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Two Subgingival Plaque Sampling Strategies Used With RNA-Probes

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Introduction

Of a total of about 500 bacterial species colonizing the oral cavity, some are narrowly assoziated with periodontal destruction [Moore & Moore 1994]. Actinobacillus actinomycetemcomitans, Tannerella forsythensis, Porphyromonas gingivalis, and Treponema denticola belong to the periodontal pathogens [Socransky et al. 1998]. It has been shown that Actinobacillus actinomycetemcomitans (AA) has an important role in the etiology of aggressive periodontal disease [Bragd et al. 1985, Newman et al. 1976]. AA is a microaerophilic, facultative anaerobic, and Gram-negative coccoid rod belonging to the family of Pasteurellaceae. Periodontal disease associated with AA in many cases cannot be treated reliably and predictively by mechanical removal of the subgingival biofilm alone [Christersson et al. 1985, Kornman et al. 1985, Mombelli et al. 1994]. Thus, the detection of AA is a significant factor contributing to the decision whether mechanical antiinfective therapy should be adjuncted by systemic antibiotics. Depending on the microbial complexes that are detected from subgingival plaque different antibiotic regimes are recommended [Beikler et al. 2004]. Microbiological testing prior to antiinfective therapy is recommended for the following clinical diagnoses: aggressive periodontitis, generalised severe chronic periodontitis, refractory periodontitis, and severe periodontitis associated with systemic diseases (e.g. HIV infection) [Flemmig et al. 1998]. Subgingival plaque samples should be taken from the deepest pockets exhibiting signs of activity, i.e. bleeding or suppuration. A micribiological analysis representative for the subgingival microflora of the whole oral cavity is relevant for adjunctive systemic antibiotic therapy of certain forms of periodontitis. Therefore also due to commercial reasons the analysis of pooled plaque sampled from several sites is recommended [Flemmig et al. 1998]. Thus, in this study the results of separate microbiological RNA-probe analyses of subgingival plaque samples from 3 different sites should be compared with the results from a pooled sample.

Material and Methods

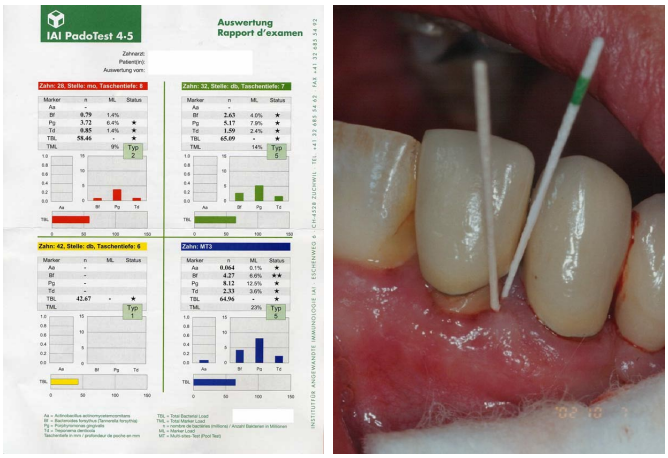

Abb.1

Patients

- 158 patients (72 female), 47.4 ± 10.6 years of age
- Two indications for microbiological examination:
  - diagnosis of untreated aggressive, generalised severe chronic periodontitis or periodontitis as manifestation of systemic disease
  - reevaluation after combined non-surgically mechanical and systemic antibiotic treatment of A.a.-associated periodontitis (after antibiotic therapy)

Clinical examinations

- At 6 sites per tooth PD and CAL-V using a rigid periodontal probe (PCPUNC 15, Hu Friedy, Chicago IL, USA)
- reference for CAL-V measurement CEJ or margin of restoration, BOP 30 sec. after probing

Microbiological examination

- 3 deepest pockets in 3 different quadrants; after removing the supragingival plaque, drying the test site by air and held dry using cotton rolls
- Simultaneous insertion of 2 sterile paper points to the bottom of the pocket and removal after 10 seconds
- One paper point of each site was put into a separate transportation vial, the other was pooled with paper points of the respective 2 other sampling sites (MT3)
- Analysis: RNA probe test kit (IAI Pado Test 4.5®, Institut für angewandte Immunologie, Zuchwil, Schweiz) with a detection limit of 103,3 for Actinobacillus actinomycetemcomitans (AA), Porphyromonas gingivalis (PG), Tannerella forsythensis (TF), Treponema denticola (TD)
- 76 patients received antiinfective therapy (oral hygiene instructions, professional tooth cleaning, supra- and subgingival scaling at all teeth within 24 hours according to the concept of "full-mouth-disinfection")
- In all 76 patients AA had been detected subgingivally before therapy and, thus mechanical therapy was combined with the systemic administration of 375mg and 250mg metronidazole 3 times daily/7days.

Statistical analysis

- All bacterial counts underwent logarithmic transformation
- Two variables were analysed for each periodontal pathogen:
  - log-transformed bacterial counts prevalence, i.e. detection of the pathogen or not
  - Prevalence of a microorganism was assessed if at least in one of the 3 samples the respective pathogen had been detected.
- Prevalence for separate analysis and MT3 were compared using Wilcoxon signed ranks tests for paired samples
- Statistical analyses were done using SystatTM for Windows Version 10, Systat Inc. Evanston, USA

Results

- The mean log-transformed number of bacteria was higher in pooled samples than the mean value of the results of the separate samples of all tested pathogens (p<0.001).
- However, for all 4 pathogens analysis failed to detect statistically significant differences between the separate samples and the mt3 regarding the detection frequency. These findings were observed over all samples as well as after evaluation of samples before and after separately.

	PD	CAL-V
All (n=158)	7.17 ± 1.9	8.00 ± 2.00
Before therapy (n=82)	8.07 ± 1.49	8.45 ± 1.59
After therapy (n=76)	6.19 ± 1.81	7.43 ± 2.23
ρ	<0.001	<0.001

Tab.1 Clinical parameters

	Seperate Analysis	MT3	P
AA	1.01 ± 1.43	1.92 ± 2.37	<0.001
TF	4.02 ± 2.41	5.28 ± 2.38	<0.001
PG	4.04 ± 2.46	5.20 ± 2.48	<0.001
TD	3.88 ± 2.30	4.81 ± 2.47	<0.001

Tab.2 Log-transformed Bacterial Counts for Separate and Pooled Analysis (MT3)

AA		Seperate analysis		
		Not detected	detected	Total
MT3	Not detected	68	23	96
	detected	26	41	67
Total		94	64	158

Tab.3 Prevalence of AA, TF, PG,TD for Separate and Pooled Analysis (MT3) No statistically significant difference between separate analysis and MT3 detected (p=0.668)

TF		Seperate analysis		
		Not detected	detected	Total
MT3	Not detected	15	11	26
	detected	10	122	132
Total		25	133	158

Tab.3 Prevalence of AA, TF, PG,TD for Separate and Pooled Analysis (MT3) No statistically significant difference between separate analysis and MT3 detected (p=0.827)

PG	Seperate analysis

		Not detected	detected	Total
MT3	Not detected	14	10	24
	detected	13	121	134
Total		27	131	158

Tab.3 Prevalence of AA, TF, PG,TD for Separate and Pooled Analysis (MT3) No statistically significant difference between separate analysis and MT3 detected (p=0.505)

TD		Seperate analysis		
		Not detected	detected	Total
MT3	Not detected	14	10	24
	detected	17	117	134
Total		31	127	158

Tab.3 Prevalence of AA, TF, PG,TD for Separate and Pooled Analysis (MT3) No statistically significant difference between separate analysis and MT3 detected (p=0.178)

Conclusions

Pooling of subgingival plaque samples increased the bacterial counts per analysis compared to separate samples and thus may increase the probability to detect existing pathogens. However, this observation had no statistically significant effect on the detection frequency of the tested pathogens.

Literature

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Two Subgingival Plaque Sampling Strategies Used With RNA-Probes

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[1] Section of Periodontology, Department of Conservative Dentistry, Clinic for Oral, Dental and Maxillofacial Diseases, University Hospital Heidelberg

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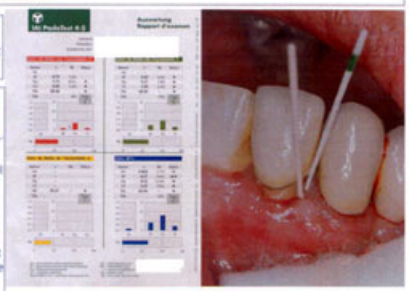
Objective

Comparison of the results of microbiological RNA-probe analysis after subgingival plaque sampling according to 2 different strategies.



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p	≤0.001	≤0.001

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Acknowledgement

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Results III

Tab.2 Log-transformed Bacterial Counts for Separate and Pooled Analysis (MT3)

	Separate Analysis	MT3	P
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MT3	Not detected	15	11	26
	detected	10	122	132
Total		25	133	158

No statistically significant difference between separate analysis and MT3 detected (p=n.s.0.82)

PG		Separate analysis		
		Not detected	detected	Total
MT3	Not detected	14	10	24
	detected	13	121	134
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No statistically significant difference between separate analysis and MT3 detected (p=n.s.0.5)

TD		Separate analysis		
		Not detected	detected	Total
MT3	Not detected	14	10	24
	detected	17	117	134
Total		31	127	158

No statistically significant difference between separate analysis and MT3 detected (p=n.s.0.08)