

Commercial DNA Probes Reveal Colonization Patterns of Periodontopathogens in Chronic Periodontitis

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In the present study, detection patterns of subgingival *Porphyromonas gingivalis* (*Pg*), *Prevotella intermedia/nigrescens* (*Pi/n*), *Tannerella forsythia* (*Tf*) and *Treponema denticola* (*Td*) were analysed, using commercially available species-specific DNA probes (DMDX/PathoTek, Switzerland) in 16 untreated periodontitis patients (5–7 non-adjacent sites per subject) before and one month after mechanical non-surgical periodontal therapy. The detection patterns of the pathogens at the two time points were studied using configural frequency analysis (CFA) and Stuart-Maxwell χ^2 test. All subjects showed favourable clinical treatment responses. Prevalence of all microbes followed was significantly reduced after treatment. CFA identified: (i) two statistically significant configural types at both time points: co-detection of *Pg*, *Pi/n*, *Tf*, *Td*, and none of the four pathogens detectable (Holm's criteria $p = 0.003$); and (ii) one statistically significant configural anti-type post-treatment: reduced detection pattern of *Pi/n* alone (Holm's criteria $p = 0.004$). For many of the pre-treatment sites with *Tf*, *Pg*, *Pi/n*, and *Td* configural type, all these periodontopathogens were undetectable one month after treatment ($p < 0.001$, McNemar's post hoc analysis with Bonferroni adjustment). Within the limitations of this study, the results indicated a co-detection of subgingival periodontopathogens *Pg*, *Pi/n*, *Tf*, and *Td*. Analysis of the subgingival colonisation of these microbes might be a useful adjunct in the assessment of periodontal therapeutic outcomes.

Key words: Dental plaque/microbiology, periodontitis/microbiology, periodontitis/therapy, statistical model

INTRODUCTION

Chronic periodontitis is an infectious disease resulting from intricate host–parasitic interactions at the soft-hard tissue border of the tooth-supporting apparatus (American Academy of Periodontology, 1996). The role of specific periodontopathogens in this disease process has been noted, dating back to the late 1970s (Socransky and Haffajee, 1994). Culture-based and molecular genetic techniques are frequently used to detect the fastidious micro-organisms that colonise the subgingival sites

in humans (Zambon, 1996). Various commercial test systems or laboratories are in operation in different parts of the world for detection of periodontopathogens (Conrads, 2002). In view of the possible immense complexity of the microbial etiology of periodontal diseases, attention has been directed towards studying the interactions between members of the subgingival microbial biofilm (Simonson et al, 1992). Research evidence accumulated in recent years has pointed towards certain periodontopathogens tending to co-exist in subgingival niches of subjects with various perio-



Table 1 Global K-th order log-linear analysis

Marginals fitted	Residual chi-squares			Component chi-squares		
	df	L	p	df	L	p
Baseline						
<i>Pg, Pi/n, Tf, Td</i>	11	173.490	0.000			
<i>Pg + Pi/n, Pg + Tf, Pg + Td, Pi/n + Tf, Pi/n + Td, Tf + Td</i>	5	17.458	0.004	6	156.032	< 0.001
<i>Pg + Pi/n + Tf, Pg + Pi/n + Td, Pg + Tf + Td, Pi/n + Tf + Td</i>	2	0.003	0.955	3	17.455	< 0.001
<i>Pg + Pi/n + Tf + Td</i>	0	0	1	2	0.003	> 0.999
One month post non-surgical therapy						
<i>Pg, Pi/n, Tf, Td</i>	11	149.571	0.000			
<i>Pg + Pi/n, Pg + Tf, Pg + Td, Pi/n + Tf, Pi/n + Td, Tf + Td</i>	5	1.993	0.850	6	147.578	< 0.001
<i>Pg + Pi/n + Tf, Pg + Pi/n + Td, Pg + Tf + Td, Pi/n + Tf + Td</i>	2	0.003	0.9593	3	1.990	< 0.75
<i>Pg + Pi/n + Tf + Td</i>	0	0	1	2	0.003	> 0.999

dental diseases (Socransky et al, 1998; Jin et al, 1999).

Various statistical analysis protocols have been applied to the processing of subgingival microbial data (Cohen, 1993; Ali et al, 1994; Socransky et al, 1998). Many studies employed statistic protocols focusing on two-by-two contingency, correlation or cluster analysis of target species. Configural frequency analysis (CFA) has been recommended as an appropriate statistical test, whereby configurations of the target micro-organisms in the subgingival environment (present, absent or in various combinations) could be potentially studied (Cohen, 1993). The main objective of CFA is to search for types and antitypes in the data. Types are the combinations of variables that exhibit frequencies (observed) that are statistically more frequent than what would be expected. Antitypes are the combinations of the variables that exhibit frequencies (observed) that are statistically less frequent than what would be expected. These types and antitypes draw investigators' attention to exceptional microbial combinations.

While cluster analysis is another commonly used statistical method for handling data of a large number of oral microbial variables (Socransky et al, 1998), the methodology has inherent limitations. The optimistic assumption, which is also the limitation of the method, is that clusters must be formed. Cluster analysis method ignores the possibility of, and cannot identify, disease-causing agents that may prefer not to form clusters with other agents, i.e. the anti-type(s). However anti-type(s) could be detected by CFA. Furthermore, in cluster analysis, the statistical significance of the assumed clusters is not directly tested (Cohen, 1993). In more recent years, researchers have taken the monitoring of periodontopathogens forward by investigating the concurrent clinical conditions and markers of host responses in periodontitis patients (Jin et al, 1999, 2002; Darby et al, 2001) and by studying the relationship between prediction and assessment of periodontal therapeutic outcomes and the detection of combinations of periodontopathogens at various stages of periodontal therapy (Fujise et al, 2002).

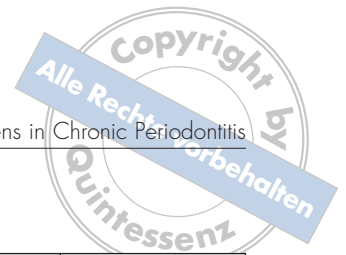


Table 2 First-order configurations (model: *Pg*, *Pi/n*, *Tf*, *Td*) at baseline

Configurations ^a				Observed frequency	Expected frequency	z	p	Holm's Criteria p	
<i>Pg</i>	<i>Pi/n</i>	<i>Tf</i>	<i>Td</i>						
-	-	-	-	19	1.30	-	0.0000	0.0031	type
+	-	-	-	3	3.51	-	1.0643 ^b		
-	+	-	-	1	1.53	-	1.0924 ^b		
-	-	+	-	0	2.05	-	0.2510 ^b		
-	-	-	+	1	2.80	-	0.4519 ^b		
+	+	-	-	0	4.14	-	0.0287 ^b		
+	-	+	-	4	5.53	0.893	0.3720		
+	-	-	+	7	7.54	0.397	0.6916		
-	+	+	-	0	2.42	-	0.1717 ^b		
-	+	-	+	0	3.30	-	0.0691 ^b		
-	-	+	+	2	4.41	-	0.3529 ^b		
+	+	+	-	0	6.52	2.861	0.0042	> 0.0036	
+	+	-	+	2	8.89	2.619	0.0088		
+	-	+	+	3	11.87	2.776	0.0055		
-	+	+	+	0	5.20	2.580	0.0099		
+	+	+	+	43	14.01	8.475	0.0000	0.0033	type

^a "+" = detectable, or "-" = not detectable by DNA probes

^b p-level computed using exact binomial

The current study used CFA in studying subgingival microbial data obtained through the application of a commercially available DNA probe method. DNA probes have the benefits of targeting a limited range of species with reasonable sensitivity and specificity, and the commercial availability of this testing method facilitates its use in clinical practice. The microbes monitored were four frequently targeted periodontopathogens, namely *Porphyromonas gingivalis* (*Pg*), *Prevotella intermedia/nigrescens* (*Pi/n*), *Tannerella forsythia* (*Tf*), and *Treponema denticola* (*Td*).

These microbes were studied in subgingival plaque samples of chronic periodontitis patients before and one month after non-surgical mechanical periodontal therapy. The null hypotheses of the current study were: (i) detection of each of the four target periodontopathogens in subgingival niches of chronic periodontitis patients would be independent of each other; and (ii) non-surgical mechanical periodontal therapy would not alter the detection frequencies and, in turn, the configurations of the four periodontopathogens studied.



STUDY DESIGN AND RESULTS

Subjects, Sampling and Examination of Samples

Microbiological samples from 85 subgingival sites of 16 untreated Chinese adults (4 females, 11 smokers) aged 32–55 years (mean 43.4 ± 7.0 years) with chronic periodontitis were studied. Ethical Committee approval was granted for this treatment study, and no untreated control group was considered. Clinical and other findings (Jin et al, 2002) have been published. From each subject, 5–7 non-adjacent sites were sampled: 3–4 periodontitis sites [probing depth (PD) ≥ 5.0 mm, attachment loss (AL) ≥ 3.0 mm, radiographic crestal bone loss (BL) present], 1–2 gingivitis sites [gingival index (GI) ≥ 2 , positive bleeding on probing (BOP), PD ≤ 4.0 mm, AL ≤ 1.0 mm, BL absent], and 1 clinically healthy site [GI ≤ 1 , BOP absent, PD ≤ 3.0 mm, AL ≤ 1.0 mm, BL absent]. Samples were collected using a sterile, medium-sized paper point (Johnson and Johnson, New Brunswick, NJ, USA), which was gently inserted into the depth of the sampling site after drying the tooth and careful removal of all supragingival plaque. The paper point was left in place for 10 seconds and then was stored dry, in labelled sterile vials at -20°C . The microbiological samples were shipped chilled (4°C) in batches, within 4 weeks after sampling, for laboratory analysis (DMDx/PathoTek, ANAWA Laboratories, Zürich, Switzerland) for *Pg*, *Pi/n*, *Tf* and *Td* using four species-specific DNA probes. *Tf* was identified by a recombinant DNA probe, while for *Pg*, *Pi/n* and *Td* genomic probes were used (Conrads, 2002). The *Pi* probe, as indicated in materials provided by DMDx/PathoTek, cross-reacts with 'Type II *Pi*', i.e. the current *Pn* ATCC-33563-type strain. Therefore, positive detection by this probe would be regarded as detection of *Pi/n*. The lowest detection levels of the commercial DNA probes used in the current study are 1000 cells/sample (French et al, 1986). Following sampling, each patient received a course of non-surgical periodontal therapy within a 6-week period as described previously (Jin et al, 2000); clinical details about the treatment responses in these patients appeared in a previous report (Jin et al, 2002). No antibiotics were prescribed and no patient reported, in response to questioning, antibiotic use for medical reasons over the treatment period. Subgingival plaque samples were collected from

the same 85 sites one month after the completion of the non-surgical periodontal therapy, and these samples were handled and analysed by the same methods.

Statistical Analysis

The microbial data were pooled. The computational unit was the sampling site. Stuart-Maxwell χ^2 test (Fleiss and Everitt, 1971) was used to analyse the change of microbial profiles, i.e. positive detection or not, of the four target species *Pg*, *Pi/n*, *Tf* and *Td* alone and in all possible combinations ($n = 16$, see Table 2) at baseline and one month post non-surgical therapy. Post hoc analysis based on McNemar's statistic with Bonferroni adjustment was carried out to test for significant microbial changes from baseline to one month post-treatment. The level of significance chosen was 0.05.

CFA was then applied to the data set (Cohen, 1993). The protocol described by von Eye (1990) was followed. A global K-th order log-linear analysis was performed to search for the appropriate statistical types. SPSS 11.0 for Windows software was used to calculate the expected frequency and component χ^2 p-value for the marginals fitted for models for CFA analysis. In brief, the first set of marginals fitted describes the model for mutual independence among all variables (the first-order model). The second, third and fourth models test, respectively, models based on two-, three-, and four-way interactions among the variables (Cohen, 1993). The statistical types were selected, based on the component of χ^2 p-value, and these were then put through the corresponding configuration analysis according to which types and/or anti-types were identified.

The configuration analysis was conducted as follows. Firstly, the observed periodontopathogen configurations were put through a log linear regression analysis, based on the appropriate model detected, the model(s) with component of χ^2 p-value < 0.05 . Then the expected frequency values for the different configurations were obtained. Based on that, the absolute z and then the p-values of the configurations were calculated. For situations where $|z|$ could not be calculated, exact binomial approach using the observed and expected frequencies was employed to calculate the p-value (Cohen, 1993). The final p-values of the CFA were measured using Holm's Criteria (Cohen,



1993). A significant configural type was a configuration with a significant Holm's Criteria p with larger observed than expected frequency, while the contrary would be a significant anti-configural type, or anti-type.

Clinical Parameters

The clinical parameters of the subjects before and one month after the non-surgical periodontal therapy were as described earlier (Jin et al, 2002). In brief, the subjects had a mean of 26.0 ± 2.3 teeth, with $34.8 \pm 18.9\%$, $44.9 \pm 13.7\%$ and $20.3 \pm 13.1\%$ healthy, gingivitis or periodontitis sites respectively. They showed favourable treatment responses to the single course of non-surgical periodontal therapy in terms of significant reductions of plaque levels ($90.3 \pm 13.9\%$ to $58.5 \pm 34.1\%$, $p < 0.01$), BOP ($70.2 \pm 20.1\%$ to $44.0 \pm 25.4\%$, $p < 0.01$), overall mean probing pocket depth (PPD) reduction (from 4.8 mm to 3.8 mm) and PPD reduction in sampling sites (from 5.7 mm to 4.5 mm, $p < 0.01$).

Microbial Parameters

The prevalence of *Pg*, *Pi/n*, *Tf* and *Td* was significantly ($p < 0.001$, Fisher exact test) reduced after treatment: 72.9%/35.3%, 55.3%/28.2%, 62.4%/17.6% and 68.2%/20.0% (baseline/one month after treatment respectively). Study of the profiles of *Pg*, *Pi/n*, *Tf* and *Td* for individual subgingival sites at baseline and one month after non-surgical therapy revealed 11 out of the possible 16 patterns. In brief, patterns of detection of *Tf* alone and *Pi/n* + *Tf*, *Pi/n* + *Td*, *Pg* + *Pi/n* + *Tf* and *Pi/n* + *Tf* + *Td* co-detection were not observed at either time points. The overall Stuart-Maxwell χ^2 value of the 11×11 table ($df = 10$) was 46.77, which is considered significant, indicating a change due to treatment. When McNemar's post hoc analysis was carried out, with Bonferroni adjustment, overall sites with none of the four species detectable were significantly increased one month after non-surgical treatment, principally because most sites that at baseline had *Pg*, *Pi/n*, *Tf* and *Td* detected were found at one month after treatment to have none of these detectable ($p < 0.001$). The proportions of sites, grouped by pre-treatment clinical condition, which had none of *Pg*, *Pi/n*, *Tf* and *Td* detected at baseline and at one month post-treatment were as follows: periodontitis sites, 3/54 to 26/54

($p < 0.001$, Fisher exact test); gingivitis sites, 5/17 to 13/17 ($p = 0.015$, Fisher exact test); healthy sites, 11/14 to 13/14 ($p = 0.6$, Fisher exact test).

The global K -th order log linear analysis of the baseline bacterial data showed that the first- and the second-order, independent or two-way interactions among the variables would be appropriate statistical types. While for the one-month post- non-surgical therapy data, only the first-order, the independent model, would be the appropriate statistical type to be tested (Table 1).

As shown in Table 2, two significant statistical microbial configural types were identified, co-detection of *Pg*, *Pi/n*, *Tf* and *Td* or none of *Pg*, *Pi/n*, *Tf* and *Td* being detectable. The same situation was observed upon first-order configuration analysis of data one month after non-surgical therapy (Table 3). From the same analysis, a significant statistical microbial configural anti-type of detection of *Pi/n* alone was observed. The results indicated preferential detection patterns of all of *Pg*, *Pi/n*, *Tf* and *Td* being detected or all these four species being undetectable. While non-surgical periodontal therapy resulted in many sites with *Pg*, *Pi/n*, *Tf* and *Td* at baseline becoming negative for detection by the commercial DNA probe approach employed for all four species at one month, the two detection patterns of all four or none appear to be preferred both before and one month after therapy. However, at one month after non-surgical therapy, detection of *Pi/n* alone in subgingival plaque samples was not preferred. No more statistically significant configurations could be identified when second-order configuration analysis of data at baseline was incorporated into the model (results not shown).

For comparison, the present set of data was also subjected to cluster analysis (Socransky et al, 1998), in which the similarity between pairs of the four species was computed using a ϕ coefficient, the coefficients were scaled and then sorted using an average unweighted linkage sort. In brief, both dendrograms of cluster analysis pre- and one month post-treatment showed all four species forming one cluster with similar levels of higher or equal to 80%. The clustering profiles were: (i) pre-treatment - both *Pg* and *Td* or *Pi/n* and *Tf* similarity of 86%, overall similarity of 85%; and (ii) one month post-treatment - *Pg* and *Pi/n* similarity of 92%, *Tf* and *Td* similarity of 90%, overall similarity of 80%.



Table 3 First-order configurations (model: *Pg, Pih, Tf, Td*) at one month post non-surgical therapy.

Configurations ^a				Observed frequency	Expected frequency	z	p	Holm's Criteria p	
<i>Pg</i>	<i>Pi/n</i>	<i>Tf</i>	<i>Td</i>						
-	-	-	-	52	26.0	6.120	0.0000	0.0033	type
+	-	-	-	5	14.18	2.671	0.0076	> 0.0038	
-	+	-	-	1	10.23	3.077	0.0021	0.0036	antitype
-	-	+	-	0	5.57	2.661	0.0078		
-	-	-	+	1	6.50	2.449	0.0143		
+	+	-	-	9	5.58	1.279	0.2010		
+	-	+	-	1	3.04	-	0.8121 ^{b,c}		
+	-	-	+	0	3.55	-	0.9734 ^{b,c}		
-	+	+	-	0	2.19	-	0.8913 ^{b,c}		
-	+	-	+	0	2.56	-	0.9257 ^{b,c}		
-	-	+	+	1	1.39	-	0.8116 ^b		
+	+	+	-	0	1.20	-	0.7014 ^{b,c}		
+	+	-	+	2	1.40	-	0.3306 ^b		
+	-	+	+	1	0.76	-	0.3530 ^b		
-	+	+	+	0	0.55	-	0.0099 ^b		
+	+	+	+	12	0.30	-	0.0000 ^b	0.0033	type

^a "+" = detectable, "-" = not detectable

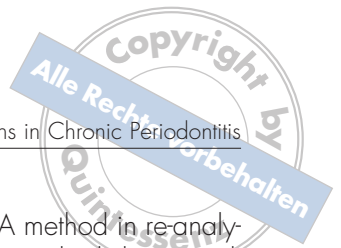
^b p-level computed using exact binomial

DISCUSSION

Genomic DNA probes (against *Pg, Pih, Td*) and recombinant DNA probes (against *Actinobacillus actinomycetemcomitans, Campylobacter rectus, Eikenella corrodens, Fusobacterium nucleatum* and *Tf*) have been widely used commercially to detect periodontopathogens in subgingival plaque in Europe, the USA and the rest of the world (e.g. Ali et al, 1994; Conrads, 2002; French et al, 1986; Jin et al, 1999, 2002; Porras et al, 2002). For instance, in 2000, about 15000 DNA-probe-based tests for periodontopathogens were performed in Germany and Switzerland (Conrads, 2002) for the guidance of periodontal

treatment. Therefore, information on the use and interpretation of results of this widely used test method would be helpful. The present report focused on a clinical approach of utilising the commercially available and widely used DNA probes for detection of periodontopathogens to explore how data generated from these tests may be used with respect to bacterial combinations to assist in the clinical management of various forms of periodontitis.

The lowest detection levels for the various DNA probes used in this study were similar to those of the DNA-DNA checkerboard hybridisation technique (Socransky et al, 1994) and the quantitative real-time PCR technique (Asai et al, 2002). The



DNA-DNA checkerboard approach can, however, distinguish between *Pi* and *Pn* (Socransky and Haffajee, 1994). Within healthy American subjects, the mean prevalence and levels of *Pi* and *Pn* at subgingival sites were similar. *Pi* and *Pn* have greater, but similarly equal, prevalence at higher quantities in American periodontally diseased subjects (Haffajee et al, 1998). However, a highly specific PCR study on 97 samples from 31 Scottish periodontitis subjects showed a similar prevalence to periodontitis American subjects of *Pi* at 40% but not of *Pn* (Riggio et al, 1998). It seems as if the preferred 'gold standard' detection method for *Pn* and this microbe's role in periodontitis are issues yet to be clarified. The present study analysed the detection of *Pi* and/or *Pn* as a group, i.e. *Pi/n*.

A recent review concluded that endodontic paper point sampling may under-represent the microbes at the base of a periodontal pocket, while curette sampling would better represent the bacteriology of the entire pocket (Loomer, 2004). The same article also drew attention to the variability in microbiology among different pockets of the same individual, which remains an unresolved issue in microbial monitoring of periodontitis patients, unless full-mouth sampling is conducted. Multiple samples from 3–4 pockets were collected in the present study. Higher proportions of putative pathogenic bacteria have been reported to be detected using paper point sampling compared with curette sampling (Renvert et al, 1992). Thus the paper point sampling technique employed should maximise the chances of the detection of the four target species.

Van der Velden and co-workers (2003) reported that smokers and non-smokers with chronic periodontitis had similar prevalence of 7 periodontopathogens, while one similar study reported, among the 5 periodontopathogens followed, higher pre-treatment prevalence of *Tf* and *Td* in smokers (Darby et al, 2005). Both studies reported that in smokers the prevalence of *Tf* remained significantly higher post-therapy with an increased prevalence of *Pi* in smokers post-treatment (van der Velden et al, 2003). The fact that 11/16 (69%) of the study cohort were smokers might imply that *Tf* and perhaps *Pi* might be more readily detectable one month after non-surgical therapy. The results of the present study should therefore be interpreted with full awareness of its limitations and the composition of its subjects.

Cohen (1993) employed CFA method in re-analysis of published subgingival microbial data in culture (Slots et al, 1986) and culture-independent (Simonson et al, 1992) studies. The anaerobic culture study showed that the presence of *Pg* (*Bg*, previously *Bacteroides gingivalis*) alone was preferred in samples from subjects with "untreated- or treated-progressive" periodontal disease, whereas the absence of *A. actinomycetemcomitans*, *Pg* and *Pi* (*Bi*, previously *Bacteroides intermedius*) configuration was not preferred (Cohen, 1993). On the contrary, absence of *A. actinomycetemcomitans*, *Pg* and *Pi* was preferred in samples from patients with "no-disease or treated-non-progressive periodontal disease". The culture (Slots et al, 1986), ELISA (Simonson et al, 1992) and DNA probe (present study) approaches in periodontal microbiological testing, as discussed by Aass et al (1994), all probably do not share the same detection levels and each would have evidenced its own inherent limitations for the detection of microbes studied.

A preferred combination of *Pg* and *Tf* was demonstrated by use of monoclonal antibodies and a very sensitive indirect immunofluorescence technique (Gmür et al, 1989). Socransky and co-workers (1998) published a study using the checkerboard DNA-DNA hybridization detection system for detection of bacteria in microbial samples collected from every mesiobuccal site in healthy subjects and periodontitis patients. A total of 13261 samples were processed and cluster analysis was employed. CFA was used in a focused fashion only to confirm the data from individual elements of the cluster analysis. In theory, CFA analysis should preferably be carried out before the cluster analysis. Nevertheless, a preferred configuration of *Pg*, *Tf* (*Bf*, previously *Bacteriodes forsythus*) and *Td*, the so called 'red complex', in subjects with periodontal disease was reported. The relatedness of these three species to *Pi* or *Pn*, as shown by the same study, was slightly higher than 40%. The 'red complex' has been extensively reviewed (Holt and Ebersole, 2005), but the co-detection of only the three periodontopathogens of this complex may not be the only predominant detection pattern. The relatedness of the four periodontopathogens identified in the current group of patients pre- and post-therapy, as shown by CFA or cluster analysis, was high. *Pi/n* appeared to relate closer to *Pg*, *Tf* and *Td* in plaque samples of



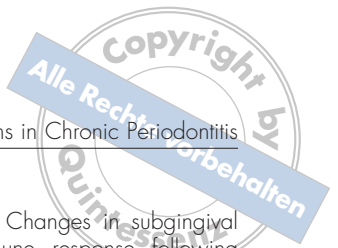
this group of predominantly male and smoking Chinese subjects than in the American cohort reported by Socransky et al (1998). What could not be observed by cluster analysis alone was the configural type of the non-detectability of *Pg*, *Pi/n*, *Tf* and *Td* at both time points and the non-preferred detection of *Pi/n* alone one-month post non-surgical periodontal therapy.

The present study dealt with both pre- and post-therapy microbial detection profiles of four putative periodontopathogens in an attempt to understand the nature of interactions among these pathogens as members of the subgingival biofilm before and following mechanical periodontal treatment, along with the changes in local environment consequent to therapy, such as reductions in pocket depths. Darby and co-workers (2001) showed that in 33 untreated adult periodontitis patients, scaling and root planing could produce statistically significant reductions of the prevalence of *Pi*, *Tf* and *Td*, detected by the PCR method, in 132 periodontitis sites studied. The frequency of *Td* post-therapy was reduced but not statistically significantly. The present study showed that mechanical therapy was successful in reducing a predominant co-detection of the four species, i.e. *Pg*, *Pi/n*, *Tf* and *Td* into a predominant nil detection of the four species. Our earlier findings suggest that persistent co-detection of the four species one month post-treatment is associated with poorer treatment outcomes (Jin et al, 2002). However, it is yet to be demonstrated if this association reflects a cause and effect relationship.

Taken together, previous studies and the present study suggest that certain periodontopathogens, particularly *Pg*, *Pi/n*, *Tf* and *Td*, if detectable in subgingival plaque samples from inflamed sites using commercially available DNA probes, tend to be co-detected, which was apparently independent of the presence of periodontal pockets, thus not substantiating the first null hypothesis of this study that the pathogens were detectable independent of each other. Interestingly, undetectable levels of pathogens were also found in untreated (and possibly non-progressive) diseased sites. The reasons for non-detection of the target pathogens might be either true absence or, more likely, presence at quantities below the detection limits, given as 1000. The non-surgical mechanical periodontal therapy seemed to produce an effect of rendering the pathogens undetectable, implying that the non-

surgical periodontal therapy could alter the detection frequencies of the four periodontopathogens. No non-treated group as a control was included, but the microbial shifts are unlikely to occur other than in response to treatment. Longitudinal microbial and clinical monitoring of diseased sites seemingly negative for the target pathogens may elucidate microbial associations and reflect the sporadic nature of periodontitis (Goodson et al, 1982). Ali and co-workers (1994), in their study using the same bacterial detection protocol, had shown that *Pg*, *Pi/n*, *F. nucleatum* and *Tf* were positively correlated in periodontally untreated Sudanese with adult chronic periodontitis. Lotufo and co-workers (1994), using a different DNA probe detection method, showed *Pg* occurrence at subgingival sites to be associated with *Tf*. A study using 16 S rRNA gene-detected multiplex PCR protocol, reported the simultaneous detection of *Pi* and *Tf* in various sites, including 38 subgingival plaque samples from untreated periodontitis patients (Conrads et al, 1999). Both species were present in 13% of the subgingival specimens, while 55% of the specimens were negative for *Pi* and *Tf*, but no statistical tests were performed to examine the association between the two bacteria. The present study revealed the co-detection of *Pg*, *Pi/n*, *Tf* and *Td* using commercially available DNA-probe analysis, further confirming the previous observations suggestive of co-existence of certain periodontopathogens, possibly because of nutritional co-dependencies or adhesion (Wyss, 1992).

The CFA method of analysing periodontopathogen-detection patterns, despite its potential to be used to promote understanding and interpretation of otherwise quite complicated microbial data, is not without limitations. The pooled microbial data of the present study was, however, sufficiently powerful to allow for analysis. Commercial periodontopathogen-detection services could potentially generate databases that could, when studied, improve understanding of the preferred co-inhabitant patterns of periodontopathogens. Knowledge of the co-inhabitant patterns of periodontopathogens in periodontally diseased and periodontally healthy conditions could be of use to clinicians in their daily practice in the treatment and especially in the delivery of supportive periodontal care of chronic periodontitis, if they use diagnostic commercial DNA periodontopathogen-detection services.



In conclusion, using a commercial DNA-probe-based detection method, the periodontopathogens *Pg*, *Pi/n*, *Tf* and *Td* were shown to exhibit a tendency to be co-detected in periodontal sites all four together or not at all. Non-surgical periodontal therapy was shown to change a large proportion of sites from being positive for *Pg*, *Pi/n*, *Tf* and *Td* to all *Pg*, *Pi/n*, *Tf* and *Td* being below detectable levels. However, in certain sites the co-detection of the four species persisted. The implications of these observations in the context of the use of commercially available DNA-probe services for detection of periodontopathogens and how results from such services may usefully inform periodontal treatment require further investigation, if possible correlating the detection patterns at sites with clinical and/or host risk or biomarker data at the same sites.

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