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Azithromycin as an adjunct to scaling and root planing in the treatment of *Porphyromonas gingivalis*-associated periodontitis: a pilot study

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Abstract

Objective: To evaluate the clinical and microbiological effects of systemic azithromycin as an adjunct to scaling and root planing (SRP) in the treatment of *Porphyromonas gingivalis*-associated chronic periodontitis.

Methods: Twenty-nine patients harbouring *P. gingivalis* were randomized into test and placebo groups. Test patients received SRP plus 500 mg of azithromycin per day (3 days), and control patients received SRP plus placebo. Clinical [plaque and bleeding indexes, probing pocket depth (PPD), clinical attachment level (CAL)] and microbiological data (four-sites pooled samples, processed by culture) were collected at baseline, and 1, 3 and 6 months, post-therapy. Clinical variables were compared by ANOVA, and microbiological variables by chi-square, signed-rank and Wilcoxon tests.

Results: Fifteen test and 11 placebo patients completed the study. Mean PPD decreased 0.34 mm [95% confidence interval (CI) 0.19–0.49] in the placebo and 0.80 mm (CI 0.57–1.04) in the test group after 6 months. For mean CAL gain, the correspondent figures were 0.29 (CI 0.08–0.49) and 0.76 (CI 0.46–1.05), respectively. The frequency of detection of *P. gingivalis* decreased significantly ($p \leqslant 0.01$) in the test group after 1, 3 and 6 months.

Conclusions: Within the limitations of this study, the adjunctive use of systemic azithromycin in the treatment of *P. gingivalis* periodontitis demonstrated significant clinical and microbiological benefits when compared with SRP plus placebo.

Key words: azithromycin; periodontitis; *P. gingivalis*; microbiology; scaling and root planing

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Chronic periodontitis is defined as an inflammatory disease of the tooth-supporting tissues caused by specific micro-organisms residing in the subgingival biofilm. This chronic inflammation causes progressive destruction of the periodontal ligament and alveolar bone resulting in pocket formation, gingival recession or both (Armitage 1999). The aetiology of periodontitis is multi-factorial, but it is an infection and bacterial species are the primary aetiologic agents. Among the bacterial species present in the subgingival biofilm, only a limited number has been clearly associated with the disease (Socransky & Haffajee 1997). In this group, *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* are known as the most relevant species, due to their strong association with periodontal pathology and their pathogenic potential.

Periodontal diseases can be treated successfully by mechanical therapy. However, in certain diseases and patients, the clinical outcome may be impaired by the presence and persistence of defined periodontal pathogens. The elimination of these pathogens during

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the treatment will enhance the clinical response and minimize the risk of future attachment loss (Winkel et al. 1997). However, research has shown that the elimination of these bacterial species is not predictable after mechanical periodontal therapy (Renvert et al. 1990a, b, Winkel et al. 1997). The adjunctive use of systemic anti-microbials has been shown to add relevant benefits (van Winkelhoff et al. 1996, Herrera et al. 2002, Haffajee et al. 2003) in certain patients and conditions, such as aggressive or active forms of periodontitis, severe diseases or diseases associated with specific microbiological profiles (Lindhe & Palmer 2002). Among these specific microbiological profiles, it has been reported that *A. actinomycetemcomitans* can be predictably eradicated with the adjunctive administration of amoxicillin plus metronidazole (Pavicic & van Winkelhoff 1994). However, *A. actinomycetemcomitans*-associated periodontitis is not frequent, and this bacterial species is only frequently found in aggressive periodontitis. Conversely, *P. gingivalis* is more prevalent, especially in chronic periodontitis, and it has also been found more frequently in certain geographical locations, such as in Spain, when compared with other European (Sanz et al. 2000) or American (Herrera et al. 2008b) countries. For *P. gingivalis*-positive patients, the eradication of the pathogen may be a microbiological goal of the periodontal therapy, and the administration of systemic anti-microbial therapy could help to achieve this goal. Among the different anti-microbials available, metronidazole may be a reasonable choice, due to its anti-microbial spectrum. However, metronidazole may have a demanding dosage regime, which may require (when used in the treatment of periodontitis) between 200 and 500 mg, two to four times per day, during 5–14 days (Herrera et al. 2002), and the lack of compliance of some patients may be associated with poorer outcomes (Loesche et al. 1993). In addition, adverse effects, including the interference with alcoholic drinks, may make difficult for some patients the use of this drug (Sharma et al. 2009).

To overcome some of the described limitations of metronidazole, azithromycin may be a good alternative. It is a macrolide antibiotic with a very simple dosage regime (administration once a day, 500 mg, during 3 consecutive days) and limited side effects. This short dosage regime improves patient compliance. Azithromycin has a wide anti-microbial spectrum with in vitro activity against aerobic and anaerobic gram-negative microorganism (Retsema et al. 1987, Willians et al. 1992, Muller et al. 2002) and a long half-life in human serum and periodontal tissues (Foulds et al. 1990, Malizia et al. 1997, Blandizzi et al. 1999, Gomi et al. 2007a). Azithromycin is found in high concentrations in fibroblasts and phagocytes (McDonald & Pruul 1991) and is carried to areas of inflammation as a result of chemotactic effects exerted on the phagocytes (Schentag & Ballow 1991), thus targeting the drug at those sites. Different in vitro (Pajukanta 1993) and in vivo (Herrera et al. 2000) studies have demonstrated the efficacy of azithromycin against *P. gingivalis*.

In the last years, different studies have reported the effects of administering azithromycin in the treatment of periodontitis (Smith et al. 2002, Mascarenhas et al. 2005, Dastoor et al. 2007, Gomi et al. 2007b, Haffajee et al. 2007, Haas et al. 2008, Pradeep et al. 2008, Yashima et al. 2009) reporting different clinical and microbiological outcomes. To our knowledge, no study has assessed the effects of the adjunctive administration of azithromycin plus scaling and root planing (SRP) in *P. gingivalis*-positive patients. Our hypothesis is that patients with *P. gingivalis* will benefit, in terms of reduction in probing pocket depth (PPD) and gain in clinical attachment levels (CAL), together with the elimination of *P. gingivalis* from the adjunctive use of azithromycin, because this drug is effective against this target pathogen, and the effects of mechanical therapy on *P. gingivalis* are not predictable.

Hence, the aim of this pilot investigation was to compare the clinical and microbiological effect of the adjunctive use of azithromycin or placebo with non-surgical periodontal therapy in the treatment of chronic periodontitis patients harbouring *P. gingivalis*.

Material and Methods

This was a pilot, double-blind, placebo-controlled, randomized clinical trial. The protocol was approved by the local ethical committee at the Hospital Clínico San Carlos, Madrid, Spain.

Patients

A microbiological screening to detect *P. gingivalis*-positive patients was performed in moderate chronic periodontitis patients, seeking for periodontal treatment at the Postgraduate Clinic of Periodontology, Faculty of Odontology, University Complutense, Madrid, Spain. The screening period lasted from January 2003 to July 2004.

Patients had to fulfill the following inclusion criteria: (i) older than 30 years, (ii) untreated moderate chronic periodontitis (Armitage 1999) with radiographic evidence of generalized alveolar bone loss $>30\%$, (iii) presence of at least one pocket with PPD >5 mm per quadrant with bleeding on probing (BoP), (iv) presence of at least three teeth per quadrant and (v) detection of *P. gingivalis* in subgingival samples taken at the screening visit and processed by culture.

Patients were excluded if (i) they had received periodontal treatment in the last 3 years, (ii) severe periodontitis with more than one tooth with a site with PPD >7 mm per quadrant except if it was scheduled for extraction, (iii) antibiotic intake in the month previous to the screening visit, (iv) pregnant or lactating females, (v) chronic diseases as diabetes, (vi) necrotizing periodontal diseases, (vii) HIV infection, (viii) use of non-steroidal anti-inflammatory drugs or (ix) intolerance or allergy to any of Zitromax® (Pfizer, Alcobendas, Madrid, Spain) components.

Microbiological samples

From each quadrant, the most accessible site with the deepest PPD and BoP was selected. Clinical variables (presence of plaque, bleeding on sampling, PPD and gingival recession) were specifically recorded at these sites, in addition to full-mouth clinical recording. Samples were taken with two consecutive sterile medium paper-points (Maillefer, Ballaigues, Switzerland) per site. Subgingival plaque was sampled after the removal of all supragingival plaque and debris (Wikstrom et al. 1991). Before sampling, the sites were isolated from the saliva by applying cotton rolls and then gently dried with compressed air, in order to avoid contamination. The paper-points were kept in place for 10 s and were then transferred into a screw-capped vial, containing 1.5 ml of RTF (Syed & Loesche 1972). Samples were transferred to the microbial laboratory within 2 h, where they were homogenized by vortexing for 30 s (Dahlen et al. 1990), and serially diluted in PBS.

At the laboratory, aliquots of 0.1 ml were plated manually for the detection of *A. actinomycetemcomitans* on the specific medium Dentaid-1 (Dentaid, Cerdanyola del Vallés, Spain) (Alsina et al. 2001). These plates were incubated for 3 days in air with 5% CO_2 at 37°C. Suspected isolates were identified on the basis of colony morphology (small colony, 1 mm in diameter, with a dark border and a ''star'' or ''crossed cigars'' shaped inner structure) and positive catalase reaction. Sample dilutions were also plated onto a non-selective blood agar plate (Blood Agar Base II®, Oxoid, Basingstoke, UK), supplemented with haemine (5 mg/l), menadione (1 mg/l) and 5% sterile horse blood. After 7–14 days of anaerobic incubation (80% N_2, 10% CO_2 and 10% H_2), total counts and counts of representative colonies (those with colony morphologies compatible with target pathogen morphology) were performed in the most suitable plates, those harbouring between 30 and 300 colonies. Suspected colonies were further identified by microscopy, studying gram staining and enzyme activity (including *N*-acetyl-β-D-glucosaminidase, α-glucosidase, α-galactosidase, α-fucosidase, esculin, indole and trypsin-like activity). Counts were transformed in colony-forming units per millilitre of the original sample. Total anaerobic counts were calculated, as well as counts of the detected periodontal pathogens (*A. actinomycetemcomitans*, *Tannerella forsythia*, *P. gingivalis*, *Prevotella intermedia/nigrescens*, *Parvimonas micra*, *Campylobacter rectus* and *Fusobacterium nucleatum*). In addition to the quantitative microbiological data, the frequency of detection and proportions for each bacterial species were also calculated.

To assess microbiological adverse effects, the overgrowth of other species, mainly super-infecting or opportunistic bacteria, such as enterics, was monitored, especially in Dentaid-1 plates.

Study visits

Screening visit

A full-mouth periodontal evaluation was performed in order to assess the inclusion/exclusion criteria and to select the sampling sites. For consecutive patients fulfilling the inclusion criteria, the purpose of the study was explained and they were asked to participate by signing an Internal Review Board-approved written consent. They were informed that participation would only occur if they were positive for *P. gingivalis*. If negative, they were conventionally treated. If the microbiological sample was positive, patients were scheduled for the baseline visit. The microbiological sample of the screening visit was then considered as the baseline sample.

Baseline visit

All clinical outcome variables were recorded using the Florida Probe System® (Florida Company, Gainsville, FL, USA) by one single trained (2 years of previous experience with Florida probe) and calibrated operator (A. O.). Intra-examiner calibration was performed twice, before and during the study, by assessing PPD and CAL in duplicate, with a degree of agreement within ± 1 mm higher than 85% at both tests.

A baseline visit was appointed and the following outcome variables were assessed:

- PPD and recession (REC) in millimetres at six sites per tooth in all teeth, excluding third molars. CAL calculated as the sum of PPD and REC.
- BoP, as present/absent 30 s after probing, and plaque index (PlI), as present/absent, visually or detected with the probe, at six sites per tooth in all teeth except third molars.
- Relative attachment level (RAL) was evaluated at four selected sites (those selected for microbiological sampling) with the stent probe (Florida Probe®) and the use of a customized acrylic stent.

At this visit, standardized oral hygiene instructions were explained, including the use of a manual toothbrush and inter-dental brushes (Vitis Access soft® and Interprox®, Dentaid).

Treatment visits

Within 15 days from baseline, the first appointment for SRP was scheduled, and the treatment was performed under local anaesthesia by a post-graduate student (A. O.) using an ultrasonic device and hand curettes. Treatment was carried out in two 1.5-h appointments, within 7 days. Oral hygiene instructions were reinforced at each visit and the adjunctive use of a 0.12% clorhexidine and 0.05% cetyl-pyridinium chloride rinse (Perio Aid Tratamiento®, Dentaid) was prescribed twice a day for 15 days, with 15 ml for 30 s, immediately after rinsing with water after brushing.

After the last SRP session, the adjunctive medication was prescribed. By means of a computer-generated randomization list, stratified for smokers (> 10 cigarettes per day) and non-smokers (non-smokers, former smokers or smokers of < 10 per day), sealed envelopes provided identification numbers for the patients, which were associated with the numbers of the blisters containing either the drug or the placebo. Subjects in the test group received a blister containing three 500 mg azithromycin tablets, and the control group received identical blisters with three placebo tablets. Test and control tablets were identical in colour, form and size. Codes were not open until the study was finished. The subjects were instructed to take one tablet in the presence of the operator, and to take the other two tablets the following 2 days at same moment of the day and in the hours away from the meals.

Follow-up visits

One month after treatment, the first follow-up visit was scheduled. Microbiological samples were taken as described previously (from the same sites selected at baseline), clinical parameters were recorded both full mouth and at the selected sites and subjects were asked if they have had any kind of problem with the medication and if they had taken the remaining two pills. In addition, oral hygiene instructions were reinforced. New follow-up visits were scheduled 3 and 6 months after treatment, for microbiological sampling and clinical evaluation.

Statistical analyses

Primary outcome variable was PPD changes. Secondary outcome variables include changes in CAL, BoP, RAL and microbiological variables, especially the presence/absence of *P. gingivalis*. PlI was evaluated as a control variable.

Clinical variables (PlI, BoP, PPD, CAL and RAL) were calculated by patient and visit and then by group. In addition, PPD was divided in two categories (1–3 and 4–6 mm) and the

proportions of each category were calculated at each visit. After evaluating the normality of the distribution (assessing skewness and kurtosis, and the Kolmogorov–Smirnov test), and whether significant differences existed between variances (*F*-test), ANOVA and the multiple rank test were used to compare the baseline visit with the 1-, 3- and 6-month visits (intra-group comparisons), and ANCOVA was selected to compare both groups, either at baseline or in changes baseline follow-up visits (inter-group comparison), including baseline values of the examined variable, smoking and gender as cofactors.

Four microbiological variables were used: total anaerobic counts, frequency of detection of target pathogens, counts of each studied pathogen and proportions of flora of each pathogen. Total anaerobic counts were log transformed to fit a normal distribution and the statistical evaluation was carried out as described for the clinical variables. Frequencies of detection were compared using the chi-square test in the inter-group assessment, at baseline and at each follow-up visit, or by the McNemar test for intra-group assessment, in changes between baseline and follow-up visits. Proportions of flora and log-transformed pathogen counts were compared using the Wilcoxon signed-rank test (intra-group) or by the Wilcoxon rank-sum test for inter-group assessment.

The level of statistical significance was set at $p < 0.05$. However, because multiple comparisons were carried out for the inter-group assessment, the Bonferroni correction was used. Thus, the inter-group assessment of changes baseline with every follow-up visit include three comparisons, the level was set $p < 0.0167$. When the p value was in between the corrected level and 0.05, it was considered as a tendency towards significance.

Sample size calculation was performed for the primary outcome variable, changes in PPD, considering a standard deviation of 0.8 mm and a desirable difference between groups of 1.02 mm, with a 90% of power, resulting in sample of 14 patients per arm (Herrera et al. 2002, Haffajee et al. 2003, Guerrero et al. 2005). However, this study was considered as a pilot study, because (to our knowledge) no previous studies have been performed on *P. gingivalis*-positive patients.

Demographic variables at baseline were compared by means of *t*-test (age) or Fisher's exact test (smoking and gender).

Results

After the microbiological screening of 40 patients, 29 patients were included in the study and scheduled for the baseline visit (see Fig. 1 for the flowchart of the study and Table 1 for the main characteristics of the patient sample at baseline). At the end of the baseline evaluation, 15 patients were randomized to the test group (seven males and eight females; eight smokers and seven non-smokers; mean age 46.6, range 38–62). All of them were treated according to the protocol and complied with the follow-up visits, with the exception of two patients (one male and one female, both smokers) that could not attend the 3-month visit. Fourteen patients were assigned to the placebo group, but one left the study after a partial baseline evaluation and the data were excluded. Out of the 13 patients who were treated (eight males and five females; six smokers and seven non-smokers; mean age 46.9, range 37–65), two patients provided only data at the baseline visit (one non-smoker male had to take a systemic antibiotic due to another disease and one non-smoker female left the study after the treatment phase was finished, due to personal reasons). Data from 11 patients were available at 1-month and 6-month visits, because two patients (smokers male) of this group missed the 3-month visit. An intention-to-treat analysis was carried out.

Clinical variables

Mean values at each study visit are shown in Table 2, and changes between baseline and each follow-up visit are shown in Table 3.

PPD

Both groups were comparable at baseline, with mean PPD ranging between 2.84 and 2.99 mm. The proportion of the sites with PPD of 4–6 mm ranged between 24 and 29%. Mean PPD significantly decreased in the test group ($p < 0.001$) after 1, 3 or 6 months. The reduction amounted 0.78 mm [95% confidence interval (CI) 0.59–0.97] after 6 months in the test group and 0.38 mm (CI 0.16–0.60) in the placebo group, being the difference between groups statistically significant ($p = 0.009$).

The proportion of pockets in the 1–3 mm category increased in both groups, reaching statistical significance in the test group ($p < 0.001$) for all follow-up visits. The increase was 10% after 6 months in the placebo group and 24% in the test group, with statistically significant inter-group differences in the changes ($p = 0.005$). The reduction in the percentage of pockets in 4–6 mm category was 20% in the test group after 6 months, while the placebo group showed 11%. Inter-group differences were significant ($p = 0.003$).

CAL and RAL

Both groups were comparable at baseline, with mean CAL ranging 3.46–3.56 mm. A significant gain in mean CAL was observed ($p = 0.004$) when comparing baseline with the other study visits in the test group. Changes in the placebo group were not significant. Significant inter-group differences were detected for changes at baseline–1 month ($p = 0.015$) and tendencies were observed for changes after 3 ($p = 0.026$) and 6 months ($p = 0.016$).

Placebo patients demonstrated an increase in RAL at every follow-up visit, totaling a loss of 0.23 mm after 6 months. Conversely, a relative gain was observed in the test group. No significant inter- or intra-group differences were detected.

PlI

Mean PlI showed a tendency towards significant differences at baseline ($p = 0.022$) due to the higher levels in the test group (88.5 *versus* 63.4%). Statistically significant reductions were observed in both groups after treatment ($p < 0.001$ for each follow-up visit), although the 6-month levels were still higher in the test group (32.9%) than in the placebo group (15.4%), and differences were not significant. No significant inter-group differences were detected.

BoP

Mean BoP showed significant differences at baseline ($p = 0.004$) due to the higher levels in the test group (54.5 *versus* 38.6%). Significant reductions were observed in both groups after treatment ($p < 0.001$), ranging from 35

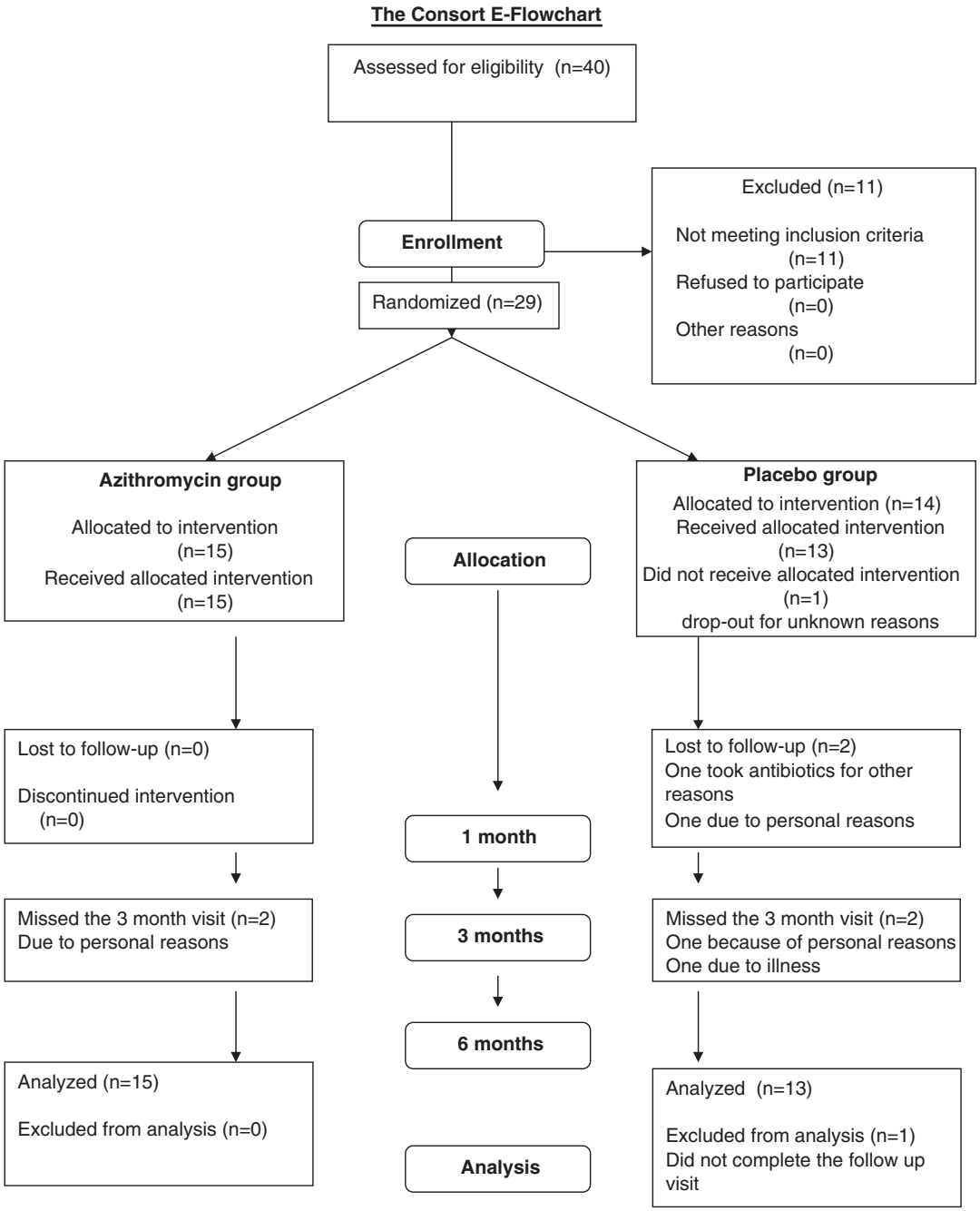

Fig. 1. The consort E-flowchart.

to 39% in the test group and 19–24% in the placebo group. No significant inter-group differences in the changes detected.

Adverse events

One patient in the test group reported diarrhoea probably associated with the study medication. No adverse events were reported in the control group.

Table 1. Demographic features of the selected sample

	Placebo	Test	*p* Value
n	13	15	
Females (*n*)	5	8	0.4757*
Smokers (n)	6	8	1.000*
Age mean (range) (years)	47.1 (36–65)	46.6 (38–62)	0.929†

*Fisher's exact test.
†Student's *t*-test.

Table 2. Mean values, standard error (SE) and 95% confidence intervals (CI) of clinical variables at study visits and intra-group comparison (ANOVA and multiple rank test)

Variables	Visit	Test					Placebo				
		mean	SE	95% CI		ANOVA	mean	SE	95% CI		ANOVA
PPD (mm)	Baseline	2.99	0.11	2.83	3.14	$p<0.001$, baseline *versus* other visits	2.84	0.14	2.65	3.04	$p = 0.2597$, no differences
	1 month	2.18	0.11	2.03	2.34		2.62	0.15	2.41	2.84	
	3 months	2.07	0.12	1.90	2.24		2.42	0.17	2.18	2.66	
	6 months	2.18	0.11	2.03	2.34		2.57	0.15	2.35	2.78	
CAL (mm)	Baseline	3.46	0.18	3.21	3.71	$p = 0.0039$, baseline *versus* other visits	3.56	0.31	3.12	4.00	$p = 0.5349$, no differences
	1 month	2.73	0.18	2.48	2.98		3.53	0.33	3.05	4.01	
	3 months	2.58	0.19	2.31	2.85		2.91	0.37	2.38	3.44	
	6 months	2.70	0.18	2.45	2.95		3.32	0.33	2.84	3.79	
PPD 1–3 mm (‰)	Baseline	0.66	0.03	0.63	0.70	$p<0.001$, baseline *versus* other visits	0.72	0.04	0.66	0.78	$p = 0.182$, baseline *versus* 3-month visit
	1 month	0.90	0.03	0.87	0.94		0.78	0.04	0.72	0.85	
	3 months	0.92	0.03	0.88	0.95		0.86	0.05	0.79	0.93	
	6 months	0.90	0.03	0.86	0.94		0.80	0.04	0.73	0.86	
PPD 4–6 mm (‰)	Baseline	0.29	0.02	0.25	0.32	$p<0.001$, baseline *versus* other visits	0.24	0.04	0.18	0.30	$p = 0.268$, no differences
	1 month	0.09	0.02	0.06	0.13		0.19	0.04	0.13	0.26	
	3 months	0.07	0.03	0.03	0.10		0.12	0.05	0.05	0.19	
	6 months	0.08	0.02	0.04	0.11		0.17	0.04	0.11	0.23	
PII (%)	Baseline	88.50	6.02	79.89	97.10	$p<0.001$, baseline *versus* other visits	63.41	6.70	53.78	73.04	$p<0.001$, baseline *versus* other visits
	1 month	19.09	6.02	10.48	27.69		22.24	6.36	13.10	31.38	
	3 months	27.04	6.96	17.10	36.97		15.48	6.70	5.84	25.11	
	6 months	32.90	6.02	24.29	41.50		15.45	6.36	6.31	24.59	
BoP (%)	Baseline	54.52	3.45	49.59	59.44	$p<0.001$, baseline *versus* other visits	38.62	2.65	34.81	42.42	$p<0.001$, baseline *versus* other visits
	1 month	17.92	3.45	12.99	22.84		16.54	2.51	12.93	20.15	
	3 months	17.00	3.98	11.31	22.68		16.74	2.65	12.93	20.55	
	6 months	19.25	3.45	14.32	24.17		20.31	2.51	16.70	23.92	
RAL (mm)	Baseline	8.57	0.39	8.02	9.12	$p = 0.627$, no differences	8.85	0.56	8.05	9.64	$p = 0.9179$, no differences
	1 month	8.07	0.37	7.54	8.60		8.40	0.64	7.49	9.31	
	3 months	7.92	0.40	7.35	8.49		8.78	0.67	7.82	9.74	
	6 months	8.40	0.37	7.87	8.93		9.00	0.61	8.13	9.87	

CAL, clinical attachment level; PPD, probing pocket depth in mm; PPD 1–3 mm (‰), mean proportion of pockets in the 1–3 mm category; PPD 4–6 mm (‰), mean proportion of pockets in the 4–6 mm category; PII, plaque index; BoP, bleeding on probing; RAL, relative attachment level.

Microbiological variables

Out of the 15 test patients, 15 samples were available at baseline and after 1 month, 13 after 3 months and 14 after 6 months. Out of the 13 placebo patients, 13 samples were available at baseline, 12 after 1 month and 10 samples at the 3- and 6-month visits.

Total anaerobic counts (Table 4a and b)

Comparable counts in log of colony-forming units were recovered at baseline (7.07–7.18). Both groups demonstrated reductions that amounted 0.24 in the placebo group and 0.54 in the test group (significantly different as compared with baseline). No inter-group significant differences were detected at any visit or in changes between visits.

Frequency of detection of target pathogens (Table 5)

No significant differences were detected at baseline in frequencies of detection of target pathogens. However, test patients harboured more frequently *T. forsythia* and *C. rectus*, while placebo patients had more *P. micra, P. intermedia* and *F. nucleatum* demonstrating prevalences close to 100% (as *P. gingivalis* did, as inclusion criteria). *A. actinomycetemcomitans* was detected in 23.1% in the placebo and 13.3% in the test group.

The prevalence of *P. gingivalis* decreased significantly after 1 ($p<0.001$), 3 ($p = 0.003$) and 6 months ($p = 0.001$), with a 6-month frequency of 42.9%. Non-significant reductions were observed in the placebo, with a tendency after 1 month ($p = 0.023$), and a 6-month prevalence of 80%.

P. intermedia prevalence was significantly reduced after 1 month ($p = 0.001$) in the test group, from 100.0 to 46.7%. Reductions were also observed in the placebo samples after 1 month, from 92.3 to 75%. After 3 months, prevalences rose again and were always over 80%.

T. forsythia was not detected after the treatment in the test group, at 1 ($p = 0.004$), 3 ($p = 0.010$) and 6 months ($p = 0.006$). Minor changes were observed in the placebo group.

P. micra prevalence was clearly reduced after 1 month in the test group, while an increase was observed in the placebo group. A rebound was observed later, making final values in the test group even higher than at baseline.

In the test group, *A. actinomycetemcomitans* was not detected after 1 and 6

Table 3. Mean values, standard error (SE) and 95% confidence intervals (CI) of changes of clinical variables between visits and inter-group comparison (ANCOVA, with treatment as factor and baseline value, smoking and gender as cofactors; significant cofactors are listed)

Variables	Change	Group	Mean	SE	95% CI		*p* Value	Cofactors
PPD (mm)	Baseline *versus* 1 month	Placebo	0.30	0.11	0.06	0.54	0.004	Baseline PPD
		Test	0.80	0.10	0.59	1.00		
	Baseline *versus* 3 months	Placebo	0.45	0.09	0.26	0.64	0.005	Baseline PPD, smoking
		Test	0.83	0.07	0.67	0.99		
	Baseline *versus* 6 months	Placebo	0.38	0.11	0.16	0.60	0.009	Baseline PPD
		Test	0.78	0.09	0.59	0.97		
CAL (mm)	Baseline *versus* 1 month	Placebo	0.07	0.19	− 0.32	0.46	0.015	None
		Test	0.73	0.16	0.40	1.06		
	Baseline *versus* 3 months	Placebo	0.34	0.13	0.06	0.62	0.026	Baseline CAL, smoking
		Test	0.77	0.11	0.53	1.00		
	Baseline *versus* 6 months	Placebo	0.28	0.14	0.00	0.57	0.016	Baseline CAL, smoking
		Test	0.76	0.12	0.52	1.01		
PPD 1–3 mm (‰)	Baseline *versus* 1 month	Placebo	− 0.10	0.04	− 0.18	− 0.01	0.016	Baseline ‰
		Test	− 0.23	0.03	− 0.30	− 0.16		
	Baseline *versus* 3 months	Placebo	− 0.16	0.02	− 0.19	− 0.12	0.009	Baseline ‰, smoking
		Test	− 0.22	0.01	− 0.25	− 0.19		
	Baseline *versus* 6 months	Placebo	− 0.11	0.03	− 0.17	− 0.06	0.005	Baseline ‰
		Test	− 0.23	0.02	− 0.28	− 0.18		
PPD 4–6 mm (‰)	Baseline *versus* 1 month	Placebo	0.07	0.04	0.00	0.15	0.027	Baseline ‰
		Test	0.19	0.03	0.12	0.26		
	Baseline *versus* 3 months	Placebo	0.14	0.01	0.11	0.17	0.005	Baseline ‰, smoking
		Test	0.20	0.01	0.18	0.23		
	Baseline *versus* 6 months	Placebo	0.11	0.02	0.06	0.15	0.003	Baseline ‰
		Test	0.20	0.02	0.17	0.24		
RAL (mm)	Baseline *versus* 1 month	Placebo	0.02	0.30	− 0.61	0.65	0.329	None
		Test	0.42	0.26	− 0.12	0.95		
	Baseline *versus* 3 months	Placebo	− 0.09	0.26	− 0.63	0.45	0.067	None
		Test	0.57	0.22	0.10	1.04		
	Baseline *versus* 6 months	Placebo	− 0.23	0.27	− 0.79	0.32	0.346	None
		Test	0.11	0.24	− 0.38	0.60		
PlI (%)	Baseline *versus* 1 month	Placebo	46.70	8.12	29.49	63.92	0.158	None
		Test	63.90	6.83	49.41	78.39		
	Baseline *versus* 3 months	Placebo	60.27	7.97	42.90	77.64	0.432	Baseline PlI
		Test	50.36	7.41	34.22	66.49		
	Baseline *versus* 6 months	Placebo	61.65	8.17	44.32	78.98	0.183	Baseline PlI
		Test	45.39	6.88	30.80	59.97		
BoP (%)	Baseline *versus* 1 month	Placebo	30.15	3.49	22.76	37.54	0.953	Baseline GI, gender
		Test	30.45	2.93	24.25	36.66		
	Baseline *versus* 3 months	Placebo	31.83	2.85	25.61	38.04	0.978	Baseline GI
		Test	31.71	2.65	25.93	37.48		
	Baseline *versus* 6 months	Placebo	24.55	4.66	14.67	34.43	0.331	Baseline GI
		Test	31.27	3.91	22.98	39.57		

CAL, clinical attachment level; PPD, probing pocket depth in mm; ‰ PPD 1–3 mm, mean proportion of pockets in the 1–3 mm category; ‰ PPD 4–6 mm, mean proportion of pockets in the 4–6 mm category; PlI, plaque index; BoP, bleeding on probing; RAL, relative attachment level.

months, and only one patient was positive after 3 months. Conversely, no impact of treatment was observed in the placebo group.

No significant impact was observed with regard to other target pathogens.

Significant inter-group differences were detected at the 1-month visit for *P. gingivalis* ($p = 0.004$) and *P. micra* ($p < 0.001$), and the 6-month visit for *A. actinomycetemcomitans* ($p = 0.010$).

Proportions of flora of target pathogens (Table 5)

No significant differences were detected at baseline in proportions of flora of target pathogens. However, test patients harboured higher proportion (11.4 *versus* 4.3%; tendency towards significance, $p = 0.032$) of *P. intermedia*. *P. gingivalis* represented the highest proportion of the flora in both groups (21.5% in placebo and 17.7% in test samples). Higher proportions of *P. micra* were observed in the placebo group (4.4 *versus* 1.3%). Proportions of other pathogens were similar at baseline.

Proportions of *P. gingivalis* were significantly reduced in the test group after 1 ($p = 0.002$) and 3 months ($p = 0.006$), and showed a tendency after 6 months ($p = 0.021$), with a final figure of 5.1%. In the placebo group, some non-significant reductions were observed after 1 month, but baseline values were achieved at 3 and 6 months.

The percentage of the flora of *P. intermedia* was reduced in the test group, being significant after 6 months ($p = 0.010$) with a final percentage of 2.2%. In the placebo group, an increase was initially observed, and then a return to baseline values after 6 months.

For *P. micra*, a non-significant increase in the proportions was observed in the test group after 6 months, as compared with some reductions in the placebo patients.

For other pathogens, non-significant limited changes were observed in proportions of flora.

Table 4a. Mean values, standard error (SE) and 95% confidence intervals (CI) of log of colony-forming units at study visits and intra-group comparison (ANOVA and multiple rank test)

Visit	Test					Placebo				
	mean	SE	95% CI		ANOVA	mean	SE	95% CI		ANOVA
Baseline	7.18	0.15	6.88	7.47	$p<0.066$, baseline	7.07	0.16	6.75	7.40	$p=0.511$,
1 month	6.79	0.14	6.51	7.07	*versus* 6 months	6.72	0.17	6.38	7.06	no differences
3 months	6.81	0.16	6.50	7.13		6.96	0.18	6.59	7.34	
6 months	6.62	0.15	6.33	6.92		6.92	0.19	6.53	7.32	

Table 4b. Mean values, standard error (SE) and 95% confidence intervals (CI) of changes in log of colony-forming units between visits and inter-group comparison (ANCOVA, with treatment as factor and baseline value as cofactors; significant cofactors are listed)

Change	Group	Mean	SE	95% CI		*p* Value	Cofactors
Baseline *versus* 1 month	Placebo	0.40	0.18	0.02	0.79	0.746	Baseline
	Test	0.32	0.17	−0.03	0.67		
Baseline *versus* 3 month	Placebo	0.22	0.13	−0.06	0.50	0.286	Baseline
	Test	0.42	0.13	0.16	0.69		
Baseline *versus* 6 month	Placebo	0.24	0.20	−0.17	0.66	0.261	Baseline
	Test	0.54	0.16	0.20	0.87		

Table 5. Frequency of detection (in percentage) and mean proportions of flora (in percentage) of different periodontal pathogens in subgingival samples, at every study visit

	Aa	*Pg*	*Pi*	*Tf*	*Pm*	*Cr*	*Fn*	*Capno*	*Ec*	*Eu*
Frequency of detection										
Placebo										
Baseline	23.1	100.0	92.3	15.4	69.2	15.4	92.3	7.7	0.0	0.0
1 month	16.7	66.7	75.0	16.7	91.7	16.7	100.0	8.3	0.0	0.0
3 months	20.0	80.0	100.0	20.0	70.0	20.0	100.0	10.0	20.0	0.0
6 months	40.0	80.0	80.0	10.0	60.0	30.0	80.0	10.0	10.0	0.0
Test										
Baseline	13.3	100.0	100.0	40.0	46.7	40.0	100.0	0.0	0.0	0.0
1 month	0.0	13.3	46.7	0.0	20.0	20.0	93.3	26.7	13.3	6.7
3 months	7.7	53.8	92.3	0.0	69.2	7.7	100.0	30.8	15.4	0.0
6 months	0.0	42.9	85.7	0.0	71.4	14.3	100.0	35.7	21.4	0.0
Mean proportions of flora										
Placebo										
Baseline	0.0	21.5	4.3	0.7	4.4	0.2	6.5	0.2	0.0	0.0
1 month	0.0	11.6	10.6	0.3	6.6	0.5	7.4	0.0	0.0	0.0
3 months	0.0	19.6	10.3	0.7	2.4	0.6	3.9	0.0	0.1	0.0
6 months	0.6	22.7	4.0	0.9	2.3	0.5	4.9	0.0	0.0	0.0
Test										
Baseline	0.0	17.7	11.4	1.2	1.3	0.3	4.9	0.0	0.0	0.0
1 month	0.0	0.3	3.9	0.0	0.3	0.8	4.5	0.3	0.3	0.1
3 months	1.5	2.9	5.5	0.0	3.0	1.1	8.1	0.4	0.1	0.0
6 months	0.0	5.1	2.2	0.0	5.4	0.3	7.5	0.5	0.2	0.0

Aa, *Aggregatibacter actinomycetemcomitans*; *Pg*, *Porphyromonas gingivalis*; *Pi*, *Prevotella intermedia*; *Tf*, *Tannerella forsythia*; *Pm*, *Parvimonas micra*; Cr, *Campylobacter rectus*; *Fn*, *Fusobacterium nucleatum*; *Capno*, *Capnocytophaga* sp.; *Ec*, *Eikenella corrodens*; *Eu*, *Eubacterium* sp.

Inter-group comparisons revealed that the proportions of *P. gingivalis* at the 1-month visit showed a tendency towards statistical significance ($p = 0.019$).

Discussion

The present randomized-controlled trial compared the clinical and microbiological effects of the adjunctive use of azithromycin or placebo with SRP in moderate chronic periodontitis patients harbouring *P. gingivalis*. The clinical results revealed clear differences between the test and the control groups. After 6 months, the PPD reduction was 0.80 mm in the test group and 0.34 mm in the control group. CAL gains after 6 months were 0.76 and 0.29 mm, respectively. In addition, significant differences in microbiological outcomes were also detected, specifically those related with *P. gingivalis*. Conversely, no adverse events were observed, except mild diarrhoea in one test patient.

These results, however, should be interpreted with caution due to some relevant limitations. Only 15 patients in the test group and 11 patients in the placebo group provided data for 6-month visit, and this may be considered as a limited sample size. Moreover, the drop-outs in the control group caused differences in size between both groups. Also, the effect of smoking in the results could not be properly assessed. An effort to control this variable was made by stratification in the randomization process, selecting a threshold of 10 cigarettes per day, because previous studies have failed to find significant differences in periodontal disease severity between non-smokers and patients who consumed <10 cigarettes per day (Martinez-Canut et al. 1995). Moreover, smoking was included as a cofactor in the statistical analyses. Finally, it is important to consider the lack of homogeneity between groups in terms of baseline (statistically significant, $p = 0.022$) and 6-month (non-significant) plaque levels, which may hamper the comparison between groups. This factor has been assessed previously by Kornman et al. (1994), concluding that supragingival plaque control is an essential factor in attaining certain clinical and microbial outcomes following systemic antibiotic therapy in periodontitis. In the present study, the test group achieved significant better clinical and microbiological results, despite the fact that plaque levels were higher at baseline and after 6 months than in the control patients. It can be speculated that the differences could have been higher if the plaque control was homogeneous in both groups. Other baseline difference detected corresponded to higher BoP levels in the test group ($p = 0.004$).

To the best of our knowledge, this is the first study in which azithromycin has been evaluated as an adjunct to SRP in patients with a specific microbiological profile. Other authors have followed previously a similar strategy (selection of patients with a specific microbiological profile, in order to prescribe an adequate drug) with other anti-microbials: a pre-defined proportion of spirochaetes when assessing metronidazole (Loesche et al. 1984); presence of *A. actinomycetemcomitans* or *P. gingivalis*

when assessing amoxicillin plus metronidazole (Flemmig et al. 1998); or *A. actinomyctemcomitans* and metronidazole in localized juvenile periodontitis (Saxen & Asikainen 1993). All the above-mentioned studies have in common excellent outcomes in the test groups, suggesting better results of adjunctive systemic antibiotics if the target pathogen has been identified previously (Herrera et al. 2002).

The patients selected for the present study, besides the presence of *P. gingivalis*, had the diagnosis of moderate chronic periodontitis, thus excluding severe patients that would clearly be in need of periodontal surgery after SRP. In consequence, the selected patients had a mean initial PPD of 2.99 mm for the test and 2.84 mm for the control group, which can be considered as a relatively low PPD, and hence the expected PPD reductions will be smaller when compared with other studies selecting patients with a deeper initial mean PPD. Previous studies have demonstrated that the additional benefit of antibiotics is more evident in deep sites (Herrera et al. 2002, Guerrero et al. 2005, Haffajee et al. 2007). With the administration of azithromycin adjunctive to SRP, different studies have rendered different results depending on baseline PPDs. In a clinical trial with aggressive periodontitis patients assessing clinical outcomes at 1 year (Haas et al. 2008), with initial PPDs 6.7–6.3 mm, the PPD reduction was 2.88–1.85 mm (for test and placebo patients, respectively). With baseline PPD of around 5 mm, the observed change was 1.60–1.10 mm (Yashima et al. 2009), while with around 4 mm, changes were 1.62–0.75 mm (Gomi et al. 2007b), or 1.33–0.45 mm (Mascarenhas et al. 2005). Other studies, however, have failed to find significant clinical differences when comparing SRP plus azithromycin *versus* SRP plus placebo (Smith et al. 2002) or SRP plus metronidazol, sub-anti-microbial doses of doxycycline or SRP alone (Haffajee et al. 2007). In these two studies, debridement was completed within 2 (Smith et al. 2002) or 3 weeks (Haffajee et al. 2007), while in the rest of studies that reported positive results for adjunctive azithromycin, debridement was completed within 1 week (Mascarenhas et al. 2005, Gomi et al. 2007b, Pradeep et al. 2008, Yashima et al. 2009), with the exception of one study, in which debridement was carried out in multiple visits on a quadrant/sextant basis within 14 days (Haas et al. 2008).

The influence of the strategy of debridement is a matter of controversy. In a recent consensus review, it was concluded that the quality and the chronology of the debridement may affect the results of the adjunctive use of systemic anti-microbials (Herrera et al. 2008a). When specifically evaluating the results of clinical trials with the use of adjunctive azithromycin, a recent study comparing ''full-mouth'' (within 24 h) *versus* ''partial-mouth'' (within 7 days) SRP during the effective half life of systemically administered azithromycin concluded that both treatment protocols were equally effective clinically and microbiologically (Yashima et al. 2009). In the present study, periodontal treatment was completed in two visits within 7 days and systemic administration of azithromycin started on the last treatment day, as recommended in a recent consensus (Herrera et al. 2008a, Sanz & Teughels 2008). As azithromycin concentration in inflamed periodontal tissues decreases from 50% after 7 days to 20% after 14 days (Gomi et al. 2007a), not exceeding this time period when completing debridement seems to be important to enhance positive results, and it seems reasonable to try to complete treatment within 7 days rather than 14, to benefit from the much higher concentration of drug found in periodontal tissues. In addition, the recommended administration of azithromycin provides an easy dosage, while the reported adverse events are very low (in 0.7% of the patients) (Contopoulos-Ioannidis et al. 2001). In the present study, only one patient reported mild diarrhoea related to azithromycin intake.

The selection of azithromycin dosage may be controversial, because the approved dosages in the United States (5-day regime, first dose of 500 mg and then 250 mm daily) and in Europe (3-day regime of 500 mg daily) were different. Both regimens have demonstrated clinical benefits when compared with 10-day courses of other antibiotics in the treatment of respiratory infections, while the 3-day regime has proven better results as compared with the 5-day course, when bacterial cure rate was analysed (Casey & Pichichero 2005). As the present study was conducted in Europe, the 3-day, 500 mg/day, regime was selected, which is also the most commonly evaluated in the periodontal literature (Smith et al. 2002, Dastoor et al. 2007, Gomi et al. 2007b, Haffajee et al. 2007, Haas et al. 2008, Yashima et al. 2009), while less studies have evaluated the 5-day regime (Mascarenhas et al. 2005).

Uncontrolled use of anti-microbials is of great health concern due to the increasing bacterial resistance, thus resulting in different bacterial antibiotic susceptibility profiles in different European countries according to more or less prescription control (van Winkelhoff et al. 2005). In order to optimize the use of anti-microbials to only those subjects who would benefit most, all the subjects included in the present study harboured *P. gingivalis* irrespective of the other bacteria detected. After treatment, *P. gingivalis* detection was significantly reduced in the test group at 1-, 3- and 6 months; conversely, in the control group, the decrease in *P. gingivalis* was less pronounced after 1 month and rebounded at 3 and 6 months. Previous studies also found significant reductions of *P. gingivalis* after 1 and 3 months (Sefton et al. 1996, Mascarenhas et al. 2005, Gomi et al. 2007b, Yashima et al. 2009), with rebounds after 5 months (Sefton et al. 1996), 6 months (Gomi et al. 2007b) or 9 months (Yashima et al. 2009), while others did not find any significant impact (Haffajee et al. 2008). With regard to *T. forsythia*, in our study, the frequency of detection was 40% at baseline, and it was not detected at any follow-up visit, as opposite to the placebo group. In two other studies, significant reductions after 12 months (Haffajee et al. 2008) and 6 months (Mascarenhas et al. 2005) were observed in the test groups. In another study (Gomi et al. 2007b), *T. forsythia* was not detected after 1 and 3 months, but it was found at 6 months. Recently, the study by Yashima et al. (2009) detected *T. forsythia* at all time periods in all study groups.

However, microbiological variables should be interpreted with caution because they have not been clearly defined in the literature; thus, it is not easy to select a clear outcome variable, and a description of a combination of variables is usually reported (total flora, frequency of detection, proportions and counts of different pathogens). However, in the present study, microbiological inclusion criteria were used (presence of *P. gingivalis* in culture) and thus an outcome variable appears as evident: presence or absence of

P. gingivalis after treatment. However, there is a clear need to clearly define microbiological outcome variables and to design studies with an appropriate size to evaluate those variables.

In the present study, improved clinical and microbiological outcomes were attained in the test group using adjunctive azithromycin, including PPD reduction, CAL gain and reductions in the frequency of detection of *P. gingivalis*, *A. actinomycetemcomitans* and *T. forsythia*. These results together with the recommended easy dosage and limited side effects make the use of this antibiotic recommendable in the treatment of periodontitis patients. Hence, we can conclude that within the limitations of the present study, patients with untreated moderate chronic periodontitis harbouring *P. gingivalis* in their subgingival biofilm may benefit from the systemic administration of azithromycin as an adjunct to SRP.

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Clinical Relevance

Scientific rationale for study: Periodontitis is a chronic oral infection associated with a limited number of pathogens, being *P. gingivalis* one of the most relevant. The eradication of this bacterial species during periodontal treatment will enhance the clinical response and minimize the risk of future attachment loss. The adjunctive use of systemic antimicrobials (such as azithromycin) has been shown to add relevant benefits in the treatment of periodontal diseases associated with specific microbiological profiles.

Principal findings: SRP plus the adjunctive use of azithromycin resulted in additional clinical and microbiological benefits compared with SRP plus placebo. Patients in the test group showed a significant decrease in PPD and gain in CAL after 6 months. The frequency of detection of *P. gingivalis*, *Aggregatibacter actinomycetemcomitans* and *T. forsythia* was also significantly reduced in the test group.

Practical implications: In *P. gingivalis* periodontitis, SRP plus the systemic administration of azithromycin may result in additional clinical and microbiological benefits.