J Clin Periodontol 2011; 38: 838–846 doi: 10.1111/j.1600-051X.2011.01766.x

Journal of Clinical Periodontology

Clinical and microbiological effects of azithromycin in the treatment of generalized chronic periodontitis: a randomized placebo-controlled clinical trial

Eduardo Sampaio, Marcelo Rocha, Luciene Cristina Figueiredo, Marcelo Faveri, Poliana Mendes Duarte, Eisla Alline Gomes Lira and Magda Feres

Department of Periodontology, Dental Research Division, Guarulhos University, Guarulhos, São Paulo, Brazil

Sampaio E, Rocha M, Figueiredo LC, Faveri M, Duarte PM, Lira EAG, Feres M: Clinical and microbiological effects of azithromycin in the treatment of generalized chronic periodontitis: a randomized placebo-controlled clinical trial. J Clin Periodontol 2011; 38: 838–846. doi: 10.1111/j.1600-051X.2011.01766.x.

Abstract

Aim: To evaluate the effects of systemic azithromycin (AZM) as an adjunct to scaling and root planing (SRP) in the treatment of generalized chronic periodontitis (ChP).

Methods: Forty subjects were randomly assigned to receive SRP alone or combined with AZM (500 mg/day) for 5 days ($n = 20$/group). Clinical and microbiological examinations were performed at baseline, 6 months and 1-year post-SRP. Nine plaque samples per subject were analysed by checkerboard DNA–DNA hybridization for 40 bacterial species. Differences between groups were assessed using the Mann–Whitney test and over time using Friedman and Dunn's tests.

Results: No statistically significant differences were observed between groups for any parameters evaluated at 1-year post-treatments. Both therapies equally reduced the mean probing depth (PD) (SRP: 3.83 ± 1.92, AZM: 3.45 ± 1.74) and improved the mean clinical attachment (SRP: 2.35 ± 1.70, AZM: 2.68 ± 1.76) in sites with initial PD ⩾ 7 mm (primary outcome variable) between baseline and 1 year. The mean counts and proportions of several periodontal pathogens were reduced, and those of host-beneficial species were increased after treatments. Nonetheless, an important recolonization with red complex species was observed in both groups over the course of the study.

Conclusion: The data of the present study suggest no adjunctive benefit of AZM in the treatment of generalized ChP.

Key words: periodontal disease; azithromycin; chronic periodontitis; scaling and root planing; microbiology

Accepted for publication 16 June 2011

Antibiotics are one of the most valuable tools in the treatment of infectious diseases, including periodontitis. Of the antibiotics tested as adjuncts in the treatment of periodontal infections to date, the association of metronidazole (MTZ) and amoxicillin (AMX) administered 3 times/day each, for 7–14 days, seems to be the most effective therapy (Guerrero et al. 2005, Xajigeorgiou et al. 2006, Cionca et al. 2009, Matarazzo et al. 2008, Mestnik et al. 2010). One of the problems related to this drug regimen is the need to take several pills per day for long periods of time, which may contribute towards reducing compliance (Greenberg 1984) and, consequently, the benefits of treatment.

More recently, azithromycin (AZM), a member of a new subclass of macrolide, the azalides, has emerged as a promising drug due to its good pharmacological properties. AZM is a semi-synthetic, bacteriostatic, wide-spectrum antibiotic, rapidly absorbed by cells such as leucocytes and fibroblasts,

Conflict of interest and source of funding statement

The authors declare that they have no conflict of interests.

This study was supported by Research Grant #308308/2009-3 from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil).

which helps to quickly bring the drug to the site of inflammation and to maintain its concentration 10–100 times higher in tissues than in serum (Hoepelman & Schneider 1995). In addition, AZM is slowly released to the tissues, which increases its half-life (Gladue et al. 1989, Gladue & Snider 1990). This favourable pharmacokinetic property allows AZM to be administered only once a day (500 mg) for short periods of time (from 3 to 5 days) (Henry et al. 2003). This simple dosage protocol and the low incidence of side-effects reported with the use of this antibiotic facilitate patient adherence to treatment, which represents a major advantage of AZM over other commonly used antibiotics in periodontics.

Few randomized-controlled clinical trials have reported different degrees of success with the use of AZM in the treatment of mild to moderate chronic periodontitis (ChP) (Smith et al. 2002, Haffajee et al. 2007, 2008, Oteo et al. 2010) or aggressive periodontitis (AgP) (Haas et al. 2008). However, only two previous studies have evaluated the adjunctive benefit of this antibiotic in the treatment of smokers (Mascarenhas et al. 2005) or non-smoker subjects (Yashima et al. 2009) with advanced ChP. In addition, to date, no study has systematically evaluated the potential of AZM in altering the subgingival microbial profile of subjects with generalized advanced periodontal infection.

Therefore, the aim of this study was to evaluate the clinical and microbiological effects of AZM in the treatment of subjects with generalized ChP. The hypothesis tested was that the adjunctive use of this antibiotic in the initial periodontal therapy would lead to better clinical and microbiological outcomes, in comparison with scaling and root planing (SRP) alone.

Material and Methods

Experimental design, allocation concealment and treatment protocol

In this double-blinded, randomized, parallel-designed and placebo-controlled clinical trial, each subject was given a code number during the enrolment visit and the study coordinator (L. C. F.) used a computer-generated table to allocate them to one of the two therapeutic groups: control – SRP+placebo (once a day for 5 days) or test – SRP+AZM (500 mg once a day for 5 days) (Hoepelman & Schneider 1995, Henry et al. 2003).

During the initial phase, all subjects received instruction on proper home-care techniques and were given the same dentifrice (Colgate Total, Colgate Palmolive Co., São Bernardo do Campo, SP, Brazil) to use during the study period. Subsequently, they received full-mouth SRP performed under local anaesthesia in four to six appointments of approximately 2 h each. The treatment of the entire oral cavity was completed in a maximum period of 2 weeks. SRP was performed by two trained periodontists (E. S. and M. R.) using mainly manual instruments. They were randomized according to the different treatment groups so that each clinician treated the same number of patients in each group. The end point for each SRP appointment was ''smoothness of the scaled roots'', which was checked by the study coordinator. The antibiotic/placebo therapies started immediately after the last session of mechanical instrumentation. Pharmedica Pharmacy (São Paulo, SP, Brazil) prepared the antibiotic and placebo capsules. Forty identical opaque plastic bottles with 5 capsules each (20 bottles with AZM 500 mg and 20 with placebo) were sent to the study coordinator, who marked the code number of each subject on each bottle, according to the therapy assigned. All the capsules in the placebo and antibiotic bottles were identical. The coded bottles were given to the examiners, who at no time during the study had access to information about the contents of the bottles or subjects' assignment to therapies. In addition, all study personnel, including the biostatisticians (M. Fa., M. Fe.) and participants, were blinded to treatment assignment. Code breaking was performed after the final statistical analysis. All subjects received microbiological and clinical monitoring at baseline, 6 months and 1-year post-therapies. This study protocol was approved by the Guarulhos University's Ethics Commmitte in Clinical Research.

Sample size calculation

The ideal sample size to ensure adequate power for this clinical trial was calculated considering differences of at least 1 mm between groups for clinical attcahment level (CAL) changes in sites with initial probing depth (PD) $\geqslant$ 7 mm and assuming a standard deviation of 1.0 mm (Matarazzo et al. 2008). Based on these calculations, it was defined that 17 subjects per group would be necessary to provide an 80% power with an α of 0.05. Considering an attrition of about 15%, 20 subjects were included in each group.

Subject population and inclusion/exclusion criteria

Forty subjects with untreated ChP were selected by one of the researchers (P. M. D.) from the population referred to the Periodontal Clinic of Guarulhos University (Guarulhos, SP, Brazil). Detailed medical, periodontal and dental histories were obtained. Subjects who fulfilled the inclusion criteria were invited to participate in the study. All eligible subjects were informed of the nature, potential risks and benefits of their participation in the study and signed a Term of Informed Consent. All subjects were in good general health and were diagnosed with generalized ChP based on the current classification of the American Academy of Periodontology (Armitage 1999). The inclusion criteria were as follows: $>$30 years of age, at least 15 teeth (excluding third molars) and at least 30% of the sites with PD and CAL $\geqslant$ 5 mm and bleeding on probing (BOP), a minimum of three non-contiguous inter-proximal sites with PD and CAL $\geqslant$ 7 mm and two other non-contiguous inter-proximal sites with PD and CAL $\geqslant$ 6 mm. The exclusion criteria were as follows: previous subgingival periodontal therapy, pregnancy, nursing, systemic diseases that could affect the progression of periodontal disease, long-term administration of anti-inflammatories, need for antibiotic coverage for routine dental therapy, antibiotic therapy in the previous 6 months and allergy to AZM.

Compliance and adverse events monitoring

On the last day of medication (fifth day after the completion of SRP), the subjects were asked to return to the clinic and bring the medication bottles, which were checked for any possible remaining pills. During this visit, subjects answered a questionnaire about any self-perceived side-effects of the medication/placebo. One study assistant (E. A. G. L.) conducted this inquiry and was also responsible for calling

the subjects everyday to monitor compliance.

Clinical monitoring

The clinical monitoring was performed by two calibrated examiners as described previously (Araujo et al. 2003) and the standard error of measurement was calculated. The inter-examiner variability was 0.25 mm for PD and 0.29 mm for CAL. The mean intra-examiner variability was 0.22 mm (PD) and 0.27 mm (CAL) for the first examiner (M. R.) and 0.23 mm (PD) and 0.26 mm (CAL) for the second examiner (E. S.). One examiner carried out all clinical measurements in a given subject and treatment was performed by the second clinician. Subjects were clinically monitored at baseline, 6 months and 1-year post-therapy. Visible plaque (0/1), gingival bleeding (0/1), BOP (0/1), suppuration (0/1), PD (distance in mm from the gingival margin to the bottom of the sulcus/pocket) and CAL (distance in mm from the cementoenamel junction to the bottom of the sulcus/pocket) were measured at six sites per tooth (mesiobuccal, buccal, distobuccal, distolingual, lingual and mesiolingual) in all teeth, excluding the third molars. The PD and CAL measurements were recorded to the nearest millimetre using a North Carolina periodontal probe (Hu-Friedy, Chicago, IL, USA).

Microbiological monitoring

Nine subgingival plaque samples [three in each of the following categories: shallow (PD $\leqslant$ 3 mm), intermediate (PD 4–6 mm) and deep (PD $\geqslant$ 7 mm)] were collected per subject at baseline, 6 months and 1-year post-SRP from non-contiguous inter-proximal sites. After the clinical parameters had been recorded, the supragingival plaque was removed and the subgingival samples were taken using individual sterile mini-Gracey curettes (#11–12) and placed in separate Eppendorf tubes containing 0.15 ml of TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.6). One hundred microlitres of 0.5 M NaOH was added to each tube and the samples were dispersed using a vortex mixer. Counts of 40 bacterial species were determined in each sample, using the checkerboard DNA–DNA hybridization technique (Socransky et al. 1994, Mestnik et al. 2010), in the Laboratory of Microbiology of Guarulhos University.

Primary and secondary outcome variables

This study compared the clinical and microbiological effects of two different periodontal therapies. The primary outcome variable was the difference between groups at 6 months and 1 year for the mean CAL change in sites with initial PD $\geqslant$ 7 mm. The secondary outcome variables were differences between groups for the following parameters: the mean PD change in sites with initial PD $\geqslant$ 7 mm; the mean CAL and PD changes in the full mouth and in sites with an initial PD between 4 and 6 mm and $\leqslant$ 3 mm; the mean number and percentage of sites with PD $\geqslant$ 5 mm (with or without BOP); the mean full-mouth PD and CAL, percentage of sites with BOP, plaque accumulation, gingival bleeding and suppuration at 6 months and 1-year post-treatment; and the mean levels and proportions of the 40 bacterial species analysed (individually or as complexes).

Statistical analysis

Each individual clinical parameter as well as the mean counts ($\times 10^5$) of each bacterial species evaluated were computed per subject and then across subjects in both groups. The changes in PD and CAL in sites with initial PD $\leqslant$ 3, 4–6 and $\geqslant$ 7 mm or percentage of sites with PD $<$ or $\geqslant$ 5 mm (with/without BOP) were averaged separately within the PD categories per subject and then across subjects in each group. In addition, the percentage of the total DNA probe counts was determined initially in each site, and then per subject and across subjects in both treatment groups. The significance of differences between the two groups for age, clinical and microbiological parameters was assessed using the Mann–Whitney *U*-test. Friedman and Dunn's multiple comparison tests were used to detect significant differences within each group over the course of the study. Adjustments for multiple comparisons (Socransky et al. 1991) were performed when the 40 bacterial species were evaluated simultaneously. The chi-square test was used to compare the differences in the frequency of gender. The level of significance was set at 5%.

Results

Subject retention, compliance and adverse effects

Subject recruitment occurred between April and October 2009. Figure 1 presents the flow diagram of the study design. One subject per group did not return for the 1-year follow-up visit. Intention-to-treat analyses were performed in these two subjects (their 6-month data were carried forward).

No remaining pills were observed in the medication bottles on the last day of medication intake, and all subjects reported full adherence to the prescribed course of the antibiotic/placebo treatments. Adverse events were reported by four subjects from the test group and three from the control group, including diarrhoea (test group, $n = 2$), headache or dizziness (test group, $n = 1$; control group, $n = 2$), excessive sleepiness ($n = 3$ per group); metallic taste (test group, $n = 2$; control group, $n = 3$) and general unwellness ($n = 1$ per group). All subjects reported that the medications did not cause any major disturbance in their daily routine and that they would start the treatment again if necessary.

Clinical findings

Table 1 presents the demographic and clinical data for both treatment groups at all time points. No statistically significant differences were observed between groups for any parameter evaluated at baseline ($p > 0.05$). Both treatments led to a statistically significant improvement in all clinical parameters, and no significant differences were detected between them at any time point ($p > 0.05$).

Table 2 shows the reductions in the mean PD and CAL between baseline and the follow-up visits for the full-mouth analysis as well as for initially shallow (PD $\leqslant$ 3 mm), intermediate (PD 4–6 mm) or deep sites (PD $\geqslant$ 7 mm). Both treatments were equally effective in improving these two parameters, with no statistically significant differences between them ($p > 0.05$).

The mean percentage and mean number of sites presenting PD $<$ 5 mm or $\geqslant$ 5 mm, with or without BOP during the course of the study, are presented in Table 3. There was a statistically significant reduction in the mean percentage and number of sites with PD $\geqslant$ 5 mm in both groups, without statistically significant differences between them at all

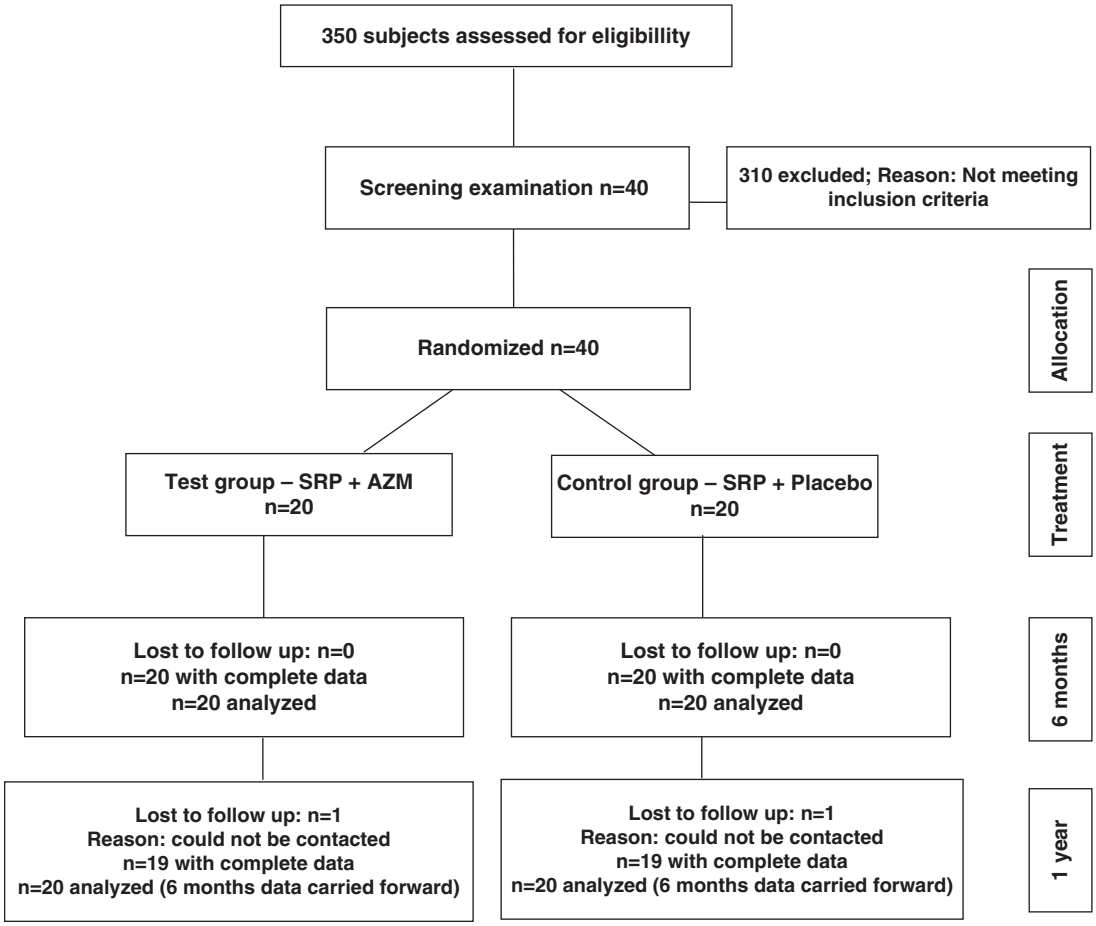

Fig. 1. Flow chart of the study design.

time points. At 1-year post-treatments, the mean numbers of sites with PD $\geqslant$ 5 mm (with+without BOP) were approximately 15 in the control group and 18 in the AZT group.

Microbiological findings

All subjects were colonized by *Tannerella forsythia*, *Porphyromonas gingivalis* and *Treponema denticola*, and no statistically significant differences were observed in the individual mean counts, proportions or prevalence of the 40 bacterial species evaluated between groups at baseline (data not shown).

Figures 2 and 3, respectively, show the mean counts ($\times 10^5$) and proportions of the 40 bacterial species evaluated at all visits. The statistical analysis presents differences between baseline and the follow-up visits for each treatment group and between groups at 1-year post-treatment. At 6 months post-treatment, the counts and proportions of *T. forsythia* and *P. gingivalis* were statistically significantly reduced in both groups, as well as *T. denticola* counts in the control group. At 1-year post-treatment, no statistically significant differences were observed between treatments for counts and proportions of the 40 bacterial species evaluated (Figs 2 and 3). Some recolonization with red complex pathogens occurred in both groups. In terms of prevalence, no *P. gingivalis* and *T. forsythia* were detected in four subjects per group at 1 year, while *T. denticola* was not found in three and four subjects from the control and the test groups, respectively (data not shown). Moreover, the two treatments were equally effective in reducing the counts, and to a lesser extent, the proportions, of some orange complex pathogens (Figs 2 and 3).

Only minor changes were observed in the mean counts of the majority of host-compatible microorganisms, such as *Actinomyces* species, purple, yellow and green complexes, after therapies (Fig. 2). As expected, the proportions of these species increased after treatments and *Actinomyces gerencseriae* and *Actinomyces naeslundii* 1 remained in statistically significant higher proportions at 1 year, in comparison with the baseline, in both groups (Fig. 3).

The proportions of the different microbial complexes are presented in Fig. 4. Both therapies led to a statistically significant reduction in the proportions of the red complex at 6 months, which remained reduced in comparison with baseline values up to 1 year ($p < 0.05$), although a relevant recolonization with these species was observed between 6 months and 1-year post-treatments. SRP alone significantly reduced the proportions of the orange complex at all follow-up visits ($p < 0.05$), while this reduction was statistically significant only at 1 year in the test group. Overall, the proportions of the complexes considered beneficial increased after treatments. These changes were statistically significant at 6 months and 1-year post-treatments for the *Actinomyces* species

Table 1. Demographic characteristics and the mean (± SD) full-mouth clinical parameters at baseline and at follow-up visits

Variable	Time point	Treatment groups	
		SRP ($n = 20$)	SRP+AZM ($n = 20$)
Gender (male/female)	Baseline	11/9	13/7
Age (years)	Baseline	43.52 ± 5.90	44.40 ± 7.42
Smokers	Baseline	5	5
% Sites with			
PD ⩽ 3 mm	Baseline	31.50 ± 11.82	32.63 ± 17.27
PD 4–6 mm	Baseline	46.50 ± 13.87	45.26 ± 11.72
PD ⩾ 7 mm	Baseline	22.50 ± 12.92	21.05 ± 11.49
PD (mm)	Baseline	5.02 ± 0.66[a]	4.82 ± 0.84[a]
	6 months	3.36 ± 0.38[b]	3.24 ± 0.41[b]
	1 year	3.34 ± 0.50[b]	3.36 ± 0.44[b]
CAL (mm)	Baseline	5.74 ± 0.83[a]	5.51 ± 0.94[a]
	6 months	4.70 ± 0.83[b]	4.43 ± 0.81[b]
	1 year	4.69 ± 0.89[b]	4.44 ± 0.77[b]
% of sites with			
Plaque accumulation	Baseline	82.70 ± 12.91[a]	81.39 ± 15.42[a]
	6 months	35.47 ± 19.02[b]	38.20 ± 16.87[b]
	1 year	36.36 ± 27.09[b]	38.65 ± 14.16[b]
Gingival bleeding	Baseline	42.73 ± 25.50[a]	44.45 ± 24.05[a]
	6 months	15.55 ± 9.04[b]	14.78 ± 11.11[b]
	1 year	11.41 ± 7.48[b]	11.85 ± 8.73[b]
Bleeding on probing	Baseline	81.93 ± 12.86[a]	75.78 ± 24.51[a]
	6 months	12.31 ± 8.83[b]	09.91 ± 7.24[b]
	1 year	9.47 ± 7.71[b]	10.01 ± 5.52[b]
Suppuration	Baseline	7.95 ± 8.44[a]	10.95 ± 14.06[a]
	6 months	0.07 ± 0.32[b]	0.13 ± 0.54[b]
	1 year	0.33 ± 0.55[b]	0.32 ± 0.52[b]

The significance of differences between baseline and the follow-up visits was assessed using Friedman and Dunn's multiple comparison tests (different letters indicate significant differences between time points). The significance of differences between groups at each time point was assessed using the Mann–Whitney *U*-test ($p > 0.05$).

SD, standard deviation; SRP, scaling and root planing; AZM, azithromycin; PD, probing depth; CAL, clinical attachment level.

in both groups and for the purple complex in the AZM group, as well as for the yellow complex at 6 months in the control
group. The only statistically significant difference observed between groups at all time points was a higher proportion of the purple complex (*Veillonella parvula*+*Actinomyces odontolyticus*) in the AZM-treated subjects at 6 months.

Discussion

This study evaluated the clinical and microbiological effects of SRP with or without adjunctive AZM in the treatment of patients with generalized ChP. The hypothesis tested, that the greatest benefits would be achieved with the aid of AZM, was rejected. The two treatments equally improved the mean CAL at initially deep sites (primary outcome variable) and other clinical and microbiological parameters evaluated, but no important differences were observed between groups at any time point.

Two research groups have evaluated previously the clinical effects of systemic AZM in the treatment of advanced ChP in non-smokers (Yashima et al. 2009) or smokers (Mascarenhas et al. 2005). In contrast to the present results, Yashima et al. (2009) reported a statistically significant greater clinical attachment gain in subjects receiving SRP+AZM than in those receiving only SRP, at 1-year post-treatments. One of the reasons for these conflicting results may be the differences in the outcomes of the control treatment. In the present study, CAL gain at 1 year in the test group was quite similar (≅ 1.02 mm) to that reported by Yashima et al. (2009) (≅ 1.0 mm), but SRP alone elicited a higher CAL gain in our study (≅ 1.04 mm), compared with their result (≅ 0.7 mm). A similar phenomenon occurred in the study of Mascarenhas et al. (2005), in a smoker population. However, it is important to emphasize that smokers do not generally respond well to mechanical treatment.

One could speculate that the lack of additional benefits of AZT in the present study might be due to the excellent results of the control treatment, such as the profound reduction in PD in initially deep sites observed in the SRP-treated subjects (3.8 mm), which was considerably above the values reported previously by our group (Carvalho et al. 2004, Matarazzo et al. 2008, Feres et al. 2009, Mestnik et al. 2010), in a comprehensive review (Cobb 2002) or in a meta-analysis (Hung & Douglass 2002). One possible explanation for these differences is the inclusion criterion of a minimum of three non-contiguous inter-proximal sites with PD and CAL ⩾ 7 mm and two other sites with PD and CAL ⩾ 6 mm used in the present study, which resulted in the selection of subjects with very advanced disease. As a result, this population had approximately 5% of sites with PD ⩾ 10 mm (data not shown), as opposed to < 1% in most of our previous studies. This fact directly influences the magnitude of clinical improvements, especially the mean reduction in PD in initially deep pockets. On the other hand, the number of residual sites with PD ⩾ 5 mm at 1-year post-treatments was quite high in both groups (≅ 15–18 sites), and similar to that observed in subjects treated with SRP in a previous study of generalized aggressive periodontitis (Mestnik et al. 2010). Therefore, it may be concluded that although the magnitude of overall improvements in both groups of this study was large, this was most probably due to a high number of ''very deep sites'' converted into ''deep sites'', rather than an outstanding clinical result from the two treatments applied.

Few randomized clinical trials have also evaluated the effects of AZM in the treatment of mild to moderate ChP (Smith et al. 2002, Haffajee et al. 2007, Oteo et al. 2010). In general, these studies reported modest additional benefits with the use of this antibiotic. Haffajee et al. (2007) showed a tendency towards greater, although not statistically significant, improvements in the mean PD and CAL in subjects with mild ChP receiving SRP plus AZM in comparison with those treated with SRP only, at 6 months and 1-year post-treatment. Smith et al. (2002) reported fewer residual sites (PD > 5 mm) in subjects who took AZT in comparison with

those receiving SRP alone, up to 5 months, although the significance of these differences was not clear. Oteo et al. (2010) also studied the effects of AZM in the treatment of subjects with mild periodontal destruction (50% of smokers) and Haas et al. (2008) in the treatment of young patients with aggressive periodontitis. Both studies found some additional benefits of the antibiotic in reducing PD, but not in terms of clinical attachment gain at 6 months or 1-year of follow-up, respectively.

According to the clinical findings of this study, the microbiological data indicated that the effect of SRP+AZM in altering the subgingival microbial profile was similar to that observed with SRP alone. Both therapies elicited a statistically significant reduction in the counts and proportions of several periodontal pathogens and a concomitant increase in the proportions of some host-compatible species. At 1-year post-treatment, the subgingival microbial profiles were more compatible with health in both groups, in comparison with the baseline. However, the three red complex pathogens were not detected in very few subjects per group after treatments (data not shown), and an important recolonization with these pathogens occurred over the course of the study. These species accounted for $\cong$ 12% of the 40 species evaluated post-treatment, which is expected for an SRP treatment (Matarazzo et al. 2008, Feres et al. 2009, Mestnik et al. 2010). However, this is quite an unexpected result for a systemic antibiotic therapy, and is probably one of the major reasons for the lack of an additional clinical effect of AZM observed in this study. In previous investigations by our group, one of the greatest advantages of using a systemic antimicrobial agent in conjunction with SRP, such as MTZ+AMX (Matarazzo et al. 2008, Mestnik et al. 2010), has been the statistically significant lower remaining proportions of red complex species.

The data from the literature regarding the benefits of AZM in reducing periodontal pathogens are somehow controversial. While some authors observed an advantage of AZM+SRP, over SRP only, in reducing the prevalence of some red complex pathogens, such as *P. gingivalis* and *T. forsythia* (Oteo et al. 2010), others did not (Gomi et al. 2007, Yashima et al. 2009). However, most of these studies evaluated the presence or absence of few

Table 2. Changes in the mean PD (± SD) and CAL (± SD) from baseline to 6 months and from baseline to 1 year

	Time point	Treatment groups	
		SRP	SRP+AZM
Full-mouth			
ΔPD (mm)	6 months	− 1.71 ± 1.71	− 1.54 ± 1.62
	1 year	− 1.77 ± 1.80	− 1.41 ± 1.68
ΔCAL (mm)	6 months	− 1.05 ± 1.54	− 1.05 ± 1.56
	1 year	− 1.04 ± 1.65	− 1.02 ± 1.62
Initially shallow sites (PD ⩽ 3 mm)			
ΔPD (mm)	6 months	− 0.23 ± 0.68	− 0.14 ± 0.65
	1 year	− 0.21 ± 0.63	− 0.01 ± 0.71
ΔCAL (mm)	6 months	0.01 ± 1.07	0.10 ± 0.90
	1 year	0.17 ± 1.10	0.17 ± 0.94
Initially intermediate sites (PD 4 − 6 mm)			
ΔPD (mm)	6 months	− 1.67 ± 1.00	− 1.66 ± 1.01
	1 year	− 1.74 ± 1.02	− 1.14 ± 1.16
ΔCAL (mm)	6 months	− 1.10 ± 1.25	− 1.18 ± 1.21
	1 year	− 1.15 ± 1.31	− 1.14 ± 1.16
Initially deep sites (PD ⩾ 7mm)			
ΔPD (mm)	6 months	− 3.65 ± 1.78	− 3.56 ± 1.54
	1 year	− 3.83 ± 1.92	− 3.45 ± 1.74
ΔCAL (mm)	6 months	− 2.29 ± 1.56	− 2.62 ± 1.56
	1 year	− 2.35 ± 1.70	− 2.68 ± 1.76

The significance of differences between groups at each time point was assessed using the Mann–Whitney *U*-test ($p > 0.05$).
SD, standard deviation; SRP, scaling and root planing; AZM, azithromycin; PD, probing depth; CAL, clinical attachment level.

Table 3. Mean percentage and mean number (± SD) of sites with PD < 5 mm or PD ⩾ 5 mm, with or without BOP, at baseline and at follow-up visits

PD category	Time point	Treatment groups	
		SRP, mean % (mean number)	SRP+AZM, mean % (mean number)
PD < 5 mm	Baseline	44.52 ± 13.18 (55.73 ± 18.44)[a]	48.45 ± 19.85 (60.31 ± 29.07)[a]
	6 months	81.76 ± 20.89 (102.68 ± 31.54)[b]	86.78 ± 8.54 (106.57 ± 18.60)[b]
	1 year	87.47 ± 12.79 (108.84 ± 24.30)[b]	84.53 ± 10.48 (103.89 ± 21.04)[b]
PD ⩾ 5 mm (without BOP)	Baseline	3.85 ± 4.50 (4.68 ± 5.29)[a]	3.93 ± 6.08 (5.00 ± 8.36)[a]
	6 months	8.61 ± 6.92 (10.42 ± 8.82)[b]	10.06 ± 6.77 (12.47 ± 9.14)[b]
	1 year	9.69 ± 10.86 (12.10 ± 14.40)[b]	11.50 ± 7.75 (14.36 ± 10.71)[b]
PD ⩾ 5 mm (with BOP)	Baseline	51.63 ± 13.65 (64.89 ± 20.25)[a]	47.61 ± 21.84 (57.84 ± 28.18)[a]
	6 months	5.50 ± 5.97 (7.00 ± 8.19)[b]	3.16 ± 3.40 (4.10 ± 5.03)[b]
	1 year	2.84 ± 3.15 (3.47 ± 3.89)[b]	3.96 ± 3.11 (4.89 ± 4.14)[b]

The significance of differences between baseline and the follow-up visits was assessed using Friedman and Dunn's multiple comparison tests (different letters indicate significant differences between time points). The significance of differences between groups at each time point was assessed using the Mann–Whitney *U*-test ($p > 0.05$).
SD, standard deviation; SRP, scaling and root planing; AZM, azithromycin; PD, probing depth; BOP, bleeding on probing.

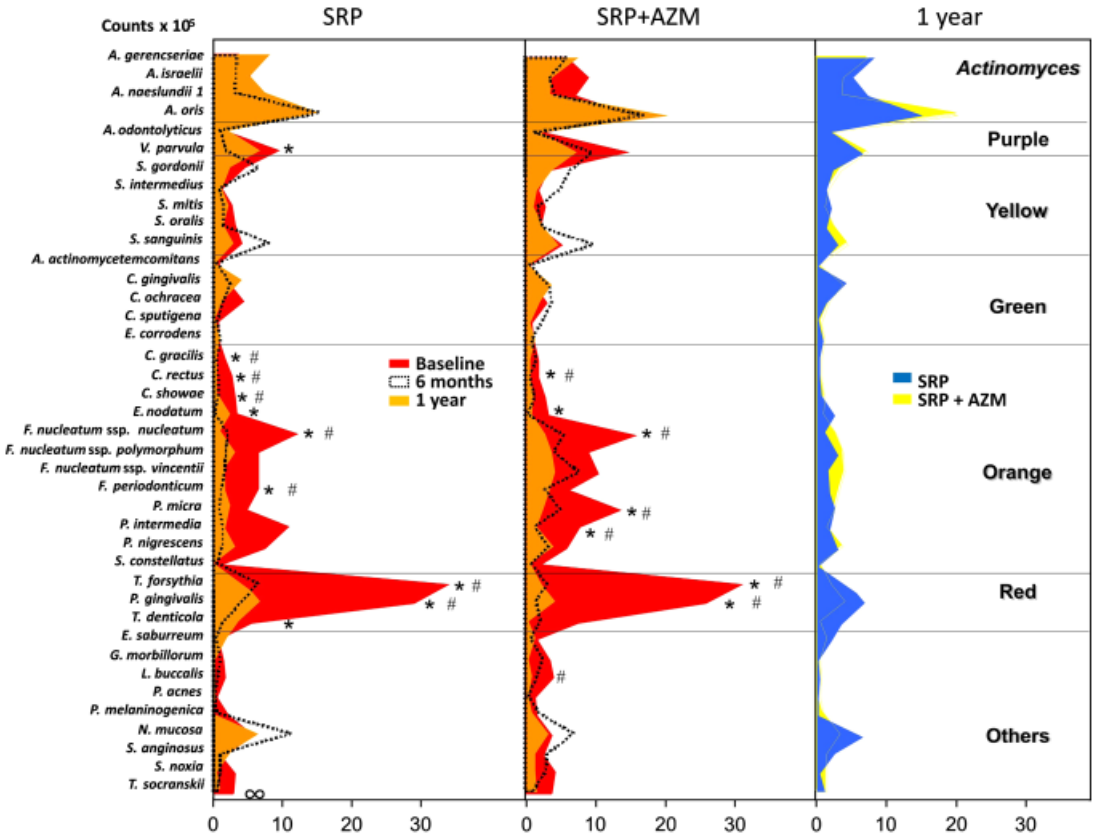

Fig. 2. Mean counts ($\times 10^5$) of the 40 test species at baseline, 6 months and 1-year post-therapy in each treatment group. The species were ordered according to the microbial complexes described by Socransky et al. (1998). The significance of differences within each group over the course of the study was assessed using the Friedman and Dunn's multiple comparison tests ($^*p<0.05$ between baseline and 6 months; # $p<0.05$ between baseline and 1 year). The significance of differences between groups at 1-year post-therapy was assessed using the Mann–Whitney *U*-test ($p>0.05$). SRP, scaling and root planing; AZM, azithromycin.

species in a limited number of sites, which makes the comparison with the data of the present study more difficult. The only clinical trial that has also systematically evaluated the changes occurring in the microbial profile after AZM administration was conducted by Haffajee et al. (2008) in subjects with mild to moderate ChP. Subjects receiving AZM showed some additional microbiological benefits at 2 weeks after treatment. However, in agreement with the data of the present study, the authors could not detect any important difference in the levels and proportions of the 40 bacterial species evaluated between SRP alone or with AZM at 6 months and 1 year of observation.

Concluding remarks

Although AZM has been pointed out as a promising drug for the treatment of different infections due to its good pharmacological properties and easy dosage regimen, the data from the present study suggested no additional effect of this antibiotic in the treatment of ChP, beyond that attained with mechanical debridement alone. The effects of other drugs, such as MTZ or MTZ+AMX, seem to be much more encouraging (Guerrero et al. 2005, Cionca et al. 2009, 2010, Haffajee et al. 2007, 2008, Matarazzo et al. 2008, Mestnik et al. 2010). However, as a few randomized clinical trials have reported some modest benefits with the use of this antibiotic in the periodontal treatment, especially for smokers and mild/moderate ChP, future studies comparing the effects of AZM and other antimicrobials could be elucidative. Nonetheless, it is important to note that the only clinical trial that directly compared the effect of AZM with another systemic antibiotic, MTZ, detected a statistically significant clinical advantage for MTZ+SRP in comparison with SRP only, but not for AZM+SRP (Haffajee et al. 2007). The mechanism of action of AZM may partially explain its lack of efficacy in treating periodontal infection. As opposed to MTZ and AMX, which are bactericidal drugs, AZM is bacteriostatic. It has recently been suggested that rapid reductions in the levels and proportions of pathogens and an increase in the beneficial/pathogen species ratio are necessary to achieve major clinical benefits over time (Teles et al. 2006). This goal might be more difficult to achieve with bacteriostatic antibiotics, such as AZM, other macrolides and tetracyclines.

In conclusion, taken together, the clinical and microbiological data of the present study suggest no adjunctive benefit of systemic AZT in the treatment of generalized ChP.

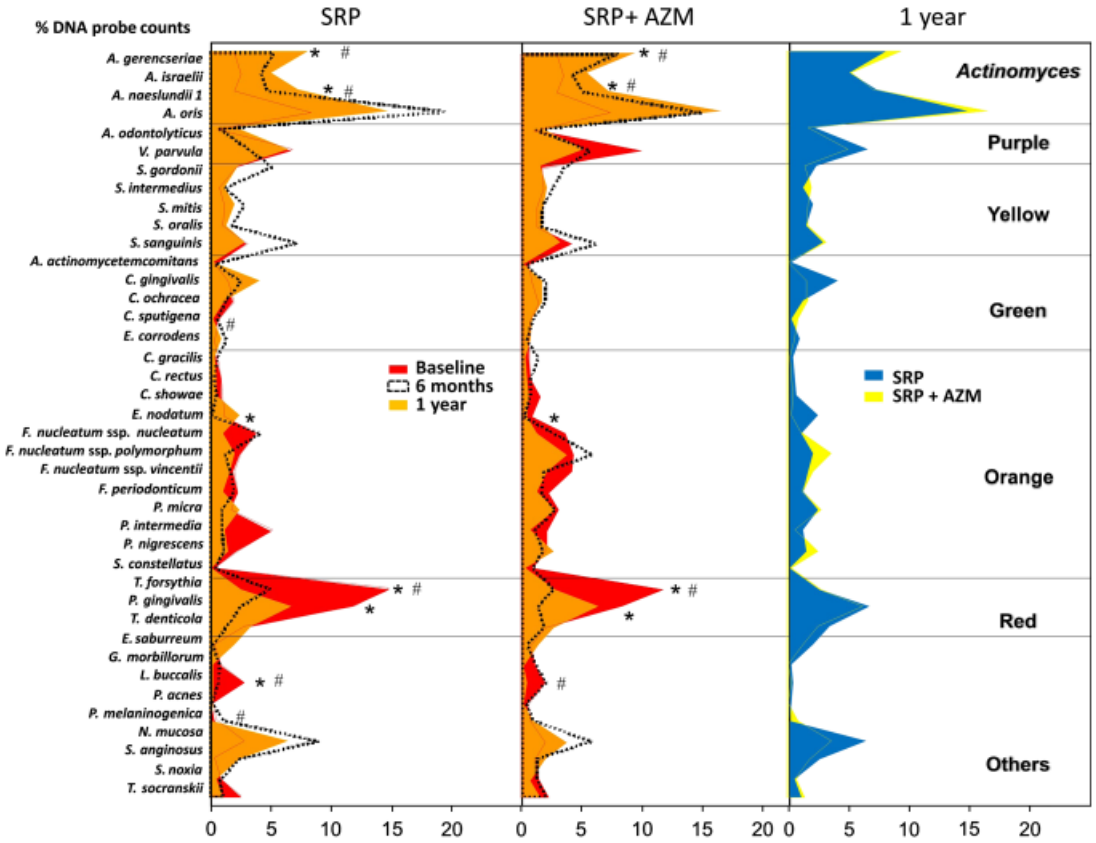

Fig. 3. Mean percentage of DNA probe counts of the 40 test species at baseline, 6 months and 1-year post-therapy in each treatment group. The species were ordered according to the microbial complexes described by Socransky et al. (1998). The significance of differences within each group over the course of the study was assessed using the Friedman and Dunn's multiple comparison tests (*$p<0.05$ between baseline and 6 months; # $p<0.05$ between baseline and 1 year). The significance of differences between groups at 1-year post-therapy was assessed using the Mann–Whitney *U*-test ($p>0.05$). SRP, scaling and root planing; AZM, azithromycin.

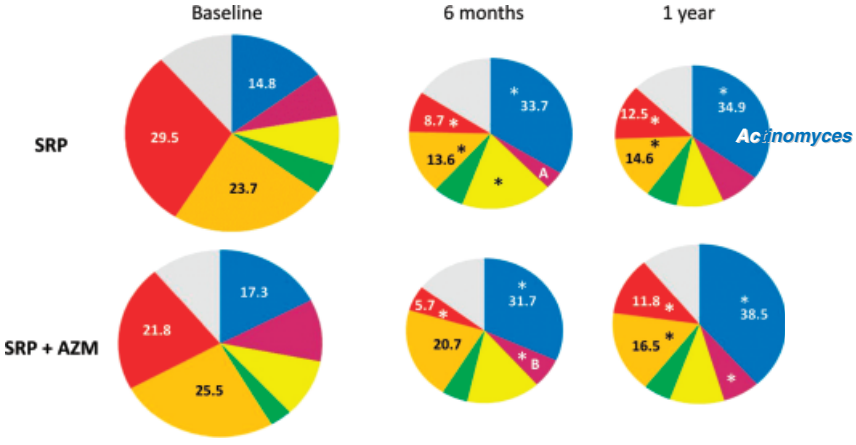

Fig. 4. Pie charts of the mean proportion of each microbial complex at baseline, 6 months and 1-year post-therapy in each treatment group. The areas of the pies were adjusted to reflect the total microbial counts. The colours represent different microbial complexes (Socransky et al. 1998) and *Actinomyces* species (in blue). The significance of differences within each group over the course of the study was assessed using the Friedman and Dunn's multiple comparison tests (*$p<0.05$ between baseline and 6 months or baseline and 1 year). The significance of differences between treatment groups at each time point was assessed using the Mann–Whitney *U*-test (different letters indicate significant differences between time points, $p<0.05$). SRP, scaling and root planing; AZM, azithromycin.

References

Araujo, M. W., Hovey, K. M., Benedek, J. R., Grossi, S. G., Dorn, J., Wactawski-Wende, J., Genco, R. J. & Trevisan, M. (2003) Reproducibility of probing depth measurement using a constant-force electronic probe: analysis of inter- and intraexaminer variability. *Journal of Periodontology* **74**, 1736–1740.

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

Carvalho, L. H., D'Avila, G. B., Leao, A., Haffajee, A. D., Socransky, S. S. & Feres, M. (2004) Scaling and root planing, systemic metronidazole and professional plaque removal in the treatment of chronic periodontitis in a Brazilian population. I. Clinical results. *Journal of Clinical Periodontology* **31**, 1070–1076.

Cionca, N., Giannopoulou, C., Ugolotti, G. & Mombelli, A. (2009) Amoxicillin and metronidazole as an adjunct to full-mouth scaling and root planing of chronic periodontitis. *Journal of Periodontology* **80**, 364–371.

Cionca, N., Giannopoulou, C., Ugolotti, G. & Mombelli, A. (2010) Microbiologic testing and outcomes of full-mouth scaling and root planing with or without amoxicillin/metronidazole in chronic periodontitis. *Journal of Periodontology* **81**, 15–23.

Cobb, C. M. (2002) Clinical significance of non-surgical periodontal therapy: an evidence-based

perspective of scaling and root planing. *Journal of Clinical Periodontology* **29** (Suppl. 2), 6–16.

Feres, M., Gursky, L. C., Faveri, M., Tsuzuki, C.O & Figueiredo, . L. C. (2009) Clinical and microbiological benefits of strict supragingival plaque control as part of the active phase of periodontal therapy. *Journal of Clinical Periodontology* **36**, 857–867.

Gladue, R. P., Bright, G. M., Isaacson, R. E. & Newborg, M. F. (1989) In vitro and in vivo uptake of azithromycin (CP-62,993) by phagocytic cells: possible mechanism of delivery and release at sites of infection. *Antimicrobial Agents Chemotherapy* **33**, 277–282.

Gladue, R. P. & Snider, M. E. (1990) Intracellular accumulation of azithromycin by cultured human fibroblasts. *Antimicrobial Agents Chemotherapy* **34**, 1056–1060.

Gomi, K., Yashima, A., Nagano, T., Kanazashi, M., Maeda, N. & Arai, T. (2007) Effects of full-mouth scaling and root planing in conjunction with systemically administered azithromycin. *Journal of Periodontology* **78**, 422–429.

Greenberg, R. N. (1984) Overview of patient compliance with medication dosing: a literature review. *Clinical Therapeutics* **6**, 592–599.

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 Periodontololgy* **32**, 1096–1107.

Haas, A. N., de Castro, G. D., Moreno, T., Susin, C., Albandar, J. M., Oppermann, R. V. & Rösing, C. K. (2008) Azithromycin as an adjunctive treatment of aggressive periodontitis: 12-months randomized clinical Trial. *Journal of Clinical Periodontololgy* **35**, 696–704.

Haffajee, A. D., Patel, M. & Socransky, S. S. (2008) Microbiological changes associated with four different periodontal therapies for the treatment of chronic periodontitis. *Oral Microbiology and Immunology* **23**, 148–157.

Haffajee, A. D., Torresyap, G. & Socransky, S. S. (2007) Clinical changes following four different periodontal therapies for the treatment of chronic periodontitis: 1 year results. *Journal of Clinical Periodontololgy* **34**, 243–253.

Henry, D. C., Riffer, E., Sokol, W. N., Chaudry, N. I. & Swanson, R. N. (2003) Randomized double-blind study comparing 3- and 6-day regimens of azithromycin with a 10-day amoxicillin-clavulanate regimen for treatment of acute bacterial sinusitis. *Antimicrobial Agents Chemotherapy* **47**, 2770–2774.

Hoepelman, I. M. & Schneider, M. M. E. (1995) Azithromycin: the first of the tissue-selective azalides. *International Journal of Antimicrobial Agents* **5**, 145–167.

Hung, H. C. & Douglass, C. W. (2002) Meta-analysis of the effect of scaling and root planing, surgical treatment and antibiotic therapies on periodontal probing depth and attachment loss. *Journal of Clinical Periodontology* **29**, 975–986.

Mascarenhas, P., Gapski, R., Al-Shammari, K., Hill, R., Sohren, S. & Fenno, J. C. (2005) Clinical response of azithromycin as an adjunct to non-surgical periodontal therapy in smokers. *Journal of Periodontology* **76**, 426–436.

Matarazzo, F., Figueiredo, L. C., Cruz, S. E., Faveri, M. & Feres, M. (2008) Clinical and microbiological benefits of systemic metronidazole and amoxicillin in the treatment of smokers with chronic periodontitis: a randomized placebo-controlled study. *Journal of Clinical Periodontology* **35**, 885–896.

Mestnik, M. J., Feres, M., Figueiredo, L. C., Duarte, P. M., Lira, E. A. G. & Faveri, M. (2010) Short-term benefits of the adjunctive use of metronidazole plus amoxicillin in the microbial profile and in clinical parameters of subjects with generalized aggressive periodontitis. *Journal of Clinical Periodontology* **37**, 353–365.

Oteo, A., Herrera, D., Figuero, E., O'Connor, A., González, I. & Sanz, M. (2010) Azithromycin as an adjunct to scaling and root planing in the treatment of *Porphyromonas gingivalis*-associated periodontitis: a pilot study. *Journal of Clinical Periodontology* **37**, 1005–1015.

Smith, S. R., Foyle, D. M., Daniels, J., Joyston-Bechal, S., Smales, F. C., Sefton, A. & Williams, J. A. (2002) Double-blind placebo controlled trial of azithromycin as an adjunct to non-surgical treatment of periodontitis in adult: clinical results. *Journal of Clinical Periodontology* **29**, 54–61.

Socransky, S. S., Haffajee, A. D., Smith, C. & Dibart, S. (1991) Relation of counts of microbial species to clinical status at the sampled site. *Journal of Clinical Periodontology* **18**, 766–775.

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

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

Teles, R. P., Haffajee, A. D. & Socransky, S. S. (2006) Microbiological goals of periodontal therapy. *Periodontology 2000* **42**, 180–218.

Xajigeorgiou, C., Sakellari, D., Slini, T., Baka, A. & Konstantinidis, A. (2006) Clinical and microbiological effects of different antimicrobials on generalized aggressive periodontitis. *Journal of Clinical Periodontology* **33**, 254–264.

Yashima, A., Gomi, K., Maeda, N. & Arai, T. (2009) One-stage full-mouth versus partial-mouth scaling and root planing during the effective half-life of systemically administered azithromycin. *Journal of Periodontology* **80**, 1406–1413.

Address:
Magda Feres
Centro de Pós-Graduação e Pesquisa-CEPPE
Universidade Guarulhos
Praça Tereza Cristina
229 Centro
07023-070 Guarulhos
SP
Brazil
E-mail: mferes@ung.br

Clinical Relevance

Scientific rationale for the study: AZM is indicated as a promising antibiotic due to its good pharmacological properties. However, data from the few studies that evaluated the clinical effects of AZM in treating periodontitis are controversial and its potential to alter the microbial profile of subjects with generalized ChP is still not defined.

Principal findings: SRP only or with AZM did not differ in their abilities to improve clinical or microbiological parameters and at 1-year post-treatments, subjects in both groups still presented several sites in need of further treatment.

Practical implications: AZM may not provide adjunctive benefits for the treatment of generalized ChP.