Short-term effects of systemic antibiotics during periodontal healing

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Objectives: To investigate the short-term effects of nonsurgical therapy (scaling and root planing, SRP) on the subgingival microbiota in chronic (CP) and aggressive (AP) perio - dontal disease. **Method and Materials:** Ninety-seven CP and AP subjects underwent full-mouth SRP on 2 consecutive days. AP patients were randomly assigned to either receive systemic metronidazole plus amoxicillin (AP+AB) or were treated mechanically alone (AP). Pathogens were identified with 16S rRNA oligodeoxynucleotide probes and dot-blot hybridization before and at days 2, 3, 4, 7, 10, and 21 of healing. CP subjects were treated by scaling and root planing along with placebo tablets. **Results:** Initially, AP cell counts were 69.9- (*Porphyromonas gingivalis*), 10.2- (*Aggregatibacter actinomycetemcomitans*), 5.7- (*Tannerella forsythia*), and 3.3-fold (*Prevotella intermedia*) enhanced compared to CP cell counts. Following SRP, immediate elimination occurred in single individuals of all three treatment groups at day 2. After SRP plus antibiotic therapy (AP+AB), the prevalence scores dropped beyond the levels of AP and CP, beginning at day 7, and remained low until day 21 ($P \leq .05$). Clinical healing statistically benefited from SRP with no differences among the three treatment groups. **Conclusion:** Nonsurgical therapy resulted in both a suppression and early elimination of single taxa immediately after completion of active treatment. Systemic antibiotics significantly accelerate the suppression of the periodontal microflora, but have limited effect on the elimination of target isolates during healing.
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Key words: aggressive periodontitis, early healing, periodontal pathogens, rRNA probes, suppression, systemic antibiotics

Aggressive periodontitis represents a complex disease entity with specific genetic determinants affecting its pathogenesis and therapy outcomes.[1–3] The rationale for the use of systemic antibiotics in periodontitis is to reduce or even eliminate suspected pathogens that exist within the relatively impervious subgingival biofilm.[4] Data from systematic reviews elucidated that the clinical outcomes probing pocket depth, clinical attachment level change, and risk for additional clinical attachment level loss may benefit from antimicrobial therapy.[5] Recently, these suggestions have been confirmed for systemic amoxicillin plus metronidazole in a randomized, placebo-controlled clinical trial at the 2- and 6-month posttreatment level.[6] In addition to these clinical parameters, the combination of mechanical debridement and the use of antimicrobials intertwine in a downregulation of the underlying inflammatory actions within the periodontal tissues.[7,8]

Evidence is rising that the elimination of single bacterial taxa from the subgingival environment below detectable levels is a prerequisite for long-term clinical improvement.[9]

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However, because the microflora associated with aggressive periodontitis varies significantly from patient to patient, the response to an antibiotic can be expected to vary as well.[10,11] Not all periodontal patients benefit equally from antibiotics because they display different microflora and harbor individual susceptibilities against key periodontal microbiota. Elimination or persistence of periodontal pathogens after antibiotic usage is an unpredictable event depending on the individual host-pathogen relationship. For example, the highly leukotoxic serotype b variant of *Aggregatibacter actinomycetemcomitans* is uniquely associated with aggressive periodontitis.[12,13]

There are conflicting data reporting either persistence or elimination of target pathogens following antibiotic therapy.[14,15] It is of special concern whether the persistence of microbes after antibiotic usage is a result of initial elimination followed by a subsequent recolonization or an inappropriate antibiotic action in an imbalanced host-pathogen environment.[16] Because most periodontal pathogens are endogenous to humans, the use of antibiotics may result in suppression rather than elimination. Reemergence of individual taxa on the subgingival platform after therapy is a complex result of a multilevel regulation with less controlled effects on resulting bacterial profiles.[17]

Although systemic antibiotic therapy enhances resolution of advanced adult periodontitis, microbial data on a short-term basis identifying the exact time point of elimination immediately after antibiotic therapy are limited. Knowledge of the early microbial responses to antibiotics would advance our understanding of microbial results deriving from long-term trials, thus ensuring the safe and efficacious usage of systemic antibiotics.

With this background, the purpose of the present trial was *(1)* to display the short-time effects of nonsurgical therapy on the subgingival microbiota in chronic and aggressive periodontal disease, and *(2)* to test the hypothesis that the time course of subgingival recolonization during healing after administration of systemic antibiotics as adjuncts to periodontal therapy is different from mechanical debridement alone.

METHOD AND MATERIALS

Patients

Sixty-eight patients with aggressive periodontitis (AP) and 29 subjects with chronic periodontitis (CP) were recruited from a private practice in North Rhine–Westphalia, Germany, between July 2005 and September 2006. AP patients were younger (AP, 42.4 ± 7.5 years; CP, 62.5 ± 10.2 years) and exhibited more severe disease radiographically, with evidence of intrabony defects exceeding more than 50% of the root length, versus 30% in CP subjects.[18] Other inclusion criteria were probing depths greater than 5 mm at eight sites or more within each quadrant, generalized severe periodontal tissue destruction, loss of periodontal support inconsistent with age, persistent gingival inflammation, increasing probing depths, and progressing tooth mobility. Exclusion criteria included smoking, pregnancy, periodontal therapy, or antibiotics in the previous 6 months; any systemic condition that might have affected the progression or treatment of periodontitis; and the need for antibiotic premedication before dental therapy.

A written informed consent was obtained from each volunteer. A subject insurance (Ecclesia No. 94543-01) covered all possible risks resulting from the clinical trial. The study protocol was approved by the Institutional Review Board of the Medical Faculty of the University of Münster, Germany, on July 11, 2002, and registered under 2IXBuch.

Periodontal intervention

The experimental protocol of the study displaying the schedule for the clinical and microbial examinations is outlined in Fig 1. All patients were monitored at baseline and at days 2, 3, 4, 7, 10, and 21 following scaling and root planing (SRP) under local anesthesia. The clinical examination included measurement of probing depth (PD) at six sites per tooth, clinical attachment level (CAL) at six sites per tooth, the Gingival Index (GI) and Plaque Index (PI),[19] and bleeding on probing (BOP) (data not shown).

AP patients were randomly assigned by the first author (R.B.) to receive scaling and root planing with either placebo tablets (n = 32 subjects) or 3 × 500 mg amoxicillin plus

3 × 250 mg metronidazole (n = 36 individuals)—systemically as adjuncts to therapy for 7 days. Blinding of the CP patients (n = 29) and the examining clinician was accomplished by administering placebos along with nonsurgical therapy. Compliance to medication was assessed by asking the patients to return with the remaining tablets at each subsequent visit. The random allocation sequence for AP subjects was concealed, with numbers distributed to the patients by the first author (R.B.) before intervention and collected at the end of the trial. Both the second author (G.C.), assessing the primary outcome variable, and the third author (T.S.), who performed the interventions and evaluated the secondary outcome measures, were blinded to group assignment.

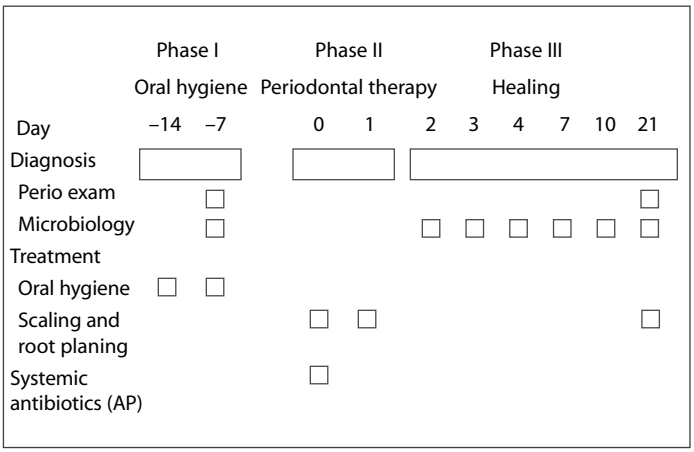

Fig 1 Experimental protocol of the study displaying the schedule for the clinical and microbial examinations.

Microbial sampling

Unpooled subgingival plaque samples were harvested at four individual sites (one in each quadrant) with a probing depth of greater than 5 mm in each subject. After the area was isolated with a cotton roll and gently air dried, supragingival deposits were carefully removed with a Gracey 11/12 curette tip (Hawe Neos). Subgingival plaque samples were assessed by inserting a sterile endodontic paper point (Roeko; International Organization for Standardization [ISO] 15) to the bottom of the periodontal pocket for 10 seconds. The subgingival plaque samples were immediately transferred into empty 1.5 mL Eppendorf vials and stored at –70°C.

Microbial analyses

The DNA probes (LCL Biokey) were developed in a computerized comparison against 9,000 bacterial 16S ribosomal RNA (rRNA)/DNA sequences and then tested empirically against the bacterial strains listed below, as well as against the human genome. They proved to be more than 99.99% specific for *A actinomycetemcomitans* ATCC 33384^T, UP6, UP57, JP2, plus 37 clinical isolates; *Tannerella forsythia* (formerly *Bacteroides forsythus*) ATCC 43037^T plus 5 clinical isolates; *Porphyromonas gingivalis* ATCC 33277^T, 381, W83, plus 27 clinical isolates, and *Prevotella intermedia* ATCC 25611^T plus 24 clinical isolates. Thirty strains of other oral taxa served as negative controls. Nucleic acids were isolated by the aid of a QIAamp Blood & Tissue kit (Quiagen) or simple boiling. Dot-blot hybridization was performed according to the manufacturer and standard procedures.[20] To ensure coverage of low cell numbers (eg, after antimicrobial therapy), a multiplex polymerase chain reaction (PCR) was performed for *P intermedia* and *T forsythia*.[21]

Statistical analysis

Data analysis and statistical tests were performed on the patient level with the results presented as mean values ± standard deviations. The microbial data were given as cell counts per microliter sample. Intragroup comparison of the clinical and microbial parameters before and after completion of therapy was analyzed by the Wilcoxon signed rank test. Intergroup differences between AP+AB, AP, and CP were subjected to the Kruskal-Wallis test. For general validity, cell counts were used as the primary outcome variables and clinical conditions as the secondary outcome variables. The PD and CAL measurements (reproducibility ±1 mm greater than 95%) were performed by the same calibrated examiner throughout the study. The α level for significance was evaluated using the adjusted Bonferroni probability for multiple comparisons. Statistical significance was defined as $P < .05$.

Table 1 Treatment-induced changes of clinical parameters*

Parameter/ group	Time†	Mean (SD)	Outset to 3 w	Range	Change‡	Change after 3 w§	
PD							
1 = AP+AB	1	6.0 (1.0)		5.0–7.8			
	2	3.6 (0.7)	2.4 (0.6)	2.9–5.0	0.008[d]		
2 = AP	1	5.4 (0.8)		4.0–6.3			
	2	3.7 (0.7)	1.7 (0.7)	2.5–5.0	0.005	1 vs 2	.07
3 = CP	1	5.8 (0.9)		4.3–7.0		1 vs 3	.12
	2	3.9 (0.7)	1.9 (0.9)	3.0–5.0	0.005	2 vs 3	.91
CAL							
1 = AP+AB	1	6.7 (1.5)	5.3–10.0				
	2	4.6 (1.2)	2.1 (0.7)	3.3–7.0	0.008		
2 = AP	1	5.9 (1.0)	4.0–7.3				
	2	4.6 (0.9)	1.3 (0.5)	3.5–6.0	0.007	1 vs 2	.03
3 = CP	1	6.0 (1.2)		4.3–8.3		1 vs 3	.02
	2	5.0 (1.0)	1.0 (0.7)	4.0–6.5	0.005	2 vs 3	.34
GI							
1 = AP+AB	1	1.9 (0.7)	1.3–2.8				
	2	0.1 (0.2)	1.8 (0.7)	0–0.8	0.008		
2 = AP	1	1.5 (0.6)	0.3–2.3				
	2	0.1 (0.2)	1.4 (0.6)	0–0.5	0.005	1 vs 2	.20
3 = CP	1	1.6 (0.6)	0.8–2.5			1 vs 3	.23
	2	0.3 (0.2)	1.3 (0.5)	0–0.5	0.005	2 vs 3	.62
PI							
1 = AP+AB	1	1.1 (0.6)		0.5–2.5			
	2	0.2 (0.3)	0.9 (0.6)	0–0.8	0.01		
2 = AP	1	0.3 (0.4)		0–1.3			
	2	0.3 (0.4)	0.03 (0.4)	0–1.0	0.86	1 vs 2	.003[?]
3 = CP	1	0.6 (0.5)		0–1.5		1 vs 3	.006[?]
	2	0.3 (0.4)	0.3 (0.4)	0–1.0	0.03	2 vs 3	.16

*In each treatment group, clinical parameters were different before and after therapy (except PI in AP).
†1 = Baseline, 2 = 3 weeks.
‡Wilcoxon signed rank test, exact *P* value.
§Kruskal-Wallis test.
[?]Significant after adjustment to Bonferoni.

RESULTS

Periodontal conditions

AP and CP patients demonstrated a comparable disease severity, in general, as evidenced by initial pocket depth and attachment loss. The significant change of PD and CAL scores in AP+AB (PD change 2.4 ± 0.6 mm, CAL change 2.1 ± 0.7 mm) compared to AP and CP indicated the additional benefit of the periodontal status from antibiotic therapy after SRP. All three patient population groups exhibited significant treatment effects, as evidenced by changes in PD, CAL, GI, and PI (*P* = .012 to .005, intragroup comparison). However, at 3 weeks, there were no differences between groups for any parameter except PI (intergroup comparison, *P* = .003 to .006) (Table 1). For each category, the clinical situation at baseline and following therapy is displayed in Figs 2 to 4.

Subgingival prevalence

The prevalence scores of the four target bacteria were markedly elevated in all periodontal subjects with baseline levels for *A actinomycetemcomitans* of 88.9% (AP+AB; n = 36 subjects), 90.0% (AP; n = 32 patients), and 60.0% (CP; n = 29 individuals), compared to healthy periodontal conditions. The initial levels for *P gingivalis* were 77.8% (AP+AB) and 70.0% (AP and CP). *T forsythia* scores at baseline reached 77.8% (AP+AB), 80.0% (AP), and 70.0% (CP). *P intermedia* displayed values of 88.9% (AP+AB), 90.0% (AP), and

Fig 2a AP+AB patient. Generalized aggressive periodontitis with multiple inflammatory cellular infiltrates and fistula at the maxillary central incisors and right lateral incisor.

Fig 2b AP+AB patient. Accelerated healing with loss of interdental soft tissues and papillae according to the advanced intrabony defect anatomy.

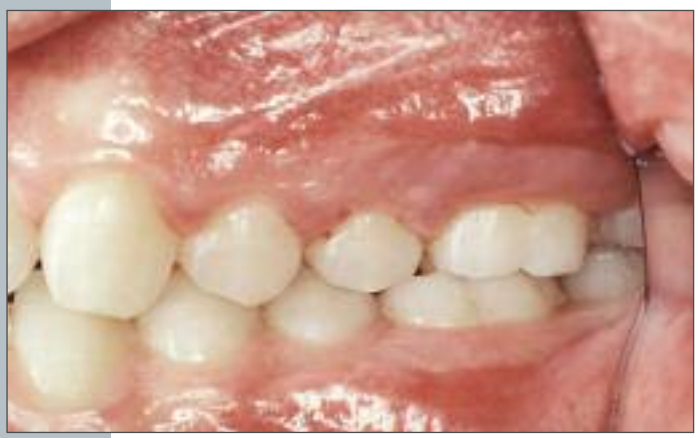

Fig 3a AP patient. Pretherapy redness, edematous swelling, and discrete bleeding of the gingiva around the maxillary left first premolar, second premolar, and first molar.

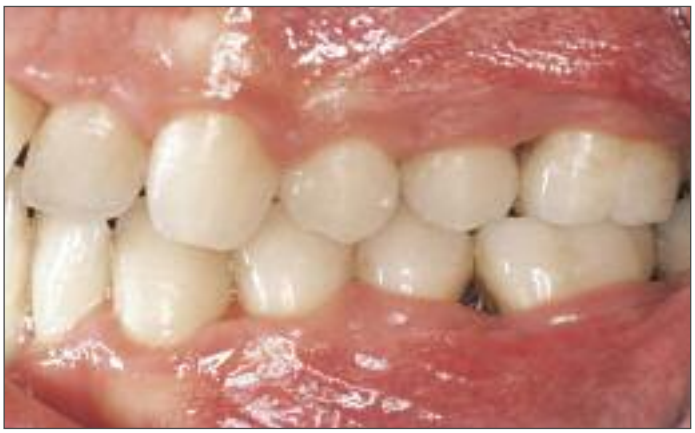

Fig 3b AP patient. Stabilization of the periodontal tissues after 3 weeks of healing with reorganization of the interdental papillae.

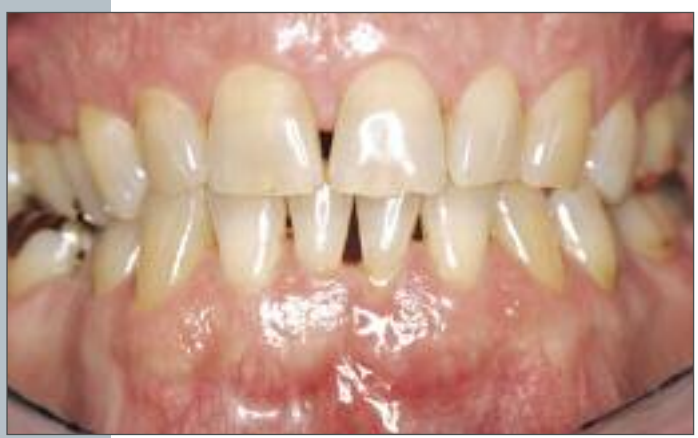

Fig 4a CP patient. Chronic periodontitis with promoted bone loss around the mandibular central incisors and gingival collagen fiber breakdown.

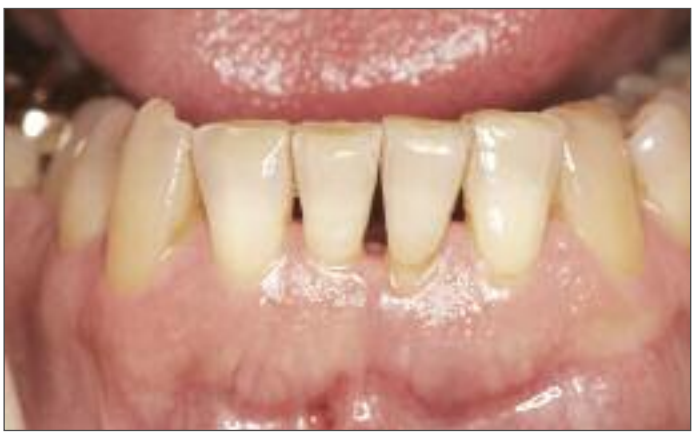

Fig 4b CP patient. Soft tissue healing with complete gingival fiber remodeling 3 weeks after nonsurgical therapy.

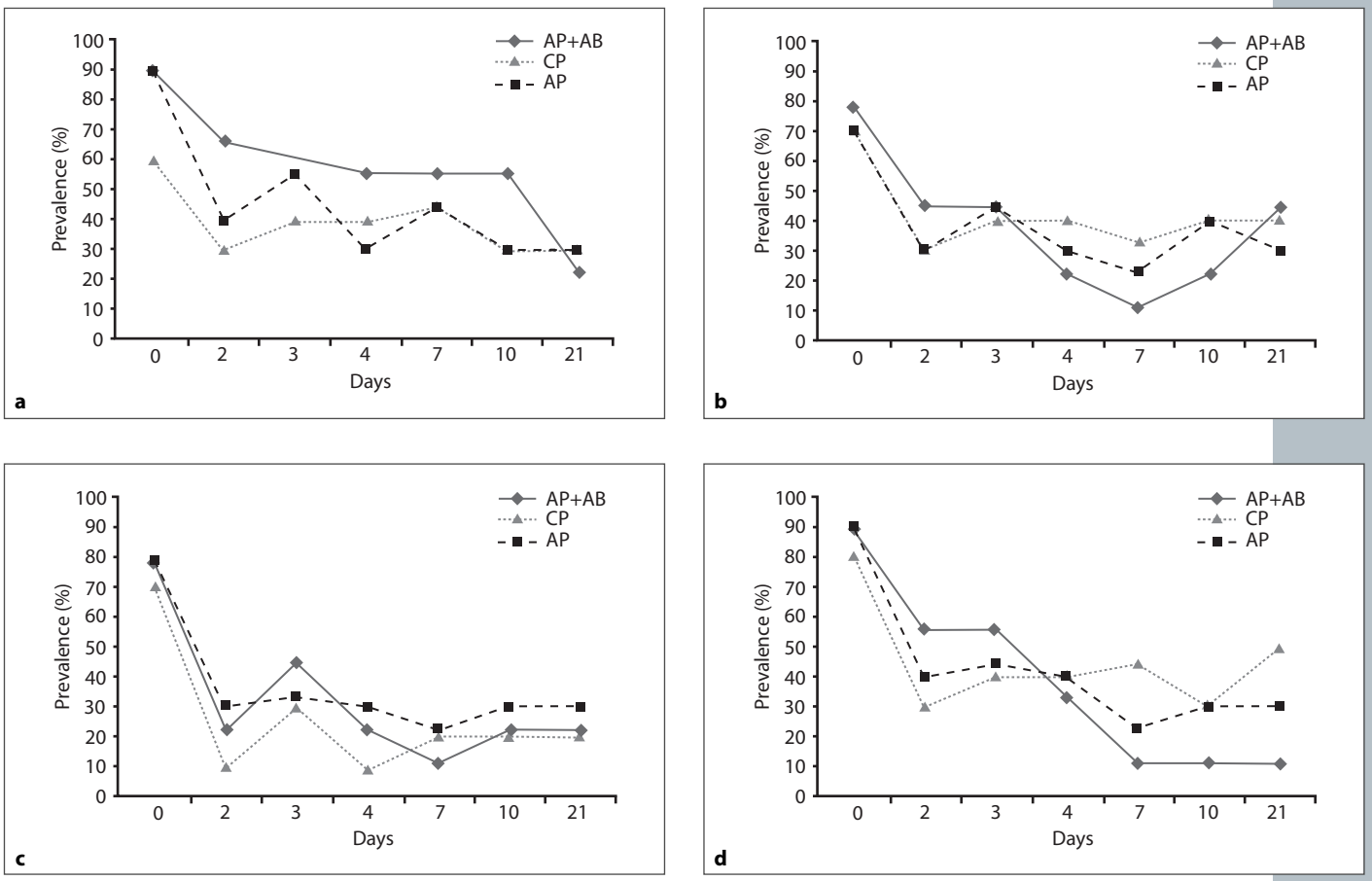

Fig 5 Subgingival prevalence of four selected microorganisms before and during 3 weeks of healing after nonsurgical periodontal therapy in chronic periodontitis (CP), aggressive periodontitis (AP), and aggressive periodontitis treated with antibiotics (AP+AB). (*a*) *A actinomycetemcomitans*, (*b*) *P gingivalis*, (*c*) *T forsythia*, (*d*) *P intermedia*. 0, 2, 3, 4, 7, 10, 21 = Days of healing. The post-treatment levels in AP+AB significantly dropped beyond the scores of AP and CP (except *P gingivalis*).

80.0% (CP). At day 2 of antibiotic therapy, the posttreatment levels continuously declined until day 7. After 3 weeks, the prevalence levels in AP+AB dropped beyond the scores of AP and CP (except *P gingivalis*) (Fig 5).

Subgingival cell counts

There was a marked reduction of subgingival *A actinomycetemcomitans* cell counts in both AP+AB and AP after therapy, but not in CP. The change was significant in AP+AB after 3 weeks compared to AP and CP. Intergroup differences were not statistically significant for all four pathogens examined (Table 2, Fig 6).

For *P gingivalis*, in AP+AB, the reduction after therapy of 125.6 ± 329.3 × 10^3/μL (P = .012) was significant. In AP, the change of subgingival cell counts was less pronounced and reached no significance. CP subjects displayed no change after 3 weeks (see Table 2, Fig 6).

The change of *T forsythia* after therapy from baseline was significant in AP+AB. A slight, but not significant, decrease of cell counts occurred in AP. In CP patients, no significant effect from therapy was obvious (see Table 2, Fig 6).

A strong decrease of subgingival *P intermedia* cell counts was observed in AP+AB. In AP, the change amounted to 20.3 ± 42.1 × 10^3/μL (P = .106), but was not significant. CP individuals experienced a modest change after 3 weeks (see Table 2, Fig 6). There were no reported adverse events from the antibiotic usage or adverse effects from periodontal therapy.

Table 2 Differences of subgingival cell counts (× $10^3/\mu L$) before and after periodontal therapy within 3 weeks of healing*

	1 = AP + AB (n = 36)		2 = AP (n = 32)		3 = CP (n = 29)		Change after 3 w (*P*)§		
	Mean	SD	Mean	SD	Mean	SD	(1 vs 2)	(1 vs 3)	(2 vs 3)
Aa	9.11*	15.81†	6.40	15.75	−1.15‡	4.41	.27	.02	.08
Change after 3 w	.01•		.07		.68				
Pg	125.61	329.26	100.65	316.00	−0.30	4.11	.05	.04	.58
Change after 3 w	.01•		.07		.83				
Tf	8.44	15.91	6.75	15.52	0.50	2.17	.36	.16	.34
Change after 3 w	.02•		.03		.24				
Pi	14.72	20.47	20.25	42.10	4.85	15.76	.25	.19	.67
Change after 3 w?	.01•		.11		.36				

(Aa) A actinomycetemcomitans, (Pg) P gingivalis, (Tf) T forsythia, (Pi) P intermedia. AP + AB = aggresive periodontitis + antibiotics, AP = aggressive periodontitis, CP = chronic periodontitis.
*Differences of mean cell counts x $10^3/\mu L$, †standard deviation, ‡Negative changes = Cell count increment compared to baseline, §Wilcoxon Signed Rank test, • = significant after adjustment to Bonferoni, ?Kruskal-Wallis test.

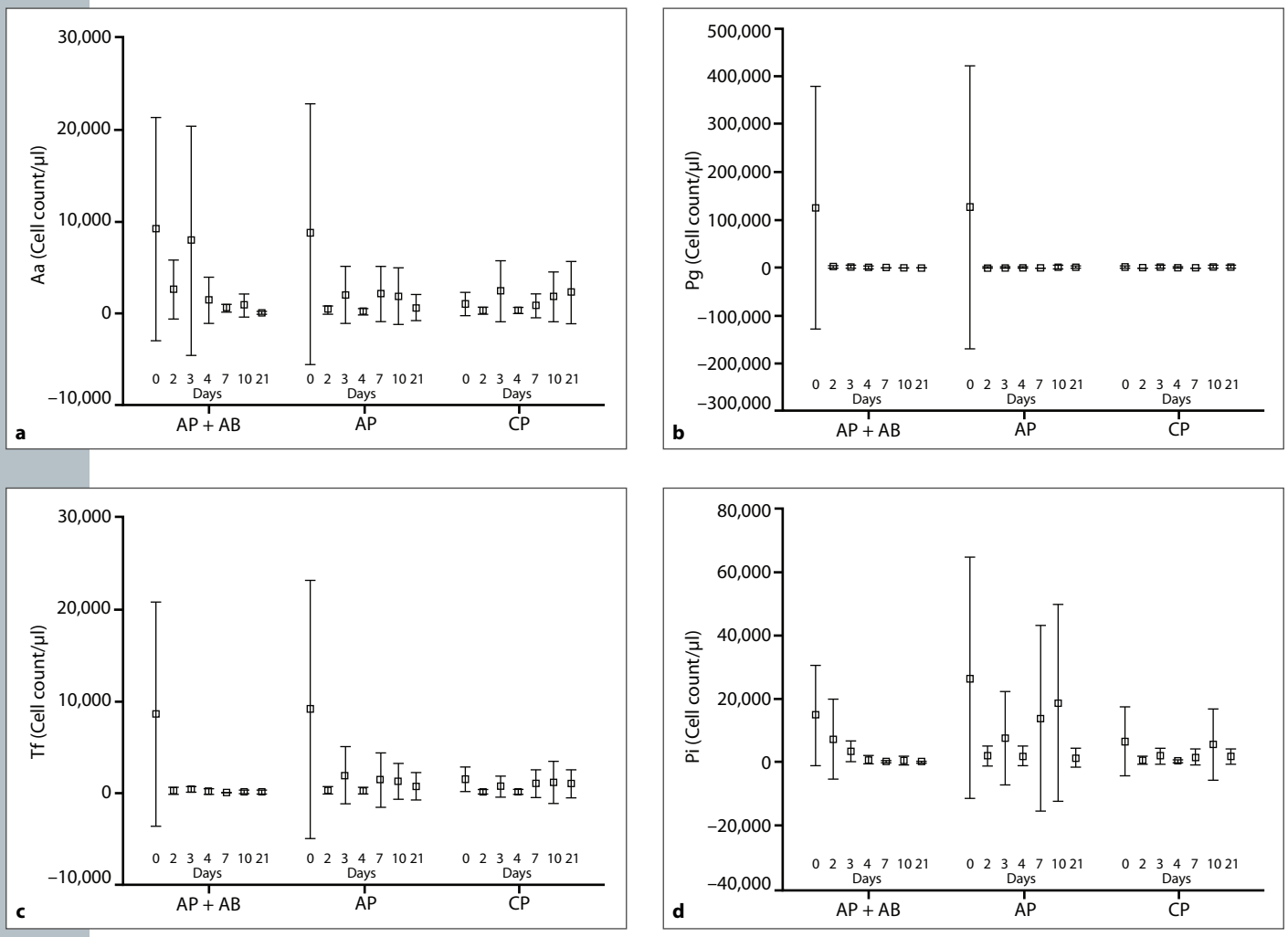

Fig 6 Error bar charts of subgingival cell counts of key periodontal microorganisms in chronic periodontitis (CP), aggressive periodontitis (AP), and aggressive periodontitis treated with antibiotics (AP+AB) at baseline and for each of the three weekly reexamination visits after periodontal therapy. (*a*) *A actinomycetemcomitans*, (*b*) *P gingivalis*, (*c*) *T forsythia*, (*d*) *P intermedia*. Between treatment groups, change after 3 weeks was not statistically different after adjustment to Bonferoni.

DISCUSSION

The results of the present study suggest that in aggressive periodontitis early periodontal healing is promoted by systemic antibiotics that accelerate the reduction of subgingival cell counts within the dental biofilms after subgingival debridement, beginning within the second day of healing. Treatment of the periodontal conditions without antibiotics also induced a decrement of pathogens in both chronic and aggressive periodontitis. The enhanced benefit from antibiotic therapy in AP compared to CP is initiated after 7 days of healing, with an improvement that remained stable during the 3-week period.

At baseline, untreated AP patients revealed an increased subgingival prevalence of all four pathogens compared to CP individuals. The prevalence levels ranked between 77.8% for *P gingivalis* and 90.0% for *A actinomycetemcomitans*. The increased prevalence scores in AP agree with profiles reported from recurrent periodontitis and generalized severe periodontitis.[22,23] However, the presence of periodontopathogens is not precisely discriminating between AP and CP.[12] Enhanced prevalence profiles of pathogens, especially *A actinomycetemcomitans*, *P gingivalis,* and *P intermedia* before periodontal therapy are not necessarily associated with worse outcome measures. Thus, the detection frequency of subgingival bacteria before SRP is not indicative to predict healing of periodontal pockets.[24] With adjunctive antibiotic therapy, the prevalence profiles in AP declined and followed the responses in CP ranking between 11.1% and 50.0% within the 3-week period. These findings support the hypothesis that the microbial treatment plan in AP patients comprises the reduction of subgingival taxa to low levels rather than the elimination of a single species. Because the initial prevalence scores may determine the final levels during maintenance, we advocate this concept in AP subjects to lower the microbial trigger with systemic antibiotics and normalize the host-pathogen relationship.[15]

Subgingival *P gingivalis* cell counts in untreated periodontitis reached a 69.9-fold increment in AP compared to CP exceeding the threshold of $\geq 10^5$ per μL plaque sample for periodontal destruction.[9,25] *A actinomycetemcomitans* (10.2-fold), *T forsythia* (5.7-fold), and *P intermedia* (3.3-fold) cell counts in AP did not reach the concentrations associated with ongoing periodontal breakdown. With antibiotic usage, this study demonstrated a significant change of subgingival cell counts of all four pathogens at day 2 of periodontal healing that fell below the levels of CP after 7 days. This is in accordance with microbial responses examined at day 7, day 10, and day 28 of healing following SRP plus antibiotics with a cell count reduction below the level of 10^3 per μL plaque sample or 5% of vital pathogens.[26–29] However, the proposed elimination of the microbiota from the subgingival platforms targeted with the antibiotics during healing was incomplete. Because most of the pathogens are endogenous to the oral cavity, antibiotics may suppress rather than eliminate the microflora, especially as they are biofilm protected.[15] Moreover, these data further elucidate that *Po gingivalis* is the prime candidate in periodontal therapy.

Cultivable microbes in periodontal patients usually grow on a high proportion level.[30] In untreated AP subjects, the cultivable flora in subgingival lesions reaches 10^6 to 10^8 colony-forming units (CFUs) per μL plaque.[31] The initial cell counts of the predominant periodontopathogens determine the usage and regimen of antibiotics, and are therefore appropriate to evaluate upper threshold levels in clinical decision-making conditions. Utilizing 16S rRNA oligodeoxynucleotide probes and dot-blot hybridization, subgingival cell counts ranked between 10^3 and 10^5 (total counts per μL plaque fluid). With a factor of 100 to 1,000, they fall below those reported from trials where culturing techniques have been employed. PCR techniques that enable a differentiation of cell counts at low threshold levels are relevant to estimate low bacterial profiles as they emerge in our trial during early healing.[21]

In the context of current evidence, the data presented here support the following concepts: *(1)* nonsurgical therapy resulted in both suppression and early elimination of single taxa immediately after completion of active

treatment at day 2 of periodontal healing; *(2)* systemic antibiotics significantly accelerate the suppression of the periodontal microflora, with the maximum response after 7 days; and *(3)* the proposed elimination of targeted pathogens cannot be achieved.

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