



Prognosis of Periodontitis Recurrence After Intensive Periodontal Treatment Using Examination of Serum IgG Antibody Titer Against Periodontal Bacteria

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Chronic periodontitis is associated with systemic diseases such as atherosclerosis. In this study, we evaluated the efficacy of serum IgG antibody titer to periodontal bacteria for prognosis of periodontitis recurrence during supportive periodontal therapy (SPT) phase. The 139 patients during SPT phase were selected and divided to two groups as follows: "Stable" and "Recurrence" group at SPT phase for case-control study: "High IgG titer" and "Normal IgG titer" group before transition to SPT phase for cohort study. We examined whether clinical findings or serum IgG antibody titers to periodontal bacteria are risk factors for the development of periodontitis recurrence. Case-control study showed that

there were significant differences between the stable and recurrence groups in age and number of teeth. The serum IgG antibody titer to *Eikenella corrodens* FDC1073, *Porphyromonas gingivalis* SU63, and *Campylobacter rectus* ATCC33238 was significantly higher in the recurrence group. Next, we found, that the recurrence ratio in the high IgG titer group to Gram-negative obligate anaerobe, *Prevotella intermedia*, *Treponema denticola*, and *C. rectus* was significantly higher than that of the normal IgG titer group. Taken together, serum IgG antibody titer test is useful in the prognosis of periodontitis recurrence during the SPT phase. J. Clin. Lab. Anal. 24:1–8, 2010.

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Key words: serum IgG antibody titer; periodontitis recurrence; supportive periodontal therapy

INTRODUCTION

Chronic periodontitis is a polymicrobial infectious disease (1) and the disease may result in loss of teeth by inflammation-mediated bone resorption. More than 300 individual cultivable species of microbes have been identified in the human mouth (2,3). Recurrence of periodontitis caused by insufficient periodontal maintenance may lead to poor oral health, and result in tooth loss. Therefore, in order to prevent the recurrence of the disease after periodontal treatment, it is important to establish the efficient methods for prediction. Recently, many researchers have reported that chronic periodontitis resulting from persistent low-grade infection of Gram-negative bacteria is associated with increased atherosclerosis, diabetes mellitus, and other systemic diseases disseminated through blood stream (4,5).

Therefore, as the infection control is very important for general health, it should be evaluated by appropriate laboratory clinical tests focused on microbial infection.

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The microbiological examinations for periodontitis have been available to dental clinicians since the end of the 1980s (6). It has been generally accepted that infection with periodontal bacteria leads to humoral immunological responses and elevates the levels of serum IgG antibody to the bacteria (7,8). There are various reports regarding the usefulness of the serum IgG antibody titer against periodontal bacteria to evaluate the treatment effects for periodontitis (9,10). As serum IgG antibody levels correspond to the amount of periodontal bacteria, the effects of treatments focused on elimination of bacteria could be evaluated by decrease of serum IgG titer to the pathogens.

Supportive periodontal therapy (SPT) is an integral part of periodontal treatment, and is essential to prevent the recurrence of the disease in susceptible individuals, because periodontitis is frequently recurrent even after the intensive treatment (11). In general, clinically, several risk factors for the susceptibility of periodontitis recurrence are evaluated during the SPT phase, including: (i) the prevalence of residual periodontal pockets, (ii) tooth loss, (iii) the systemic conditions in each patient, and (iv) environmental or behavioral factors such as smoking (12). Basically, these factors should be considered and evaluated together for prognosis of periodontitis recurrence. Determining the risk for periodontitis recurrence during SPT phase would help the clinician to customize the frequency and contents of SPT visits. As chronic periodontitis is an infectious disease, it is important to evaluate the infection levels of periodontal pathogens. However, the current test for evaluating the level during SPT phase is not clinically useful, so establishment of convenient diagnosis system for the prognosis of periodontitis recurrence is needed.

In this study, we propose a new method for the prognosis of periodontitis recurrence during SPT phase using measurements of serum IgG antibody titer against periodontal pathogens. To show the clinical usefulness of serum IgG antibody titer for prognosis of the disease, we analyzed the relationship of several clinical data and serum IgG antibody titer to periodontitis recurrence during SPT. This examination will help to identify the most appropriate approach to SPT for individual patients to prevent the periodontitis recurrence. We believe our approach contributes to promotion of general health in the future.

MATERIALS AND METHODS

Study Population

The subjects included 139 (male: 34, female: 105, average age: 61.4 ± 10.4) chronic periodontitis patients at the Department of Periodontics and Endodontics, Okayama University Hospital of Medicine and Dentistry.

The patients received intensive periodontal treatment followed by SPT for more than 1 year.

Informed consent was obtained from each subject, and the protocol for the evaluation of serum IgG titer has been approved by the institutional review board. The intensive periodontal treatment include scaling, root planning, under infiltration anesthesia, and periodontal surgeries at one or more sites. SPT procedures included re-motivation, plaque control guidance, scaling and root planning, and removal of local environmental factors at intervals of a few months. Patients with systemic diseases such as diabetes were excluded from this study because of the elevated risk factors for periodontal diseases. A detailed breakdown of the criteria for inclusion and exclusion in this study is presented below.

Inclusion Criteria

1. Adult patients with chronic periodontitis.
2. Patients with chronic periodontitis, treated by means of scaling and root planning and/or periodontal surgery, and in SPT phase for at least 1 year.
3. Patients systemically healthy, and without relevant chronic medication intake.

Exclusion Criteria

1. Pregnant women or in lactation.
2. Systemic antibiotic intake. Frequent use of anti-inflammatory drugs.
3. Patients with systemic diseases.
4. Three or more periodontal pockets with ≥ 6 mm
5. Additionally, other habits, such as smoking, were recorded by a directed interview, as well as any relevant systemic condition or medication intake.

Preparation of Bacterial Antigens

Ultrasonic extract antigens were used for antigen samples of periodontal bacteria. The bacteria were allowed to reach maturity in pure cultures, using agar plate and liquid media, and diluted with phosphate-buffered saline solution (PBS). After the bacterial cells were sonicated to destroy cellular membranes, each bacterial solution sonicated were centrifuged at 12,000g for 20 min to obtain the supernatants. These bacteria included: *Aggregatibacter actinomycetemcomitans* Y4, *A. actinomycetemcomitans* ATCC29523, *A. actinomycetemcomitans* SUNY67, *Capnocytophaga ochracea* S3, *Eikenerra corrodens* FDC1073, *Fusobacterium nucleatum*



1 ATCC25586, *Prevotella intermedia* ATCC33563,
 2 *P. intermedia* ATCC25611, *Porphyromonas gingivalis*
 3 FDC381, *P. gingivalis* SU63, *Treponem denticola*
 4 ATCC35405, and *Campylobacter rectus* ATCC33238.

7 Measurement of the Serum IgG Antibody Titer to Periodontal Bacteria

9 The levels of serum IgG antibody titer against
 periodontal bacteria were measured before transition
 11 to SPT phase, and once or twice a year during SPT
 phase.

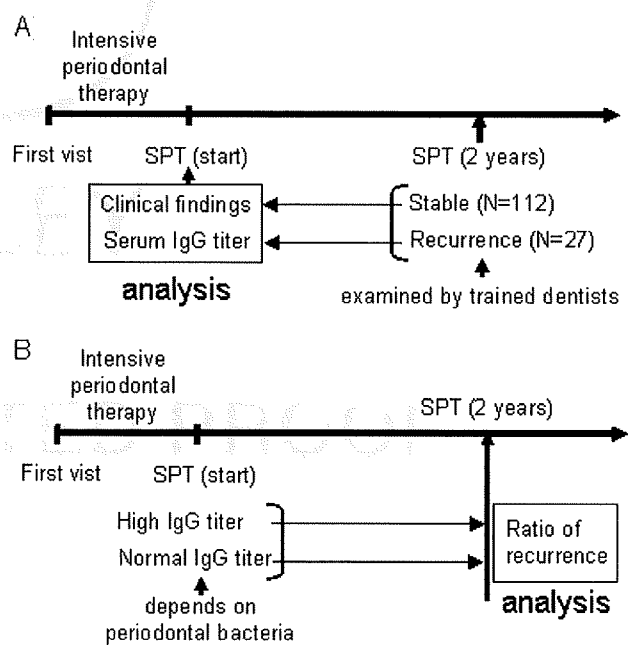
13 The amount of serum IgG that bound to each
 pathogenic bacteria antigen causing periodontitis was
 15 measured by ELISA as described previously (8). Briefly,
 each antigen was diluted to 10 µg/ml with 0.1 M
 17 carbonate buffer (pH 9.6). A portion of this diluted
 solution (100 µl) was then added to each well in a flat-
 19 bottomed microtiter plate (Greiner Co., Ltd., Frick-
 enhausen, Germany) and the plate was stored overnight
 21 at 4°C. Each well with immobilized antigen was washed
 three times with PBS (pH 7.4) containing 0.05% Tween-
 23 20 (PBST). Subsequently, a diluted serum sample
 (3,100-fold dilution with PBST) was added to each well.
 25 After incubation at 37°C for 2 hr, each well was washed
 three times with PBST and bound/free (B/F) separation
 27 was carried out. Next, a 100 µl portion of 1:5,000 diluted
 alkaline phosphatase-conjugated goat antihuman IgG
 29 (Jackson Immuno Research Laboratories, Inc., Balti-
 more, MD) was added to each well. After incubation at
 31 37°C for 2 h, each well was washed three times with
 PBST and B/F separation was carried out. Thereafter,
 33 50 µl of *p*-nitrophenyl phosphate (Wako Pure Chemical
 Industries, Ltd., Osaka, Japan) adjusted to 1 mg/ml with
 35 10% diethanolamine buffer (pH 9.8) was added to each
 well as substrate. The plate was then incubated at room
 37 temperature for 10–20 min. The enzymatic reaction was
 terminated by adding 50 µl of 3N NaOH and optical
 39 density (measurement at 405 nm; reference at 490 nm)
 was measured in a Micro ELISA Auto Reader (Bio-Rad
 41 Laboratories, Hercules, CA).

43 The sera from ten healthy subjects (age: 20–29 yr)
 were pooled and used as the calibrator of analysis.
 45 Using serial dilutions (1:12.5, 1:50, 1:200, 1:800, 1:3,200,
 1:12,800, and 1:51,200) of this pooled control plasma,
 standard titration curves were prepared. The absorbance
 47 of each sample after reaction was defined as ELISA unit
 (EU), so that 100 EU corresponds to 1:3,200 dilution of
 49 the calibrator sample. For clinical use, the following
 formula was applied to the EU to calculate the
 51 diagnostic standardized value: standardized value =
 (IgG titer of patient – mean IgG titer of healthy
 53 subjects) / 2 standard deviation (SD) determined by mean
 IgG titer of ten healthy subjects.

Classification of Subjects and Statistical Analysis

55 At 2 years during SPT after periodontal healing,
 57 subjects were classified into a “Recurrence group” (with
 59 recurrence or progression of periodontitis) and a “Stable
 group” (without recurrence or progression of periodontal
 61 disease) for a case-control study (Fig. 1A). Patients with
 63 three or more deepening periodontal pockets with a depth
 of 3 mm or more after the transition to SPT phase were
 65 judged to be “with periodontitis recurrence or progression,”
 based on the report of Levine et al. (13). Trained dentists
 67 performed the examination of clinical findings (age, number
 of teeth, plaque control record (PCR), bleeding on probing
 (BOP), and periodontal pocket depth by pocket probing),
 69 and a supervisory doctor checked it so that there was no
 difference in technique among attending dentists. PCR was
 71 examined using O’Leary plaque index (14). Significant
 differences between each group were analyzed by Mann-
 73 Whitney *U*-test.

75 Secondly, subjects were classified into “High IgG titer”
 and “Normal IgG titer” group in serum IgG antibody titer
 77 against periodontal bacteria at the



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Fig. 1. Experimental protocol (A) A case-control study. At 2 years during SPT after intensive periodontal treatment, subjects were classified into a “Recurrence group” (with recurrence or progression of periodontitis, *N* = 112) and a “Stable group” (without recurrence or progression of periodontal disease, *N* = 27). Significant differences between each group were analyzed by Mann-Whitney *U*-test. (B) A cohort study. At the beginning of the SPT phase, subjects were classified into “High serum IgG titer” and “Normal serum IgG titer” group in each strain of periodontal bacteria. Significant differences of periodontitis recurrence ratio within 2 years after intensive periodontal treatment between each group were analyzed by Pearson’s χ^2 test.



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beginning of the SPT phase for a cohort study (Fig. 1B). Patients exhibiting IgG antibody titer levels significantly ($>2\sigma$) above the average among healthy volunteers are defined as having high-level serum IgG antibody titer against periodonopathic bacteria. Significant differences of periodontitis recurrence ratio between each group were analyzed by Pearson's χ^2 test.

For statistical analysis, computer software Statview 5.0 (Abacus Concepts, Inc., Berkeley, CA) was used.

11 RESULTS

13 Clinical Findings of Patients Before SPT Phase

Chronic periodontitis of all patients were treated by intensive periodontal treatment. The healing was evaluated by trained dentists using routine periodontal examination methods (periodontal pocket depth, BOP, and X-ray). A total of 139 patients during SPT phase were analyzed for case-control study (Stable group: 112, Recurrence group: 27). Clinical findings of patients before SPT phase are summarized in Table 1. There were no significant differences between the stable and recurrence group in the score of their PCR, BOP, and even averaged probing pocket depth. On the other hand, there were significant differences between the stable and recurrence groups in their age and number of teeth (age, $P = 0.026$; number of teeth, $P = 0.025$; Mann-Whitney U -test).

31 Statistical Differences Between the Stable and Recurrence Group in Serum IgG Antibody Titer Before Transition to SPT Phase

In 12 strains from 8 bacterial species, average of serum IgG antibody titer against all of periodontal bacteria before transition to SPT phase in the recurrence group was higher than that of the stable group (Fig. 2). Especially, the levels of serum IgG antibody titer to several periodontal bacteria were statistically higher in the recurrence group than that of the stable group before transition to SPT phase (*A. actinomycetemcomitans* Y4,

$P = 0.020$; *E. corrodens* ATCC1073, $P = 0.040$; *P. gingivalis* SU63, $P = 0.020$; *C. rectus* ATCC33238, $P = 0.025$; Mann-Whitney U -test). The serum IgG antibody titer against *T. denticola* ATCC35405 was also clearly higher in the recurrence group than in the stable group ($P = 0.081$; Mann-Whitney U -test) before transition to SPT phase.

Statistical Differences Between the High and Normal Serum IgG Titer Group in Periodontitis Recurrence

In a cohort study, the patients were categorized into two groups according to their serum IgG antibody titer levels associated with the eight known periodontal bacteria. In the "normal" group, the level of serum IgG antibody titer was observed to be lower than 1.0 against each type of bacteria at the beginning of the SPT phase. In the "high" group, the level of serum IgG antibody titer exceeds 1.0 against periodontal bacteria. As shown in Table 2, importantly, we found that there were no significant differences between the Normal and High serum IgG antibody titer group in all clinical findings. From these clinical data, we confirmed to become healthy clinically in both groups by active periodontal treatment. Furthermore, we observed the tendency that the recurrence ratio of the high serum IgG titer group was higher than that of the normal group (Normal group: 14.9–19.0 %, High group: 20.5–36.8 %). Especially, the recurrence ratio of the high IgG titer group to three obligate anaerobic bacteria was statistically higher than that of the normal titer group (*P. intermedia* ATCC25611, $P = 0.021$; *T. denticola* ATCC35405, $P = 0.039$; *C. rectus* ATCC33238, $P = 0.048$; Pearson's χ^2 test). In addition, the recurrence ratio of the high titer group against *P. gingivalis* SU63 was higher than that of the normal titer group, although there was no statistical difference ($P = 0.083$; Pearson's χ^2 test). Furthermore, we examined the combined recurrence ratio in high IgG antibody titer against 12 periodontal bacteria, and the periodontitis recurrence ratio of the high titer group was

43 TABLE 1. Clinical Findings at the Beginning of SPT Phase

	Stable group (N = 112)	Recurrence group (N = 27)	P-value
Age (yr)	60.2 ± 10.6	67.0 ± 8.1	0.026*
Number of teeth	22.0 ± 6.3	17.2 ± 8.2	0.025
PCR (%)	21.5 ± 15.1	22.4 ± 13.0	0.775
BOP (%)	11.3 ± 11.0	13.9 ± 8.7	0.224
Pocket depth (mm)	2.30 ± 0.3	2.50 ± 0.5	0.158
SPT period (month)	48.9 ± 12.4	51.8 ± 12.5	0.362

Clinical findings excluding SPT period were examined at the beginning of SPT phase. PCR, Plaque control record; BOP, Bleeding on probing. *Significant difference ($P < 0.05$, Mann-Whitney U -test) between stable and recurrence group. Values represent the mean ± standard deviation (SD).



Prognosis of Periodontitis Recurrence 5

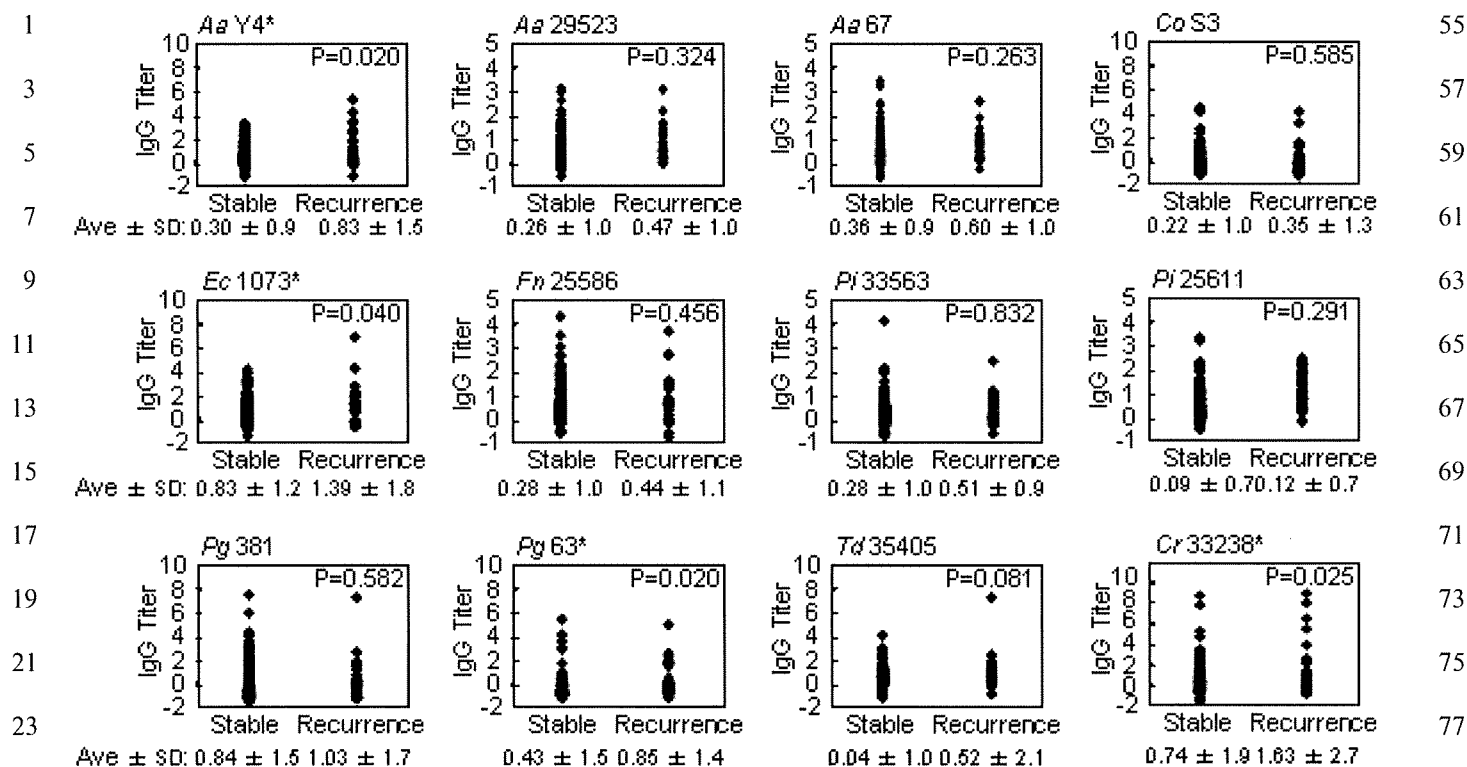


Fig. 2. The levels of serum IgG antibody titer against 12 periodontal bacteria. The significant differences between "Stable" and "Recurrence" group were analyzed using the Mann-Whitney *U*-test. Each dot represents an individual data tested by ELISA assay. The Y-axis (IgG Titer) in each panel denotes the value determined as (serum IgG titer tested by ELISA)–(mean titer calculated using that of healthy subjects)/(2 SD calculated using that of healthy subjects) as described in Materials and Methods section. Ave, average of IgG Titer; each data have calculated and shown as average ± SD. **P* < 0.05. Aa, *A. actinomycetemcomitans*; Co, *C. ochracea*; Ec, *E. corrodens*; Fn, *F. nucleatum*; Pi, *P. intermedia*; Pg, *P. gingivalis*; Td, *T. denticola*; Cr, *C. rectus*.

greater than that of the normal titer group (High titer group: 21.6 % (*N* = 97), Normal titer group: 14.3% (*N* = 42), *P* = 0.339, Pearson's χ^2 test).

DISCUSSION

Periodontal disease is a common chronic infection caused by Gram-negative bacteria such as *P. gingivalis* and *P. intermedia* (1). Recurrence of periodontitis may lead to poor oral health, and result in tooth loss. Therefore, in order to prevent the recurrence of the disease after periodontal treatment, it is important to establish the efficient methods for patients. Recently, epidemiological research provides strong evidence that periodontitis is a risk factor for systemic diseases such as cardiovascular disease (5,6). A number of studies have reported that periodontal infection would be a risk factor for progression of myocardial infarction and stroke (15,16). Therefore, persistent low-grade infection by chronic periodontitis is also a focus for physicians.

This study is a part of our ongoing efforts to elucidate the clinical usefulness of serum IgG antibody titer to periodontal bacteria. In general, it is well recognized

that periodontitis is a multifactorial disease (17–19). For example, a young patient developing periodontitis might be most likely a carrier of one or more genetic factors. Patients may also have one or more chronic systemic diseases associated with an increased risk for periodontitis. Therefore, it is difficult to identify the factors contributing to the onset, progression, and the recurrence of periodontitis following periodontal therapy.

Good control of supragingival plaque is important to prevent the periodontitis recurrence in SPT phase, after intensive periodontal treatment. However, our results have shown that the predictive value of routine periodontal parameters (PCR, BOP, and pocket probing depth) is relatively low (Table 1). Periodontal examinations we performed routinely did not provide clear predictions for the recurrence of periodontitis. This is not unexpected because routine periodontal examinations such as BOP and pocket probing depth primarily indicate the past reaction to inflamed periodontal tissue. As shown in Table 1, among the factors relating to the periodontitis recurrence during SPT phase, we found age of patients is one of the risk factors in the recurrence. With age, metabolism, restoration ability, and preventive ability of



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TABLE 2. Clinical Findings After Periodontitis Treatment and Recurrence Ratio During SPT					
	Strains	Examination	Normal IaG titer	High IgG titer	P-value
1					55
3					57
5	Facultative anaerobic	<i>Aa</i> Y4			
7					61
9					63
11					65
13					67
15					69
17					71
19					73
21					75
23					77
25	Obligate anaerobic	<i>Pi</i> ATCC25611*			79
27					81
29					83
31					85
33					87
35					89
37					91
39					93
41					95
43					97
45					99
47					101
49					103
51					105
53					107



1 TABLE 2. Continued

3	Strains	Examination	Normal IgG titer	High IgG titer	P-value
5		BOP (%)	11.8	13.6	0.65
5		Pocket depth (mm)	2.26	2.42	0.13
7		Serum IgG Ab. Titer	0.02	3.67	<0.0001
7		Recurrence ratio (%)	14.9	29.7	0.048

6 Data were analyzed by Mann-Whitney *U*-test for clinical findings and Pearson's χ^2 test for Recurrence ratio between "Normal" and "High" IgG
 9 titer group. *, $P < 0.05$: The recurrence ratio in "High" IgG Titer group is significantly higher. *Aa*, *A. actinomycetemcomitans*; *Ec*, *E. corrodens*;
 11 *Pi*, *P. intermedia*; *Pg*, *P. gingivalis*; *Td*, *T. denticola*; *Cr*, *C. rectus*.

13 periodontal tissue cells are reduced irreversibly. There-
 15 fore, the risk of periodontitis recurrence might increase
 with the age of patients indirectly.

17 There have been reports that measurement of serum
 19 IgG antibody titer was useful for diagnosing period-
 21 ontitis or judging the treatment effects (15). However,
 23 during the SPT phase following active periodontal
 25 treatment, the usefulness of the levels of serum IgG
 27 antibody titer was still unknown. We have proposed a
 29 new insight for the prognosis of periodontitis recurrence
 31 during SPT phase using serum IgG antibody titer. In
 this study, we analyzed the usefulness of the levels of
 serum IgG antibody titer in predicting the recurrence of
 periodontitis during SPT phase by multiple classification
 analysis. We used sonic extracts of whole bacterial cells
 as antigens for ELISA. As the bacterial antigens include
 various components, mainly protein, lipopolysaccharide
 (LPS), and DNA, the serum IgG antibody titer against
 periodontal bacteria reflects total results of antibody
 responses (8).

33 Periodontitis is a bacterial infectious disease (17). The
 35 humoral responses against bacteria are largely different
 37 among individuals. The immunological response against
 39 specific bacteria should be clinically useful for evaluating
 41 the risk of periodontitis recurrence. Figure 2 shows the
 43 levels of serum IgG antibody titer against 12 periodontal
 45 bacteria before transition to SPT phase in the stable and
 47 recurrence group. Interestingly, although the levels of
 49 serum IgG antibody titer against all periodontal bacteria
 were variable, we found that the serum IgG antibody titer
 against several bacteria (*A. actinomycetemcomitans* Y4,
E. corrodens ATCC1073, *P. gingivalis* SU63, and *C. rectus*
 ATCC33238) was significantly higher within the recur-
 rence group than the stable group when in transition to
 SPT phase. These findings indicate that serum IgG
 antibody titer might be useful clinically as a diagnostic
 marker of periodontitis recurrence during SPT phase.

51 From another viewpoint, we examined the differences
 53 of the periodontitis recurrence ratio between the high
 and normal serum IgG antibody titer group when
 transition to SPT as a companion study. Interestingly,
 we observed the tendency that the recurrence ratio of the

high serum IgG titer group was higher than that of the
 normal group as shown in Table 2. Especially, we found
 the recurrence ratio of the high titer group against
 several periodontal bacteria (*P. intermedia* ATCC25611,
T. denticola ATCC35405, and *C. rectus* ATCC33238)
 was statistically higher than that of the normal titer
 group. Furthermore, we examined the combined recur-
 rence ratio in high IgG antibody titer against 12
 periodontal bacteria. Interestingly, we found that the
 periodontitis recurrence ratio of the high titer group was
 greater than that of the normal titer group. The
 combined periodontal bacteria might provide an effec-
 tive clinical prognosis of periodontitis recurrence. Our
 findings indicate that the serum IgG antibody titer
 might be useful as a predicting marker of periodontitis
 recurrence during SPT phase. Also, Tolo et al. reported
 that the level of serum IgG antibody titer against
P. gingivalis increases before absorption of alveolar bone,
 and could predict the progression of periodontitis (20).
 This report supports our concept.

According to recent studies, chronic periodontitis,
 persistent low-grade infection of Gram-negative bacteria,
 is associated with increased atherosclerosis, heart
 disease, diabetes mellitus, and other systemic diseases
 through the blood stream (4,5,21). So poor oral health
 may have profound effect on general health; therefore, it
 is important to prevent the recurrence of periodontitis
 for health promotion practice.

We believe that SPT is effective for preventing the
 recurrence of periodontitis. In this study, we wanted to
 find the primary risk factors of periodontitis recurrence
 in patients after periodontal treatment. From multiple
 classification analysis on clinical findings and serum IgG
 antibody titers before transition to SPT phase, we
 elucidated the predictive markers for the recurrence of
 periodontitis in view of humoral immune responses to
 periodontal infection. We propose the attention should
 be focused on the levels of serum IgG antibody to
 periodontal bacteria when transition to SPT phase. Our
 findings show that elevated serum IgG antibody titer is
 an important marker to predict the periodontitis
 recurrence during the transition to SPT phase.



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UNCORRECTED PROOF

