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ORIGINAL ARTICLE

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Clinical effects of scaling and root planing in adults infected with *Actinobacillus actinomycetemcomitans*

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Abstract The periodontal pathogen *Actinobacillus actinomycetemcomitans* can frequently be isolated from subgingival plaque of adults with chronic inflammatory periodontal disease and individuals with plaque-induced gingivitis. Problems with the persistence of the organism after thorough debridement of root surfaces have been reported. In the present study clinical effects of the hygienic phase of periodontal therapy in ten adult patients with moderate or advanced periodontitis harbouring *A. actinomycetemcomitans* were analysed. Since proper analysis of highly correlated data within a given patient is crucial for appropriate interpretation, a major objective of this study was to compare the results of different models derived from logistic regression of clinical and microbiological factors on gain or loss of clinical attachment under different assumptions. Subgingival samples from every tooth present were obtained before and 6 weeks after thorough subgingival scaling, and selectively cultivated for the organism. A relevant gain of clinical attachment of 2 mm or more was observed at a total of 36% of periodontitis sites after scaling. Overall, loss of attachment of 2 mm or more was observed at 8% sites. Most loss occurred at sites with gingival enlargement (15%), whereas 3% periodontitis sites lost 2 mm or more. In multivariate analyses erroneously assuming either independence of data or correctly considering the correlated structure of observations attachment gain was mainly associated with deep probing depths at the outset. Presence or absence of *A. actinomycetemcomitans* before or after therapy was not included into the periodontitis models. Also, loss of attachment of 2 mm or more after subgingival scaling was not influenced by the organism. A direct comparison of the results obtained with both approaches of logistic regression may be helpful in the assessment of the influence of the magnitude of correlation of the data on the regression coefficients.

Keywords *Actinobacillus actinomycetemcomitans* · Periodontal disease · Hygienic phase · Attachment gain · Attachment loss · Multivariable logistic regression · GEE methods

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Introduction

The clinical effects of nonsurgical, mechanical periodontal therapy have been studied in great detail [1, 2, 4, 17, 21, 22, 31, 33]. Unanimously, sites with initially shallow probing depths respond to scaling and root planing with little or no change in probing depth and a slight loss of clinical attachment, whereas thorough instrumentation in moderately deep pockets in the 4–6 mm range may result in reductions in probing depth and some gain in clinical attachment of up to 1 mm. Gain in attachment may be even more pronounced in deeper pockets of 7 mm or more. It should be remembered, however, that some patients may have an overall poor response [9].

One of the strongest and most convincing associations with destructive periodontal diseases has been provided by the Gram-negative, facultatively anaerobic bacterium *Actinobacillus actinomycetemcomitans* [12]. In recent years its central role in the initiation and progression of localized juvenile periodontitis [3, 19] and, to a lesser extent, its association with adult forms of periodontitis [20, 34, 35] have been established. In general, recovery after therapy appears to expose the patient to an enhanced risk for further periodontal deterioration [5, 23, 32], and microbiological diagnosis has been advocated to identify especially adult patients with persisting subgingival infection with the organism [6, 20, 36].

Similar to other periodontal organisms, the intraoral distribution of *A. actinomycetemcomitans* is widespread.

Besides several extracrevicular niches, especially non-diseased periodontal sites may be colonised by the organism [26, 29]. In a previous analysis of data acquired in adult patients with moderate or advanced periodontitis harbouring *A. actinomycetemcomitans* [27], persistence of relatively high numbers of the organism of more than 10^4 colony-forming units per subgingival plaque sample after non-surgical periodontal therapy seemed to interfere with clinically relevant attachment gain. However, this observation might be due to a considerable number of relatively shallow sites actually with no chance for attachment gain after scaling and root planing but being colonised with *A. actinomycetemcomitans*. Thus, the major objective of the present study was to re-examine the clinical short-term effects of the hygienic phase of periodontal therapy and to identify factors probably being associated with the clinical outcome, while considering the various periodontal conditions at all periodontal sites examined. Since proper analysis of correlated data within a given patient is crucial for appropriate interpretation [10, 25, 28], a second objective was to compare the results of models derived from logistic regression of clinical and microbiological factors on gain or loss of clinical attachment under different assumptions.

Material and methods

Recruiting procedures, clinical and microbiological examinations as well as therapy have been published in a previous publication [27]. Ten adult, systemically healthy patients suffering from chronic periodontitis took part. A total of 120 periodontally diseased patients had been screened for the following inclusion criteria: (i) presence of *A. actinomycetemcomitans* in a pooled subgingival plaque sample from the deepest pockets in every quadrant of the dentition or cheek, dorsum of tongue or saliva sample; (ii) moderate or advanced chronic periodontitis; (iii) no periodontal or antibiotic therapy within the past 4 months; (iv) no pregnancy or lactation; (v) no current or previous medication known to affect the periodontal tissues; (vi) no allergy to metronidazole, amoxicillin or ciprofloxacin. Patients were between 28 and 60 years of age. Informed consent for participation this study was obtained after briefing on the procedures, risks and benefits of the planned therapy.

Clinical examinations were carried out at six sites of every tooth present. The degree of gingival inflammation and the amount of supragingival plaque were assessed by the gingival (GI) and plaque index (PLI) systems [18, 37]. Periodontal probing depth (PPD) and clinical attachment levels (CAL) were measured with a straight periodontal probe (PUNC15, Hu Friedy, Leimen, Germany). Thereafter, bleeding upon probing within 20 s was recorded. According to various combinations of the periodontal probing parameters, four different periodontal conditions could be differentiated: (i) periodontitis sites (n=456) were characterised by a PPD in excess of 3 mm and loss of clinical attachment of more than 2 mm; (ii) healthy (apart from gingival inflammation) sites (n=662) were characterised by a PPD of 1–3 mm and 0–2 mm attachment loss; (iii) sites with gingival enlargement (n=73) had a PPD in excess of 3 mm but 0–2 mm attachment loss, whereas (iv) recession sites (n=313) were shallow (PPD 1–3 mm) with attachment loss of 3 mm or more.

One week after the clinical examination, microbiological sampling was carried out. Subgingival plaque was sampled from every tooth at the site with the worst periodontal condition. First, the site with the deepest pocket was chosen. If two or more sites had the same PPD, CAL was considered, and, if necessary, BOP and GI. Supragingival plaque was removed with a curette, and three sterile fine paper points were inserted into the pocket until resistance was felt or the paper point bent. The paper points were removed after 10 s and transferred to 1 ml half-strength Ringer's solution. All samples were immediately processed in the laboratory. Samples were diluted in tenfold steps, and aliquots spread on TSBV-agar plates [38] for selective cultivation of *A. actinomycetemcomitans*. Plates were incubated in air supplemented with 10% CO_2 at 36°C for 5 days. Presumptive identification of the organism was based on colony morphology, a positive catalase and a negative oxidase test. After subcultivation of representative colonies, definite identification was accomplished using established biochemical tests [39]. Based on the dilution factor and considering the Poisson distribution, the limit for detection was below 30 colony-forming units (CFU) per millilitre transport fluid with 95% confidence.

A traditional periodontal treatment schedule was applied. In four weekly sessions, the patient's oral hygiene was improved, carious lesions treated and overhanging fillings removed or contoured. Thereafter, in 2–4 sessions of 30 min each, a thorough subgingival scaling and root planing took place under local anaesthesia. Patients rinsed for 2 weeks with a 0.1% solution of chlorhexidine-digluconate and were re-evaluated clinically and microbiologically 6 weeks after completion of the hygienic phase of periodontal therapy.

Statistical analysis

Descriptive data presented in the text and figures are based on individual sites as statistical unit. Hypothesis testing was, however, done under the assumption of non-independence of data. Primary outcome variables of the present study were relevant gain or loss of clinical attachment of 2 mm or more. This figure was based on a previous reliability study of probing measurements [23] in which a measurement error for CAL assessment of 0.50 mm was calculated. In order to identify clinical and microbiological factors increasing the likelihood of these events, different logistic regression models were considered. In general, the analysis was performed according to recommendations outlined by Hosmer and Lemeshow [13]. As a first step, models assuming independence of observations were constructed. Recently, it was demonstrated that conventional logistic regression models assuming the level of response depending on the individual subject usually raise considerable problems with the interpretation of the model as well as a desired generalisation of observations [28]. Such models were, therefore, not considered in the present study. Univariate logistic analyses were followed by a multivariate, backward stepping, model-building process. Variables were removed at $p>0.2$. Thereafter, biologically plausible interactions between significant variables were allowed to enter the model. A thorough analysis of diagnostics was performed considering the summary measures of goodness-of-fit of the models (Pearson's X^2 and deviance) as well as the Hosmer-Lemeshow statistic. As a final step, Generalised Estimating Equation (GEE) methods were employed in order to adjust for the correlation between sites in a given patient [10, 16, 40]. An exchangeable working correlation structure was used. Significance of odds ratios was tested by the Wald test in which the estimated regression coefficient from the model divided by its robust GEE standard error estimate was assumed to follow a standard normal distribution. All calculations and graphical display were performed on a personal computer using SAS/STAT 8.0 (SAS Institute, Cary, NC, USA) and SYSTAT 8.0 for WINDOWS, (SYSTAT, Evanston, IL, USA).

Results

A total of 1,504 periodontal sites were followed in the ten patients. At baseline, a mean (± standard deviation) GI of 1.2±1.0 and a mean PLI of 0.8±1.0 was observed. These figures dropped, 6 weeks after scaling, to 0.6±0.8 and

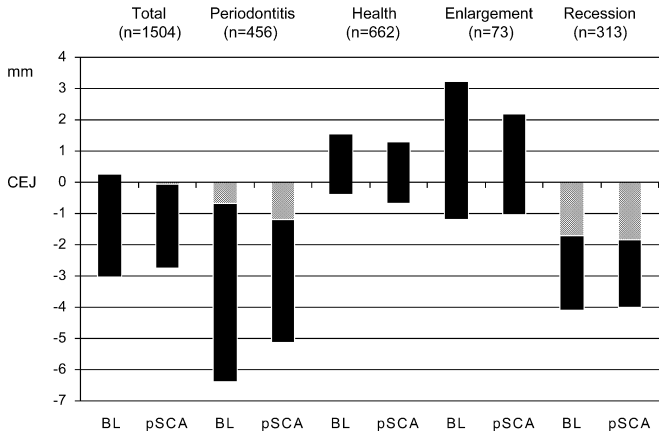

Fig. 1 Change in probing parameters 6 weeks after subgingival scaling according to various periodontal conditions. *Black bars* periodontal probing depth, *grey bars* gingival recession, *CEJ* cemento-enamel junction

0.5±0.7. Fifty-one percent of sites bled after probing at baseline and 36% after scaling. Mean PPD at the outset was 3.29±1.97 mm, and mean attachment loss 3.02±2.96 mm. Six weeks after subgingival scaling, overall PPD was reduced by 0.62±1.36 mm, whereas 0.28±1.53 mm attachment gain was observed, on average. Figure 1 shows that attachment gain was virtually confined to periodontitis sites. For this category, mean PPD was reduced from 5.71±1.59 mm to 3.95±1.77 mm. Pocket reduction was mainly due to a mean attachment gain of 1.27±1.79 mm. Healthy sites lost 0.30±1.05 mm attachment, on average, whereas sites with gingival enlargement underwent a mean decrease in pocket depth of 1.19±1.31 mm. On average, no change was observed at sites with gingival recession (Fig. 1).

Out of 298 subgingival sites sampled for selective cultivation of *A. actinomycetemcomitans*, 51% harboured the organism at baseline (between 9 and 96% sites in individual patients) and 42% after scaling (between 7 and 91%). At baseline, 68% periodontitis sites, 17% healthy sites, 50% sites with gingival enlargement and 56% recession sites were culture-positive. Mean log-transformed counts of *A. actinomycetemcomitans* CFU in culture-positive samples ranged between 2.42±0.96 in healthy sites, and 3.69±1.41 and 3.70±1.26 in periodontitis and recession sites, respectively. After scaling, the organism was detected at 50% sites formerly diagnosed as periodontitis sites, 22% healthy sites, 38% former sites with gingival enlargement and 48% former recession sites (mean counts of CFU in positive samples between 2.41±1.26 in healthy and 3.67±1.26 in recession sites).

A relevant gain of clinical attachment of 2 mm or more was observed at 15% sites after scaling. Whereas 36.2% of periodontitis sites gained 2 mm or more, among periodontal conditions other than periodontitis a total of 6.4% sites also gained 2 mm or more. Overall, loss of attachment of 2 mm or more was observed at 8% sites. Most losses occurred at sites with gingival enlargement (15.1%), whereas only 2.9% periodontitis sites lost 2 mm or more [11].

In a search for baseline and postoperative factors with an impact on significant gain in clinical attachment, periodontitis sites were considered in the first place. The code sheet of the variables is shown in Table 1. In univariate analyses (Table 2), baseline and postoperative PPD, PLI, and BOP were significantly associated with attachment gain of 2 mm or more. *A. actinomycetem-*

Table 1 Code sheet for variables

Variable	Examination	Category	Abbreviation
Periodontal probing depth	Baseline	1–3 mm	PPDBL_1
		4–6 mm	PPDBL_2
		>6 mm	PPDBL_3 [a]
	Post scaling	1–3 mm	PPDPS_1
		4–6 mm	PPDPS_2
		>6 mm	PPDPS_3 [a]
Clinical attachment level	Baseline	0–2 mm	CALBL_1
		3–5 mm	CALBL_2
		>5 mm	CALBL_3 [a]
	Post scaling	0–2 mm	CALPS_1
		3–5 mm	CALPS_2
		>5 mm	CALPS_3 [a]
A. actinomycetemcomitans count	Baseline	Log CFU 0–2	CFUBL_1
		Log CFU 2–4	CFUBL_2
		Log CFU >4	CFUBL_3 [a]
	Post scaling	Log CFU 0–2	CFUPS_1
		Log CFU 2–4	CFUPS_2
		Log CFU>4	CFUPS_3 [a]
Gingival index	Post scaling	0–1	GIPS_0
		2–3	GIPS_1 [a]
Plaque index	Post scaling	0–1	PLIPS_0
		2–3	PLIPS_1 [a]
Bleeding on probing	Post scaling	0	BOPPS_0
		1	BOPPS_1 [a]

[a] In all models the reference is the highest category

Table 2 Univariate logistic regression models for CAL gain of 2 mm or more after scaling in periodontitis sites assuming independent observations

Variable	β [a]	s.e. (β) [b]	Log-Likelihood	G [c]	p
Constant	–0.567	0.097	–298.44		
PPDBL_2	–0.649	0.221	–294.16	8.57	0.003
CALBL_2	0.186	0.201	–298.01	0.85	0.356
CFUBL_1	–0.332	0.398	–91.81	2.03	0.362
CFUBL_2	0.314	0.479			
PPDPS_1	2.423	0.619	–274.88	47.1	0.000
PPDPS_2	1.262	0.625			
GIPS_0	0.308	0.205	–298.44	2.28	0.131
PLIPS_0	1.172	0.303	–289.61	17.7	0.000
BOPPS_0	0.660	0.197	–292.79	11.3	0.001
CFUPS_1	0.713	0.490	–95.53	2.26	0.323
CFUPS_2	0.573	0.559			

[a] Estimate of coefficient for the univariate logistic regression model containing only this variable
[b] Standard error of estimated coefficient
[c] Likelihood ratio test statistic for the hypothesis that the slope coefficient is zero

Table 3 Multivariate logistic regression analysis assuming independent observations. Dependent variable: CAL gain of 2 mm or more after scaling in periodontitis sites assuming independent observations

Variable	β[a]	s.e. (β)[b]	p	ψ[c]	95% C.I.[d]
Constant	–1.440	0.256	0.000	–	–
PPDBL_2	–0.897	0.152	0.000	0.41	0.30; 0.55
PPDPS_1	1.785	0.267	0.000	5.96	3.54; 10.1
PPDPS_2	0.160	0.242	0.509	1.17	0.73; 1.88
PLIPS_0	1.233	0.328	0.000	3.43	1.82; 6.67
BOPPS_0	0.536	0.227	0.018	1.71	1.10; 2.63

For abbreviations of statistics see Table 1
[a] Estimate of coefficient for the multivariate logistic regression model
[b] Standard error of estimated coefficient
[c] Estimated odds ratio
[d] 95% confidence intervall of estimated odds ratio

Table 4 Model diagnostics of Table 3. Log-likelihood: –243.4

Likelihood ratio	110	5 DF	p =0.000
Hosmer-Lemeshow statistic	6.04	7 DF	p =0.535
Pearson's X 2	534	450 DF	p =0.004
Deviance	487	450 DF	p =0.112

comitans counts did not play a significant role, neither at baseline nor after scaling (p>0.3). The multivariate case is seen in Tables 3 and 4. Significant gain was mainly influenced by pocket depth at baseline. In moderately deep pockets of 4–6 mm, the odds were decreased by a factor of 2.4 as compared with deeper pockets. Postoperative conditions were significantly associated with attachment gain. For example, at periodontitis sites with a shallow PPD after scaling, no BOP and no supragingival plaque, an odds ratio of 35 for attachment gain of 2 mm or more could be calculated. No interaction term was included in the final model. Model diagnostics are shown in Table 4. The Hosmer-Lemeshow goodness-of-fit statistic, which is based on the decile of risk grouping strategy, was 6.04 with 7 degrees of freedom (p=0.535), indicating that the model seems to fit quite well. However, since both Pearson's X^2 and Deviance are large, a closer look on suspected problems with the model fit is warranted.

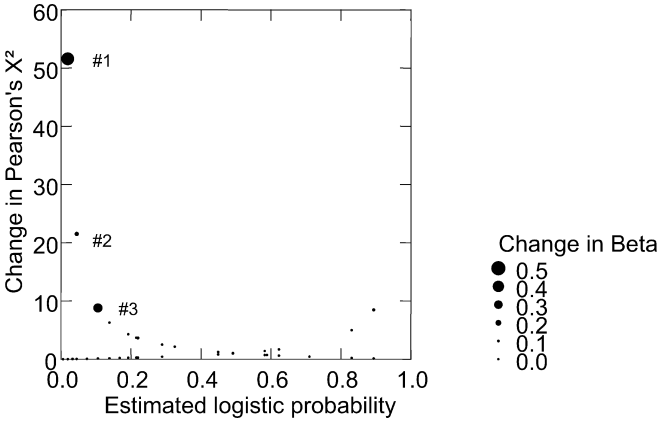

Fig. 2 Logistic regression model of Table 3. Outcome variable: CAL gain of 2 mm or more after scaling. Plot of change in Pearson's X^2 on the estimated logistic probability with the plotting symbol proportional in size to the standardised change in estimated β. Problematic covariate patterns ##1 and 2 could be identified and were subsequently eliminated

In Fig. 2 the change in Pearson's X^2 (ΔX^2) is plotted against the estimated logistic probability π. In general, the plot shows two curves. The curve going from the top left to bottom right corner corresponds to covariate patterns and y=1, i.e. attachment gain of 2 mm or more. The ordinate of these points is $(1-\pi)/\pi$. The points on the curve going from the bottom left to top right corner correspond to covariate patterns and y =0, i.e. no or minor attachment gain. The ordinate for these points is π /(1- π). The size of the symbol is proportional to the change in the estimated coefficient vector, $\Delta\beta$. In principle, a plot of change of deviance on the estimated logistic probability revealed a similar result. As can be seen in Fig. 2, a total of seven covariate patterns (corresponding to 17 sites, i.e. 3.7%) seem to fit poorly (ΔX^2>4), two or three having, in addition, a large effect on the influence diagnostic $\Delta\beta$. Covariate pattern #1 corresponds to two sites located distobuccal to tooth #48 in one patient and, in another patient, mesiobuccal to tooth #37. Attachment gains were

Table 5 Multivariate logistic regression analysis considering the correlated structure of the data. Dependent variable: CAL gain of 2 mm or more after scaling in periodontitis sites. Correlation structure: "dental common", correlation estimate: 0.011

Variable	β	s.e. (β)	p	ψ	95% C.I.
Constant	−1.698	0.611	0.005	–	–
PPDBL_2	−1.721	0.198	0.000	0.18	0.12; 0.26
PPDPS_1	3.760	0.701	0.000	42.9	10.9; 170
PPDPS_2	2.152	0.671	0.001	8.60	2.31; 32.1
PLIPS_0	1.168	0.269	0.000	3.22	1.89; 5.56
BOPPS_0	0.527	0.189	0.005	1.69	1.16; 2.44

For abbreviations of statistics see Tables 1 and 3

Table 6 Multivariate logistic regression analysis considering the correlated structure of the data. Dependent variable: CAL loss of 2 mm or more after scaling of all sites. Correlation structure: "dental common". Correlation estimate: 0.007

Variable	β	s.e. (β)	p	ψ	95% C.I.
Constant	1.643	1.026	0.109	–	–
Health	3.685	0.517	0.000	39.9	14.5; 110
Enlargement	2.997	0.658	0.000	20.0	5.51; 72.7
Periodontitis	−3.158	0.672	0.001	0.04	0.01; 0.16
PPDPS_1	−2.977	1.029	0.004	0.05	0.01; 0.38
PPDPS_2	−1.233	0.797	0.122	0.29	0.06; 1.39
CALPS_1	−5.340	0.603	0.000	0.005	0.001; 0.02
CALPS_2	−1.813	0.324	0.000	0.16	0.09; 0.31
PLIPS_0	−0.499	0.296	0.091	0.61	0.34; 1.09
BOPPS_0	0.479	0.187	0.010	1.61	1.12; 2.33

For abbreviations of statistics see Tables 1 and 3

associated with an increase of PPD from 6 to 7 mm after scaling in each case. The two sites representing pattern #2 (site 28db and site 44 dl in two different patients) correspond to slight pocket alterations of moderately deep pockets associated with attachment gain. Both patterns do not appear to be biologically plausible but rather may be due to measurement and/or recording error. Covariate pattern #3 corresponds to a decrease of PPD from 11 to 10 mm with a concomitant gain of 2 mm attachment at site 17dp. Although this pattern has a relatively large influence on the coefficient vector, it may indeed correctly describe a 6-week response to subgingival scaling. Patterns ##1 and 2 were deleted from the data set in order to assess the influence on the respective regression coefficients. Deletion of these patterns (four out of 456 sites, 0.9%) altered the regression coefficients in a substantial way (data not shown). The association between shallow PPD after scaling and attachment gain was further strengthened. P -values for the Hosmer-Lemeshow statistic, Pearson's X^2 and deviance increased to 0.742, 0.710 and 0.370, respectively, signaling considerably better fit of the new model.

Table 5 presents the multivariate logistic regression model considering the correlated structure of the data by using GEE methods. Interpretation of this model may be similar to that of the model in Tables 3 and 4. However, with regard to post-therapeutic PPD, respective associations are considerably stronger. Again, *A. actinomycetemcomitans* counts were not included in the model.

Finally, models were analysed with significant attachment loss of 2 mm or more as dependent variable. Data of all examined sites were used. The GEE model is shown in Table 6. Obviously, shallow sites, with no or minimal attachment loss were at high risk for losing clinical attachment after subgingival scaling as compared with recession sites. In contrast, the risk for periodontitis sites was minimal.

Discussion

In a recent longitudinal cohort study the intraoral distribution of *A. actinomycetemcomitans* was examined at different stages of therapy of adults with chronic periodontitis [27]. Subgingival samples were obtained from every tooth present. Also, numerous mucosal surfaces of the oral cavity were sampled. The material was selectively cultivated for *A. actinomycetemcomitans* with a very low limit of detection of about 30 CFU/sample. It was concluded that eradication from the oral cavity of this periodontal pathogen might only be possible by adjunctive administration of systemic antibiotics as, e.g. a combination of metronidazole and amoxicillin. Besides this major finding, it was observed that the likelihood of recovering *A. actinomycetemcomitans* from subgingival plaque at baseline was influenced by PPD. The odds of a culture-positive sample were increased by a factor of 3.7 in moderately deep pockets of 4–6 mm and about 10 in 7-mm or deeper pockets. After scaling, the relationship with pocket depth was largely attenuated. However, sites with attachment loss of 6 mm or more had an 83% increased odds for harbouring cultivable numbers of *A. actinomycetemcomitans*. Interestingly, attachment gains of 2 mm or more were strongly associated with baseline pocket depth and no bleeding post therapy. Only high counts of persisting *A. actinomycetemcomitans* appeared to inhibit relevant attachment gain [27]. This observation may be in contrast to results reported in some studies, in which the presence of the organism was checked only in representative, rather deep pockets [15, 23, 34], and elimination was found to be crucial for clinical improvement.

Very recently, the intraoral distribution of *A. actinomycetemcomitans* was studied in detail in 17 young adults with plaque-induced gingivitis [29]. Whereas only a few subgingival and oral mucosal surfaces were culture-positive in about half of the subjects, in other individuals an extremely wide distribution of *A. actinomycetemcomitans* was found, similar to that seen in the present patients with chronic periodontitis. In that study of subjects without periodontal destruction, PPD was also associated with presence of the organism: with every millimetre the odds to recover the pathogen increased by 35% [30]. However, most sites were in the 1–3 mm range, i.e. rather shallow. Since the size of the subgingival plaque sample cannot be standardised, it may be argued that more subgingival material is sampled from deeper pockets, from which more viable cells of the pathogen may be

recovered [20]. This fact will certainly reduce the likelihood of a false-negative result.

In the present cohort of patients with chronic periodontitis, many sites sampled for presence of *A. actinomycetemcomitans* could not be regarded typical for destructive periodontitis. Since no attachment gain is expected at shallow sites, including such sites into the analysis may in fact dilute the effect of scaling on attachment level. This is in particular true in the very case of a large proportion of these sites being actually colonised with *A. actinomycetemcomitans* before (17% of healthy sites, 50% of sites with gingival enlargement and 56% recession sites of the present study), and after therapy. When only periodontitis-affected sites were considered, *A. actinomycetemcomitans* was not associated with relevant attachment gain of 2 mm or more. It must be mentioned, however, that microbiological data were only present for 32% of periodontitis sites. In a multivariate model including all 456 sites with periodontitis and assuming (erroneously) independence of observations, attachment gain depended mainly on probing depth at the outset (the higher the baseline PPD, the higher the odds of attachment gain), whereas several post-therapeutic clinical variables (plaque, bleeding on probing, PPD) were negatively associated. This (conventional) logistic regression model has several interesting properties as, e.g. the possibility of a thorough analysis of regression diagnostics (Hosmer-Lemeshow statistic, Pearsons X^2 and deviance). Thus, model fit could easily be checked and interesting covariate patterns identified. Eventually, two covariate patterns with a large influence on the coefficient vector, which appeared to be mainly due to measurement or recording error, were eliminated, while fit of the model could be improved. However, the assumption of independence of site data is actually not valid. The (correct) GEE model revealed, therefore, some specific differences. In particular, the association of attachment gain with post-therapeutic PPD was much stronger, although overall interpretation of the model may be similar.

Attachment loss of 2 mm or more occurred at 120 sites (8%). Since microbiological data were only available for 12.5% of these sites, a proper analysis of a potential influence of *A. actinomycetemcomitans* on this event was largely prevented. Relevant attachment loss was strongly associated with no attachment loss at baseline. This is in accordance with observations made in numerous studies in the past [2, 14, 17]. An important factor which might explain this observation may be thickness of the tissue [7], i.e. thin and delicate gingiva may recede after thorough subgingival scaling. Another consequence of scaling may be remodelling and subsequent gingival recession [8]. Furthermore, it was recently suggested that the statistical phenomenon of regression towards the mean might account for a considerable portion of the observed attachment loss in shallow sites after mechanical debridement [10].

In conclusion, the present re-analysis of data acquired in patients with chronic periodontitis did not provide any evidence for a significant role of *A. actinomycetemcomitans* in the short-term therapeutic outcome after the hygienic phase of therapy. Probing depth reduction and clinical attachment gain at sites with periodontitis occurred irrespective of presence or absence of *A. actinomycetemcomitans*. Longitudinal studies have shown that observed clinical improvements might be transient if *A. actinomycetemcomitans* persists in the oral cavity [24]. Future studies should make use of appropriate statistical tools in the analysis of complex data sets in order to delineate the true clinical and microbiological long-term effects of periodontal therapy. Conventional logistic regression models under the (erroneous) assumption of independence of observations provide valuable information regarding model diagnostics and identification of interesting or problematic covariate patterns. GEE methods, on the other hand, may allow a site-specific analysis while correctly considering the correlated structure of the data. A direct comparison of the results obtained with both approaches may be especially helpful in the assessment of the magnitude of the correlation of the data and its influence on the regression coefficients.

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