Actinobacillus actinomycetemcomitans in Destructive Periodontal Disease. Three-Year Follow-Up Results

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Background: Convincing data exist that *A. actinomycetemcomitans* is an etiologic agent of periodontal disease. The purpose of this longitudinal study was to evaluate *A. actinomycetemcomitans* as a diagnostic indicator for periodontal disease in treated and periodontally maintained patients.

Methods: Following comprehensive mechanical/surgical and supportive amoxicillin plus metronidazole therapy in 13 subjects with *A. actinomycetemcomitans*-associated destructive periodontal disease, we monitored subgingival *A. actinomycetemcomitans* at 4 individual sites in each patient up to 3 years post-therapy. The periodontal status was determined, and *A. actinomycetemcomitans* levels were quantitatively enumerated on TSBV agar in CFU/ml. Six patients with a persistence of subgingival *A. actinomycetemcomitans* at each reexamination within 3 years post-therapy were selected to be at risk for minor periodontal treatment outcomes and further recurrence of periodontal disease (test group). Seven subjects with a complete suppression of *A. actinomycetemcomitans* at each post-therapy visit served as controls.

Results: The periodontal parameters decreased from overall values of 6.39 mm (probing depth, PD) and 7.64 mm (clinical attachment level, CAL) at the outset to 3.81 mm (PD) and 5.62 mm (CAL) 2 years post-therapy (Friedman, $P \leq 0.05$). At the 3-year reexamination, the PD/CAL scores increased to 4.03/5.78 mm. Among the 6 individuals (46%) with persistence of subgingival *A. actinomycetemcomitans* at the final 3-year visit (test group), periodontal status yielded increased levels of 4.45 mm (PD) and 6.60 mm (CAL). The control subjects (n = 7) revealed lower values of 3.67 mm (PD) and 5.09 mm (CAL). However, on a patient level, during the 3-year observational trial, the periodontal status of the 13 individuals was not statistically affected by subgingival infection with *A. actinomycetemcomitans*.

Conclusions: Although in advanced periodontal disease, comprehensive mechanical and antimicrobial treatment is an appropriate regimen for sustained improvement of periodontal health, long-term control of subgingival infection with *A. actinomycetemcomitans* could not be achieved. In the maintenance care of destructive periodontitis, the persistence of *A. actinomycetemcomitans* is not a diagnostic parameter for periodontal disease. *J Periodontol 2000;71:444-453.*

KEY WORDS

Periodontal diseases/diagnosis; follow-up studies; *Actinobacillus actinomycetemcomitans*; outcome assessment; periodontal diseases/microbiology.

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There has been a renewed recognition that the morbidity and mortality of certain systemic conditions such as atherosclerosis, myocardial infarction, stroke, pneumonia, and obstetric complications are affected by periodontal infections.[1] These new links challenge periodontists to focus periodontal therapy on stringent control of periodontal bacteria in the oral cavity as a principal goal of therapy. However, reports of *Actinobacillus actinomycetemcomitans*-related effects on systemic diseases as mentioned above are outstanding. On the oral level, evidence is emerging that treatment outcomes in periodontitis patients are affected by the persistence of specific subgingival species including *A. actinomycetemcomitans, Porphyromonas gingivalis,* and *Treponema denticola*.[2-5] Potent virulence factors, accompanied with altered host response mechanisms, are attributed to minor treatment responses in *A. actinomycetemcomitans*-associated periodontitis subjects.[6-8] Therefore, systemically administered antibiotics are recommended to eliminate *A. actinomycetemcomitans* from subgingival and adjacent intraoral areas.[9] The question arises whether it is possible to achieve a complete elimination of selected species from the subgingival environment and the oral cavity by sys-

temic release of antimicrobials as an adjunct to periodontal therapy. However, data suggest that resistance of *A. actinomycetemcomitans* may occur even to a combination of amoxicillin and metronidazole, which are acting synergistically.[10] Because pathogens do reemerge after periodontal treatment, it is of special interest to evaluate the effect of subgingival recurrence with selected species on the clinical conditions following therapy.[11]

Thus, the objective of the present longitudinal study was to compare clinical and microbial data in patients with severe periodontal disease representing either subgingival suppression or recurrence of the periodontal microorganism *A. actinomycetemcomitans*. The hypothesis was examined that in destructive adult periodontitis, the periodontal treatment response is negatively affected by a persistence of subgingival *A. actinomycetemcomitans* over a 3-year maintenance period.

MATERIALS AND METHODS

Subjects

A total of 13 subjects (7 women, 6 men) from the Department of Periodontology, ranging in age from 28 to 62 years, with severe destructive *A. actinomycetemcomitans*-associated periodontitis and evidence of prior attachment loss were selected to enter into the study. The average age of the patients was 39.6 ± 10.6 years at the last visit. All subjects had at least 22 teeth. Exclusion criteria included pregnancy; periodontal therapy or antibiotics in the previous 3 months; any systemic condition that might have affected the progression or treatment of periodontitis and the need for pre-medication for therapy. No subject with localized juvenile periodontitis or acute necrotizing ulcerative gingivitis was included in the study. The baseline demographic and clinical characteristics of the 13 periodontal patients enrolled in the study are presented in Table 1.

Table 1.

Baseline Demographic and Clinical Characteristics of the 13 Periodontitis Subjects

	Test	Control	Total
Subjects (n)	6	7	13
Sex (m/f)	3/3	3/4	6/7
Age (years)	47.0 ± 9.84*	37.9 ± 9.9	39.6 ± 10.6
Number of sites examined	46	56	102
% of sites with CAL <4mm (n)	2.17 (1)	1.79 (1)	1.96 (2)
% of sites with CAL 4-6mm (n)	71.74 (33)	48.21 (27)	58.82 (60)
% of sites with CAL >6mm (n)	26.09 (12)	50.0 (28)	39.22 (40)

* Mean ± SD.

Periodontal Monitoring

Subjects were screened for suitability and, if accepted, were asked to sign informed consent forms. All patients were monitored at baseline and at 3-month intervals in the first year post-therapy. In the second and third year of maintenance, clinical examinations were performed once a year. Probing depth (PD), as the distance between the gingival margin and the bottom of the periodontal pocket, as well as clinical attachment level (CAL), as the distance from the cemento-enamel junction (CEJ) to the bottom of the periodontal pocket, were assessed at 6 sites per tooth (mesiobuccal, buccal, distobuccal, distolingual, lingual, mesiolingual) using a straight rigid periodontal probe‡ with a 3-3-2-3 mm calibration and a 0.4 mm diameter tip. PD and CAL measurements (reproducibility ± 1 mm greater than 95%) were performed by the same calibrated examiner. The presence of supragingival plaque was assessed at 6 sites per tooth according to the plaque index (PI).[12] The gingival condition was examined at 6 surfaces per tooth using the gingival index (GI).[13] Gingival crevicular fluid readings§ were assessed at the interproximal area of the periodontal defect. Following removal of supragingival plaque with a sterile curet, the diseased site was isolated with cotton rolls and gently air dried; and a paper strip‖ was carefully inserted at the entrance of the periodontal pocket according to the method of Brill.[14]

Microbial Examinations

Subgingival plaque was sampled at 4 sites with PD >5 mm. After isolating the area with a cotton roll and gently air drying, supragingival deposits were carefully removed with a curet tip.¶ Subgingival plaque samples were assessed by inserting a sterile endodontic paper point# to the bottom of the periodontal pocket for 10 seconds. The subgingival plaque samples were immediately transferred into an Eppendorf vial with 500µl one-quarter concentrated, ice-cold, filter-sterilized Ringer's solution. We then suspended the samples for 10 seconds in an ultrasonic unit.** For quantitative enumeration of *A. actinomycetemcomitans* in the subgingival plaque samples (detection limit: 100

‡ PCP 11, Hu-Friedy, Chicago, IL.
§ Periotron 6000, Siemens, Bensheim, Germany.
‖ Harco, Tustin, CA.
¶ Hu-Friedy.
# Roeko, Langenau, Germany.
** Sonorex RK 82, Bandelin Electronic KG, Berlin, Germany.

cells/ml) and the cheek smears, 0.1 ml of the transport medium was diluted to 10^{-1} and 10^{-2} and spread on freshly prepared TSBV agar.[15] The TSBV agar consisted of 4% trypticase soy agar with 1 g of yeast extract per liter at pH 7.2. The agar was cooled to 56°C, then horse serum (10%), filter sterilized bacitracin (75 mg/l), and vancomycin (5 mg/l) were added. The agar sheets were kept at 4°C and used within 7 days. To identify and determine the biotype, the sheets were incubated in a CO_2 (5%) enriched incubator for 3 days at 35°C. Catalase-positive, small, convex colonies with star-shaped inner structures adhering to the agar were identified as *A. actinomycetemcomitans* colonies. The evaluation of *A. actinomycetemcomitans* on TSBV agar was expressed quantitatively in colony forming units (CFU/ml).

Periodontal Treatment

All individuals were enrolled in an oral hygiene program with weekly prophylaxis and repeated motivation and instruction in self-performed oral hygiene. Under local anesthesia,†† subgingival scaling and root planing (SRP) was assessed at sites with probing depths (PD) exceeding 4 mm. Additionally, at periodontal defects with PD >6 mm, surgical access was achieved according to the modified Widman flap technique and carried out by one single dentist (RB) in 3 to 4 sessions. After intrasulcular incision, a mucoperiosteal flap was raised beyond the mucogingival border to access the periodontal defect. Following removal of the interradicular inflammatory granulation tissue, the denuded root surfaces were mechanically scaled and root planed and repeatedly rinsed with 0.1% chlorhexidine digluconate solution.‡‡ No osseous surgery was performed. Flaps were replaced as close as possible to their initial position to completely cover the periodontal defect and fixed with interdental sutures. To eliminate *A. actinomycetemcomitans* from the subgingival environment and the oral cavity, systemic amoxicillin at 3 × 500mg§§ and metronidazole at 3 × 250mg‖‖ were prescribed for 7 days during SRP and surgical periodontal therapy. The postsurgical follow-up included removal of the sutures and careful cleaning of the treated periodontal sites 1 week post-therapy. During the first and second postoperative week, a 0.1% chlorhexidine digluconate solution was administered to the patients twice daily for 2 minutes. The patients were enrolled in a periodontal maintenance program and monitored on a 3- to 6-month recall schedule, including repeated oral hygiene instruction and a full-mouth tooth cleaning according to their individual needs.

Study Design

At baseline, the full range of periodontal parameters was assessed as described above. Reexaminations were performed on all subjects at 3, 6, 12, 24, and 36 months after surgical periodontal therapy. The clinical status and microbial samples were taken by the first author (RB) for each surgical site at baseline and within the maintenance period (Fig. 1). Six patients with a persistence of subgingival *A. actinomycetemcomitans* occurring over the 3-year observation period served as the test group (persistence of *A. actinomycetemcomitans*). Seven individuals in whom *A. actinomycetemcomitans* was eliminated from the subgingival environment served as successfully treated controls.

Statistical Analysis

Data analysis and statistical tests were performed on a patient-level basis using statistical software.¶¶ For each individual, the mean and standard deviations (S.D.) for each periodontal and microbial parameter were calculated. Significant changes in the clinical parameters and *A. actinomycetemcomitans* scores over the 36-month maintenance period were subjected to Friedman analysis of variance. Differences between the test (persistence) and control group (suppression) were analyzed by Mann-Whitney-Wilcoxon. Statistical significance was determined at an alpha level of 0.05.

†† Xylocain Spezial 2%, Astra Chemicals, Sverige, Sweden.
‡‡ Chlorhexamed fluid, Procter & Gamble, Schwalbach, Germany.
§§ Ratiopharm GmbH, Blaubeuren, Germany.
‖‖ Artesan GmbH, Lüchow, Germany.
¶¶ SAS Institute Inc., Cary, NC.

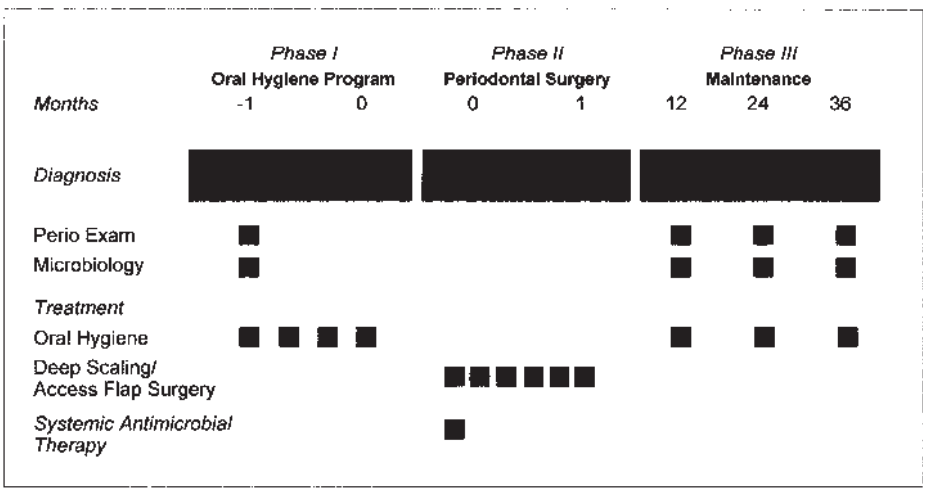

Figure 1.
Experimental protocol of the study, displaying the schedule for clinical and microbial examinations.

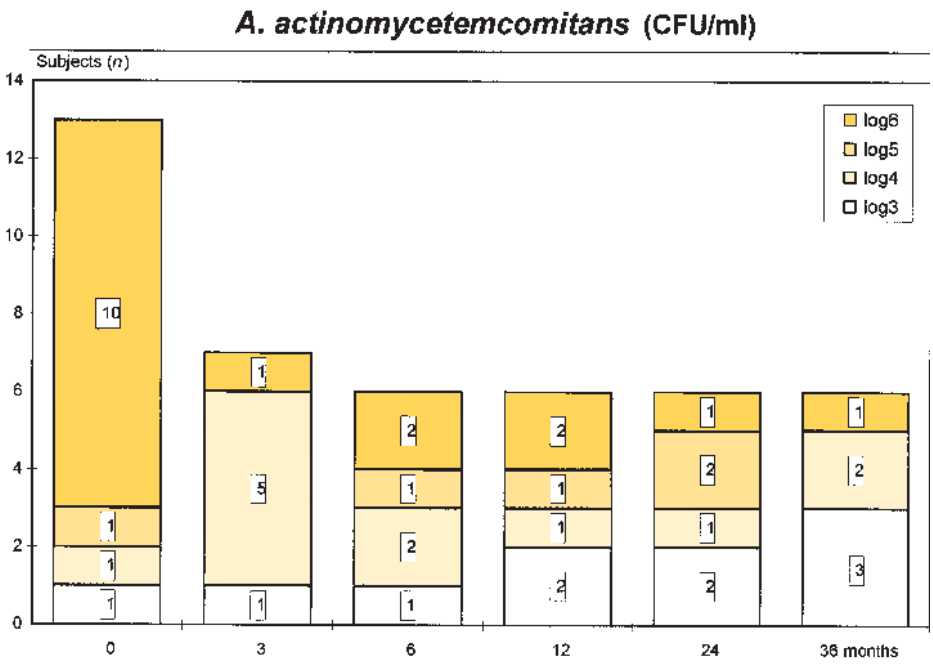

Figure 2.
Frequency of A. actinomycetemcomitans *persistence in destructive periodontitis patients (n = 13 subjects,* A. actinomycetemcomitans *in CFU/ml).*

RESULTS

Microbiology

At baseline, all subjects were positive for subgingival *A. actinomycetemcomitans*. At 3 months post-therapy, *A. actinomycetemcomitans* persisted in 7 patients (54%); in 6 individuals, *A. actinomycetemcomitans* was suppressed below detectable levels. At the 6-month, 1-, 2- and 3-year visit, *A. actinomycetemcomitans* persisted in 6 subjects (46%). The suppression of *A. actinomycetemcomitans* continued in 7 individuals over the 3-year maintenance period. At the outset in 10 subjects, subgingival *A. actinomycetemcomitans* was detected in concentrations of 10^6 CFU/ml; among the 3 other patients, *A. actinomycetemcomitans* was distributed between 10^3 and 10^5 CFU/ml. In subjects with a persistence of *A. actinomycetemcomitans* (54%) 3 months post-therapy, the CFU levels ranged between 10^4 and 10^6 CFU/ml. At the 6- and 12-month reexamination, 2 individuals (15%) yielded concentrations of 10^6 CFU/ml. At the 2-year recall appointment, only 1 patient was positive for *A. actinomycetemcomitans* (10^6 CFU/ml). Lower *A. actinomycetemcomitans* scores between 10^3 and 10^5 CFU/ml were detected in 4 subjects at the 6- and 12-month visit and in 5 individuals 2 and 3 years after treatment (Fig. 2).

Clinical Parameters

Throughout the 3-year observation, the periodontal conditions revealed no statistically significant differences between both 3-year patient categories (Mann-Whitney, $P \leq 0.05$). The average gingival crevicular fluid readings (GCF) decreased from baseline levels of 129.9 ± 38.8 to 93.4 ± 42.2 at the 3-month visit. At the 6-month recall appointment, the GCF value was 100.5 ± 50.7 and remained stable during the 3-year period. The GCF values ranged from 88.65 ± 35.6 and 95.4 ± 55.1 between the 1- and 3-year reexamination (test group). In the control subjects, the GCF scores dropped from 147.1 ± 34.3 to 107.6 ± 41.8 at the 3-month recall. Within the first year, the GCF scores were 93.0 ± 45.4 and 86.5 ± 45.3. At the 2- and 3-year appointments, the GCF levels were 94.6 ± 47.3 and 103.1 ± 49.1, respectively. In the test group, the gingival index (GI) was 2.0 ± 0.8 prior to therapy and decreased to 0.9 ± 0.5 three months post-therapy. The GI scores ranged between 0.7 ± 0.5 and 0.9 ± 0.7 during the 3-year observation period. The controls showed baseline GI values of 2.4 ± 0.6 that decreased to 0.9 ± 0.7 at the 3-month reexamination and to 0.6 ± 0.7 at the 3-year visit. The plaque index (PI) exhibited no differences between test and control subjects and revealed moderate scores between 0.5 ± 0.7 and 0.7 ± 0.7 during the 3-year trial (data not shown).

The baseline and 3-year follow-up PD and CAL of each of the 13 periodontitis patients in both test (*A.a.* persistence) and control groups (*A.a.* suppression) are summarized in Tables 2 and 3. The comparison of the individual patient levels revealed an inconsistent decrease in PD and CAL scores following periodontal therapy. Frequency and percentage calculations of pre- and post-therapy PD and CAL measurements displayed an apparent trend for less reduction in individuals with a persistence of *A. actinomycetemcomitans* compared to subjects with suppression of *A. actinomycetemcomitans* at sites >6 mm. In the test group, the percentage of monitored PD sites in the category >6 mm decreased from 26.1% at the outset to 9.3% three years post-therapy, whereas in the controls, the reduction was 48.0% (50.0% at baseline, 2.0% after 3 years). CAL sites >6 mm in the test group dropped from 73.9% to 37.2% and from 69.6% to 14.3% in the controls (Fig. 3).

Patient means of PD and CAL parameters following comprehensive flap surgery and amoxicillin and metronidazole therapy suggested a trend to more improved treatment outcomes when *A. actinomycetemcomitans*

could be continuously suppressed during the 3-year observation period (test group) as compared to control measurements. However, although both of the grouped individuals strongly benefited from scaling and root planing plus antimicrobial treatment (Friedman analysis, $P<0.05$, Table 4), the amount of change in CAL and PD measurements following therapy was not statistically different (Mann-Whitney, $P<0.05$). The changes from baseline to each of the post-therapy reexaminations are displayed in Figures 4 and 5. The real change occurs between baseline and 3 months, with little improvement after that. With an estimated measurement error of rigid periodontal probes at ± 1 mm (95% confidence), these results suggest that, on a patient level, persistent subgingival infection with *A. actinomycetemcomitans* alone had no significant impact on the post-therapy periodontal conditions.

DISCUSSION

Recolonization with periodontal pathogens following treatment of periodontal disease is a commonly observed phenomenon.[16] Therefore, subsequent microbial control of periodontally compromised patients with advanced bone loss is challenging. Differences in microbial species between clinically healthy and diseased periodontal sites are well documented. But even today, the effect of microbial factors on the clinical condition and development of inflammatory periodontal lesions is subject to controversial discussion. For example, the most current multivariate models of adult periodontal disease that include microorganisms have correlation coefficients in the order of 0.3 to 0.4 for the presence, absence, or level of specific microbes believed to be important pathogens.[17-19] However, the microbial contribution should not be minimized because no infectious disease, such as periodontal dis-

Table 2.

Baseline and 3-Year Follow-Up of PD in 13 Periodontitis Patients

	PD (Means ± SD)					
	Months					
	0	3	6	12	24	36
Persistence *Subj. No.*						
1	5.38 ± 1.60	3.38 ± 1.06	3.63 ± 1.30	3.88 ± 1.13	4.01 ± 1.10	4.25 ± 2.12
2	5.50 ± 0.93	2.25 ± 0.71	2.38 ± 0.74	2.25 ± 0.71	2.75 ± 0.46	3.75 ± 1.16
3	6.25 ± 1.75	3.05 ± 0.54	2.75 ± 0.46	2.75 ± 0.46	3.10 ± 0.40	3.13 ± 0.35
4	6.00 ± 0.58	4.43 ± 1.13	4.43 ± 1.27	6.29 ± 2.06	4.00 ± 1.41	5.57 ± 2.07
5	7.17 ± 1.33	4.50 ± 1.64	4.05 ± 1.41	3.67 ± 1.51	4.40 ± 2.30	4.33 ± 1.21
6	6.33 ± 0.52	5.33 ± 1.21	5.33 ± 1.21	4.83 ± 1.17	5.36 ± 1.08	5.67 ± 0.82
	0	3	6	12	24	36
Suppression *Subj. No.*						
7	5.63 ± 1.06	2.63 ± 0.74	2.88 ± 0.64	2.75 ± 0.46	3.12 ± 0.57	3.25 ± 1.16
8	8.00 ± 1.93	3.50 ± 1.41	4.13 ± 1.46	4.25 ± 2.12	4.63 ± 1.51	3.94 ± 1.38
9	5.63 ± 1.19	2.88 ± 0.64	3.13 ± 0.35	3.13 ± 0.35	3.25 ± 0.46	3.38 ± 0.52
10	7.88 ± 1.25	4.25 ± 1.67	4.63 ± 1.30	4.63 ± 1.60	5.00 ± 1.20	4.38 ± 1.85
11	4.50 ± 0.93	3.13 ± 0.35	2.90 ± 0.44	2.75 ± 0.46	3.13 ± 0.64	3.63 ± 0.74
12	7.88 ± 1.73	2.63 ± 0.52	2.75 ± 0.46	3.13 ± 0.64	3.13 ± 0.99	3.13 ± 0.64
13	7.00 ± 0.76	3.63 ± 1.41	3.63 ± 1.30	3.75 ± 1.16	3.63 ± 1.19	4.00 ± 1.41

Patient means = mean values of 4 sampled sites.

ease, can occur without the presence of the infectious agent.[20] There is growing evidence that, among microbial factors, *A. actinomycetemcomitans* is of special interest.[21-23] In early childhood, an intrafamilial transmission of *A. actinomycetemcomitans* into the oral cavity from parents or family members to the infant occurs. Thus, with aging and periodontal pocket development, *A. actinomycetemcomitans*-associated periodontal diseases are emerging. However, studies of pathogenesis in periodontology indicate that the chronic or destructive character of the disease is dependent on the level of site-specific immunoregulation in the infected periodontium compromised by selected environmental factors.[24-27] In a short-term adult periodontitis study, the presence of subgingival *A. actinomycetemcomitans* did not correlate with the clinical outcomes following SRP treatment alone.[28] At baseline, *A. actinomycetemcomitans* occurred in 47% of the plaque samples; six weeks following treatment, the frequencey scores were 37%. When considering the acquired data, *A. actinomycetemcomitans* at most of the sampled sites was present below the calculated threshold levels and did not affect clinical attachment level changes. Thus, in chronic adult periodontal disease, control of *A. actinomycetemcomitans* is not a priority and, in addition, antibiotics should not be prescribed.

Our data from an adult population with destructive periodontal disease revealed a persistence of *A. actinomycetemcomitans* in 6 (46%) out of 13 subjects. Reemergence of *A. actinomycetemcomitans* following periodontal therapy might be related to transmission from family members as carriers of the pathogen during the 3-year period. To avoid any negative side effects resulting from patients' non-compliance to antibiotic medication, the subjects enrolled in the present study

Table 3.

Baseline and 3-Year Follow-Up of CAL in 13 Periodontitis Patients

	CAL (Means ± SD)					
	Months					
	0	3	6	12	24	36
Persistence *Subj. No.*						
1	6.38 ± 1.51	5.00 ± 1.93	5.50 ± 1.77	5.63 ± 1.77	6.12 ± 1.70	6.50 ± 1.85
2	6.38 ± 0.92	3.25 ± 1.28	3.63 ± 0.92	3.00 ± 1.07	3.88 ± 0.83	5.38 ± 2.97
3	8.25 ± 1.98	6.13 ± 1.25	5.63 ± 1.60	5.63 ± 1.19	5.52 ± 1.10	5.75 ± 0.46
4	8.43 ± 1.51	6.71 ± 1.50	6.86 ± 1.77	8.29 ± 2.06	5.75 ± 1.72	7.14 ± 2.48
5	7.67 ± 1.03	5.17 ± 2.14	4.42 ± 1.65	4.64 ± 1.75	5.60 ± 2.07	5.00 ± 1.41
6	9.67 ± 1.51	9.17 ± 1.47	9.17 ± 1.72	8.67 ± 1.63	9.32 ± 1.88	9.83 ± 1.72
	0	3	6	12	24	36
Suppression *Subj. No.*						
7	7.13 ± 1.25	5.83 ± 0.74	5.63 ± 1.30	5.13 ± 1.25	5.42 ± 1.31	5.75 ± 1.49
8	9.75 ± 2.05	7.00 ± 1.41	7.75 ± 1.58	7.38 ± 1.19	8.00 ± 1.51	7.14 ± 1.50
9	5.88 ± 1.25	3.75 ± 1.16	3.88 ± 0.83	4.00 ± 1.07	4.25 ± 1.28	4.13 ± 0.99
10	8.50 ± 1.07	6.13 ± 2.03	5.86 ± 1.35	5.75 ± 1.49	6.13 ± 0.99	5.63 ± 1.30
11	5.50 ± 0.93	3.94 ± 0.82	3.75 ± 0.71	3.50 ± 0.93	3.88 ± 0.83	3.75 ± 1.04
12	8.25 ± 1.49	3.88 ± 0.99	3.75 ± 1.16	3.88 ± 1.46	3.75 ± 1.28	3.38 ± 0.52
13	7.50 ± 0.76	5.38 ± 1.41	5.63 ± 0.74	5.63 ± 0.52	5.50 ± 1.20	5.88 ± 1.36

Patient means = mean values of 4 sampled sites.

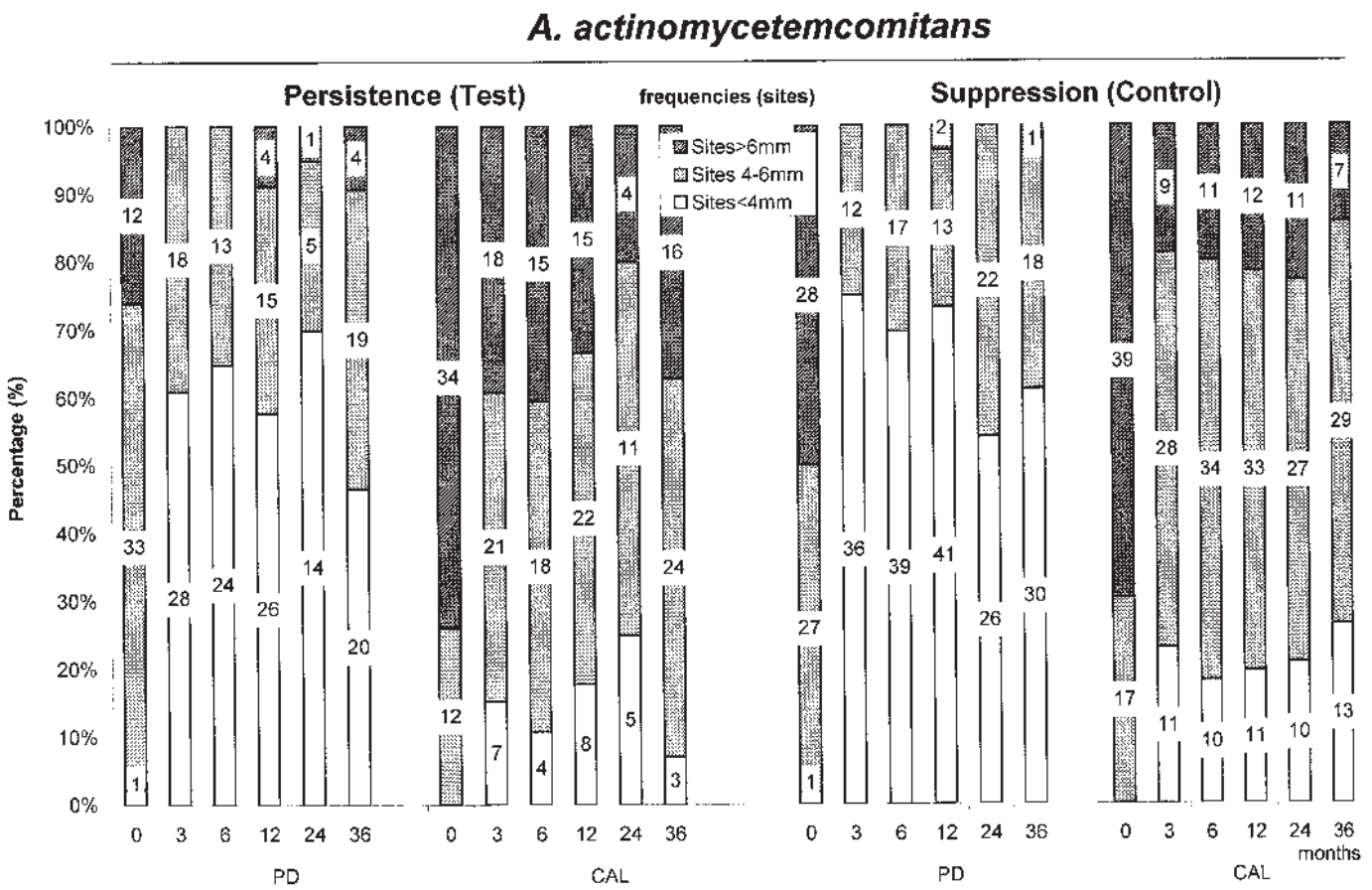

Figure 3.
Three-year survey of PD and CAL in individuals with persistence (test) or suppression (control) of A. actinomycetemcomitans *(frequencies and percentages of pre- and post-therapy PD and CAL measurements).*

Table 4.

Three-Year Course of PD and CAL (Friedman analysis of variance)

	Cases	Chi-Square	D. F.	Significance
Probing depths (PD)				
Suppression	5	17.7879	5	0.0032*
Persistence	5	13.0417	5	0.0030*
Clinical attachment level (CAL)				
Suppression	5	12.7647	5	0.0257*
Persistence	5	11.8776	5	0.0183*

* $P<0.05$.

were asked to bring their tablets during SRP and surgical treatment sessions to count the number of remaining tablets. However, at the 3-month reexamination, 7 patients (54%) were positive for *A. actinomycetemcomitans,* with CFU levels between 10^4 and 10^6 CFU/ml. Patient no. 13, who revealed low *A. actinomycetemcomitans* scores of 10^3 CFU/ml, showed no *Aa* between the 3- and 6-month reexamination. This is in accordance with Mombelli et al.,[4,5] who reported that *A. actinomycetemcomitans* could be eliminated from the pocket if it is detected in low numbers. On the other hand, we could not support the observation that a minor improvement of clinical outcomes in subjects with persistence of *A. actinomycetemcomitans* is associated with enhanced subgingival concentrations that exceed calculated threshold levels for periodontal disease.[3,7] For example, patient no. 6, who had a persistent infection, did not respond to periodontal therapy, although the subgingival *A. actinomycetemcomitans* levels

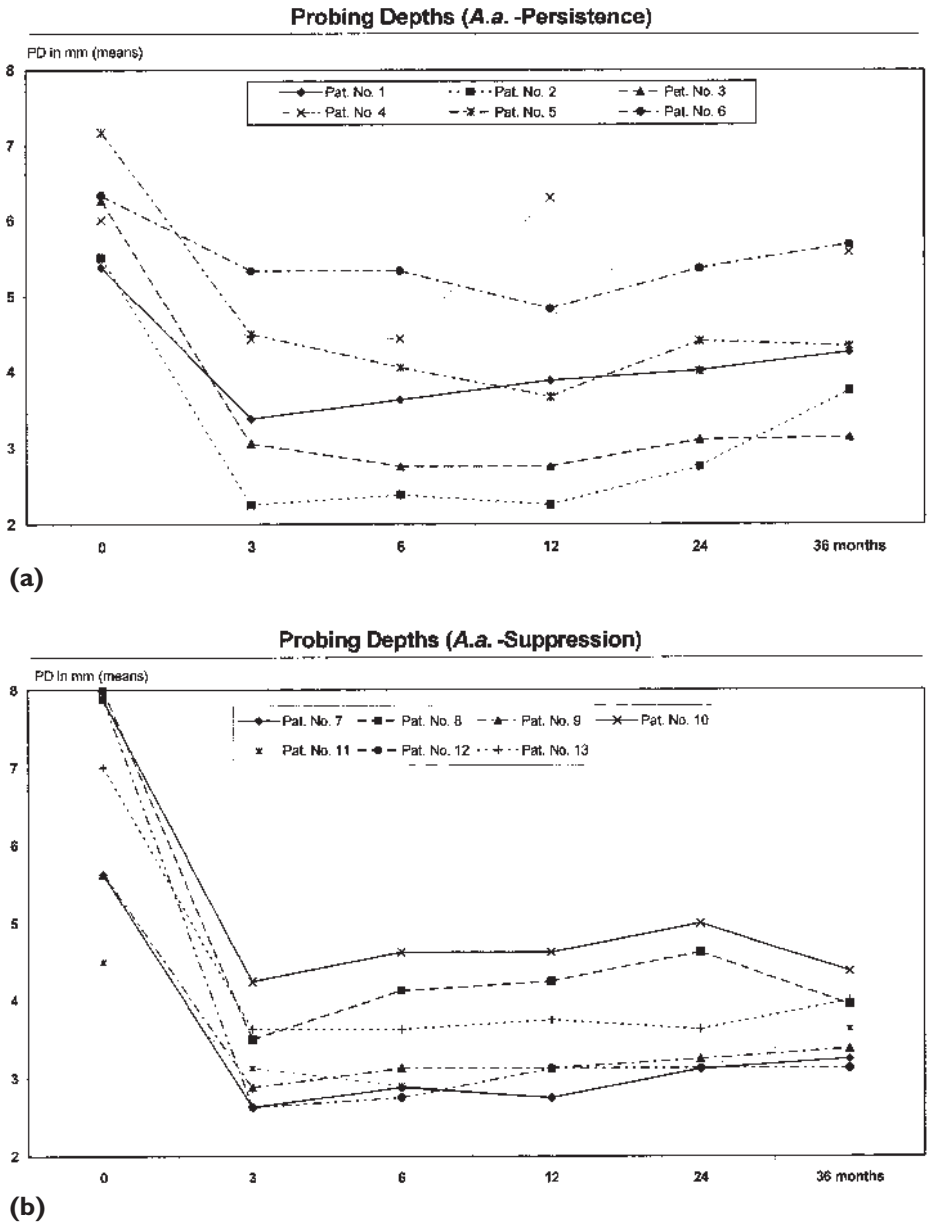

Figure 4.
Pre- and post-therapy comparisons of mean probing depths (PD) in patients with persistence ***(a)*** *and in subjects with suppression* ***(b)*** *of subgingival* A. actinomycetemcomitans *over a 3-year period.*

reached only moderate concentrations of 10^4 CFU/ml over the 3-year observation period.

The periodontal parameters, as a whole, significantly dropped as a result of periodontal therapy. Our data did not show any significant differences between subjects in whom *A. actinomycetemcomitans* was completely suppressed and individuals with a 3-year persistent subgingival infection. The trend to inconsistent treatment responses in patients with persistent *A. actinomycetemcomitans* might be due to the findings that the persistence is often associated with identical genotypes of *A. actinomycetemcomitans*. Treatment-induced changes of *A. actinomycetemcomitans* serotype strains are extremely rare.[29] Polymerase chain reaction (PCR) analysis studies of *A. actinomycetemcomitans* following SRP treatment with adjunctive amoxicillin plus metronidazole administration reported persistence frequencies of only 10% at the 3-month visit.[30] It is of special concern that these data are derived from adult periodontitis patients with an average age of 51.6 years, rendering the comparison to our destructive patient group (39.6 years) difficult. The cultural detection of *A. actinomycetemcomitans* as the gold standard on the selected medium has to be reconsidered. Recently, PCR was compared to selective cultivation in regards to sensitivity and specifity.[31] However, we doubt that false-positive results emerge following conventional cultivation. In accordance with Flemmig et al.,[31] false-negative results occur predominantly in plaque samples harboring *A. actinomycetemcomitans* scores less than 10^3 CFU/ml regardless of the microbial analysis system.

In contrast to other trials on *A. actinomycetemcomitans*-associated periodontal disease,[2,11,30,32] our microbial and clinical data were generated in individuals with a history of destructive periodontal disease. In these limited patient categories, the post-therapy subgingival recurrence of *A. actinomycetemcomitans* could not be considered as a risk factor for further periodontal tissue breakdown. We assume that additional selected factors, i.e., the reappearance of toxic, highly virulent *A. actinomycetemcomitans* strains[29] or the clonal character of *A. actinomycetemcomitans* infection, might contribute to the extent and severity of the disease process.[33]

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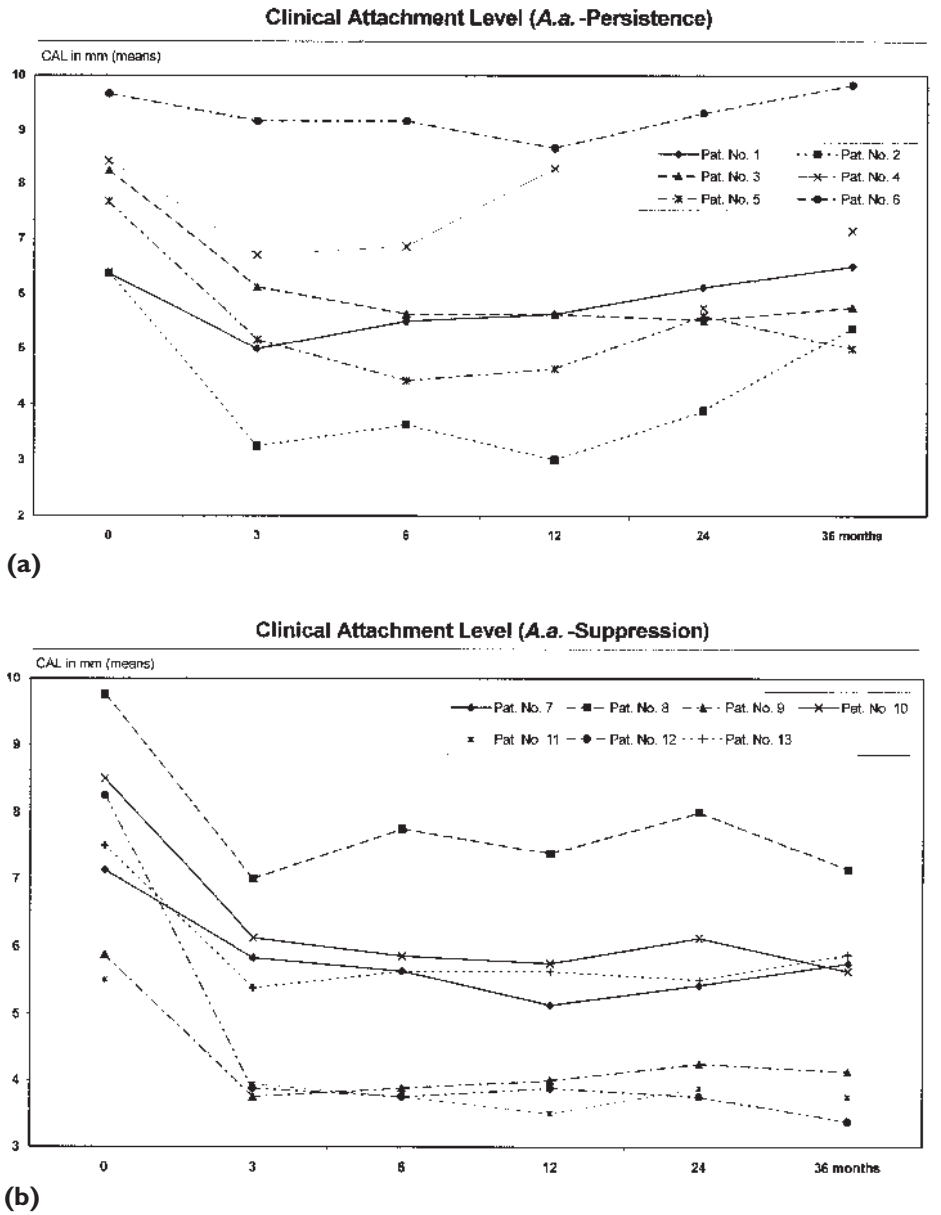

Figure 5.
Mean pre- and post-therapy clinical attachment levels (CAL) in individuals with a 3-year persistence ***(a)*** *of* A. actinomycetemcomitans *and in subjects with suppression* ***(b)*** *of* A. actinomycetemcomitans *following mechanical and antimicrobial periodontal therapy.*

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