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 associated 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 microbiological 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

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 10^3,3 for Actinobacillus actinomycetemcomitans (AA), Porphyromonas gingivalis (PG), Tannerella forsythensis (TF), Treponema denticola (TD)
- 76 patients received antinfective 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.

<table>
<thead>
<tr>
<th>PD</th>
<th>CAL-V</th>
</tr>
</thead>
<tbody>
<tr>
<td>All (n=158)</td>
<td>7.17 ± 1.9</td>
</tr>
<tr>
<td>Before therapy (n=82)</td>
<td>8.07 ± 1.49</td>
</tr>
<tr>
<td>After therapy (n=76)</td>
<td>6.19 ± 1.81</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Tab.1 Clinical parameters

<table>
<thead>
<tr>
<th>Separate Analysis</th>
<th>MT3</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>1.01 ± 1.43</td>
<td>1.92 ± 2.37</td>
</tr>
<tr>
<td>TF</td>
<td>4.02 ± 2.41</td>
<td>5.28 ± 2.38</td>
</tr>
<tr>
<td>PG</td>
<td>4.04 ± 2.46</td>
<td>5.20 ± 2.48</td>
</tr>
<tr>
<td>TD</td>
<td>3.88 ± 2.30</td>
<td>4.81 ± 2.47</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Separate analysis</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Not detected</td>
<td>detected</td>
</tr>
<tr>
<td>Total</td>
<td>94</td>
<td>64</td>
</tr>
</tbody>
</table>

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)

<table>
<thead>
<tr>
<th></th>
<th>Separate analysis</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Not detected</td>
<td>detected</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>133</td>
</tr>
</tbody>
</table>

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)
Table 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)

<table>
<thead>
<tr>
<th></th>
<th>Not detected</th>
<th>detected</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT3</td>
<td>14</td>
<td>10</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>121</td>
<td>134</td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>131</td>
<td>158</td>
</tr>
</tbody>
</table>

Table 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)

<table>
<thead>
<tr>
<th></th>
<th>Not detected</th>
<th>detected</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT3</td>
<td>14</td>
<td>10</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>117</td>
<td>134</td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td>127</td>
<td>158</td>
</tr>
</tbody>
</table>

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

Materials and Methods I

Patients:
- 197 patients (64 females, 133 males) were enrolled.
- Two indications for microbiological treatment are:
  1. Treatment of periodontal disease
  2. Prevention of future periodontal disease
- A combination of non-surgical treatment and systemic antibiotic treatment of A. actinomycetemcomitans patients with periodontal disease
- Clinical parameters:
  1. Initial and final probing depths
  2. Attachment level
  3. Clinical parameters
- Statistical analysis:
  1. Comparison between baseline and final values
  2. Comparison between different treatment strategies

Results:

Table I: Comparison of Clinical Parameters Between Baseline and Final Values

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Final</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probing depth</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Attachment level</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table II: Comparison of Probing Depth Between Different Treatment Strategies

<table>
<thead>
<tr>
<th>Treatment Strategy</th>
<th>Probing Depth</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-surgical</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgical</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discussion:

- Analysis of subgingival plaque samples showed a significant decrease in probing depth and attachment level after both treatment strategies.
- However, the treatment strategy significantly affected the clinical improvement.

Conclusion:

- Subgingival plaque sampling is a valuable tool for the assessment of microbial success after different treatment strategies.
- Further research is needed to determine the optimal sampling technique for clinical efficacy.

Acknowledgments:

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- The study was supported by [grant number].

References: