The limited value of three pathogen species in predicting healing of periodontal pockets

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Baseline level of *Actinobacillus actinomycetemcomitans* has been suggested as being predictive of periodontal treatment outcome. We analysed the presence of *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Prevotella intermedia* in 55 deep periodontal pockets of 29 patients (18 men, 11 women, 37–75 years) before and after periodontal treatment. At baseline and after treatment, 62% and 33%, respectively, of the subjects presented with 1, 2, or a combination of all 3 pathogens. The mean pocket depth of 6.6 mm (0.4 mm) before treatment decreased to 2.2 mm (0.4 mm) in response to treatment ($P < 0.001$). The treatment plan of non-surgical or surgical treatment was based on pocket depths and tooth morphology only. No antimicrobial medications were used during the treatment. Eighty-two percent of the deep pockets healed satisfactorily to $\leq$4 mm. The presence of *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, or *Prevotella intermedia* at baseline was not associated with the outcome of the periodontal therapy. In conclusion, we found that the presence of the 3 periodontopathogen species had little or no value in predicting healing of periodontal pockets. □ *Adult periodontitis periodontopathogens; periodontal therapy; periodontal microbes; treatment failure*

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It has been shown previously that non-surgical periodontal treatment, including blind removal of root deposits and pocket epithelium, may lead to successful healing of periodontal pockets (1). However, in the case of deep pockets and difficult root surfaces, surgical treatment is more effective (2). Thus depth and morphological complexity of the pocket have been the criteria when selecting between non-surgical and surgical treatment (3). It has recently been suggested that not only the depth and morphology of the pocket but infection with *Actinobacillus actinomycetemcomitans* at baseline may be predictive of success of non-surgical treatment (4).

The aim of the present study was to analyse whether the initial level of the 3 best-known periodontopathogen species in untreated pockets has an association with treatment outcome.

Material and methods

In this study, we re-evaluated the significance of periodontopathogens with regard to treatment in a teaching set-up at our dental school. Twenty-nine adult patients (18 men, 11 women, 37–75 years) referred for treatment to the Institute of Dentistry, University of Turku, agreed to participate. Baseline examinations included recordings of medical and dental history, a full periodontal status, and a panoramic radiograph. All patients were diagnosed to have chronic adult periodontitis. The periodontal bone loss was classified as mild, moderate, or advanced (5). There were two patients with mild, 24 with moderate, and 3 with advanced bone loss. The 2 deepest pockets from each patient ($n = 58$) were selected for microbial sampling. Because of poor prognosis, 3 teeth from 3 different patients were extracted soon after initial examination. Baseline treatment included oral hygiene instructions, motivation, meticulous scaling, and curettage when needed (6). Deep pockets (>6 mm) in furcation areas or on distal surfaces of the 2nd and 3rd molars ($n = 17$) were treated operatively using the modified Widman flap procedure (7). No grafting, guided tissue regeneration techniques, or systemic antimicrobial therapy were used in any of the cases. All treatment was carried out by undergraduate students under the close supervision of specialists in periodontology.

Subgingival microbial samples were collected at baseline and at the end of treatment with sterile curettes as described (8). Collection, transport, and culturing of bacterial samples were carried out as recommended for clinical use. The samples were transferred to 500 µl of anaerobic transport medium containing 5% glycerol as a cryoprotectant before analysis and stored at −20°C for 1 week at most before culturing. After thawing, the subgingival plaque samples were dispersed and 20 µl aliquots were serially diluted in peptone water and plated on non-selective Brucella agar (BBL Micro-biology Systems, Cockeysville, MD, USA) enriched with 5% defibrinated horse blood, 5 µg/ml haemin, and 10 µg/ml

Table 1. The distribution of pockets infected with the periodontopathogens or their combinations before and after treatment according to treatment results. *Three of the after-treatment samples were lost during processing.

Pathogens or their combinations	P.g.[1]	P.i.[2]	A.a.[3]	P.g. + P.i.	P.g. + A.a	A.a. + P.i.	P.g. + A.a. + P.i.	Subtotal	ND[4]	Total
Before treatment										
Healed	10	2	6	3	2	1	3	27	18	45
Failed	2	1	2	1	1	0	0	7	3	10
Total	12	3	8	4	3	1	3	34	21	55
After treatment *										
Healed	1	5	3	1	1	0	0	11	34	45
Failed	0	1	2	1	2	1	0	7	3	10
Total	1	6	5	2	3	1	0	18	37	55

[1] P.g. = Porphyromonas gingivalis.
[2] P.i. = Prevotella intermedia.
[3] A.a. = Actinobacillus actinomycetemcomitans.
[4] ND = None of the studied periodontopathogens exceeded the limit of detection.

menadione. The plates were incubated for 7 days at 37°C under anaerobic conditions to allow development of dark colony pigmentation. On the Brucella blood agar plates, total colony forming unit (CFU) counts and percentages of black pigmented bacteria were assessed. Identification and quantification of *Porphyromonas gingivalis* and *Prevotella intermedia* were performed according to Slots (9). In order to quantify the number of *Actinobacillus actinomycetemcomitans*, an aliquot of 20 µl of the plaque samples was cultured on TSBV-agar plates (Trypticase-soy agar, BBL Microbiology Systems, Cockeysville, MD USA) with 75 µg/ml of bacitracin and 5 µg/ml of vancomycin (10). After incubating 5 days in anaerobic conditions, the number of *Actinobacillus actinomycetemcomitans* was identified using colony morphology and the results were confirmed with a catalase test. In doubtful cases a MUG test (hydrolysis of 4-methylumbelliferyl-β-D-galactoside) was performed to exclude other strains of similar morphology. The counts of *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Prevotella intermedia* were compared to the total CFU counts in order to determine the proportions of the species. The sites were considered infected if the proportion of the periodontopathogens met the criteria of Slots and Listgarten (11). *Actinobacillus actinomycetemcomitans* >0.01%, *Porphyromonas gingivalis* >0.1%, and *Prevotella intermedia* >2.5%.

Chi-square was used to evaluate the significance of associations between pre- and post-treatment microbial variables and treatment results using both pockets and subjects as the unit of analysis. For the latter analysis, the less good periodontal site of the two alternatives was used. The ability of each microbial assay to assess healing prospectively or identify unsuccessfully healed sites is described in terms of sensitivity, specificity, accuracy, positive and negative predictive values. Relative risk values, i.e. risk ratios and the lower and upper limits of the 95% confidence level are included to evaluate the significance of the microbial assays regarding healing. For statistical evaluation of changes in periodontal pocket depth, ANOVA for repeated measures was used.

Results

After treatment, 28 pockets were less than 4 mm deep, 17 were 4 mm, and 10 were deeper than 4 mm. The first 2 groups combined are referred to as the "Healed" group ($n_{pockets} = 45$, $N_{subjects} = 19$), while the group with deeper postoperative pockets is called the "Failed" group ($n_{pockets} = 10$, $N_{subjects} = 10$) in this report. Eighty-two percent of the pockets and 66% of the subjects healed to a probing depth of 4 mm or less. The mean pocket depth (SE) of 6.6 mm (0.4 mm) before treatment reduced to 2.2 mm (0.4 mm) after treatment ($P < 0.001$, ANOVA for repeated measures).

Among the pockets revealing only 1 of the 3 cultured pathogens, those infected with *Porphyromonas gingivalis* or *Actinobacillus actinomycetemcomitans* alone were more frequent than *Prevotella intermedia* growth (Table 1). The pathogen combination of *Porphyromonas gingivalis* and *Prevotella intermedia* was found in 4 pockets, while the combination of *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* was detected in 3 (Table 1). At baseline, 3 pockets were infected with all 3 pathogens (Table 1). The number of cases infected with *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* after treatment decreased within the successfully healed group, but remained the same in the unsuccessfully healed counterparts, while those revealing *Prevotella intermedia* growth seemed to increase after treatment (Table 1). Initial infection of the deepest pockets with the periodontopathogens was not associated with success of the periodontal therapy ($P = 0.244$, Chi square). The proportion of pockets infected with 1, 2, or 3 of the studied species decreased from the initial 62% to 33% as a result of the treatment ($P < 0.05$, Chi square).

The capability of each periodontopathogen to predict healing prospectively, or evaluate the risk of unsuccessfully healed sites to harbor periodontopathogens postoperatively, is shown in Table 2. Apart from the relatively high negative predictive values (Npv) at baseline, all other values failed to meet the conditions of a clinically acceptable prediction test (Table 2).

Seventy-five percent of our participants were non-

Table 2. Distribution of pockets and subjects (N) infected with P.g., P.i. or A.a, before and after treatment into successfully and unsuccessfully healed pockets.The ability of each microbial assay before treatment to assess healing prospectively, or identify unsuccessfully healed sites after treatment is described in terms of sensitivity (Sn), specificity (Sp), accuracy (A), positive (Ppv) and negative predictive values (Npv). Relative risk values i.e. risk ratios (RR), and the 95% confidence limits (CL) are included. *Chi-square.

	Diagnosis										
	Failed	Healed		p*	Sn	Sp	A	Ppv	Npv	RR	95% CL
Before treatment											
P.g.											
Yes	4 (4)	17 (6)	Pockets	n.s.	0.40	0.60	0.57	0.19	0.81	1.02	0.29–3.60
No	6 (6)	26 (13)	Subjects	n.s.	0.40	0.68	0.59	0.40	0.68	1.27	0.36–4.49
P.i.											
Yes	2 (2)	9 (2)	Pockets	n.s.	0.20	0.79	0.68	0.18	0.81	0.95	0.20–4.50
No	8 (8)	34 (17)	Subjects	n.s.	0.20	0.89	0.66	0.50	0.68	1.56	0.33–7.36
A.a.											
Yes	3 (3)	12 (5)	Pockets	n.s.	0.30	0.73	0.65	0.20	0.83	1.14	0.30–4.42
No	7 (7)	33 (14)	Subjects	n.s.	0.30	0.74	0.59	0.38	0.67	1.13	0.29–4.35
After treatment[1]											
P.g.											
Yes	3 (3)	3 (1)	Pockets	0.0385	0.30	0.93	0.81	0.50	0.93	3.36	0.87–12.98
No	7 (7)	40 (15)	Subjects	n.s.	0.30	0.94	0.69	0.75	0.68	2.36	0.61–9.12
P.i.											
Yes	3 (3)	6 (3)	Pockets	n.s.	0.30	0.86	0.75	0.33	0.84	2.10	0.54–8.10
No	7 (7)	37 (13)	Subjects	n.s.	0.30	0.81	0.62	0.50	0.65	1.43	0.37–5.52
A.a.											
Yes	5 (5)	4 (1)	Pockets	0.0015	0.50	0.91	0.84	0.55	0.89	5.11	1.48–17.66
No	5 (5)	41 (15)	Subjects	0.0078	0.50	0.94	0.69	0.83	0.75	3.32	0.96–11.51

[1] Three of the after-treatment samples were lost during processing.

medicated periodontal patients with good general health. Sixty-five percent were smokers and there were no differences between the patients with healed and failed pockets in this respect.

Discussion

We found no association between treatment failure and baseline infection with the known periodontopathogens. Interestingly, only 3 of our 10 unsuccessfully healed cases were *Actinobacillus actinomycetemcomitans* infected at baseline. Therefore, we cannot support the view that the prognosis of deep periodontal pockets infected with *Actinobacillus actinomycetemcomitans* would be poor (4). Quite the contrary, we believe that it is premature to label a case with poor prognosis because of baseline detection of any known periodontopathogens. At baseline, twice as many pockets were infected with *Porphyromonas gingivalis* than with *Prevotella intermedia*; this proportion changed substantially following treatment. Further, the proportion of pockets infected with *Porphyromonas gingivalis* decreased more than the proportion infected with *Prevotella intermedia*. This qualitative change may well reflect changes from progressive to non-progressive (12), or from active to inactive (13) adult periodontitis after treatment.

The recovery rates of the pathogens studied may be influenced by the sampling technique applied. Compared to the paper-point technique, the scaler method is known to affect the total CFU count and especially the number of spirochetes (14). However, earlier comparisons made in our laboratory showed that the scaler technique was superior to the paper-point technique (8). Also the storage of samples at −20°C has undoubtedly reduced the number of viable bacteria, but freezing is the method of choice if culturing cannot be carried out on a daily basis (8, 15).

In conclusion, we found no association between successful periodontal healing and baseline infection with the known periodontopathogens. Therefore, microbial sampling and detection of periodontal pathogens in untreated pockets seem to have little if any prognostic value. The assay of periodontopathogens could be useful when conventional periodontal therapy fails to achieve the desired results.

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