Acta Odontologica Scandinavica, 2010; 68: 115–121

informa
healthcare

ORIGINAL ARTICLE

Comparison of two different microbiological test kits for detection of periodontal pathogens

RALUCA COSGAREA, AMELIE BÄUMER, BERNADETTE PRETZL, SVEN ZEHACZEK & TI-SUN KIM

Department of Operative Dentistry, Section of Periodontology, University Hospital Heidelberg, Heidelberg, Germany

Abstract

Objective. The aim of this study was to compare the outcome of two different microbiological tests for detection of *Aggregatibacter actinomycetemcomitans* (A.a.), *Porphyromonas gingivalis* (P.g.), *Tannerella forsythia* (T.f.) and *Treponema denticola* (T.d.). ***Material and methods.*** A total of 69 adult patients with severe chronic (sCP) or aggressive periodontitis (AgP) participated in the study. Microbiological samples were examined for A.a., P.g., T.f. and T.d. using an RNA probe test (PADO) and a real-time polymerase chain reaction test (MERI). ***Results.*** For all periodontal pathogens under investigation, the median bacterial counts detected with PADO were smaller compared to those detected with MERI. P.g., T.f. and T.d. could be found in the majority of all patients with both tests. With MERI, A.a. was detected more often (24.6%) than with PADO (18.8%). Only 10.1% of the patients tested positive for A.a. with both tests. ***Conclusion.*** Both tests showed a high percentage of agreement for P.g., T.f. and T.d., but exhibited marked differences in the detection of A.a.

Key Words: *Gene probe test, kappa statistics, microbiology, prevalence-and-bias-adjusted kappa, periopathogens*

Introduction

Identification of target microorganisms in periodontal patients with the help of commercially available gene probe tests has become a valuable diagnostic tool to help plan an efficient adjunctive antibiotic therapy.

The establishment and progression of periodontal diseases are based on the presence of high levels of periodontal pathogens in the sulcular fluid [1]. Over 400 species of microorganism can be detected in periodontal pockets, but only a few are discussed currently in the aetiology and pathogenesis of periodontal diseases. The majority of patients diagnosed with chronic periodontitis show a favourable treatment response after mechanical therapy, which primarily aims at the decontamination of infected root surfaces. The most important constituents of anti-infective therapy are improvement of oral hygiene, scaling and root planing and periodontal surgery. Removal of the biofilm and thus reduction of the total bacterial counts in the pockets of periodontal patients by means of personal and professional plaque removal is a prerequisite to stop the progression of the disease. Nevertheless, in certain patients this treatment regime may not be sufficient to prevent further destruction of tissue and periodontal breakdown. Especially if patients are diagnosed with aggressive periodontitis, mechanical removal of plaque and bacterial biofilm does not always guarantee successful management of the periodontal infection [2]. For these patients, the use of an adjunctive antibiotic therapy may be indicated [3].

In 1996, the World Workshop in Periodontics described *Aggregatibacter actinomycetemcomitans* (A.a.), *Porphyromonas gingivalis* (P.g.) and *Tannerella forsythia* (T.f.) as microorganisms that play a key role in the pathomechanisms of periodontal destruction [4]. Since then, *Prevotella intermedia*, *Treponema denticola* (T.d.), *Fusobacterium nucleatum*, *Parvimonas micra* and *Eikenella corrodens* have been added as important periodontopathogens [2]. Identification of these microorganisms in periodontal patients with the help of commercially available gene probe tests has become a clinical valuable tool in treatment planning and in the selection of adjunctive

Correspondence: Ti-Sun Kim, Sektion Parodontologie, Poliklinik für Zahnerhaltungskunde, Mund-Zahn-Kieferklinik, INF 400, 69120 Heidelberg, Germany. Tel: +49 6221 566022. Fax: +49 6221 567054 . E-mail: ti-sun_kim@med.uni-heidelberg.de

(Received 30 June 2009; accepted 28 November 2009)

ISSN 0001-6357 print/ISSN 1502-3850 online
DOI: 10.3109/00016350903514848

antibiotics. In patients who tested positive for A.a., it could be shown that the mechanical removal of the subgingival biofilm alone did not ensure a reliable and predictable treatment outcome [5–7]. Furthermore, it was shown that A.a. plays a crucial role in the aetiology of severe chronic periodontitis, aggressive periodontitis [8–10] and periodontitis as a manifestation of Papillon Lefèvre syndrome [11]. These patients have to be managed with an adequate therapy protocol, including adjunctive administration of systemic antibiotics, as recommended by the American Academy of Periodontology (2000, 2001).

The aim of this study was to compare the outcome of two different commercially available microbiological tests which are commonly used in a clinical setting to detect A.a, P.g., T.f. and T.d. in patients with severe chronic (sCP) and aggressive periodontitis (AgP).

Material and methods

Patients

A total of 69 adult subjects participated in this prospective study. Between 2004 and 2007, patients were recruited before (n = 45) or after (n = 24) undergoing anti-infective therapy at the Section of Periodontology, Department of Operative Dentistry, University Hospital Heidelberg. To be included, patients had to fulfil the criteria for clinical diagnosis of AgP or sCP. Patients who had received antibiotic therapy within the last 6 months or who needed antibiotic prophylaxis before dental treatment were excluded. The diagnoses AgP and sCP were defined according to the International Workshop for a Classification of Periodontal Diseases and Conditions [12]. In the patients awaiting anti-infective therapy, microbiological testing was conducted to identify A.a.-positive patients, which was a prerequisite for an adjunctive antibiotic treatment. All of the patients who had received anti-infective therapy had a history of a subgingival microbiological sample that had tested positive for A.a. prior to their anti-infective treatment [IAI Pado-Test 4.5® RNA probe test kit (PADO); Institut für Angewandte Immunologie, Zuchwil, Switzerland]. In the follow-up of these patients, microbiological sampling was repeated to confirm complete eradication of A.a.

Informed written consent to participate in the study was obtained from the patients.

Clinical examinations

The periodontal parameters were assessed by a calibrated examiner (Section of Periodontology, University Hospital Heidelberg). Pocket probing depth (PPD) and vertical clinical attachment (CAL-V) were measured to the nearest millimetre using a rigid periodontal probe (PCPUNC15; Hu Friedy, Chicago, IL) at six sites per tooth. Bleeding on probing (BOP) was recorded as the percentage of teeth with signs of bleeding 30 s after probing. The cement–enamel junction (CEJ) was defined as a reference point for the assessment of CAL-V. If the CEJ had been destroyed by restorative treatment, the reference point was represented instead by the most apically located margin of the restoration. Additionally, the gingival bleeding index (GBI) [13] and plaque control record [14] were assessed at four sites per tooth.

Microbiological examination

The microbiological examination was accomplished with two commercially available test kits. With the PADO RNA probe test kit, four periodontal pathogens were detected: A.a., P.g., T.f. and T.d. This test uses oligonucleotide probes complementary to conserved fragments of the 16S rRNA gene that encodes the rRNA, which forms a subunit of the bacterial ribosome. The detection threshold of this test is 10^3 for *A.a.* and 10^4 for P.g., T.f. and T.d. The second test used was a commercially available real-time polymerase chain reaction (PCR) test [Meridol® Paro Diagnostik (MERI); Gaba GmbH, Lörrach, Germany]. The detection threshold of this test is 10^2. The real-time PCR directly records the reaction process of amplification. In addition to the specific primers, the real-time PCR uses a further species-specific DNA fragment (TaqMan probe). This TaqMan probe binds within the target sequence. During duplication of the target sequence, the TaqMan probe is split off from the target sequence and destroyed by the exonuclease activity of the Taq polymerase. In this breakdown of the probe, a fluorescent signal is released which is measured online and immediately recorded by means of automatic laser detection in the reactor vessel. The intensity of the fluorescent signal is thus a measure of the amount of the product formed, and is directly proportional to the initial amount of the periodontal pathogen in the patient samples.

Microbiological sampling was performed after assessment of the periodontal chart according to the joint statement of the German Society of Periodontology and the German Society of Dental, Oral and Maxillofacial Medicine following the manufacturer's test protocol. The four deepest pockets in four different quadrants were selected for the sampling procedure [6,15]. After removal of supragingival plaque using cotton pellets, the test site was dried and kept dry with cotton rolls. Two sterile paper points were inserted simultaneously to the bottom of the selected pocket. After 10 s the paper points were removed and placed in two separate transportation

Table I. Descriptive statistics for PPD and CAL-V of the test site teeth (MT4m). All values shown are in millimeters.

	Test site 1		Test site 2		Test site 3		Test site 4	
	PPD	CAL-V	PPD	CAL-V	PPD	CAL-V	PPD	CAL-V
Mean	7.4	7.9	7.2	7.9	6.6	6.9	6.7	6.9
SD	1.8	2.6	2.1	2.6	2.1	2.6	1.9	2.4
Min	4	2	4	3	2	3	2	2
Max	14	14	13	15	14	16	12	14

MT4: microbiological analysis of material pooled from 4 different test sites (Multi Site 4).

vials: one for the PADO test and the other for the MERI test. The paper points from the other selected pockets were placed similarly in the same/respective vials and the samples were pooled.

Statistical analysis

Sampled data were collected using a software program for table calculation (Microsoft Excel) and later transferred into a scientific statistical software program (SPSS Version 12.0; SPSS Inc., Chicago, IL) for further analysis focusing on special strategies for method comparisons. For statistical analysis, special methods to describe the extent of agreement between the two microbiological test kits were used (kappa-coefficient). Confidence intervals of kappa values were calculated with the formulas provided by Fleiss [16]. According to the suggestions of Byrt et al. [17] as well as Sim and Wright [18], prevalence and bias adjustment for kappa was done by additionally calculating prevalence-and-bias-adjusted kappa (PABAK) values.

Comparison of bacterial counts between both test kits was performed with a Wilcoxon signed rank test for paired observations, as distribution analysis revealed a significant deviation from a normal distribution for all bacterial species under investigation. For a two-tailed test, alpha was set to 0.05.

To analyse the distribution of differences between the two test kits, Bland–Altman plots including limits of agreement were calculated after logarithmic transformation of the bacterial counts (lg = log 10), referring to 10^6 counts +1 [19].

Results

Clinical data of patients

A total of 69 patients (45 females; age range 26–71 years; mean age 46.3 ± 9.9 years) were recruited for the study between 2004 and 2007. Of these patients, 49 (28 females) had been diagnosed with generalized sCP and 20 (17 females) with AgP. Mean PPD and CAL-V were 3.76 ± 2.05 and 4.2 ± 2.4 mm, respectively. Descriptive statistics for PPD and CAL-V of the test sites are listed in Table I. Mean ± SD values were 38.16% ± 20.39% for plaque control record, 11.8% ± 18.7% for GBI and 28.95% ± 22.31% for BOP.

Microbiological examination

Dichotomous classification and kappa statistics. Results of the microbiological examination were classified dichotomously into 'positive' or 'negative' samples. Table II summarizes the prevalences of positive results for all the periodontal pathogens under investigation, depending on the test kit that had been applied. For all periodontal pathogens targeted by the PADO and MERI tests, the distribution of positive and negative test results was not congruent when comparing the two kits. Thus, the number of patients who tested positive with both kits simultaneously was always lower than the prevalence of positive results with a single kit. For T.f. and T.d., 89.9% and 88.4% of all patients, respectively tested positive with both kits. For P.g., 69.6% of all patients were positive with the PADO and MERI tests simultaneously. For A.a., only 10.1% of the samples tested positive with both test kits. Unweighted kappa values (Table III) showed fair agreement for A.a. ($\kappa = 0.322$), moderate agreement for T.f. ($\kappa = 0.410$) and T.d. ($\kappa = 0.506$) and good agreement for P.g. ($\kappa = 0.689$). Analysis of subgroups depending on clinical categories ('prior to anti-infective therapy', $n = 45$; 'follow-up', $n = 24$) also revealed a higher percentage of positive test results for all microorganisms with the MERI test compared to the PADO test in both subgroups. Furthermore, in follow-up patients, prevalences of all microorganisms under investigation tended to be lower compared to those in patients prior to anti-infective therapy (Table II).

PABAK values. After adjustment for differences in prevalence and bias, PABAK values (Table III) varied from 0.536 to 0.855, indicating moderate agreement for A.a. (PABAK = 0.536), good agreement for P.g.

Table II. Agreement between PADO and MERI tests in patients who tested positive for A.a., P.g., T.f. and T.d. Values are given as numbers of patients, with percentages in parentheses.

	PADO test	MERI test	MERI and PADO tests
All patients (n = 69)			
A.a.	13 (18.8)	17 (24.6)	7 (10.1)
P.g.	50 (72.5)	54 (78.3)	48 (69.6)
T.f.	63 (91.3)	66 (95.7)	62 (89.9)
T.d.	63 (91.3)	64 (92.8)	61 (88.4)
Patients prior to anti-infective therapy (n = 45)			
A.a.	12 (26.7)	14 (31.1)	6 (13.3)
P.g.	40 (88.9)	43 (95.6)	40 (88.9)
T.f.	44 (97.8)	45 (100.0)	45 (100.0)
T.d.	43 (95.6)	44 (97.8)	42 (93.3)
Follow-up patients (n = 24)			
A.a.	1 (4.2)	3 (12.5)	1 (4.2)
P.g.	10 (41.7)	11(45.8)	8 (33.3)
T.f.	19 (79.2)	21 (87.5)	18 (75.0)
T.d.	20 (83.3)	20 (83.3)	19 (79.2)

Table III. Kappa statistics and PABAK for all pathogens under investigation.

	Unweighted κ	Standard error	95% CI
A.a.	0.322	0.149	0.031–0.613
PABAK	0.536	0.102	0.337–0.735
P.g.	0.689	0.103	0.487–0.892
PABAK	0.768	0.077	0.617–0.919
T.f.	0.410	0.254	–0.088–0.908
PABAK	0.855	0.062	0.733–0.977
T.d.	0.506	0.213	0.090–0.923
PABAK	0.855	0.062	0.733–0.977

(PABAK = 0.768) and very good agreement for T.f. (PABAK = 0.855) and T.d. (PABAK = 0.855).

Comparison of bacterial counts. Since distribution analysis revealed a significant deviation from normality, bacterial counts measured with the PADO and MERI tests were compared with a Wilcoxon signed rank test for paired observations. The results of this analysis are listed in Table IV. For all periodontal pathogens under investigation, the MERI test revealed higher median bacterial counts compared to the PADO test; the differences proved to be statistically significant for P.g., T.f. and T.d.

Discussion

The goal of this study was to compare the outcome of two different commercially available microbiological tests regarding the detection of A.a., P.g., T.f. and T.d. in patients with sCP and AgP in order to obtain further information about the reliability of these microbiological tests. Since a gold standard was not used in this study, the comparison between the two methods was only relative to each other. The inclusion of defined bacterial samples is part of a study protocol we are currently designing to find out which of the two test systems gives results closest to the correct detection.

If microbiological samples from periodontal patients are analysed, the considerable variability in prevalences of these microorganisms may also depend on the protocol of the microbiological test, which includes both the sampling and detection methods that were applied [20–23]. From study of the literature it is well known that identification of periodontal pathogens, especially A.a., can be very challenging, due to the lack of sensitivity and reproducibility of certain microbiological tests [24,25] and the fact that A.a. often invades the inflamed tissues, which means that this microorganism can be present even if it is not harvested with a

Table IV. Difference in bacterial counts between the PADO and MERI tests.

	A.a.		P.g.	
	PADO test	MERI test	PADO test	MERI test
No. of patients who tested positive	13	17	50	54
Range ($\times 10^6$)	0.09–0.447	0.00025–4.1	0.02–12.88	0.00025–71.0
Median ($\times 10^6$)	0.02	0.07	3.47	9.90
Wilcoxon test	$P > 0.05$		$P < 0.0001$[a]	
	T.f.		T.d.	
	PADO test	MERI test	PADO test	MERI test
No. of patients who tested positive	63	66	63	64
Range ($\times 10^6$)	0.09–10.1	0.0014–72.0	0.02–5.69	0.0025–35.0
Median ($\times 10^6$)	2.30	3.80	1.11	4.60
Wilcoxon test	$P < 0.0001$[a]		$P < 0.0001$[a]	

[a]Statistically significant difference.

subgingival plaque sample. The two test kits that were compared in this study show considerable differences regarding the methods for assessing estimated numbers of target bacteria in the samples. Thus, the manufacturer of the oligonucleotide probe of the PADO test claims that only living bacteria are probed, whereas the real-time PCR of the MERI test targets, and therefore counts, both living and dead bacteria. This may be one of the explanations for the fact that, with the MERI test, significantly higher bacterial counts could be found compared to the PADO test. Nevertheless, as enzymes in crevicular fluid are able to cleave and degrade nucleic acids, further bacterial compounds may flow out with the fluid. Furthermore, for the two test kits that were investigated in this study, the manufacturers give different thresholds for the lowest detection limit: for the PADO test, lowest detection limits are 10^3 (A.a.) and 10^4 (P.g., T.f. and T.d.), whereas the MERI test is labelled to be more sensitive according to the manufacturer's information (lowest detection limit for all pathogens: 10^2).

Besides the lower detection threshold, the strategy of sample drawing may have a significant impact on the test result. Thus, if two different test kits are compared, the sampling strategy has to be standardized. A generally accepted standard sampling strategy for clinical routine is to draw one sample from the deepest pockets of each quadrant, i.e. four samples per patient [6]. If the samples are taken from sites with typical signs of inflammation (BOP, suppuration) and several subgingival plaque samples are pooled, the likelihood of detection of A.a. can be increased further [6,11]. In the current study, testing for A.a. was performed with two different commercially available tests. The first microbiological assay is a commercially available oligonucleotide probe test that has been applied in a series of scientific studies [24,26–29]. The probe of this assay is complementary to conserved regions of the 16S rRNA gene, which encodes the rRNA that forms the small subunit of the bacterial ribosome. The Institut für Angewandte Immunologie, Zuchwil, Switzerland translates the results of this test into millions of bacteria by arbitrarily deciding that one bacterium is equivalent to 10^4 copies of ssrRNA.

A precondition for the detection of periodontal pathogens is the taking of a subgingival sample that contains the relevant microorganism with the maximum likelihood. However, it can be very difficult to obtain such a sample, especially if the target pathogen shows a topical appearance within the dentition, as is known for patients who have been infected with A.a. [30]. The necessary number of samples varies between different microorganisms. It could be demonstrated that up to 25 samples are required to identify A.a. [30,31]. In the routine periodontal treatment setting, the taking of such a high number of samples can be a problem, primarily due to economic reasons. Therefore, the drawing of four different samples is a widespread clinical standard [6]. A further increase in the chances of finding A.a. in the selected samples can be obtained by pooling several subgingival plaque samples.

In our study, samples were taken in the course of regular supportive periodontal therapy (SPT) or before the start of full-mouth disinfection from four different subgingival areas (preferably from those sites with the highest PPD values and signs of active inflammation) and analyzed with the two gene probe tests. Since it is known that persons are usually infected with only one specific serotype of A.a. [32], and it has also been reported that the subgingival serotype is normally identical with the serotype in saliva [33], it might make sense to add saliva

specimens or microbiological samples from the dorsum of the tongue or the oral mucosa to the material that is sent to the laboratory for further analysis. Nevertheless, there will still remain a certain risk of obtaining a false-negative result.

The PADO test is based on the detection of sequences within the bacterial 16S rRNA gene that are specific for the detected pathogen. The oligonucleotide probe that targets this gene is marked radioactively and shows no cross-reactivity with related microorganisms. With the use of these probes, reactions with homologous sequences of different species that are closely related to the target organism are possible. Probes that have originally been cloned for the DNA of A.a. show cross-reactions with *Haemophilus aphrophilus*, *Haemophilus influenzae*, *Haemophilus parahaemolyticus* and *Haemophilus parainfluenzae [34]*.

A final conclusion concerning the clinical relevance of the different results when comparing the PADO and MERI tests is not possible, as a reference test with a defined bacterial sample was not part of this study. Therefore, it could not be verified whether A.a. was not present in the samples of the patients who tested negative or if it was present but could not be detected (false-negative result). On the other hand, especially near the lowest detection threshold, the risk of obtaining false-positive results with both the PADO and MERI tests has to be discussed. Thus, the results of the current study also underline the conclusion of a recent literature review on microbial testing by Shaddox and Walker [35], who pointed out that the available techniques for the detection of periopathogens are still limited.

The two commercially available microbiological tests showed incongruencies regarding the identification of all four periodontal pathogens, with the MERI test finding more patients with a positive diagnosis and higher bacterial counts. Unweighted kappa and PABAK analysis showed that, for A.a., the agreement between both microbiological test kits was much weaker compared to P.g., T.f. and T.d.

Acknowledgements

The study was supported by the Institut für Angewandte Immunologie, Zuchwil, Switzerland and GABA GmbH, Lörrach, Germany, by partially providing the test-kits and performing the analysis for a reduced price. There are no conflicts of interest relevant to the contents of the article for any author.

References

[1] Socransky SS, Haffajee AD. Microbial mechanisms in the pathogenesis of destructive periodontal diseases: a critical assessment. J Periodontal Res 1991;26:195–209.

[2] Loomer PM. Microbiological diagnostic testing in the treatment of periodontal diseases. Periodontol 2000 2004;34:49–56.

[3] Van Winkelhoff AJ, Rodenburg JP, Goené RJ, Abbas F, Winkel EG, de Graaff J. Metronidazole plus amoxicillin in the treatment of Actinobacillus actinomycetemcomitans associated periodontitis. J Clin Periodontol 1989;16:128–31.

[4] Zambon JJ. American Academy of Periodontology Consensus report periodontal disease. Microbial factors. Ann Periodontol 1995;67:879–925.

[5] Müller HP, Lange DE, Müller RF. Failure of adjunctive minocycline-HCl to eliminate oral Actinobacillus actinomycetemcomitans. J Clin Periodontol 1993;20:498–504.

[6] Mombelli A, Gmür R, Gobbi C, Lang NP. Actinobacillus actinomycetemcomitans in adult periodontitis. I. Topographic distribution before and after treatment. J Periodontol 1994;65:820–6.

[7] Ehmke B, Moter A, Beikler T, Milian E, Flemmig TF. Adjunctive antimicrobial therapy of periodontitis: long-term effects on disease progression and oral colonization. J Periodontol 2005;76:749–59.

[8] Newmann MG, Socransky SS, Savitt ED, Propas DA, Crawford A. Studies of the microbiology of periodontosis. J Clin Periodontol 1976;47:373–9.

[9] Bragd L, Dahlen G, Wikström M, Slots J. The capability of Actinobacillus actinomycetemcomitans, Bacteroides gingivalis and Bacteroides intermedius to indicate progressive periodontitis: retrospective study. J Clin Periodontol 1987;14:95–9.

[10] Tonetti MS, Mombelli A. Early onset periodontitis. Ann Periodontol 1999;4:39–52.

[11] Schacher B, Baron F, Ludwig B, Valesky E, Noack B, Eickholz P. Periodontal therapy in siblings with Papillon-Lefèvre syndrome and tinea capitis: a report of two cases. J Clin Periodontol 2006;33:829–36.

[12] Armitage GC. Development of a classification system for periodontal diseases and conditions. Ann Periodontol 1999;4:1–6.

[13] Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. Int Dent J 1975;25:229–35.

[14] O'Leary TJ, Drake RB, Naylor JE. The plaque control record. J Periodontol 1972;43:38.

[15] Mombelli A, McNabb H, Lang NP. Black-pigmenting gram-negative bacteria in periodontal disease. II. Screening strategies for detection of P. gingivalis. J Periodontol Res 1991;26:308–13.

[16] Fleiss JL. The measurement of interrater agreement. Statistical methods for rates and proportions. Hoboken, NJ: Wiley & Sons; 2003. p. 150–61.

[17] Byrt T, Bishop J, Carlin JB. Bias, prevalence and kappa. J Clin Epidemiol 1993;46:423–9.

[18] Sim J, Wright CC. The kappa statistic in reliability studies: use, interpretation and sample size requirements. Phys Ther 2005;85:257–68.

[19] Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;i:307–10.

[20] Kamma JJ, Nakou M, Manti FA. Microbiota of rapidly progressive periodontitis lesions in association with clinical parameters. J Periodontol 1994;65:1073–8.

[21] Kamma JJ, Nakou M, Baehni PC. Clinical and microbiological characteristics of smokers with early onset periodontitis. J Periodontal Res 1999;34:25–33.

[22] Loesche WJ, Syed SA, Schmidt E, Morrison EC. Bacterial profiles of subgingival plaques in periodontitis. J Periodontol 1985;56:447–56.

[23] Moore WEC, Moore LVH. The bacteria of periodontal diseases. Periodontol 2000 1994;5:66–77.

[24] Dannewitz B, Pohl S, Eickholz P, Kim TS. Clinical and microbiological effects of a combined mechanic-

antibiotic therapy in patients with Actinobacillus actinomycetemcomitans-associated periodontitis. Am J Dent 2007;20:153–6.

[25] Krigar DM, Kaltschmitt J, Krieger JK, Eickholz P. Two subgingival plaque sampling strategies used with RNA-probes. J Periodontol 2007;78:72–8.

[26] Brochut PF, Marin I, Baehni P, Mombelli A. Predictive value of clinical and microbiological parameters for the treatment outcome of scaling and root planing. J Clin Periodontol 2005;32:695–701.

[27] Eguchi T, Koshy G, Umeda M, Iwanami T, Suga J, Momura Y, et al. Microbial changes in patients with acute periodontal abscess after treatment detected by PadoTest. Oral Dis 2008;14:180–4.

[28] Kamma JJ, Baehni PC. Five-year maintenance follow-up of early-onset periodontitis patients. J Clin Periodontol 2003;30:562–72.

[29] Luterbacher S, Mayfield L, Brägger U, Lang NP. Diagnostic characteristics of clinical and microbiological tests for monitoring periodontal and peri-implant mucosal tissue conditions during supportive periodontal therapy (SPT). Clin Oral Implants Res 2000;11:521–9.

[30] Christersson L, Fransson C, Dunford R, Zambon JJ. Subgingival distribution of periodontal pathogenic microorganisms in adult periodontitis. J Periodontol 1992;63:418–25.

[31] Christersson L, Zambon J. Suppression of subgingival Actinobacillus actinomycetemcomitans in localized juvenile periodontitis by systemic tetracycline. J Clin Periodontol 1993;20:395–401.

[32] Saarela M, Asikainen S, Jousimies-Somer H, Asikainen T, von Troil-Lindén B, Alaluusua S. Hybridization patterns of Actinobacillus actinomycetemcomitans serotypes a-e detected with an rRNA gene probe. Oral Microbiol Immunol 1993;8:111–15.

[33] Lindhe J. Treatment of localized juvenile periodontitis. In: Genco R, Mergenhagen S, editors. Host parasite interactions in periodontal disease. Washington DC: American Society for Microbiology; 1982. p. 27.

[34] Savitt E, Keville M, Peros W. DNA probes in the diagnosis of periodontal microorganisms. Arch Oral Biol 1990;35 (Suppl):153–9.

[35] Shaddox LM, Walker C. Microbial testing in periodontics: value, limitations and future directions. Periodontol 2000 2009;50:25–38.