

# Use of DNA Probes in the Diagnosis and Treatment of Periodontitis – A Case Series

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## ABSTRACT

Aggressive periodontitis is characterized by rapid attachment and bone loss with no underlying systemic disease and is associated with specific bacteria like *Actinobacillus actinomycetemcomitans* (Aa) and *Porphyromonas gingivalis* (Pg). In this case series 25 patients were diagnosed with aggressive periodontitis by the aid of DNA probes for Aa and Pg and other periodontal pathogens. The use of DNA probes for the detection of periodontal pathogens may aid in the diagnosis and treatment of aggressive periodontitis. Clinical experience suggests that lowering periodontal pathogens to undetectable levels could improve the long-term stability of periodontal health.

**Key words:** periodontitis, periodontal diseases, diagnosis, treatment

## Introduction

Aggressive periodontitis as defined by the 1999 International Workshop for a Classification of Periodontal Diseases and Conditions is characterized by the following:

1. Patients that have no underlying systemic disease.
2. Have rapid attachment and bone loss.
3. Have a family history of the disease.

Secondary features may include elevated proportions of *Actinobacillus actinomycetemcomitans* (Aa) and in some cases a high level of *Porphyromonas gingivalis* (Pg)<sup>1</sup>. A correct diagnosis is essential in the treatment of aggressive periodontitis. The elimination or significant reduction of periodontal pathogens is one of the major treatment goals. The use of the DNA probes for diagnosis of aggressive periodontitis is well documented<sup>2-6</sup>. It appears to be a good alternative to culturing, with faster results<sup>2,7</sup>. One of the drawbacks of this technique is that it is limited to the known periodontal pathogens that the specific DNA probes is designed for.

DNA probes could also be used to monitor sites before and following therapy, in order to validate the desired outcome of reduction of the subgingival pathogens to undetectable levels<sup>8-10</sup>. Several studies linked periodontally affected sites with elevated levels of periodontal patho-

gens<sup>11-18</sup>. Further studies have shown that sites left populated with periodontal pathogens like Pg and *Tannerella forsythensis* (Tf) (formerly *Bacteroides forsythus*) lost more clinical attachment and had more bleeding on probing<sup>9,19</sup>. In addition, it was demonstrated that sites that improve clinically have lower levels of Pg<sup>20</sup>.

In this report 25 cases of aggressive periodontitis that were treated in the practice of one of the authors (GZ) are documented. Diagnosis of aggressive periodontitis was based on clinical and radiographic evaluation of the periodontal condition, the absence of underlying systemic disease, and by utilizing DNA probes to analyze their subgingival microbial flora. DNA probes were also used to monitor those profiles throughout treatment. Three case reports will be presented to demonstrate the use of DNA probes in conjunction with periodontal therapy, to assure reduction of periodontal pathogens to undetectable levels, and thus stabilizing the periodontal condition of the patients.

## Materials and Methods

Twenty-five patients were treated in a Center for Periodontology. They were referred for evaluation and

treatment of their periodontal condition by their general dentists. In addition to a regular clinical and radiographic evaluation subgingival plaque samples were obtained and their microbial profile was analyzed in the following way:

Immediately after sampling, paper points were dipped into 50  $\mu$ l of guanidinium thiocyanate buffer<sup>21-23</sup> in tight-sealing 100  $\mu$ l screw-cap tubes. The samples were heated to 70 °C for 10 minutes, thoroughly vortexed and stored frozen until analysis (no longer than 2 weeks). A microbiologist, blinded to treatment allocation, performed the analysis of the samples.

For analysis, the samples were diluted and aliquots of each were applied on 5 nylon membranes mounted in dot-blot manifolds (Inotech AG). The membranes were processed by standard procedures<sup>24</sup>. Each one was then hybridized to one of five<sup>22</sup>, P-labelled, specific probes for the small subunit ribosomal RNA's (ssRNAs) of Aa, Tf, Pg, *Treponema denticola* (Td) and to a universal bacterial probe (UP). Probes Aa, Bf, Pg and UP were obtained

from Microprobe (Microprobe Corporation, Bothell, WA, USA), probe Td was designed by IAI (Institute of Applied Immunology). Hybridization and wash conditions were set as recommended by the manufacturer of the probes (Microprobe)<sup>25</sup>. A processing control was attained by sampling a known amount of lysed *Escherichia coli* on each membrane. Blots were quantified by direct counting in a Trace-96 system (Inotech AG). No calibration curves were necessary because the cpm readings turned out to be linear from  $2 \times 10^4$  to  $600 \times 10^6$  bacteria.

Denatured reference standards for each of the 4 bacteria were applied along with the samples to each membrane. Counts for each bacterial species were determined by comparison to the homologous standard. To determine total bacteria, a pool of the 4 standards, consisting of quantified dilutions of plasmids containing a cloned full DNA copy of the ssrRNA of each of the 4 bacterial species were used. Counts were transferred in to 'millions of bacteria' by using an arbitrarily set standard of each bacterium being equivalent to  $10^4$  copies of ssrRNA.

TABLE 1  
PERIODONTAL PATHOGENS AND ANTIBIOTIC REGIMEN (A-R) IN THE TREATED CASES

Cases	Periodontal pathogens (at baseline)	A-R 1	Periodontal pathogens (after SRP)	A-R 2	Periodontal pathogens (at maintenance)	A-R 3
1	A.a, Pg	X 1	A.a	X 2	∅	∅
2	A.a	X 1	∅	∅	∅	∅
3	A.a	X 1	∅	∅	∅	∅
4	A.a, Pg, Pi, Tf	X 1	A.a, Pg	X 2	∅	∅
5	Pg, Pi, Tf	X 4	Pg	X 4	∅	∅
6	A.a, Pg	X 1	∅	∅	∅	∅
7	Pg, Pi, Tf	X 4	Pg, Tf	X 4	∅	∅
8	A.a, Pg, Pi, Tf	X 1	A.a, Pi, Tf	X 2	∅	∅
9	A.a, Pg	X 1	A.a	X 3	∅	∅
10	Pg, Tf, Td	X 4	∅	∅	∅	∅
11	A.a, Pg	X 1	A.a, Pg	X 2	∅	∅
12	A.a	X 3	A.a	X 3	∅	∅
13	A.a, Pg, Tf	X 1	Pg	X 4	∅	∅
14	A.a, Pg	X 1	A.a	X 3	∅	∅
15	A.a	X 3	A.a	X 3	∅	∅
16	Pg, Pi, Tf	X 4	∅	∅	∅	∅
17	A.a, Pg, Pi, Tf	X 1	A.a, Pg	X 2	A.a	X 3
18	A.a	X 3	∅	∅	∅	∅
19	Pg, Pi, Tf	X 4	∅	∅	∅	∅
20	Pg, Pi, Tf	X 4	Pg	X 4	∅	∅
21	A.a, Pg	X 1	∅	∅	∅	∅
22	A.a, Pg, Pi, Tf	X 1	A.a, Pg	X 2	A.a	X 3
23	A.a, Pg, Pi, Tf	X 1	Pg, Pi	X 4	∅	∅
24	A.a, Pg, Tf	X 1	A.a, Tf	X 2	A.a	X 3
25	Pg, Pi, Tf	X 4	Pg, Pi	X 4	∅	∅

A.a – *Actinobacillus actinomycetemcomitans*, Pg – *Porphyromonas gingivalis*, Pi – *Prevotella intermedia*, Tf – *Tannerella forsythensis*,

X 1 – Amoxicillin 1000 mg daily plus Metronidazole 800 mg daily, 1 week

X 2 – Augmentin 1000 mg daily plus Metronidazole 800 mg daily, 1 week

X 3 – Doxycyclin 200 mg daily, 1 week

X 4 – Metronidazole 800 mg daily, 1 week

## Case 1

The patient (43 year-old female) presented in the office for an initial periodontal examination in February 1993. Her chief complaint was constant gingival pain, swelling and bleeding on probing. She denied any systemic disease, allergies, or taking any medication on a regular basis.

### Intraoral examination

The intraoral examination revealed unsatisfactory restorative treatment. The gingiva in maxilla and mandible was edematous and bulbous (Figure 1). The gingival margins were generalized rolled, and bled spontaneously or upon light probing. Gingival recession was evident throughout (Figure 1). The probing depth (PD) in the areas of # 3, 8, 9, 12–14, 19–21, 28–30 ranged from 8–10 mm. Purulent exudate was present in localized sites. The oral hygiene index (OHI)<sup>26</sup> was 50%, bleeding on probing (BOP) was generalized (100%).



Fig. 1. Facial view baseline, case #1.

### Radiographic analysis

Generalized horizontal bone loss ( $\geq 50\%$ ) with localized vertical defects in the area of the mandibular incisors and 1<sup>st</sup> molars (both jaws) was observed in the radiographs.

### Microbiology

The microbiological analysis of the subgingival plaque samples collected from the deepest sites revealed elevated levels of Aa ( $2 \times 10^3$ ) as well as Pg ( $1 \times 10^3$ ; Table 1).

### Diagnosis – aggressive periodontitis

#### Treatment phase

The patient was informed about the etiology of periodontal disease and was instructed and motivated in self-performed plaque control. During the initial treatment phase supragingival plaque was removed, and the teeth were polished. Scaling and root planing (SRP) was performed under local anesthesia with lidocaine hydrochloride and epinephrine (Xylestesin-S, 3M ESPE). Following SRP the patient was put on a systemic antibiotic regimen

of Amoxicillin (Ratiopharm GmbH) 1000 mg daily and Metronidazole (Aventis Pharma) 800 mg daily for one week.

Ten weeks post SRP subgingival plaque samples were collected. The result of the DNA analysis showed elevated levels of Aa ( $1 \times 10^3$ ; Table 1).

Subgingival deep scaling and root planing (SRP) under local anesthesia was repeated and the patient was put again on a systemic antibiotic regimen (Augmentin 1000 mg daily and Metronidazole 800 mg daily for one week) were repeated.

In order to reduce probing pocket depths, and to ensure thorough removal of subgingival deposits, flap surgery was performed in the areas of the localized defects. Surgical treatment was covered by systemic use of Doxycyclin 200 mg once a day for a period of 10 days. The patient refused to have any type of regenerative treatment. Ten weeks after medication subgingival plaque samples were collected and analyzed for detection of periodontal pathogens. The results of the analysis were negative.

### Maintenance phase

The patient was enrolled in a 3-month interval maintenance program. Subgingival plaque samples were collected and analyzed microbiologically. The analysis of the plaque samples was performed once a year, starting six months after completion of the treatment. The results were negative for periodontal pathogens (Table 1). PDs had decreased to 2–3 mm. The OHI improved (8%), and BOP was reduced (5%).



Fig. 2. Facial view ten year recall, case #1.

Due to the patient's excellent plaque control, the patient recall schedule was adjusted to every 6 months. The PDs (3 mm), OHI (6–10%) and BOP (0–5%) remained stable throughout the maintenance phase to date during the 10 years of follow-up (Figure 2).

## Case 2

The patient (31 year-old female) was referred in December 1993 for periodontal treatment by her general dentist. The medical history was negative for any chronic



Fig. 3. Facial view at baseline, case #2.

disease and no medications were taken on a regular basis. She was not aware of any drug allergies. Previous attempts by her general dentist to reduce PPDs by performing gingivectomies had failed. Therefore the patient was referred to the periodontist.

#### Intraoral examination

The intraoral examination revealed localized discolored gingiva. The gingival margins were rolled. Generalized gingival recession was evident (Figure 3). The PDs of the incisors, 1<sup>st</sup> molars and canines of both jaws ranged between 8–10 mm. The oral hygiene index (OHI) was 10%, BOP was (5%).

#### Radiographic analysis

Horizontal bone loss with localized vertical defects that extended up to 50% of the root length could be observed on several teeth (Figure 4).

#### Microbiology

The microbiological analysis of the subgingival plaque samples collected from the deepest sites revealed elevated levels of Aa ( $1 \times 10^4$ ; Table 1).

#### Diagnosis – aggressive periodontitis.

##### Treatment phase

The patient was informed about the etiology of periodontal disease and was instructed in plaque control methods.

Scaling and root planing (SRP) was performed with local anesthesia which was followed by a systemic antibiotic regimen (Amoxicillin 1000mg daily and Metronidazole 800mg daily for one week).

Ten weeks later subgingival plaque samples were collected again. The result of the DNA analysis was negative for periodontal pathogens (Table 1).

After microbiological examination, in the area of the maxillary and mandibular molars. No augmentive procedures were performed. Doxycyclin 200 mg was given for 10 days post-operatively.

##### Maintenance phase

The patient was enrolled in a supportive periodontal therapy (SPT) program. Subgingival plaque samples were collected and analyzed microbiologically. The analysis of the plaque samples was performed once a year, starting six months after completion of the treatment. The results were negative for periodontal pathogens (Table 1). PDs had decreased to 2–3mm. The OHI improved to 4%, and BOP was reduced 0%. The gingiva appeared firm and pink (Figure 5).

The patient was placed on a 6 months recall schedule. The PDs (2–3 mm), OHI (4–6%) and BOP (0–2%) re-

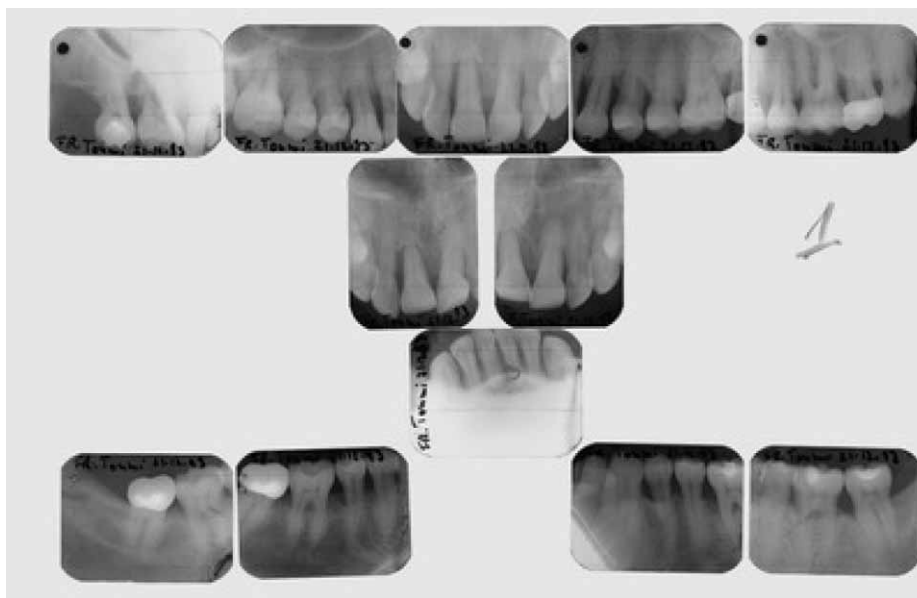


Fig. 4. Full mouth radiographs at baseline, case #2.



Fig. 5. Facial view 8-year recall, case #2.

remained stable throughout the maintenance phase. Additional gingival recessions did not occur during a follow-up period of 9 years.

### Case 3

The patient (36 year-old female) presented for an initial periodontal examination in July 1995. The chief complaint was constant gingival pain, swelling and bleeding. She denied any systemic disease, allergies, or taking any medication on a regular basis.

#### *Intraoral examination*

The intraoral examination revealed unsatisfactory restorative treatment. The gingiva in maxilla and mandible was edematous and bulbous. The gingival margins appeared »rolled«. Localized gingival recession was present. The papillae were edematous and bled upon light probing (Figure 6). Probing depths up to 10mm were detected in the areas of the canines and first molars of both jaws with out purulent exudate. The oral hygiene index (OHI) was 20% and BOP was 30%.



Fig. 6. Facial view at baseline, case #3.

#### *Radiographic analysis*

Radiographic analysis (Figure 7) revealed generalized horizontal bone loss with localized vertical defects that

extended to the apical third of the roots ( $\geq 50\%$ ). A panoramic radiograph of this patient is presented in Figure 7.



Fig. 7. Panoramic radiograph at baseline, case #3.

#### *Microbiology*

The microbiological analysis of the subgingival plaque samples collected from the deepest sites revealed elevated levels of Aa ( $2 \times 10^4$ ; Table 1).

#### *Diagnosis – aggressive periodontitis.*

##### *Treatment phase*

The patient was educated about the etiology of periodontal disease and was instructed and motivated in self-performed plaque control. Scaling and root planing was performed under local anesthesia and followed by a systemic antibiotic regimen (Amoxicillin 1000 mg daily and Metronidazole 800 mg daily for one week).

Ten weeks post SRP subgingival plaque samples were collected. The result of the DNA analysis was negative for periodontal pathogens (Table 1).

After this microbiological examination, flap surgery was performed in the areas of the localized defects. Doxycyclin 200 mg once a day for a period of 10 days was administered post-operative.

##### *Maintenance phase*

The patient was enrolled in a periodontal maintenance program. Subgingival plaque samples were collected and analyzed microbiologically once a year, start-



Fig. 8. Facial view 8-year recall, case #3.

ing six months after completion of the treatment. The results were negative for periodontal pathogens (Table 1). PDs had decreased to 2–3mm. The OHI improved to 10%, and BOP was reduced to 5%.

The patient was on a 6 months recall schedule. The PDs (2–3 mm), OHI (10%) and BOP (0–5%) remained stable throughout the maintenance phase. No further gingival recession was detected during the 8-year follow-up period (Figure 8).

## Discussion

This case series presents various treatment modalities for aggressive periodontitis patients with the common aspect that the patients were monitored for periodontal pathogens levels by DNA probes. It was observed in these 25 additional cases of aggressive periodontitis treated in the authors practice that ensuring undetectable levels of periodontal pathogens by using a combination of non-surgical, antimicrobial and surgical treatment modalities could prevent future periodontal destruction could be prevented and long-term stability achieved.

Our clinical experience confirms the findings of Shiloah et al. (1998), who observed that sites infected with

Pg or Tf showed more attachment loss at the one year follow-up irrespective of the treatment modality used<sup>19</sup>. The follow-up periods of all 25 cases ranged from 3–10 years. All patients showed stability during this time period. In the treatment of periodontitis, especially when dealing with aggressive forms of the disease that fact that periodontitis is a bacterial infection needs to be addressed. Reducing a amount of microbial periodontal pathogens to undetectable levels should be a goal of treatment, thus allowing the hosts immune system to better cope with the infection<sup>27</sup>. DNA probes are a fast way to determine periodontal pathogen levels and help to indicate necessary treatment adjustments.

## Conclusion

This case series suggests that the use of DNA probes to detect periodontal pathogens, may provides the clinician with the opportunity to decide at immediately if supplemental antibiotic therapy is necessary, in addition to non-surgical or surgical therapy and to monitor periodontal pathogen level during maintenance to assure periodontal stability.

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## UPOTREBA DNA-SONDI U DIJAGNOSTICI I TRETMANU PARODONTITISA – SERIJA SLUČAJEVA

### S A Ž E T A K

Agresivni parodontis karakterizira brzi gubitak pričvrstka i kosti bez prisustva sistemnih bolesti, a povezan je s specifičnim bakterijama kao što su *Actinobacillus actinomycetemcomitans* (Aa) i *Porphyromonas gingivalis* (Pg). U ovoj seriji od 25 slučajeva kod pacijenata je dijagnosticiran agresivni parodontitis pomoću DNA sonda za Aa i Pg i ostale parodontopatogene. Rezultati pokazuju da upotreba DNA sonda za detekciju parodontopatogenih bakterije može pomoći u dijagnozi i terapiji agresivnog parodontitisa. Pored toga, prema našim kliničkim iskustvima smanjenje parodontnih patogena rezultira dugoročno stabilno parodontno zdravlje.