The most commonly detected pathogens were members of the *Streptococcus mitis* and the *Fusobacterium nucleatum* groups. In our study, although we could not analyze tissue samples, we analyzed bacterial DNA from oral and plasma specimens. We found that the positive rate of DNA from *P. gingivalis* and *T. denticola* was high (100%) in patients with severe PD. Consistent with the other studies,²⁴ our study showed that the high positive rate of these bacteria possibly plays a significant role in the pathogenesis of PD. Moreover, detection of DNA specific oral pathogens and of the co-stimulation of the toll-like receptor (TLR)-2-

CD14 complex in samples from ruptured aneurysms suggests that oral pathogens could disseminate into the systemic circulation, localize in aneurysms, and elicit TLR-mediated inflammation.^{9,10} Previous studies^{24,25} suggested that bacterial dissemination from oral tissue into the systemic circulation and peripheral tissue infections contribute to the pathogenesis of IAs. Delbosc et al²⁶ reported that neutrophil activation in human abdominal aortic aneurysms was associated with neutrophil extracellular trap (NET) formation in the intraluminal thrombus, which led to the release of cell-free DNA. Although *P. gingivalis* trapped by NETs were more common in the plasma of patients with abdominal aortic aneurysms. Thus, in our study, we examined the relationship between periodontal bacteria and IAs. Although Salhi et al²⁷ suggested that the presence of PD bacteria in serum is a risk factor for aneurysmal progression, we found that PD bacteria DNA in oral specimens was present even in control subjects without IAs similar to that in ruptured patients. However, the positive rate of DNA for periodontal bacteria contained in the oral specimen was significantly higher in the severe group than in the slight PD group. Ruptured IAs were more commonly observed in the severe PD group, suggesting that ruptured IAs may be triggered by local inflammation caused by severe PD via oral PD bacteria. There was no significant difference in plasma IgG titers against 4 strains of periodontal pathogens in any of our patients or controls. We propose that the IgG titers reflected those not only of the current but also prior infections and of cell-mediated



immunity. We could not identify the rupture-specific bacteria in the current study.

In our rat rupture IA model,¹⁵ we demonstrated that vascular walls prone to rupture had increased macrophage infiltration, high expression of MMP-9, and an imbalance of the MMP-9/TIMP-2 ratio. Moreover, decreased expression of procollagen I in the vascular wall was indicative of IAs.²⁸ Our study demon-

strated that the plasma MMP-9 levels and the MMP-9/TIMP-2 ratio were higher and procollagen I was lower in patients with high BOP rates.

The rates of hypertension or smoking were not significantly different between patients with unruptured and ruptured IAs (Table 1). Conversely, in severe PD patients, the rates of both smoking and hypertension were significantly higher than in patients with slight PD (Table 2 and **Supplemental Figure 1B**), suggesting that smoking and hypertension are associated with severe PD. Regarding ruptured patients (**Supplemental Figure 1A**), 36% had hypertension combined with smoking, 27% had hypertension alone, and 9% reported smoking history alone, indicating that 73% presented these risk factors. Thus, combination of hypertension with severe PD in regular smokers might indicate a crucial risk factor leading to aneurysm rupture. Hypertension or smoking habits in ruptured IAs have been highlighted by Etminan et al.²⁹ However, a large-scale study by Hallikainen et al¹² showed there were no significant relationships between SAH, hypertension, and current smoking or smoking history, despite the strong relationship between SAH and periodontitis and severe periodontitis. Our findings suggest that severe PD may be a potential risk factor for SAH and that the subgingival inflammatory response induced by periodontal bacteria may be attributable to SAH. Although these findings are consistent partly with another study,^{12,29} we further need to clarify the direct association between local inflammation by severe PD and systemic inflammation.

Gingivitis and periodontitis are treatable conditions. The prevention of PD involves reducing the formation of bacterial plaques in the oral cavity by physical and chemical forces. Oral or periodontal bacteria may infect the aneurysm wall and play a role in the pathophysiology of IAs; however, our study demonstrated no SAH-specific pathogens. To distinguish the mechanisms underlying the systemic inflammatory response from a local inflammation, we need further studies, such as an analysis of the exosomes secreted from periodontitis.

Limitations

There are some limitations in our study. The sample size was small. The high grade SAH patients intubated prior to admission were excluded. Thus, it is unclear whether risk factors, such as hypertension and smoking, could be adjusted for. Consequently, we could not obtain aneurysmal samples that are large enough for the evaluation of bacterial DNA, the expression of MMP-9, or the MMP-9/TIMP-2 ratio. Numerous other factors have been shown to participate in the pathophysiology of IAs, such as family history, multiple aneurysms and measured and unmeasured confounding factors. These cannot be ruled out. Although severe PD-induced changes in plasma biomarkers may affect systemic inflammatory response and SAH, we cannot demonstrate a direct relationship between local- and systemic inflammation induced by PD bacteria. We need to clarify that

optimal oral care may prevent inflammation in diseases such as IAs.

CONCLUSION

Our study is the first to demonstrate an association between BOP in severe PD and dysregulation in the extracellular matrix of IA patients. Our findings suggest that periodontal inflammation due to local bacterial infection could lead to severe PD and the imbalance between MMP-9 and TIMP-2 in plasma and decrease the plasma level of procollagen I, which may play a crucial role in IA rupture. Hence, severe PD may be at least partly involved in the pathophysiology of IAs, and PD treatment may help to prevent IA rupture. Our present findings warrant further studies to clarify the plausible mechanisms linking severe PD and IA rupture.

Disclosures

This study was supported by Grants-in-Aid for Scientific Research: KAKENHI-15H04950 (SN), 15K10305 (JS), and 15K19972 (YT) and Grant-in-Aid for Research Activity start-up: KAKENHI 19K24013 (TM). The authors have no personal financial, or institutional interest in any of the drugs, materials, or devices described in this article.

REFERENCES

- Brown RD, Jr, Broderick JP. Unruptured intracranial aneurysms: epidemiology, natural history, management options, and familial screening. *Lancet Neurol*. 2014;13(4):393-404.
- Shivashankar R, Miller TR, Jindal G, Simard JM, Aldrich EF, Gandhi D. Treatment of cerebral aneurysms-surgical clipping or endovascular coiling: the guiding principles. *Semin Neurol*. 2013;33(5):476-487.
- Cheng F, Zhang M, Wang Q, et al. Tooth loss and risk of cardiovascular disease and stroke: a dose-response meta-analysis of prospective cohort studies. *PLoS One*. 2018;13(3):e0194563.
- You Z, Cushman M, Jenny N, Howard G. Tooth loss, systemic inflammation, and prevalent stroke among participants in the reasons for geographic and racial difference in stroke (REGARDS) study. *Atherosclerosis*. 2009;203(2):615-619.
- Fernandes CP, Oliveira FA, Silva PG, et al. Molecular analysis of oral bacteria in dental biofilm and atherosclerotic plaques of patients with vascular disease. *Int J Cardiol*. 2014;174(3):710-712.
- Haraszthy VI, Zambon JJ, Trevisan M, Zeid M, Genco RJ. Identification of periodontal pathogens in atheromatous plaques. *J Periodontol*. 2000;71(10):1-7.
- Fiehn N-E, Larsen T, Christiansen N, Holmstrup P, Schroeder TV. Identification of periodontal pathogens in atherosclerotic vessels. *J Periodontol*. 2005;76(5):731-736.
- Chiu B. Multiple infections in carotid atherosclerotic plaques. *Am Heart J*. 1999;138(5 Pt 2):S534-S536.
- Pyysalo MJ, Pyysalo LM, Pessi T, Karhunen PJ, Öhman JE. The connection between ruptured cerebral aneurysms and odontogenic bacteria. *J Neurol Neurosurg Psychiatry*. 2013;84(11):1214-1218.
- Pyysalo MJ, Pyysalo LM, Pessi T, et al. Bacterial DNA findings in ruptured and unruptured intracranial aneurysms. *Acta Odontol Scand*. 2016;74(4):315-320.
- Grau AJ, Becher H, Ziegler CM, et al. Periodontal disease as a risk factor for ischemic stroke. *Stroke*. 2004;35(2):496-501.
- Hallikainen J, Lindgren A, Savolainen J, et al. Periodontitis and gingival bleeding associate with intracranial aneurysms and risk of aneurysmal subarachnoid hemorrhage. *Neurosurg Rev*. 2019;43(2):669-679.
- Yagi K, Tada Y, Kitazato KT, Tamura T, Satomi J, Nagahiro S. Ibudilast inhibits cerebral aneurysms by down-regulating inflammation-related molecules in the vascular wall of rats. *Neurosurgery*. 2010;66(3):551-559.
- Matsushita N, Kitazato KT, Tada Y, et al. Increase in body Na⁺/water ratio is associated with cerebral aneurysm formation in oophorectomized rats. *Hypertension*. 2012;60(5):1309-1315.
- Korai M, Kitazato KT, Tada Y, et al. Hyperhomocysteinemia induced by excessive methionine intake promotes rupture of cerebral aneurysms in ovariectomized rats. *J Neuroinflammation*. 2016;13(1):165.
- Miyamoto T, Kung DK, Kitazato KT, et al. Site-specific elevation of interleukin-1 β and matrix metalloproteinase-9 in the Willis circle by hemodynamic changes is associated with rupture in a novel rat cerebral aneurysm model. *J Cereb Blood Flow Metab*. 2017;37(8):2795-2805.
- Yamaguchi T, Miyamoto T, Kitazato KT, et al. Time-dependent and site-dependent morphological changes in rupture-prone arteries: ovariectomized rat intracranial aneurysm model. *J Neurosurg*. 2019;1-9.
- Ramseier CA, Anerud A, Dulac M, et al. Natural history of periodontitis: disease progression and tooth loss over 40 years. *J Clin Periodontol*. 2017;44(12):1182-1191.
- Tulamo R, Frosen J, Junnikkala S, et al. Complement activation associates with saccular cerebral artery aneurysm wall degeneration and rupture. *Neurosurgery*. 2006;59(55):1069-1076.
- Biljana E, Boris V, Cena D, Veselka DI. Matrix metalloproteinases (with accent on collagenases). *J Cell Anim Biol*. 2011;5(7):113-120.
- Thompson R, Holmes D, Mertens R, et al. Production and localization of 92-kilodalton gelatinase in abdominal aortic aneurysms. An elastolytic metalloproteinase expressed by aneurysm-infiltrating macrophages. *J Clin Invest*. 1995;96(1):318-326.
- Martinez-Canut P. Predictors of tooth loss due to periodontal disease in patients following long-term periodontal maintenance. *J Clin Periodontol*. 2015;42(12):1115-1125.
- Duan Y, Lagman C, Ems R, Bambakidis NC. Relationship between middle cerebral parent artery asymmetry and middle cerebral artery aneurysms rupture risk factors. *J Neurosurg*. 2019;29:1-8.
- Gheorghita D, Eördegh G, Nagy F, Antal M. Periodontal disease, a risk factor for atherosclerotic cardiovascular disease. *Orv Hetil*. 2019;160(11):419-425.
- O'Boyle C, Haley MJ, Lemarchand E, et al. Ligature-induced periodontitis induces systemic inflammation but does not alter acute outcome after stroke in mice. *Int J Stroke*. 2019;22(2):175-187.
- Delbosc S, Alsac JM, Journe C, et al. *Porphyromonas gingivalis* participates in pathogenesis of human abdominal aortic aneurysm by neutrophil activation. Proof of concept in rats. *PLoS One*. 2011;6(4):e18679.
- Salhi L, Rompen E, Sakalihan N, et al. Can periodontitis influence the progression of abdominal aortic aneurysm? A systematic review. *Angiology*. 2018;70(6):479-491.
- Aoki T, Kataoka H, Ishibashi R, Nozaki K, Morishita R, Hashimoto N. Reduced collagen biosynthesis is the hallmark of cerebral aneurysm: contribution of interleukin-1beta and nuclear factor-kappaB. *Arterioscler Thromb Vasc Biol*. 2009;29(7):1080-1086.
- Etminan N, Chang HS, Hackenberg K, et al. Worldwide incidence of aneurysmal subarachnoid hemorrhage according to region, time period, blood pressure, and smoking prevalence in the population: a systematic review and meta-analysis. *JAMA Neurol*. 2019;76(5):588-597.

Acknowledgments

We thank Mina Kishida for her assistance in obtaining the magnetic resonance imaging scans and all participating individuals who provided their consent to be included in this study.

Supplemental Digital Content is available for this article at www.neurosurgeryopen-online.com.

Supplement Figure 1. The relationship among intracranial aneurysms, the severity of periodontal disease and risk factors. **A**, The relationship between intracranial aneurysms (IAs) and risk factors. **B**, The relationships between the severity of periodontal disease (PD) and risk factors.

Supplement Figure 2. The relationship between intracranial aneurysms and plasma biomarkers.

Supplemental Methods. The detailed materials and methods.

COMMENT

The authors identify an association of ruptured intracranial aneurysms with periodontal inflammation, imbalance between MMP-9 and TIMP-2, and decrease the level of procollagen I. However, no causative association can be established given the nature of this study. Additionally the small sample size prevents any conclusions to be drawn from the

multivariable analysis due to the risk of overfitting. Further research in this matter will be needed to confirm or refute these preliminary findings.

Kimon Bekelis

Hanover, New Hampshire