J Clin Periodontol 2007; 34: 566–573 doi: 10.1111/j.1600-051X.2007.01080.x

Journal of Clinical Periodontology

# *Aggregatibacter actinomycetemcomitans* as indicator for aggressive periodontitis by two analysing strategies

**Beate Schacher, Frédéric Baron, Matthias Roßberg, Martin Wohlfeil, Rita Arndt and Peter Eickholz**

Department of Periodontology, Center of Dental, Oral, and Maxillofacial Medicine (Carolinum), Hospital of the Johann Wolfgang Goethe-University Frankfurt/Main, Frankfurt, Germany

*Schacher B, Baron F, Roßberg M, Wohlfeil M, Arndt R, Eickholz P.* Aggregatibacter actinomycetemcomitans *as indicator for aggressive periodontitis by two analysing strategies. J Clin Periodontol 2007; 34: 566–573. doi: 10.1111/j.1600-051X.2007.01080.x.*

***Abstract***

**Objective:** To compare the subgingival microbiota of aggressive and chronic periodontitis (ChP) using single-site and pooled plaque samples.

**Methods:** In 60 patients with aggressive or ChP, subgingival plaque was sampled from the four deepest pockets using two sterile paper points simultaneously. One paper point from each pocket was put in a separate transport vial, the second was pooled with the three other paper points of a respective patient. The content of each vial was analysed for *Aggregatibacter actinomycetemcomitans*, *Tannerella forsythensis*, *Porphyromonas gingivalis*, and *Treponema denticola*.

**Results:** Pooled plaque samples detected higher numbers for all tested pathogens than single-site samples. Detection frequencies were similar for both strategies. Using single-site samples, *A. actinomycetemcomitans* detection rate was statistically significantly a higher in aggressive than in ChP ($p = 0.01$). *A. actinomycetemcomitans* was found in higher numbers, the other pathogens in lower numbers in aggressive than in ChP. Neither presence nor absence of one of the tested bacteria had sufficient positive or negative predictive value for aggressive periodontitis.

**Conclusion:** *A. actinomycetemcomitans* was detected in higher numbers and frequency in aggressive than in ChP. Its detection may confirm the clinical diagnosis and influence therapy. As a diagnostic test, its sensitivity and predictive value was low.

Key words: generalized severe chronic periodontitis/aggressive periodontitis; oral microbiology; real time PCR; RNA-probes; subgingival plaque: *Aggregatibacter actinomycetemcomitans*, *Tannerella forsythensis*, *Porphyromonas gingivalis*, *Treponema denticola*

Accepted for publication 21 February 2007

The pathogenesis of periodontitis is characterized by microbial challenge and the host's response to it. From the approximately 400 bacterial species colonizing periodontal pockets and a further 300 in the rest of the oral cavity, approximately one half still awaits growth in culture (Paster et al. 2001, 2006). From the other half, some bacteria are closely associated with periodontal destruction. *Aggregatibacter actinomycetemcomitans* (formerly known as *Actinobacillus actinomycetemcomitans*) (Nørskov-Lauritsen & Kilian 2006), *Porphyromonas gingivalis*, *Tannerella forsythensis*, and *Treponema denticola* are periodontal pathogens (Socransky et al. 1998, van Winkelhoff et al. 2002). Furthermore, *A. actinomycetemcomitans* has been closely associated with the aetiology of severe periodontal disease: aggressive periodontitis (AgP) (Newman et al. 1976, Bragd et al. 1985, Tonetti & Mombelli 1999) and periodontitis as manifestation of Papillon Lefèvre syndrome (Schacher et al. 2006). *A. actinomycetemcomitans* is a microaerophilic, facultative anaerobic, and Gram-negative coccoid rod belonging to the family of *Pasteurellaceae* (Nørskov-Lauritsen & Kilian 2006). Periodontal disease associated with *A. actinomycetemcomitans*

**Conflict of interest and source of funding statement**

The authors declare that they have no conflict of interests.

This study was supported by Institut für angewandte Immunologie, Zuchwil, Switzerland and Gaba GmbH, Lörrach, Germany.

in many cases cannot be treated reliably and predictively by mechanical removal of the subgingival biofilm alone (Müller et al. 1993, Mombelli et al. 1994, Takamatsu et al. 1999, Ehmke et al. 2005). Thus, the detection of *A. actinomycetemcomitans* may be an indicator for the presence of AgP and is a significant factor contributing to the decision whether mechanical antiinfective therapy should be used in conjunction with systemic antibiotics (American Academy of Periodontology 2000, 2001).

According to the joint statement of the German Society of Periodontology (DGP) and the German Society of Dental, Oral, and Maxillofacial Medicine (DGZMK), microbiological testing before antiinfective therapy is recommended for the following clinical diagnoses: AgP, severe chronic periodontitis (ChP), periodontitis exhibiting progressive attachment loss despite thorough treatment, and severe periodontitis associated with systemic diseases (e.g. HIV infection) (Beikler et al. 2005). Subgingival plaque samples should be taken from the deepest pockets exhibiting signs of activity, i.e. bleeding or suppuration. A microbiological analysis representative of the subgingival microflora of the whole oral cavity is relevant for the adjunctive systemic antibiotic therapy of certain forms of periodontitis (Beikler et al. 2004, Eickholz et al. 2005). Therefore also due to financial constraints, the analysis of pooled plaque sampled from several sites is recommended using commercially available tests (Beikler et al. 2005).

The aim of this study was to compare numbers and prevalence of *A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythensis*, and *T. denticola* in the subgingival microflora of patients suffering from aggressive and generalized severe ChP using single-site and pooled plaque samples.

## Material and Methods

### Patients

Starting in March 2005, all patients scheduled for systematic periodontal therapy at the Department of Periodontology at the Center of Dental, Oral, and Maxillofacial Medicine at the Hospital of the Johann Wolfgang Goethe-University Frankfurt/Main were screened for this study. The following inclusion criteria had to be fulfilled by the patients:

- clinical diagnosis of untreated aggressive or generalized severe ChP,
- at least 18 years of age,
- at least five teeth per quadrant,
- informed written consent.

The following criteria lead to exclusion from the study:

- need for antibiotic prophylaxis before periodontal diagnosis or treatment,
- antibiotic therapy within the last 6 months or subgingival debridement (non-surgical or surgical) within the last 12 months before microbiological sampling,
- antiinfective periodontal therapy with adjunctive systemic amoxicillin or ciprofloxacin and metronidazole.

For this study, the diagnoses aggressive (AgP) and generalized ChP were defined as follows:

*Aggressive periodontitis* (Kim et al. 2006):

- Patient is clinically healthy, i.e. systemic diseases predisposing for periodontitis are not reported (e.g. diabetes mellitus),
- radiographic bone loss $\geqslant 50\%$ at at least two different teeth,
- age $\leqslant 35$ years.

*Generalized ChP*:

- attachment loss $\geqslant 5$ mm at more than 30% of sites,
- age >35 years.

The study had been approved by the Institutional Review Board for Human Studies of the Medical Faculty of the Johann Wolfgang Goethe-University Frankfurt/Main (Study #341/04).

### Clinical examinations

At six sites per tooth (mesiobuccal, midbuccal, distobuccal, distooral, midoral, mesiooral), probing pocket depths (PPD) and vertical clinical attachment levels (PAL-V) were measured using a manual rigid periodontal probe (PCPUNC15, Hu Friedy, Chicago, IL, USA) to the nearest millimetre. As reference for PAL-V measurements, the CEJ was used. If the CEJ was destroyed by restorative treatment the margin of the restoration was taken as reference. Bleeding on probing (BOP) was recorded 30 s after probing.

### Microbiological examination

Microbiological sampling was performed within the clinical routine after clinical measurements according to the joint statement of the German Society of Periodontology (DGP) and the German Society of Dental, Oral, and Maxillofacial Medicine (DGZMK) (Beikler et al. 2005). For sampling, the four deepest pockets in four different quadrants were selected (Mombelli et al. 1991, 1994). After gentle removal of supragingival plaque, the test site was dried by air and held dry using cotton rolls. Simultaneously two sterile paper points were inserted to the bottom of the respective pocket. After 10 s the paper points were removed. One paper point of each site was put into a separate transportation vial. The other was pooled with the paper points of the respective three other sampling sites (pooled plaque sample). Hence, for each patient five transportation vials were loaded: four containing one separate paper point of each of the four sampled sites and one containing four paper points pooled from each of the four sampled sites.

For detection of *A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythensis*, and *T. denticola*, two commercially available tests were used. For the first 20 patients of each diagnosis group, an RNA probe test kit (IAI Pado-Test 4.5®) (Institut für angewandte Immunologie, Zuchwil, Switzerland) was used. This is an oligonucleotide probe test complementary to conserved regions of the 16S rRNA gene, which encodes the rRNA that forms the small subunit of the bacterial ribosome. The detection limit of this test is $10^{3.3}$ for *A. actinomycetemcomitans* and $10^4$ for *P. gingivalis*, *T. forsythensis*, and *T. denticola*, respectively. For further 10 patients of each diagnosis, a commercially available real-time PCR test (Meridol Paro Diagnostik, Gaba GmbH, Lörrach, Germany) was used. The detection limit of this test is $10^2$.

### Statistical analysis

For 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.

For the single-site samples of each of four samples per patient two calculations were performed: (1) log-transformation of the sum of the numbers of each sampled site, (2) the mean of the four log-transformed bacterial counts of each site. The log-transformed sums and means were compared with the log-transformed counts of the pooled plaque samples using the Wilcoxon signed-ranks test for paired samples.

For the strategy of analysing the four single-site samples, a patient was classified positive for a microorganism if at least one of the four samples had detected the respective pathogen (prevalence positive). Prevalence for single-site and pooled plaque samples were compared using the Wilcoxon signed-ranks test for paired samples. Agreement of both analysing strategies was estimated by calculating Cohen's $\kappa$ (Fleiss 1981).

The prevalence of each periodontal pathogen was listed according to diagnosis in four cell tables for three different detection strategies:

- single-site samples,
- pooled plaque samples,
- total prevalence, i.e. if single-site samples and/or pooled plaque samples had detected the respective pathogen, the patient was classified positive for it.

Prevalence of the respective pathogen was compared according to diagnosis (ChP/AgP). Further for each detection strategy, sensitivity, specificity, and positive and negative predictive value were calculated.

All microbiological parameters were compared also according to the respective test (RNA probe/real-time PCR).

PPD and PAL-V were used to describe the clinical status of sampled sites. All statistical analyses were carrired out using a PC program (Systat™ for Windows Version 10, Systat Inc., Evanston, USA).

## Results

### Patients

Sixty patients (33 females) suffering from untreated aggressive (AgP: 30) or generalized severe (ChP: 30) that had been identified from March 2005 until May 2006 were included in this analysis. ChP patients had fewer teeth than AgP patients (Table 1). PPD and PAL-V at the sampled teeth was *higher* in the ChP group than in the AgP group (Table 1).

*Table 1*. Patients according to diagnosis: sex, age, number of teeth, and clinical parameters

| | Generalized chronic periodontitis | Aggressive periodontitis (AgP) |
|---|---|---|
| Total number | 30 | 30 |
| Female | 16 | 17 |
| Age | 50.1 ± 7.8** | 30.3 ± 6.9 |
| Number of teeth | 25.2 ± 3.1* | 27.7 ± 3.4 |
| PPD (mm) | 8.39 ± 1.38* | 7.53 ± 1.38 |
| PAL-V (mm) | 8.98 ± 1.61* | 8.04 ± 1.69 |

**Statistically significant difference to AgP ($p < 0.005$).
*Statistically significant difference to AgP ($p < 0.05$).
PPD, probing pocket depths; PAL-V, vertical clinical attachment levels.

*Table 2*. Log-transformed bacterial counts for single-site and pooled plaque samples

| | Single-site samples | | Pooled plaque samples |
|---|---|---|---|
| | mean | sum | |
| *Aggregatibacter actinomycetemcomitans* | 1.09 ± 1.66** | 2.11 ± 2.57* | 2.01 ± 2.55 |
| *Porphyromonas gingivalis* | 4.48 ± 2.39** | 5.77 ± 2.36* | 5.42 ± 2.62 |
| *Tannerella forsythensis* | 5.18 ± 1.54** | 6.54 ± 1.02* | 6.46 ± 1.01 |
| *Treponema denticola* | 5.10 ± 1.56** | 6.36 ± 1.12* | 6.29 ± 1.05 |

**Statistically significant difference to pooled plaque sample analysis ($p < 0.001$).
*Statistically significant difference to pooled plaque sample analysis ($p < 0.05$).

### Analysing strategy

The mean log-transformed number of bacteria was higher in pooled samples than the mean value of the log-transformed number of bacteria of the separate samples for all tested pathogens ($p < 0.001$). The log-transformed sums of the numbers of bacteria after single-site samples were higher than log-transformed numbers after pooled analysis for *P. gingivalis*, *T. forsythensis*, and *T. denticola* ($p < 0.05$). However, the difference was small and the statistical significance disappeared after adjustment for multiple testing (Table 2).

Whereas *P. gingivalis*, *T. forsythensis*, and *T. denticola* were detected in the majority of patients (*P. gingivalis*: 90%, *T. forsythensis*: 98%; *T. denticola*: 98%), *A. actinomycetemcomitans* was prevalent only in 57% of the cases. Whereas, *A. actinomycetemcomitans* if detected at all was present in most cases only in one out of the four sampled sites, *P. gingivalis*, *T. forsythensis*, and *T. denticola* were detected in most cases in all four samples (Table 3). For all tested pathogens, analysis failed to detect statistically significant differences between the single-site samples and the pooled plaque samples regarding the detection frequency (Table 4). Agreement between single-site samples and pooled plaque samples was perfect for *T. forsythensis* and *T. denticola* (Cohen's $\kappa = 1.0$; Table 4c and d). However, for several patients single-site samples were positive for *A. actinomycetemcomitans* and *P. gingivalis* whereas pooled analysis was not and vice versa. This resulted in low (*A. actinomycetemcomitans*: 0.384; Table 4a) and moderate (*P. gingivalis*: 0.609; Table 4b) agreement between both analysing strategies.

The analysis failed to detect differences between the microbiological parameters according to the respective test used. However, *T. forsythensis* and *T. denticola* were detected in more patients in all four samples for real-time PCR than for RNA probe test ($p < 0.01$).

### Diagnostic value

For log-transformed bacterial counts, the analyses detected statistically significant more *A. actinomycetemcomitans* in AgP than in ChP for single-site samples ($p = 0.036$). This difference could not be confirmed for pooled plaque samples. *T. forsythensis, P. gingivalis*, and *T. denticola* were detected in higher numbers in ChP than in AgP using single-site samples. For pooled plaque

*Table 3.* Number (percent of all sampled patients) of positive samples per patient for single-site samples

| Number of positive samples | 0 (%) | 1 (%) | 2 (%) | 3 (%) | 4 (%) |
|---|---|---|---|---|---|
| *Aggregatibacter actinomycetemcomitans* | 34 (57) | 11 (18) | 6 (10) | 3 (5%) | 6 (10) |
| *Porphyromonas gingivalis* | 7 (12) | 3 (5) | 9 (15) | 4 (7) | 37 (62) |
| *Tannerella forsythensis* | 1 (2) | 2 (3) | 7 (12) | 8 (13) | 40 (72) |
| *Treponema denticola* | 1 (2) | 3 (5) | 2 (3) | 10 (17) | 44 (73) |

*Table 4.* Prevalence [*n* (%)] of single-site and pooled plaque samples

| | | Pooled plaque sample | | Total |
|---|---|---|---|---|
| | | not detected (%) | detected (%) | |
| *(a) Aggregatibacter actinomycetemcomitans* | | | | |
| Single-site samples | Not detected | 26 (43%) | 8 (13%) | 34 (56%) |
| | Detected | 10 (17%) | 16 (27%) | 26 (44%) |
| Total | | 36 (60%) | 24 (40%) | 60 |
| No statistically significant difference between single-site and pooled plaque samples detected ($p = 0.637$) | | | | |
| Cohen's $\kappa$: 0.384; standard error: 0.120 | | | | |
| *(b) Porphyromonas gingivalis* | | | | |
| Single-site samples | Not detected | 6 (10%) | 2 (3%) | 8 (13%) |
| | Detected | 4 (7%) | 48 (80%) | 52 (87%) |
| Total | | 10 (17%) | 50 (83%) | 60 |
| No statistically significant difference between single-site and pooled plaque samples detected ($p = 0.414$) | | | | |
| Cohen's $\kappa$: 0.609; standard error: 0.144 | | | | |
| *(c) Tannerella forsythensis* | | | | |
| Single-site samples | Not detected | 1 (2%) | 0 | 1 (2%) |
| | Detected | 0 | 59 (98%) | 59 (98%) |
| Total | | 1 (2%) | 59 (98%) | 60 |
| No statistically significant difference between single-site and pooled plaque samples detected ($p = 1.000$) | | | | |
| Cohen's $\kappa$: 1.000; standard error: 0.000 | | | | |
| *(d) Treponema denticola* | | | | |
| Single-site samples | Not detected | 1 (2%) | 0 | 1 (2%) |
| | Detected | 0 | 59 (98%) | 59 (98%) |
| Total | | 1 (2%) | 59 (98%) | 60 |
| No statistically significant difference between single-site and pooled plaque samples detected ($p = 1.000$) | | | | |
| Cohen's $\underline{\kappa}$: 1.000; standard error: 0.000 | | | | |

samples, this could be confirmed only for *T. forsythensis* (Table 5).

Using single-site samples, *A. actinomycetemcomitans* was detected more often in aggressive than in ChP. This observation was not confirmed by pooled plaque samples and total prevalence (Table 6). *P. gingivalis* was detected more often in chronic than in aggressive periodontitis with either analysing strategy (Table 7). *T. forsythensis* and *T. denticola* were prevalent in all but one patient (AgP). Thus, sensitivity to detect AgP by detection of *T. forsythensis* and *T. denticola* reached 97%. However, due to the high prevalence of these pathogens, positive predictive value was only 49%. There was no statistically significant difference between aggressive and ChP regarding prevalence of *T. forsythensis* and *T. denticola* (Tables 8 and 9).

## Discussion

*A. actinomycetemcomitans*, *P. gingivalis*, and *T. forsythensis* are closely associated with destructive periodontal disease (Newman et al. 1976, Bragd et al. 1985, Socransky et al. 1998, van Winkelhoff et al. 2002). There is a lot of evidence that periodontal disease associated with *A. actinomycetemcomitans* in many cases cannot be treated reliably and predictively by mechanical removal of the subgingival biofilm alone (Kornman & Robertson 1985, Müller et al. 1993, Mombelli et al. 1994, Takamatsu et al. 1999, Ehmke et al. 2005). Systemic use of certain antibiotics adjunctively to mechanical debridement may suppress *A. actinomycetemcomitans* below detection limits and lead to favourable clinical results (van Winkelhoff et al. 1989, Ehmke et al. 2005, Dannewitz et al. 2007). Thus, the detection of *A. actinomycetemcomitans* is a significant factor contributing to the decision whether mechanical antiinfective therapy should be used in conjunction with systemic antibiotics.

### Analysing strategy

*A. actinomycetemcomitans* may not be present at all sites in a patient suffering from untreated periodontal disease (Table 4, Christersson et al. 1992, Ebersole et al. 1994, Müller et al. 2001, Krigar et al. 2005). Taking subgingival samples from all teeth actually would be the most reliable way to detect *A. actinomycetemcomitans* (Mombelli et al. 1994). However, this method is too time consuming and expensive to be used in daily practice. Sampling of the deepest pocket of each quadrant has been demonstrated to quite reliably represent the subgingival presence of *A. actinomycetemcomitans* (Mombelli et al. 1994) or *P. gingivalis* (Mombelli et al. 1991) in untreated patients. Other authors recommend sampling of subgingival and supragingival plaque at the most severely affected site of each sextant (Beikler et al. 2006). Mombelli et al. (1994) as well as Beikler et al. (2006) sampled all sites and theoretically tested different sampling strategies. However, their strategies were based on single-site samples. For economic reasons in daily clinical routine the different samples taken from the deepest sites per quadrant (or sextant) are pooled. Till date it is not clear that the results of the analysis of several pooled samples yield similar results as the single-site samples of the different samples. There is evidence that pooling subgingival plaque sampled from the three deepest pockets per patient (MT3) provides at least the same detection rate as single-site samples for

*Table 5.* Log-transformed bacterial counts for single-site and pooled plaque samples according to diagnosis

| | Generalized chronic periodontitis | Aggressive periodontitis | *p* |
|---|---|---|---|
| *Aggregatibacter actinomycetemcomitans* | | | |
| Single-site samples | 0.78 ± 1.42 | 1.40 ± 1.85 | 0.036 |
| Pooled plaque samples | 1.74 ± 2.37 | 2.27 ± 2.72 | 0.372 |
| *Porphyromonas gingivalis* | | | |
| Single-site samples | 5.18 ± 2.03 | 3.78 ± 2.54 | 0.024 |
| Pooled plaque samples | 6.24 ± 1.67 | 4.60 ± 3.13 | 0.058 |
| *Tannerella forsythensis* | | | |
| Single-site samples | 5.62 ± 1.22 | 4.75 ± 1.73 | 0.032 |
| Pooled plaque samples | 6.71 ± 0.49 | 6.22 ± 1.31 | 0.040 |
| *Treponema denticola* | | | |
| Single-site samples | 5.71 ± 0.83 | 4.50 ± 1.87 | 0.009 |
| Pooled plaque samples | 6.57 ± 0.44 | 6.01 ± 1.37 | 0.052 |

*Table 6.* Prevalence of *Aggregatibacter actinomycetemcomitans* according to diagnosis

| | Generalized chronic periodontitis | Aggressive periodontitis | Total |
|---|---|---|---|
| *(a) After single-site samples* | | | |
| *A. actinomycetemcomitans* negative | 22 | 12 | 34 |
| *A. actinomycetemcomitans* positive | 8 | 18* | 26 |
| Total | 30 | 30 | 60 |
| *$p = 0.01$ | | | |
| Sensitivity: 60%; positive predictive value: 69% | | | |
| Specificity: 73%; negative predictive value: 65% | | | |
| *(b) After pooled plaque samples* | | | |
| *A. actinomycetemcomitans* negative | 19 | 17 | 36 |
| *A. actinomycetemcomitans* positive | 11 | 13* | 24 |
| Total | 30 | 30 | 60 |
| *$p = 0.601$ | | | |
| Sensitivity: 43%; positive predictive value: 54% | | | |
| Specificity: 63%; negative predictive value: 53% | | | |
| *(c) Total prevalence* | | | |
| *A. actinomycetemcomitans* negative | 16 | 10 | 26 |
| *A. actinomycetemcomitans* positive | 14 | 20* | 34 |
| Total | 30 | 30 | 60 |
| *$p = 0.121$ | | | |
| Sensitivity: 53%; positive predictive value: 62% | | | |
| Specificity: 67%; negative predictive value: 59% | | | |

*A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythensis*, and *T. denticola* (Krigar et al. 2007). This study has confirmed this observation for pooled analysis of the deepest site of each quadrant (pooled plaque samples).

In this study, the log-transformed bacterial counts of the pooled plaque sample were statistically significantly higher than the means of the log-transformed bacterial counts from single-site samples for all tested microorganisms (*A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythensis*, and *T. denticola*). These observations have been made before for the MT3 strategy (Krigar et al. 2005, 2007). However, the log-transformed sums of the numbers of bacteria after single-site samples were higher than log-transformed numbers after pooled analysis for all pathogens. However, the difference was small and the statistical significance disappeared after adjustment for multiple testing.

The detection rates for pooled plaque samples were higher than after single-site samples for all tested pathogens. However, this study failed to detect statistically significant differences between the detection rates of pooled plaque samples and single-site samples. For *A. actinomycetemcomitans*, *P. gingivalis*, and *T. forsythensis* this confirms results of a recent trial (Krigar et al. 2007). However, for *T. denticola*, Krigar et al. observed lower detection rates after single-site samples of three sampled sites compared with pooled analysis (MT3). Using the four most severely diseased sites instead of three results in a higher probability to detect *T. denticola* and may explain this difference.

The low to moderate reproducibility of microbiological testing for *A. actinomycetemcomitans* and *P. gingivalis* after subgingival plaque sampling is another interesting observation made in this study. For each site, two sterile paper points had been inserted into and been removed from the periodontal pockets at the same time before they had been separated for separate or pooled analysis. However, for some patients single-site samples were positive for one of the tested periodontal pathogens whereas pooled analysis were not and vice versa. This resulted in low (*A. actinomycetemcomitans*: 0.384) to moderate (*P. gingivalis*: 0.609) agreement between both sampling strategies that was assessed as Cohen's $\kappa$ (Fleiss 1981). Other authors have made this observation for *A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythensis*, and *T. denticola* before (Krigar et al. 2007). This observation may be explained by an uneven distribution or at least uneven concentrations of the different bacterial species within periodontal pockets. Pooled analysis of samples within a patient may be a strategy to deal with this problem. However, even pooled analysis does not seem to overcome the risk of false-negative test results: one sampling strategy detects the microorganisms whereas the other does not. However, reproducibility of single-site and pooled plaque samples analysis for *T. forsythensis* and *T. denticola* was perfect ($\kappa$: 1.0), which partially may be explained by the overall high prevalence of both bacteria. Another factor may be the sampling of four instead of three (Krigar et al. 2007) sites. Perhaps sampling of six sites may further increase reproducibility (Beikler et al. 2006).

#### Diagnostic value

Why did we use a definition for aggressive periodontitis that is different from the International Classification (Armitage 1999)? To assess the rate of periodontal destruction, documentation of the

*Table 7.* Prevalence of *Porphyromonas gingivalis* according to diagnosis

| | Generalized chronic periodontitis | Aggressive periodontitis | Total |
|---|---|---|---|
| *(a) After single-site samples* | | | |
| *P. gingivalis* negative | 1 | 7 | 8 |
| *P. gingivalis* positive | 29 | 23* | 52 |
| Total | 30 | 30 | 60 |
| *$p = 0.024$ | | | |
| Sensitivity: 77%; positive predictive value: 44% | | | |
| Specificity: 3%; negative predictive value: 13% | | | |
| *(b) After pooled plaque samples* | | | |
| *P. gingivalis* negative | 1 | 9 | 10 |
| *P. gingivalis* positive | 29 | 21* | 50 |
| Total | 30 | 30 | 60 |
| *$p = 0.006$ | | | |
| Sensitivity: 70%; positive predictive value: 42% | | | |
| Specificity: 3%; negative predictive value: 10% | | | |
| *(c) Total prevalence* | | | |
| *P. gingivalis* negative | 0 | 6 | 6 |
| *P. gingivalis* positive | 30 | 24* | 54 |
| Total | 30 | 30 | 60 |
| *$p = 0.01$ | | | |
| Sensitivity: 80%; positive predictive value: 44% | | | |
| Specificity: 0%; negative predictive value: 0% | | | |

*Table 8.* Prevalence of *Tannerella forsythensis* according to diagnosis: identical for single-site samples, pooled plaque samples, and total prevalence

| | Generalized chronic periodontitis | Aggressive periodontitis | Total |
|---|---|---|---|
| *T. forsythensis* negative | 0 | 1 | 1 |
| *T. forsythensis* positive | 30 | 29* | 59 |
| Total | 30 | 30 | 60 |

*$p = 0.317$.
Sensitivity: 97%; positive predictive value: 49%.
Specificity: 0%; negative predictive value: 0%.

*Table 9.* Prevalence of *Treponema denticola* according to diagnosis: identical for single-site samples, pooled plaque samples, and total prevalence

| | Generalized chronic periodontitis | Aggressive periodontitis | Total |
|---|---|---|---|
| *Treponema denticola* negative | 0 | 1 | 1 |
| *T. denticola* positive | 30 | 29* | 59 |
| Total | 30 | 30 | 60 |

*$p = 0.317$.
Sensitivity: 97%; positive predictive value: 49%.
Specificity: 0%; negative predictive value: 0%.

periodontal status from two time points is required. However, for most patients diagnosed with untreated periodontal disease we only have an actual status. In younger patients ($\leqslant$35 years) with severe disease ($\geqslant$50% bone loss), we may estimate rapid destruction if we set the earliest starting point of disease around puberty. Severe disease in a 50 year old may be the result of rapid destruction (starting point e.g. at 40 years) or slow destruction (starting point e.g. 20 years). Thus, we have chosen age-related definitions to provide ''true'' diagnoses of aggressive and ChP with high certainty, which is a prerequisite to evaluate detection of certain bacteria as diagnostic test.

*A. actinomycetemcomitans* was found in higher numbers in patients with aggressive than in those diagnosed with generalized severe ChP before therapy. *P. gingivalis*, *T. forsythensis*, and *T. denticola* were found in lower numbers in AgP than in ChP. However, the differences between the log-transformed numbers for both diagnoses depended on analysing strategy: After single-site samples the differences were statistically significant but not after pooled plaque samples analysis. A similar result was observed for detection rate. Whereas single-site samples detected significantly more frequently *A. actinomycetemcomitans* in AgP than in ChP, for pooled plaque samples and total prevalence (combination of single-site and pooled plaque samples) significance was not shown. Positive and negative predictive value of detection of *A. actinomycetemcomitans* as diagnostic test for AgP demonstrates values between 53% and 69%, which are too low for a reasonable test. Other authors had shown before that the detection of *A. actinomycetemcomitans* is of limited use as a diagnostic test for aggressive periodontitis (Mombelli et al. 2002). Furthermore, *P. gingivalis*, *T. forsythensis*, and *T. denticola* are much too prevalent to be used to confirm the diagnosis AgP.

It seems that analysis of *A. actinomycetemcomitans* on the species level is not sufficient to indicate AgP (Kilian et al. 2006). The highly leucotoxic clone of *A. actinomycetemcomitans* has been detected only in patients with AgP, whereas this genotype was not found in periodontally healthy subjects and in adult periodontitis (ChP) patients (Haraszthy et al. 2000). Commercially available microbiological tests should be developed to identify highly leucotoxic genotypes. Using these tests, AgP may be identified with higher certainty (Cortelli et al. 2005) and antibiotic therapy may be aimed more precisely to the particularly pathogenic bacteria. However, it is not clear yet whether the highly leucotoxic *A. actinomycetemcomitans* clone is generally associated with AgP or particularly in subjects with African ancestry (Haubek et al. 1997, Kilian et al. 2006).

Within the limitations of the present study, the following conclusions may be drawn:

- The detection of *A. actinomycetemcomitans* without further differentiation into genotypes is neither a sensitive nor a specific diagnostic test for aggressive periodontitis.
- Pooling of subgingival plaque samples from the deepest sites of each quadrant (pooled plaque samples) increased the bacterial counts per analysis compared with means of separate samples and thus may increase the probability to detect existing pathogens. However, this observation failed to have statistically significant effect on any of the investigated pathogens.

## References


American Academy of Periodontology. (2000) Parameter on progressive periodontitis. *Journal of Periodontology* **71**, 867–869.

American Academy of Periodontology. (2001) Treatment of plaque-induced gingivitis, chronic periodontitis, and other clinical conditions. *Journal of Periodontology* **72**, 1790–1800.

Armitage, G. C. (1999) Development of a classification system for periodontal diseases and conditions. *Annals of Periodontology* **4**, 1–6.

Beikler, T., Karch, H. & Flemmig, T. F. (2005) Mikrobiologische Diagnostik in der Parodontitistherapie. Gemeinsame Stellungnahme der Deutschen Gesellschaft für Parodontologie (DGP) und der Deutschen Gesellschaft für Zahn-, Mund- und Kieferheilkunde (DGZMK). *Deutsche Zahnärztliche Zeitschrift* **60**, 660–662.

Beikler, T., Prior, K., Ehmke, B. & Flemmig, T. F. (2004) Specific antibiotics in the treatment of periodontitis – a proposed strategy. *Journal of Periodontology* **75**, 169–175.

Beikler, T., Schnitzer, S., Abdeen, G., Ehmke, B., Eisenacher, M. & Flemmig, T. F. (2006) Sampling strategy for intraoral detection of periodontal pathogens before and following periodontal therapy. *Journal of Periodontology* **77**, 1323–1332.

Bragd, L., Dahlen, G., Wikström, M. & Slots, J. (1985) The capability of *Actinobacillus actinomycetemcomitans, Bacteroides gingivalis* and *Bacteroides intermedius* to indicate progressive periodontitis; retrospective study. *Journal of Clinical Periodontology* **14**, 95–99.

Christersson, L. A., Fransson, C. L., Dunford, R. G. & Zambon, J. J. (1992) Subgingival distribution of periodontal pathogenic microorganisms in adult periodontitis. *Journal of Periodontology* **63**, 418–425.

Cortelli, J. R., Cortelli, S. C., Jordan, S., Haraszthy, V. I. & Zambon, J. J. (2005) Prevalence of periodontal pathogens in Brazilians with aggressive or chronic periodontitis. *Journal of Clinical Periodontology* **32**, 860–866.

Dannewitz, B., Pohl, S., Eickholz, P. & Kim, T.-S. (2007) Clinical and microbiological effects of a combined mechanic-antibiotic therapy in patients with *Actinobacillus actinomycetemcomitans*-associated periodontitis. *American Journal of Dentistry* (in press).

Ebersole, J. L., Cappelli, D. & Sandoval, M. N. (1994) Subgingival distribution of *A. actinomycetemcomitans* in periodontitis. *Journal of Clinical Periodontology* **21**, 65–75.

Ehmke, B., Moter, A., Beikler, T., Milian, E. & Flemmig, T. F. (2005) Adjunctive antimicrobial therapy of periodontitis: long-term effects on disease progression and oral colonization. *Journal of Periodontology* **76**, 749–759.

Eickholz, P., Dannewitz, B. & Kim, T.-S. (2005) Antibiotics in periodontal therapy. *Perio* **2**, 235–251.

Fleiss, J. L. (1981) *Statistical Methods for Rates and Proportions*, 2nd edition, pp. 223–225. New York: John Wiley & Sons.

Haraszthy, V. I., Hariharan, G., Tinoco, E. M. B., Cortelli, J. R., Lally, E. T., Davis, E. & Zambon, J. J. (2000) Evidence for the role of highly leukotoxic *Actinobacillus actinomycetemcomitans* in the pathogenesis of localised juvenile and other forms of early-onset periodontitis. *Journal of Periodontology* **71**, 912–922.

Haubek, D., Dirienzo, J. M., Tinoco, E. M. B., Westergaard, J., López, V. J., Chung, C.-P., Poulsen, K. & Kilian, M. (1997) Racial tropism of a highly toxic clone of *Actinobacillus actinomycetemcomitans* associated with juvenile periodontitis. *Journal of Clinical Microbiology* **35**, 3037–3042.

Kilian, M., Frandsen, E. V. G., Haubek, D. & Poulson, K. (2006) The etiology of periodontal disease revisited by population genetic analysis. *Periodontology 2000* **42**, 158–179.

Kim, C.-K., Choi, S.-H., Kim, T.-S., Kaltschmitt, J. & Eickholz, P. (2006) The infrabony defect and its determinants. *Journal of Periodontal Research* **41**, 498–502.

Kornman, K. & Robertson, P. (1985) Clinical and microbiological evaluation of therapy of juvenile periodontitis. *Journal of Periodontology* **56**, 443–446.

Krigar, D.-M., Kaltschmitt, J., Krieger, J. K. & Eickholz, P. (2007) Two subgingival plaque sampling strategies used with RNA-probes. *Journal of Periodontology* **78**, 72–78.

Krigar, D., Kaltschmitt, J., Radek, M. & Eickholz, P. (2005) Vergleich zweier Entnahmestrategien subgingivaler Plaqueproben für mikrobiologische Gensonden-Tests. *Deutsche Zahnärztliche Zeitschrift* **60**, 219–224.

Mombelli, A., Casagni, F. & Madianos, P. N. (2002) Can presence or absence of periodontal pathogens distinguish between subjects with chronic and aggressive periodontitis? A systematic review. *Journal of Clinical Periodontology* **29** (Suppl. 3), 10–21.

Mombelli, A., Gmür, R., Gobbi, C. & Lang, N. P. (1994) *Actinobacillus actinomycetemcomitans* in adult periodontitis. I. Topographic distribution before and after treatment. *Journal of Periodontology* **65**, 820–826.

Mombelli, A., McNabb, H. & Lang, N. P. (1991) Black-pigmenting gram-negative bacteria in periodontal disease. I. Topographic distribution in the human dentition. *Journal of Periodontal Research* **26**, 301–307.

Müller, H.-P., Heinecke, A., Fuhrmann, A., Eger, T. & Zöller, L. (2001) Intraoral distribution of *Actinobacillus actinomycetemcomitans* in young adults with minimal periodontal disease. *Journal of Periodontal Research* **36**, 114–123.

Müller, H.-P., Lange, D. E. & Müller, R. F. (1993) Failure of adjunctive minocycline-HCl to eliminate oral *Actinobacillus actinomycetemcomitans*. *Journal of Clinical Periodontology* **20**, 498–504.

Newman, M. G., Socransky, S. S., Savitt, E. D., Propas, D. A. & Crawford, A. (1976) Studies of the microbiology of periodontosis. *Journal of Periodontology* **47**, 373–379.

Nørskov-Lauritsen, N. & Kilian, M. (2006) Reclassification of *Actinobacillus actinomycetemcomitans, Haemophilus aphrophilus, Haemophilus paraphrophilus* and *Haemophilus segnis* as *Aggregatibacter actinomycetemcomitans* gen. nov., comb. nov., *Aggregatibacter aphrophilus* comb. nov. and *Aggregatibacter segnis* comb. nov., and emended description of *Aggregatibacter aphrophilus* to include V factor-dependent and V factor-independent isolates. *International Journal of Systematic and Evolutionary Microbiology* **56**, 2135–2146.

Paster, B. J., Boches, S. K., Galvin, J. L., Ericson, R. E., Lau, C. N., Levanos, V. A., Sahasrabudhe, A. & Dewhirst, F. E. (2001) Bacterial diversity in human subgingival plaque. *Journal of Bacteriology* **183**, 3770–3783.

Paster, B. J., Olsen, I., Aas, J. A. & Dewhirst, F. E. (2006) The breadth of bacterial diversity in the human periodontal pocket and other oral sites. *Periodontology 2000* **42**, 80–87.

Schacher, B., Baron, F., Ludwig, B., Valesky, E., Noack, B. & Eickholz, P. (2006) Periodontal therapy in siblings with Papillon-Lefèvre syndrome and tinea capitis: a report of 2 cases. *Journal of Clinical Periodontology* **33**, 829–836.

Socransky, S. S., Haffajee, A. D., Cugini, M. A., Smith, C. & Kent Jr., R. L. (1998) Microbial complexes in subgingival plaque. *Journal of Clinical Periodontology* **25**, 134–144.

Takamatsu, N., Yano, K., He, T., Umeda, M. & Ishikawa, I. (1999) Effect of initial periodontal therapy on the frequency of detecting *Bacteroides forsythus, Porphyromonas gingivalis*, and *Actinobacillus actinomycetemcomitans*. *Journal of Periodontology* **70**, 574–580.

Tonetti, M. S. & Mombelli, A. (1999) Early-onset periodontitis. *Annali of Periodontology* **4**, 39–52.

van Winkelhoff, A. J., Loos, B. G., van der Reijden, W. A. & van der Velden, U. (2002) *Porphyromonas gingivalis* and *Bacteroides forsythus* and other putative periodontal pathogens in subjects with and without periodontal destruction. *Journal of Clinical Periodontology* **29**, 1023–1028.

van Winkelhoff, A. J., Rodenburg, J. P., Goené, R. J., Abbas, E. G., Winkel, E. G. & deGraaff, J. (1989) Metronidazole plus amoxicillin in the treatment of *Actinobacillus actinomycetemcomitans* associated periodontitis. *Journal of Clinical Periodontology* **16**, 128–131.



Address:
*Prof. Peter Eickholz*
*Poliklinik für Parodontologie*
*Zentrum der Zahn-*
*Mund- und Kieferheilkunde (Carolinum)*
*Klinikum der Johann Wolfgang Goethe-Universität Frankfurt am Main*
*Theodor-Stern-Kai 7*
60590 Frankfurt
*Germany*
E-mail: eickholz@med.uni-frankfurt.de

**Clinical Relevance**

*Scientific rationale for the study*: (1) Does pooled microbiological analysis provide similar results as single-site samples? (2) How does detection of periodontal pathogens serve as diagnostic test for aggressive periodontitis?

*Principal findings*: Regarding detection frequency, pooled analysis is as good as single-site samples. Detection of *A. actinomycetemcomitans* is not a valid diagnostic test for aggressive periodontitis.

*Practical implications*: To assess subgingival microflora in untreated periodontitis patients, pooled analysis may be recommended.