J Clin Periodontol 2006; 33: 254–264 doi: 10.1111/j.1600-051X.2006.00905.x

Journal of Clinical Periodontology

Clinical and microbiological effects of different antimicrobials on generalized aggressive periodontitis

Christiana Xajigeorgiou[1], Dimitra Sakellari[1], Theodora Slini[2], Anneta Baka[1] and Antonis Konstantinidis[1]

[1]Departments of Preventive Dentistry, Periodontology and Implant Biology Dental School and; [2]Mechanical Engineering, Aristotle University of Thessaloniki, Greece

Xajigeorgiou C, Sakellari D, Slini T, Baka A, Konstantinidis A. Clinical and microbiological effects of different antimicrobials on generalized aggressive periodontitis. J Clin Periodontol 2006; 33: 254–264. doi: 10.1111/j.1600-051X.2006.00905.x. © Blackwell Munksgaard 2006.

Abstract

Aim: To evaluate and compare the effects of adjunctive metronidazole plus amoxicillin, doxycycline and metronidazole on clinical and microbiological parameters in patients with generalized aggressive periodontitis.

Material and Methods: Forty-three patients participated in this randomized clinical trial divided into four groups. Six weeks after scaling and root planning (SRP), groups 1–3 received adjunctive metronidazole, plus amoxicillin, doxycycline and metronidazole respectively, and group 4 acted as controls. Clinical recordings concerning probing depth, probing attachment level and bleeding on probing were performed at baseline, 6 weeks after SRP and 6 months from baseline. Subgingival samples were analysed using the 'checkerboard' DNA–DNA hybridization for *Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans, Tannerella forsythia and Treponema denticola*.

Results: All treatments resulted in improvement of clinical parameters (ANOVA $p > 0.05$). Systemic administration of metronidazole plus amoxicillin or metronidazole resulted in statistically significant greater reduction of the proportion of sites $>$ 6mm than SRP (z-test, $p < 0.05$). These antimicrobials yielded a significant effect on levels of important periodontal pathogens for 6 months.

Conclusion: Adjunctive metronidazole plus amoxicillin or metronidazole alone (when *A.actinomycetemcomitans* is not involved) is effective in deep pockets of aggressive periodontitis patients.

Key words: aggressive periodontitis; amoxicillin; doxycycline; metronidazole; RCT

Accepted for publication 9 January 2006

The treatment of aggressive periodontitis (AgP) has always presented a challenge for clinicians, but there are no established protocols and guidelines for efficiently controlling the disease. The recent reports of the European Federation of Periodontology and the American Academy of periodontology which evaluated the overall contribution of systemic antimicrobials to the treatment of periodontal disease suggest that patients with AgP appear to benefit from their adjunctive use (Herrera et al. 2002, Haffajee et al. 2003). However, both reports emphasize the current inability to conclude which antimicrobial agent, dosage and duration provide the optimal clinical and microbiological effects in this group of patients, because of the wide design variability of existing studies. It is also suggested that randomized, controlled trials (RCTs) could provide reliable information concerning the effects of various antimicrobials currently used by clinicians.

The aim of the present study was to evaluate and compare the effects of adjunctive metronidazole plus amoxicillin, doxycycline, and metronidazole on clinical and microbiological parameters in patients with generalized AgP.

Materials and Methods

Subject sample

Forty-seven subjects, patients of the Clinic of the Department of Periodontology and Implant Biology, Dental School, Aristotle University of Thessaloniki, Greece, were originally recruited for the present study. Subjects were diagnosed with generalized AgP according to criteria described by the AAP (Armitage 1999). Only subjects reporting a familial distribution of the disease were included in the study. Further criteria for inclusion were as follows: presence of at least 20 teeth, absence of antibiotic intake for the last 3

months, no known allergies to antibiotics and no periodontal treatment for the previous 12 months. Pregnant or lactating women were excluded from the present study. Smoking status (smoker, non-smoker) as reported by patients was also recorded. The study was conducted according to the protocol outlined by the Research Committee, Aristotle University of Thessaloniki Greece and was approved by the Ethical Committee of the School of Dentistry.

Study design

The present study was designed as a randomized, controlled clinical trial (RCT) according to the CONSORT criteria (Altman et al. 2001). It included three experimental and one control groups characterized as group 1: scaling and root planing (SRP) plus adjunctive metronidazole and amoxicillin, group 2: SRP plus adjunctive doxycycline, group 3: SRP plus adjunctive metronidazole, and group 4: SRP. Group 4 acted as control group.

The date of patient enrolment was recorded on a numbered list. Randomization was generated using random tables. The randomization list was kept by one of the authors (A.K.) until patients were eligible for the study. The study was designed as blinded concerning the examiner (A.B.) who was not aware of the treatment that the patient had received. Analysis of subgingival samples was performed by one of the authors (C.X.) who was also unaware of the treatment that the patient had received (coded samples).

Experimental design

Upon final recruitment, subjects were scheduled for baseline sampling of subgingival plaque and for baseline full-mouth clinical recordings a week later, as described below.

All patients received oral hygiene instructions and full-mouth SRP performed per quadrant – under local anaesthesia in four sequential visits. SRP was performed by the same clinician (D.S.).

Patients were re-examined on a bi-weekly basis for recordings and reinforcement of oral hygiene.

Six weeks after completion of treatment participants were scheduled for subgingival sampling and clinical recordings. Only subjects with proven ability to perform oral hygiene as instructed (presence of plaque $<20\%$ of surfaces) continued the study. Thus, two subjects were excluded at this timepoint and their data were not included in the analysis. All patients were treated with debridement (ultrasonics and polishing with a rubber cup). Subsequently patients assigned to group 1 (MET+AMO) were administered 500 mg of metronidazole and 500 mg of amoxicillin, three times per day for 7 days, patients assigned to group 2 (DOXY) were administered 200 mg of doxycycline as a loading dose and 100 mg per day for 14 days, and patients assigned to group 3 (MET) were administered 500 mg of metronidazole three times per day for seven days. Patients assigned in group 4 did not receive any further treatment. Any adverse effects from antibiotic intake and compliance, as reported by patients, were recorded. Four months after administration of antimicrobials, thus 6 months from baseline, patients were scheduled for microbial sampling and clinical recordings. Two participants (one from group 1 and another from group 2) were unwilling to complete the study. Subject data are presented in Table 1 and the flowchart of patients throughout the study is illustrated in Fig. 1.

Clinical recordings

Clinical data were recorded at all teeth present in the dentition. The following parameters were recorded at six sites for each tooth (disto-, mid-, and mesiobuccal; mesio-, mid-, and distolingual)

(a) probing depth (PD),
(b) probing attachment level (PAL), and
(c) bleeding on probing (BOP).

All measurements were performed by one calibrated examiner (A.B.), using a manual probe (Hu-Friedy, Chicago, IL, USA).

Microbiological examination

Plaque samples were taken from the mesio-buccal surface of ten pre-selected teeth with PD >5 mm and care was taken to include teeth representative of all groups in the dentition. At all timepoints, microbial plaque samples were taken before the clinical measurements. Timepoints of sampling included baseline, 6 weeks after treatment, immediately after completion of antibiotic intake for groups 1, 2, and 3, and 6 months from baseline. After isolating with cotton rolls, drying, and removal of supragingival plaque, subgingival samples were taken by means of a sterile Gracey curette, placed in 100 μL of TE buffer (Tris HCL 10 mM, EDTA 1 mM, pH 7.5) and stored after treatment with an alkali solution (0.5 M NaOH) at -20° C. A total of 1610 samples were processed for four bacterial species, using the ''checkerboard'' DNA–DNA hybridization technique as described in detail by Socransky et al. (1994, 1998). The subgingival species used for development of digoxigenin-labelled whole genomic probes were *Porphyromonas gingivalis* (FDC 381), *Actinobacillus actinomycetemcomitans* serotype b (FDC Y4), *Tannerella forsythia* (FDC 338), and *Treponema denticola* (TD1).

Cell numbers were quantified by comparing the signal intensities of unknowns to those of standard suspensions of 10^5 and 10^6 bacterial cells, according to the following scale: 0 = absence of signal, 1 = signal corresponding to $<10^5$ bacterial cells, 2 = signal corresponding to 10^5 bacterial cells, 3 = signal corresponding to $>10^5$ bacterial cells but $<10^6$, 4 = signal corresponding to 10^6 bacterial cells, and 5 = signal corresponding to $>10^6$ bacterial cells.

Statistical analysis

The statistical analysis of the data was carried out with the statistic package SPSS version 12. PD was set as the primary outcome, and PAL and BOP as secondary outcomes of the present study. Indicators of Descriptive Statistics were used, such as frequencies, percentage, average, variance, and standard deviation for each group at all timepoints. In order to check differences among groups at all timepoints the gen-

Table 1. Subject data

	Number	Age mean ± SD	Age range	Male	Female	Smokers
Group 1 metronidazole plus amoxicillin	10	38.88 ± 8.74	22–49	5	5	3
Group 2 Doxycycline	10	38.5 ± 4.65	34–49	4	6	3
Group 3 Metronidazole	12	40.9 ± 4.57	33–48	7	5	5
Group 4 Controls	11	37 ± 5.59	28–46	6	5	4

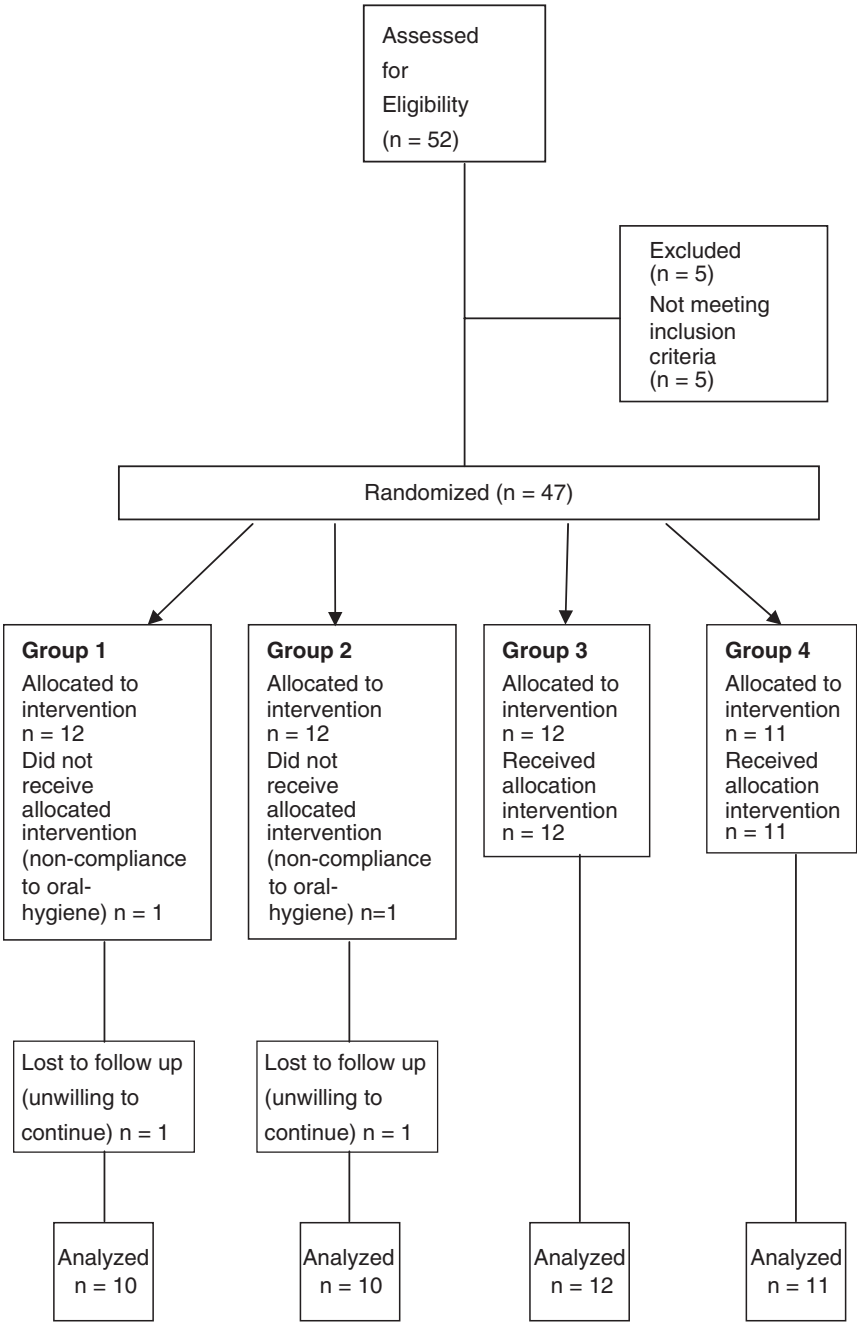

Fig. 1. Flow-chart of patients through the experimental period.

eral linear model, repeated measures procedure was applied with the patient as the observational unit. The analysis of variance (ANOVA) procedure was also implemented at each timepoint.

Levene's test for equality of error variances was applied in order to check for homogeneity of clinical parameters at baseline. Differences between timepoints within each group were separately tested with the paired samples *t*-test procedure.

Data concerning sites with PD >6 mm were analysed separately with the site as the observational unit. At each timepoint, for each group, the proportion of sites >6 mm was calculated and changes of these proportions from baseline between groups were compared using the two-sided *z*-test adjusted by the Bonferroni correction.

Microbiological data were analysed with the subject as the observational unit. The number of subjects positive for the investigated species at any of the sampled sites were estimated at all timepoints. These numbers were compared between groups at all timepoints using, the two-sided *z*-test adjusted by the Bonferroni correction.

In addition, averaged bacterial scores from each subject were consequently averaged for each group and compared at all timepoints. Differences between the four groups at baseline were tested using the Kruskal–Wallis test. In order to identify specific differences between pairs of groups at each timepoint the Mann–Whitney test was applied, while differences between timepoints within each group were tested with the Wilcoxon's signed ranks test.

The significance level was set at 0.05 for all tests.

Results

No differences were observed between the mean age of subjects of the four groups (Table 1, one-way ANOVA, $p = 0.52$).

No serious adverse effects were observed or reported from antibiotic intake other than a mild gastrointestinal discomfort in two subjects from group 1 (MET+AMO) and a metallic taste in one subject from group 3 (MET).

The results of the present study concerning clinical data with the subject as the observational unit are presented in Tables 2– 4.

Table 2 displays descriptive statistics and comparisons for all groups concerning PD. All groups were homogeneous at baseline (Levene's test) and no differences were observed between the four groups at any timepoint (one-way ANOVA $p>0.05$). When comparisons were made within each group, SRP resulted in significant reduction of PD compared with baseline, and this difference was maintained at 6 months from baseline in all four groups (paired *t*-test $p<0.05$). In addition, subjects who received adjunctive metronidazole displayed a statistically significant reduction in mean PD after antibiotic intake (Table 2, paired *t*-test $p<0.05$).

Table 3 displays descriptive statistics and comparisons for all groups concerning PAL. When comparisons were made within each group, SRP resulted in significant improvement in PAL compared with baseline, and this difference was maintained at 6 months from baseline in all four groups (Table 3 paired *t*-test $p<0.05$). No differences were observed within any of the four groups between 6 weeks after SRP and 6 months after baseline.

Table 2. Probing depth (mean+SD) of the four groups during the experimental period

	Baseline (mean ± SD)	6 weeks after SRP (mean ± SD)	6 months (mean ± SD)
Group 1 $n = 10$ metronidazole plus amoxicillin	4.63 ± 0.97 (a,b)	3.44 ± 0.48 (a)	3.12 ± 0.71 (b)
Group 2 $n = 10$ doxycycline	4.24 ± 0.57 (a,b)	3.48 ± 0.67 (a)	3.35 ± 0.76 (b)
Group 3 $n = 12$ metronidazole	4.71 ± 0.57 (a,c)	3.47 ± 0.51 (a,b)	2.86 ± 0.65 (b,c)
Group 4 $n = 11$ controls	4.21 ± 0.74 (a,b)	3.19 ± 0.59 (a)	3.52 ± 0.76 (b)

Data were homogeneous at baseline (Levene's test of homogeneity of variances $p > 0.05$).
No differences were observed between groups (ANOVA $p > 0.05$).
Data followed by the same letter differ statistically within groups (paired *t*-test $p < 0.05$).

Table 3. Probing attachment level (mean+SD) of the four groups during the experimental period

	Baseline (mean ± SD)	6 weeks after SRP (mean ± SD)	6 months (mean ± SD)
Group 1 $n = 10$ metronidazole plus amoxicillin	4.97 ± 1.01 (a,b)	4.31 ± 0.92 (a)	4.05 ± 1.34 (b)
Group 2 $n = 10$ doxycycline	5.03 ± 1.42 (a,b)	4.43 ± 1.73 (a)	4.22 ± 1.92 (b)
Group 3 $n = 12$ metronidazole	5.35 ± 1.27 (a,b)	4.61 ± 1.13 (a)	4.11 ± 1.34 (b)
Group 4 $n = 11$ controls	4.55 ± 0.72 (a,b)	3.80 ± 0.62 (a)	4.07 ± 0.59 (b)

Data were homogeneous at baseline (Levene's test of homogeneity of variances $p > 0.05$).
No differences were observed between groups (ANOVA $p > 0.05$).
Data followed by the same letter differ statistically within groups (paired *t*-test $p < 0.05$).

Table 4. Bleeding on probing (mean+SD) of the four groups during the experimental period

	Baseline (mean ± SD)	6 weeks after SRP (mean ± SD)	6 months (mean ± SD)
Group 1 $n = 10$ metronidazole plus amoxicillin	0.87 ± 0.21 (a,b)	0.22 ± 0.18 (a)	0.15 ± 0.14 (b)
Group 2 $n = 10$ doxycycline	0.81 ± 0.25 (a,b)	0.24 ± 0.23 (a)	0.14 ± 0.22 (b)
Group 3 $n = 12$ metronidazole	0.80 ± 0.36 (a,b)	0.29 ± 0.15 (a)	0.21 ± 0.31 (b)
Group 4 $n = 11$ controls	0.78 ± 0.37 (a,b)	0.33 ± 0.24 (a)	0.15 ± 0.25 (b)

Data were homogeneous at baseline (Levene's test of homogeneity of variances $p > 0.05$).
No differences were observed between groups (ANOVA $p > 0.05$).
Data followed by the same letter differ statistically within groups (paired *t*-test $p < 0.05$).

Table 4 displays descriptive statistics and comparisons for all groups concerning BOP. When comparisons were made within each group, SRP resulted in significant improvement in BOP compared with baseline, and this difference was maintained at 6 months from baseline in all four groups (Table 4 paired *t*-test $p < 0.05$). Similarly to PAL no further improvement was observed for any group between 6 weeks after SRP and 6 months after baseline.

Additional differences concerning the effect of different treatments on the primary outcome were sought with the site instead of the subject as the observational unit, analyzing the subset of pockets with PD >6 mm (Table 5).

According to the findings, subjects in groups 1 and 3 at 6 months displayed the greatest reduction from baseline in proportions of sites with PD >6 mm. Statistical analysis has shown that there was a significant difference in these changes between subjects who received adjunctive metronidazole plus amoxicillin and metronidazole alone compared with control subjects (Table 5, *z*-test, $p < 0.05$). No differences were observed between the subjects who received adjunctive doxycycline compared with control subjects (Table 5).

Number of subjects positive for investigated species are presented in Table 6. At baseline, almost all participants were positive for the investigated species. In contrast to SRP, administration of antimicrobials resulted in elimination of bacteria at the subject level immediately after completion of antimicrobial intake. Six months from baseline a recolonization or regrowth of investigated species was observed. No differences were observed between groups at any timepoint (*z*-test $p > 0.05$).

According to results of the present study concerning levels of investigated species (when present) on a subject basis (Figs 2–5), at baseline no differences were observed between the four groups (Kruskal–Wallis test). Subjects in all groups displayed high levels of the investigated species.

SRP resulted in a reduction of levels of the four species in our groups, which was not homogenous. When analyzing the effect of SRP within each group, in 11 out of the 16 comparisons a statistically significant reduction was observed (Wilcoxon's signed ranks test $p < 0.05$). *A. actinomycetemcomitans* was not significantly reduced in two groups (MET+AMO and MET), *Tannerella forsythia* in one group (controls) and *Treponema denticola* in two groups (DOXY and controls). In contrast, *P. gingivalis* was statistically significantly reduced in all four groups 6 weeks after SRP (Wilcoxon's signed ranks test $p < 0.05$).

Antibiotic intake resulted in a further reduction of the bacterial population in the three groups that received antibiotics (Figs 2–4). Immediately after completion of antibiotic intake in 10 out of the 12 comparisons within groups statistically significant differences were observed (Wilcoxon's signed ranks test $p < 0.05$) with the notable exception of *A. actinomycetemcomitans*, where there was no further reduction in groups 2 and 3 (DOXY and MET, respectively).

At 6 months after baseline, in nine out of 12 comparisons within each group a statistically significant difference was observed from baseline (Wilcoxon's signed ranks test $p < 0.05$). *A. actinomycetemcomitans* (groups DOXY and MET) and *Treponema denticola* (DOXY) did not statistically significantly differ between the two timepoints. In the control group, *P. gingivalis* was the only species where a statistically significant difference was still present at 6 months from baseline (Wilcoxon's signed ranks test $p < 0.05$).

Discussion

Currently, data in the literature suggest that systemically administered antimicrobials can enhance the effects of mechanical treatment in the treatment of AgP as assessed by clinical para-

Table 5. Change of proportions of sites with probing depth > 6 mm of the four groups during the experimental period

	Group 1 metronidazole plus amoxicillin		Group 2 doxycycline		Group 3 metronidazole		Group 4 controls	
	n (sites)	change from baseline (%)	*n* (sites)	change from baseline (%)	*n* (sites)	change from baseline (%)	*n* (sites)	change from baseline (%)
Baseline	331		165		270		222	
6 weeks after SRP	125	62.2	82	50.3	123	54.4	52	76.6
6 months	66	80 (a)	59	64.2 (b)	33	87.8 (b,c)	94	57.7 (a,c)

Data followed by the same letter display statistically significant difference of change of proportions between baseline and 6 months (*z*-test ,using the Bonferroni corrections, $p < 0.05$).

Table 6. Number of subjects positive for investigated species during the experimental period

	Porphyromonas gingivalis				*Actinobacillus actinomycetemcomitans*				*Tannerella forsythia*				*Treponema denticola*			
Groups	1 ($n = 10$)	2 ($n = 10$)	3 ($n = 12$)	4 ($n = 11$)	1 ($n = 10$)	2 ($n = 10$)	3 ($n = 12$)	4 ($n = 11$)	1 ($n = 10$)	2 ($n = 10$)	3 ($n = 12$)	4 ($n = 11$)	1 ($n = 10$)	2 ($n = 10$)	3 ($n = 12$)	4 ($n = 11$)
Baseline	10	10	11	10	10	10	10	5	10	10	11	9	10	10	10	9
6 weeks from SRP	9	10	9	8	9	10	7	5	9	10	9	6	10	11	9	4
Post antibiotic	1	4	4	ND	2	5	4	ND	2	5	3	ND	4	6	6	ND
6 months from baseline	8	8	9	9	9	10	9	5	8	10	9	3	8	10	10	4

No differences were observed between groups at any timepoint (*z*-test using the Bonferroni correction)
ND, non-determined

meters (Herrera et al. 2002, Haffajee et al. 2003). However, because of the relative absence of randomized controlled clinical trials including microbiological data, no definitive guidelines exist regarding the most effective antibiotic regime and time of administration for this group of patients. Therefore, the present study was designed in order to investigate and compare the adjunctive effects of three systemic antimicrobials on clinical and certain microbiological parameters. It was also chosen to administer antimicrobials after the initial healing period in order to enhance the effects of mechanical treatment and facilitate the efficacy of antimicrobials after the physical removal and/or disruption of the subgingival biofilm (Van Winkelhoff et al. 1996, Socransky & Haffajee 2002).

Data from the present study should be considered indicative, as they were derived from a relative small sample in each group (10–11 patients each). According to our calculations in order to detect differences of 2 ± 1 mm PD at 95% power analysis our groups should include 25 subjects each (Statmate2®, Graphpad Inc., San Diego, CA, USA). Unfortunately recruitment of these patients has proved relatively difficult probably because of the limited prevalence of this disease (Sheiham & Netuveli 2002). A further obstacle to recruitment was the exclusion of subjects, even at younger ages, who did not report a family history of the disease. Although self-reporting hereditary patterns of disease must be considered as a caveat in the current taxonomy, this major diagnostic criterion was, nevertheless, applied. According to the design of the present study, only subjects who complied well with proper oral hygiene procedures were included in the four groups and two subjects were excluded after the 6-week healing period. In addition, as mentioned in the materials and methods section, two participants were unwilling to continue the study. It is worth mentioning that the endpoint of this clinical study was set at 6 months from baseline (approximately 4 months after antimicrobial intake) because it was considered that extending the experimental period without further mechanical treatment might present risks of the disease recurring. No serious adverse effects were observed or reported from antibiotic intake other than a mild gastrointestinal discomfort in two subjects from group 1 (metronidazole and amoxicillin) and a

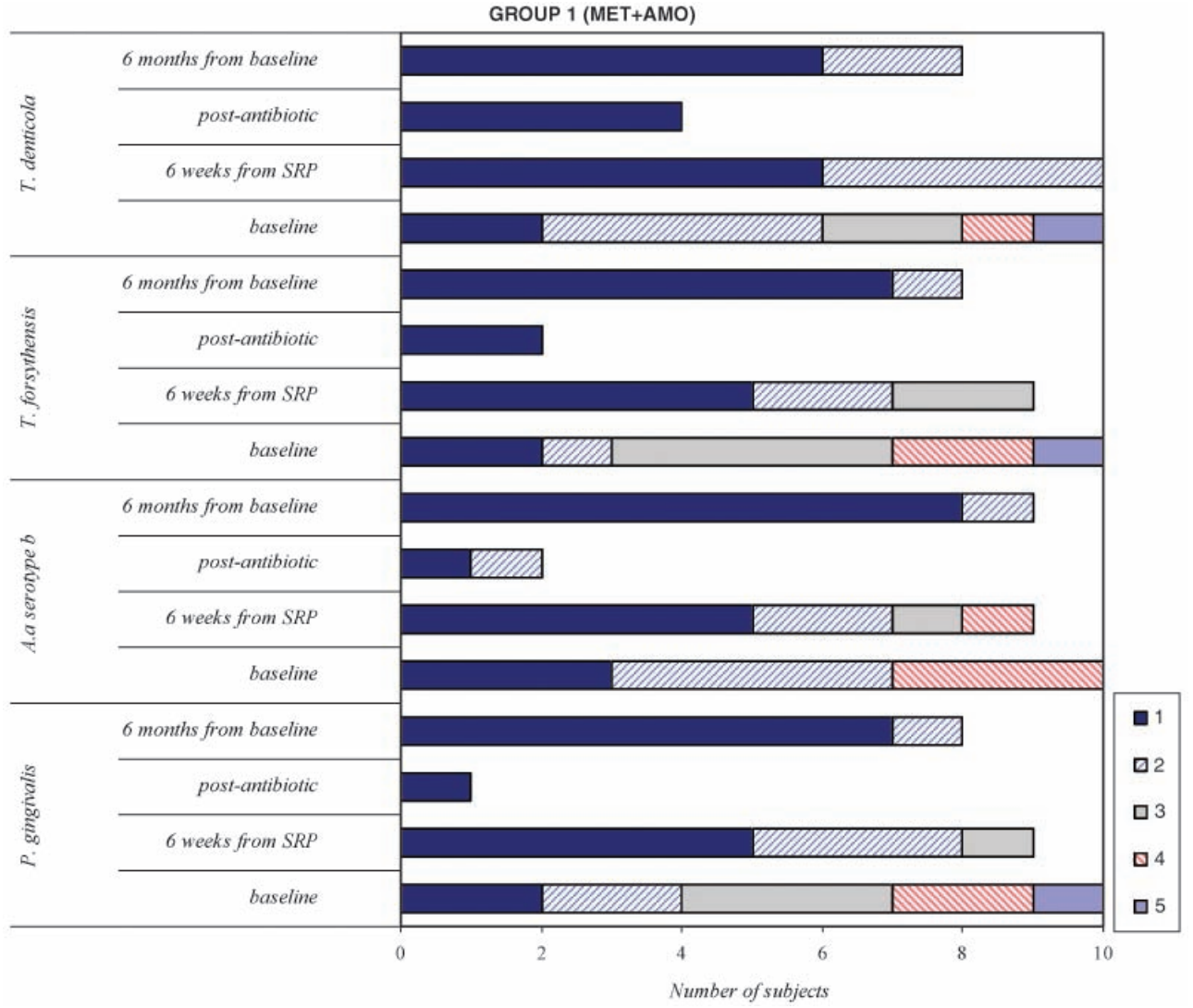

Fig. 2. Changes of levels of investigated species in group 1 (adjunctive metronidazole plus amoxicillin) during the experimental period. Values that scaled above zero were averaged for each subject and each species. At individual timepoints, the distribution of the subjects to the levels of the scale are displayed. A statistically significant decrease from baseline was observed after SRP for *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola*. and a further decrease for all species after antibiotic intake. At 6 months the difference was present for all species compared with baseline (Mann–Whitney test $p < 0.05$). SRP, scaling and root planing; MET+AMO, metronidazole and amoxicillin ($1 = < 10^5$, $2 = 10^5$, $3 = > 10^5 < 10^6$, $4 = 10^6$, and $5 = > 10^6$).

metallic taste in one subject from group 3 (metronidazole). Unfortunately, in the present study, compliance relied solely on good patient cooperation, although the literature shows that unsupervised usage of antibiotics and the assessment of compliance by interviews or counting tablets are not always objective (Loesche et al. 1993, López et al. 2000). In addition, we were unable to stratify our subjects according to smoking status because of the small subject sample.

Mean values of PD at baseline are lower than the ones reported in previous studies (Purucker et al. 2001, Sigusch et al. 2001), possibly because of the fact that five subjects were excluded, regardless of age and severity of periodontitis, on the grounds of no reported family history of the disease. The major effect on PD for all groups resulted from SRP, and no differences were observed between groups at any timepoint on the subject level (Table 2).

When analyzing data at a site level, and in particular, the proportion of sites with PD > 6 mm, a different pattern was observed. The intake of MET+AMO and MET, resulted in significant reduction of the percentages of sites > 6 mm compared with the control group (Table 5). This finding indicates a beneficial effect of these antimicrobials on deep pockets, and therefore a reduced need for surgical intervention. Findings of the present study agrees with the ones from a recently published randomized placebo-controlled clinical trial by Guerrero et al. (2005). These authors have shown that a 7-day adjunctive course of systemic metronidazole and amoxicillin resulted in an additional reduction of PD in deep pockets (>7 mm) at 6 months also in generalized AgP cases compared with controls.

High prevalences and levels of investigated species were observed at subgingival samples at baseline. On the subject level, almost all participants were positive for these species at this timepoint (Table 6) agree with studies using the

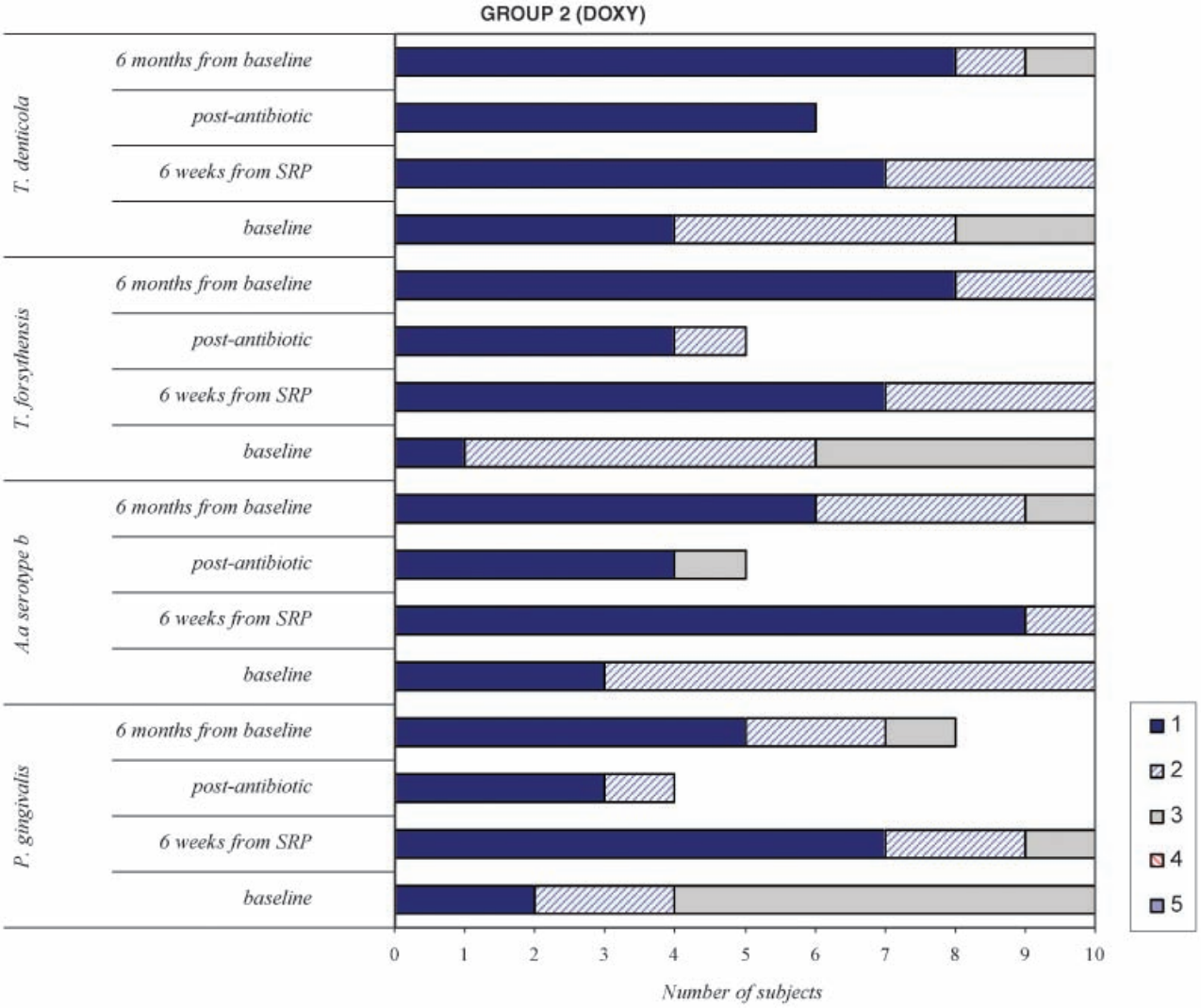

Fig. 3. Changes of levels of investigated species in group 2 (adjunctive doxycycline) during the experimental period. Values that scaled above zero were averaged for each subject and each species. At individual timepoints, the distribution of the subjects to the levels of the scale are displayed. A statistically significant decrease from baseline was observed after SRP for *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans* and *Tannerella forsythia* and a further decrease after antibiotic intake for all species but *A. actinomycetemcomitans*. At 6 months the difference was present for *P. gingivalis* and *T. forsythia* compared with baseline (Mann–Whitney test $p<0.05$). SRP, scaling and root planing; DOXY, doxycycline ($1 = <10^5$, $2 = 10^5$, $3 = >10^5 <10^6$, $4 = 10^6$, and $5 = >10^6$).

same technique (Picolos et al. 2005). In a previous study, investigating 40 subgingival species in 210 sites from 35 untreated chronic periodontitis patients of Greek origin, we reported prevalences of 27.2% and 11.4% for *A. actinomycetemcomitans* serotypes b and a, respectively (Sakellari et al. 2000). In the present study, *A. actinomycetemcomitans* serotype b was detected in 81.4% of the subjects, and therefore appears to be implicated in generalized AgP, at least in a group of patients of this origin.

The effect of SRP on the subgingival microflora has been investigated in several studies as described in recent reviews by Petersilka et al. (2002) and Umeda et al. (2004). There is a general aggreement that this procedure in addition to improving clinical parameters also reduces the microbial load and results in a shift towards a more health – compatible microflora (Mousquès et al. 1980, Haffajee et al. 1997). However, there are conflicting reports about the ability of SRP to eradicate or suppress important periodontal pathogens. *Tannerella forsythia* and especially *A. actinomycetemcomitans* have been shown to remain in periodontal pockets after non-surgical therapy (Renvert et al. 1990, Takamatsu et al. 1999). Results from the present study agrees with the above mentioned findings, since according to our data the effect of SRP on the subgingival microflora cannot be predicted in terms of prevalence and levels of the four pathogens under investigation (Table 6, Figs 2–5). Furthermore, only the effect on levels of *P. gingivalis* was maintained at 6 months from baseline (Kruskal–Wallis test $p<0.05$). The significant effect of SRP on levels of *A. actinomycetemcomitans* was not maintained at 6 months after baseline. In contrast, levels of *Tannerella forsythia* and *Treponema denticola* were neither affected at 6 weeks after SRP nor at 6 months after baseline (Fig. 5).

The administration of antimicrobials resulted in elimination of species under

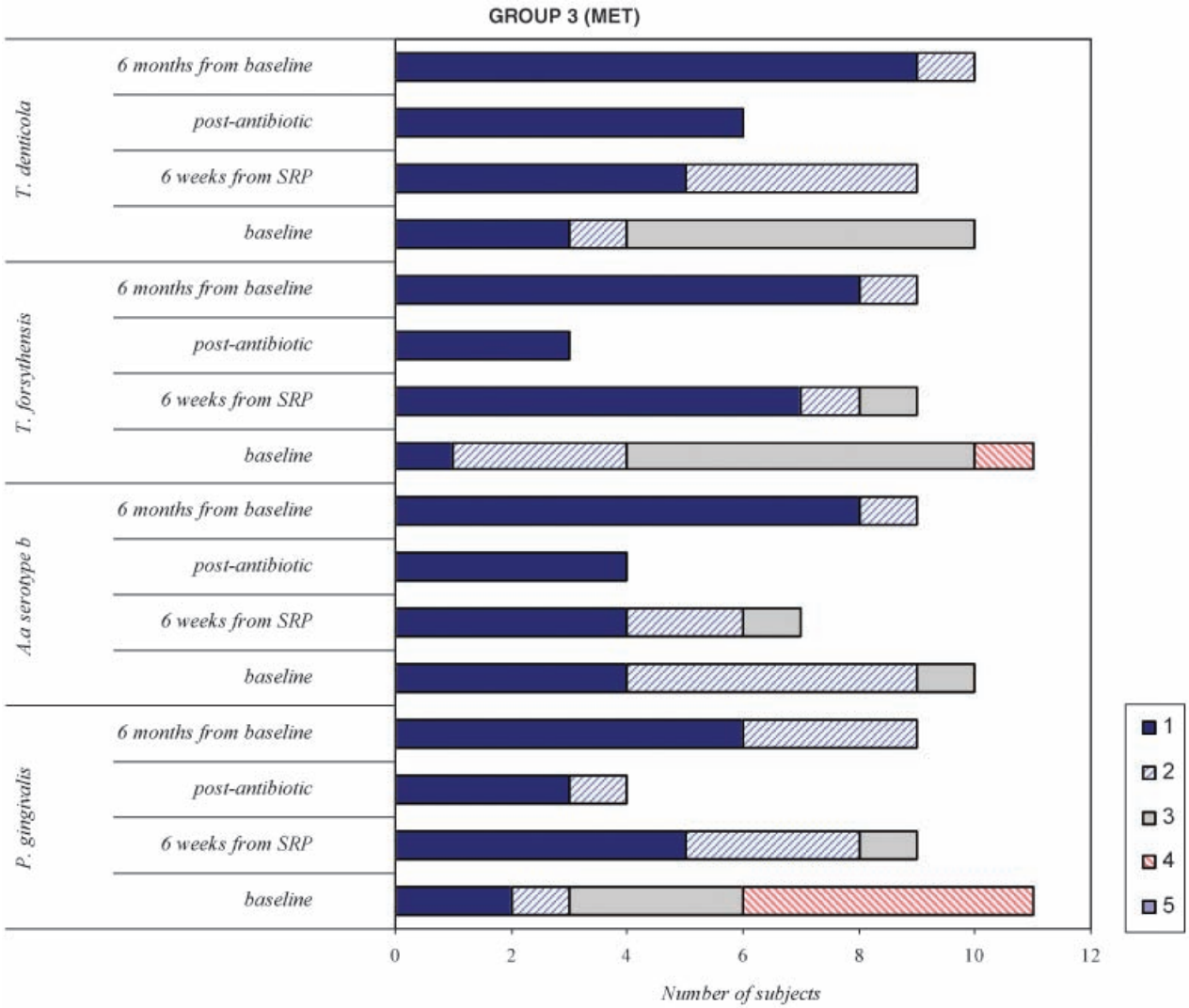

Fig. 4. Changes of levels of investigated species in group 3 (adjunctive metronidazole) during the experimental period. Values that scaled above zero were averaged for each subject and each species. At individual timepoints, the distribution of the subjects to the levels of the scale are displayed. A statistically significant decrease from baseline was observed after SRP for *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola* and a further decrease after antibiotic intake for all species but *Actinobacillus actinomycetemcomitans*. At 6 months the difference was present for all species but *A. actinomycetemcomitans* compared with baseline (Mann–Whitney test $p<0.05$). SRP, scaling and root planing; MET, metronidazole($1 = <10^5$, $2 = 10^5$, $3 = >10^5$ $<10^6$, $4 = 10^6$, and $5 = >10^6$).

investigation for several subjects in all three groups and no statistical differences were observed in the numbers of subjects remaining positive as estimated immediately after completion of antibiotic intake (Table 6).

However, the administration of MET+AMO was the only adjunctive treatment which resulted in statistically significant reductions in levels of all four species as observed immediately after completion of antibiotic intake (Fig. 2). Although SRP did not significantly affect *A. actinomycetemcomitans* in this group, the combination of these antibiotics effectively reduced this species and the reduction in levels of all investigated species was maintained until 6 months from baseline. This finding agrees with previous findings in the literature reporting reduction or elimination of important periodontal pathogens after administration of this drug combination (van Winkelhoff et al. 1992, Berglundh et al. 1998, López et al. 2000, Winkel et al. 2001). Although MET+AMO appear an effective choice when *A. actinomycetemcomitans* is involved, a 12-month study by Flemming et al. (1998) has shown persistance of *P. gingivalis*. In the present study, a higher dose of both antibiotics (500 mg of each) was administered and this fact may account for the significant impact on the subgingival microflora.

The administration of DOXY yielded conflicting results. Levels of *P. gingivalis*, *Tannerella forsythia* and *Treponema denticola* were statistically significantly reduced immediately after completion of antibiotic intake but not *A. actinomycetemcomitans* (Fig. 3). Although in this group a significant reduction of *A. actinomycetemcomitans* was observed 6 weeks after SRP, no differences were observed at 6 months compared with baseline. The antibiotic effect was also not maintained at 6 months from baseline for *Treponema denticola*.

Reports in the literature have indicated both a suppressive and a non-adjunctive effect of the tetracyclines on periodontal

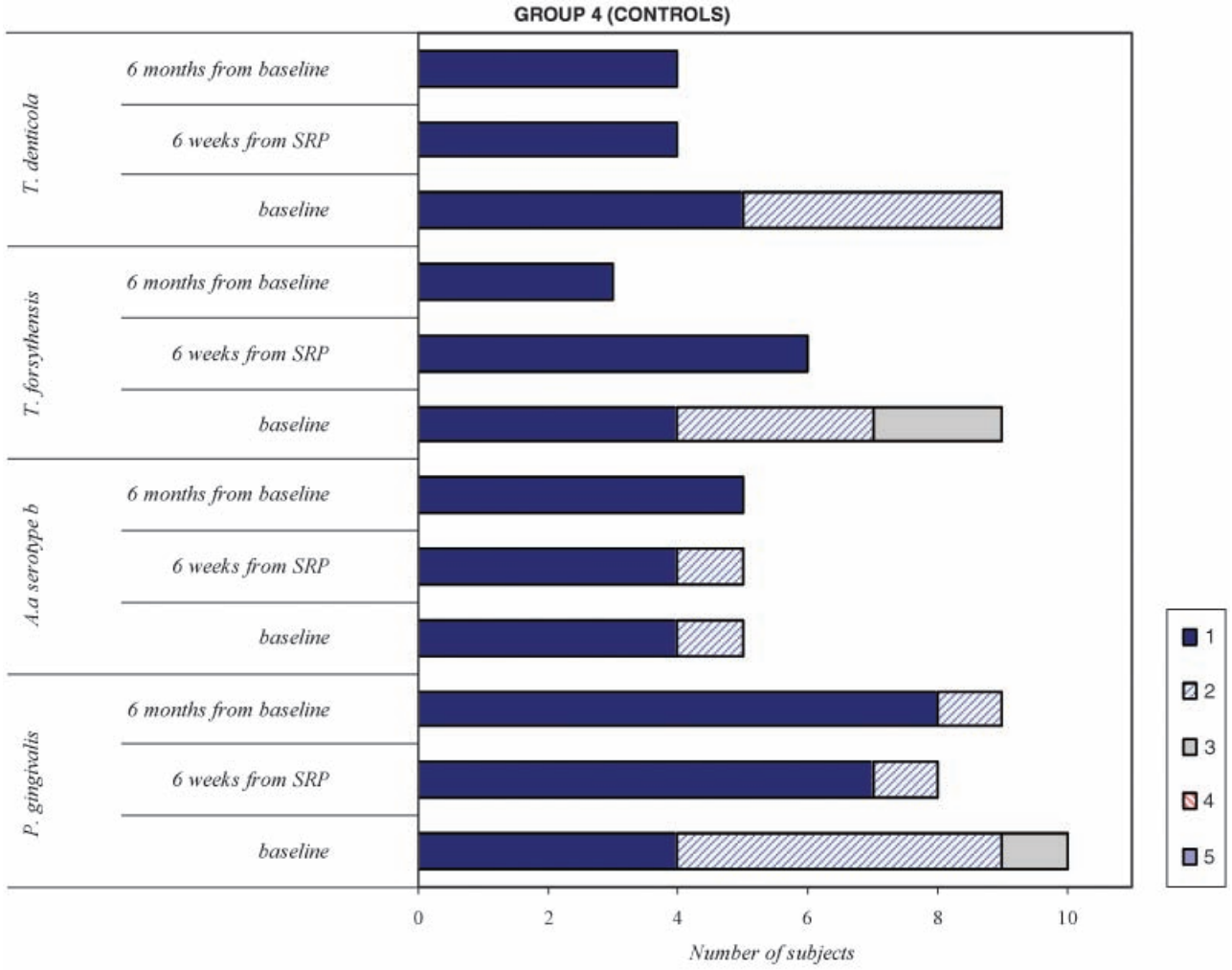

Fig. 5. Changes of levels of investigated species in group 4 (controls, SRP only) during the experimental period. Values that scaled above zero were averaged for each subject and each species. At individual timepoints, the distribution of the subjects to the levels of the scale are displayed. A statistically significant decrease from baseline was observed after SRP for *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans*. At 6 months only the decrease of *P. gingivalis* was still present compared with baseline (Mann–Whitney test $p<0.05$). SRP, scaling and root planing ($1 = <10^5$, $2 = 10^5$, $3 = >10^5 <10^6$, $4 = 10^6$, $5 = >10^6$).

pathogens (Lindhe et al. 1983, Rams & Keyes 1983, Asikainen et al. 1990, McCulloch et al. 1990, Feres et al. q1999, Sigusch et al. 2001). Furthermore, an overall increased frequency of tetracycline resistance has been recently reported in the medical and dental literature; especially, in countries with unrestricted antimicrobial use and it is suggested that bacteriostatic drugs may not be suitable for treating biofilm infections (van Winkelhoff et al. 2000, Chopra & Roberts 2001). The above mentioned data from the literature may contribute for the less favourable clinical and microbiological response of the DOXY group as reported in the present study.

The administration of MET resulted in an efficient reduction of both prevalence and levels of *P. gingivalis*, *Tannerella forsythia*, and *Treponema denticola* (Fig. 4) and the effect was preserved at 6 months from baseline. In contrast, the administration of metronidazole did not enhance the effects of SRP on *A. actinomycetemcomitans* in this group and the levels of this species did not differ at 6 months compared with baseline. Metronidazole has been proven effective against anaerobic periodontal pathogens in several studies (Loesche et al. 1984, 1991, Feres et al. 2001, Sigusch et al. 2001) but its effects on *A. actinomycetemcomitans* are somewhat controversial (Aitken et al. 1992, Loesche et al. 1992, Saxen & Asikainen 1993).

Bacterial recolonization or regrowth in the subgingival environment is anticipated after SRP, even shortly after treatment and it is suggested that in order to prevent a return to pretreatment prevalence and levels of pathogens regularly performed supportive periodontal therapy is essential (Petersilka et al. 2002). Data from the present study at 6 months from baseline indicate an increase in the prevalence (Table 6) and levels (Figs 2–5) of periodontal pathogens compared with immediately after antibiotic intake, but the effect on levels of pathogens was still maintained for the MET+AMO and the MET groups.

The present findings support the utility of microbiological testing especially in this group of patients. If adjunctive antimicrobial treatment were to be guided by microbiological findings then in the absence of *A. actinomycetemcomitans*, MET alone would appear a reasonable choice, effective in sup-

pressing anaerobic pathogens and in reducing deep pockets. In the presence of *A. actinomycetemcomitans*, the addition of AMO appears to predictably suppress the pathogenic subgingival microflora and enhance the microbiological effects of MET alone. In the current literature, a very limited number of controlled studies have tested the possible impact of microbiological analysis on patient management (Levy et al. 1993). As pointed out in a recent review by Listgarten and Loomer (2003), the absence of strong evidence supporting the incorporation of this analysis in periodontal practice does not indicate that it is meaningless and future studies should investigate if treatment based on identification of periodontal pathogens offers benefits to the therapeutic outcome. Although the present study was not designed to address this question, the findings suggest that misuse of systemic antimicrobials could be avoided by identifying key pathogens and determining their susceptibility.

In addition, a recent study by van Winkelhoff et al. (2005) has shown wide differences in the susceptibility profiles of periodontal pathogens isolated from periodontitis patients between Spain and the Netherlands. According to the authors, their findings possibly reflect differences in antibiotic misuse and patient non-compliance, which are shown to be higher in Mediterranean countries. Data from the present study derive from Greek subjects and although antimicrobial susceptibility profiles for periodontal pathogens are unavailable for our subject sample, our findings strongly support the suggestions of van Winkelhoff et al. (2005) that further studies are needed in European countries in order to investigate variations of resistance and develop country-specific guidelines for rationalized usage of antimicrobials.

Collectively, the above-mentioned data suggest a beneficial effect of adjunctive metronidazole plus amoxicillin or metronidazole in reducing the proportions of sites with PD >6 mm in AgP patients after the initial mechanical therapy compared with SRP alone. The combination of metronidazole and amoxicillin effectively reduced levels of all investigated species for the experimental period, while metronidazole did not display a significant effect on *A. actinomycetemcomitans*. Adjunctive doxycycline yielded conflicting results and therefore the administration of bacteriostatic antimicrobials might not be an appropriate choice, at least for this group of patients.

References

Aitken, S., Birek, P., Kulkarni, G. V., Lee, W. L. & McCulloch, C. A. (1992) Serial doxycycline and metronidazole in prevention of recurrent periodontitis in high risk patients. *Journal of Periodontology* **63**, 87–92.

Altman, D. G., Schulz, K. F., Moher, D., Egger, M., Davidoff, F., Elbourne, D., Gøtzsche, P. C. & Lang, T. (2001) The revised consort statement for reporting randomized trials: explanation and elaboration. *Annals of Internal Medicine* **134**, 663–694.

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

Asikainen, L. C., Jousimies-Somer, H., Kanervo, A. & Saxen, L. (1990) The immediate efficacy of adjunctive doxycycline in treatment of localized juvenile periodontitis. *Archives of Oral Biology* **35**, 231S–234S.

Berglundh, T., Krok, L., Liljenberg, B., Westfeld, E., Serino, G. & Lindhe, J. (1998) The use of metronidazole and amoxicillin in the treatment of advanced periodontal disease. *Journal of Clinical Periodontology* **25**, 354–362.

Chopra, I. & Roberts, M. (2001) Tetracycline antibiotics: mode of action, applications, molecular biology and epidemiology of bacterial resistance. *Microbiology and Molecular Biology Reviews* **65**, 232–260.

Feres, M., Haffajee, A. D., Allard, K., Som, S. & Socransky, S. S. (2001) Change in subgingival microbial profiles in adult periodontitis subjects receiving either systemically-administered amoxicillin or metronidazole. *Journal of Clinical Periodontology* **28**, 597–609.

Feres, M., Haffajee, A. D., Goncalves, C., Allard, K. A., Som, S., Smith, C., Goodson, J. M. & Socransky, S. S. (1999) Systemic doxycycline administration in the treatment of periodontal infections (I). Effect on the subgingival microbiota. *Journal of Clinical Periodontology* **26**, 775–783.

Flemming, T. F., Milian, E., Kopp, C., Karch, H. & Klaiber, B. (1998) Differential effects of systemic metronidazole and amoxicillin on *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in intraoral habitats. *Journal of Clinical Periodontology* **25**, 1–10.

Guerrero, A., Griffiths, G. S., Nibali, L., Suvan, J., Moles, D. R., Laurell, L. & Tonetti, M. S. (2005) Adjunctive benefits of systemic amoxicillin and metronidazole in non-surgical treatment of generalized aggressive periodontitis: a randomized placebo-controlled clinical trial. *Journal of Clinical Periodontology* **32**, 1096–1107.

Haffajee, A., Cugini, M. A., Dibart, S., Smith, C., Kent, R. L. Jr & Socransky, S. (1997) The effect of SRP on the clinical and microbiological parameters of periodontal diseases. *Journal of Clinical Periodontology* **24**, 324–334.

Haffajee, A., Socransky, S. & Gunsolley, J. (2003) Systematic anti-infective periodontal therapy. A systematic review. *Annals of Periodontology* **8**, 115–181.

Herrera, D., Sanz, M., Jepsen, S., Needleman, I. & Roldán, S. (2002) A systematic review on the effect of systemic antimicrobials as an adjunct to scaling and root planing in the periodontitis patients. *Journal of Clinical Periodontology* **29** (suppl. 3), 136–159.

López, N. J., Gamonal, J. A. & Martinez, B. (2000) Repeated metronidazole and amoxicillin treatment of periodontitis. A follow up study. *Journal of Periodontology* **71**, 79–89.

Levy, D., Csima, A., Birek, P., Ellen, R. P. & McCulloch, C. A. (1993) Impact of microbiological consultation on clinical decition making: a case–control study of clinical mangement of recurrent periodontitis. *Journal of Periodontology* **64**, 1029–1039.

Lindhe, J., Liljenberg, B. & Adielsson, B. (1983) Effect of long-term tetracycline therapy on human periodontal disease. *Journal of Clinical Periodontology* **10**, 590–601.

Listgarten, M. A. & Loomer, P. M. (2003) Microbial identification in the management of periodontal diseases. A systematic review. *Annals of Periodontology* **8**, 182–192.

Loesche, W. J., Giordano, J. R., Hujoel, P., Schwarcz, J. & Smith, B. A. (1992) Metronidazole in periodontitis: reduced need for surgery. *Journal of Clinical Periodontology* **19**, 103–112.

Loesche, W. J., Grossman, N. & Giordano, J. (1993) Metronidazole in periodontitis (IV). The effect of patient compliance on treatment parameters. *Journal of Clinical Periodontology* **20**, 96–104.

Loesche, W. J., Schmidt, E., Smith, B. A., Morrison, E. C., Caffesse, R. & Hujoel, P. P. (1991) Effects of metronidazole on periodontal treatment needs. *Journal of Periodontology* **62**, 247–257.

Loesche, W. J., Syed, S. A., Morrison, E. C., Kerry, G. A., Higgins, T. & Stoll, J. (1984) Metronidazole in periodontitis. I. Clinical and bacteriological results after 15 to 30 weeks. *Journal of Periodontology* **55**, 325–335.

McCulloch, C. A., Birek, P., Overall, C., Aitken, S., Lee, W. & Kulkarni, G. (1990) Randomized controlled trial of doxycycline in prevention of recurrent periodontitis in high risk patients: antimicrobial activity and collagenase inhibition. *Journal of Clinical Periodontology* **17**, 616–622.

Mousquès, T., Listgarten, M. & Phillips, R. (1980) Effect of scaling and root planing on the composition of the human subgingival microbial flora. *Journal of Periodontal Research* **15**, 144–151.

Petersilka, G. J., Ehmke, B. & Flemming, T. F. (2002) Antimicrobial effects of mechanical debridement. *Periodontology 2000* **28**, 56–71.

Picolos, D. K., Lerche-Sehm, J., Abron, A., Fine, J. B. & Papapanou, P. N. (2005) Infection patterns in chronic and aggressive periodontitis. *Journal of Clinical Periodontology* **32**, 1055–1061.

Purucker, P., Mertes, H., Goodson, J. M. & Bernimoulin, J. P. (2001) Local versus systemic adjunctive antibiotic therapy in 28 patients with generalized aggressive periodontitis. *Journal of Periodontology* **72**, 1241–1245.

Rams, T. E. & Keyes, P. H. (1983) A rationale for the management of periodontal diseases: effect of tetracycline on subgingival bacteria. *Journal of American Dental Association* **107**, 37–41.

Renvert, S., Wikstrom, M., Dahlén, G., Slots, J. & Egelberg, J. (1990) Effect of rrot debridement on the elimination of *Actinobacillus actinomycetemcomitans* and *Bacteroides gingivalis* from periodontal pockets. *Journal of Clinical Periodontology* **17**, 345–350.

Sakellari, D., Vouros, I., Konstantinidis, A. & Socransky, S. (2000) Investigation of subgingival microflora in a group of greek patients with untreated adult periodontitis. *Balkan Journal of Stomatology* **4**, 138–143.

Saxen, L. & Asikainen, S. (1993) Metronidazole in the treatment of localized juvenile periodontitis. *Journal of Clinical Periodontology* **20**, 166–171.

Sheiham, A. & Netuveli, G. (2002) Periodontal diseases in Europe. *Periodontology 2000* **29**, 104–121.

Sigusch, B., Beier, M., Klinger, G., Pfister, W. & Glockman, E. (2001) A 2-step non surgical procedure and systemic antibiotics in the treatment of rapidly progressive periodontitis. *Journal of Periodontology* **72**, 275–283.

Socransky, S. & Haffajee, A. D. (2002) Dental biofilms: difficult therapeutic targets. *Periodontology 2000* **28**, 12–55.

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

Socransky, S., Smith, C., Martin, L., Paster, B. J., Dewhirst, F. E. & Levin, A. E. (1994) Checkerboard'' DNA–DNA hybridization. *BioTechniques* **17**, 788–792.

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

Umeda, M., Takeuchi, Y., Noguchi, K., Huang, Y., Koshy, G. & Ishikawa, I. (2004) Effects of nonsurgical periodontal therapy on the mocrobiota. *Periodontology 2000* **36**, 98–120.

Winkel, E. G., Van Winkelhoff, A. J., Timmerman, M. F., Van der Velden, U. & Van der Weijden, G. A. (2001) Amoxicillin plus metronidazole in the treatment of adult periodontitis patients. A double-blind placebo-controlled study. *Journal of Clinical Periodontology* **28**, 296–305.

Van Winkelhoff, A. J., Herrera, D., Oteo, A. & Sanz, M. (2005) Antimicrobial profiles of periodontal pathoens isolated from periodontitis patients in the Netherlands and Spain. *Journal of Clinical Periodontology* **32**, 893–898.

Van Winkelhoff, A. J., Herrera, D., Winkel, E. G., Dellemijn, K., Vanderbroucke, G. & Sanz, M. (2000) Antimicrobial resistence in the subgingival microflora in patients with adult periodontitis. A comparison between The Netherlands and Spain. *Journal of Clinical Periodontology* **27**, 79–86.

Van Winkelhoff, A. J., Rams, T. E. & Slots, J. (1996) Systemic antibiotic therapy in periodontics. *Periodontology 2000* **10**, 45–78.

Van Winkelhoff, A. J., Tijhof, C. J. & de Graaff, J. (1992) Microbiological and clinical results of metronidazole plus amoxicillin therapy in *Actinobacillus actinomycetemcomitans* associated periodontitis. *Journal of Periodontology* **63**, 52–57.

Address:
Dimitra Sakellari
88 Mitropoleos str
Thessaloniki 54622
Greece
Fax: +0030 2310 999613
E-mail: dimisak@med.auth.gr

Clinical Relevance

Scientific rationale for the study: Few RCTs including microbiological data have evaluated antimicrobials as adjuncts of treatment of AgP. We compared the effects of metronidazole plus amoxicillin, doxycycline, and metronidazole *versus* mechanical treatment.

Principal findings: Metronidazole plus amoxicillin or metronidazole resulted in statistically significant greater reduction of the proportion of sites >6 mm than SRP and yielded a significant effect on important pathogens for 6 months.

Practical implications: Adjunctive metronidazole plus amoxicillin or metronidazole alone (when *A. actinomycetemcomitans* is not involved) are effective in pockets >6 mm of AgP patients.