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Adjunctive benefits of systemic amoxicillin and metronidazole in non-surgical treatment of generalized aggressive periodontitis: a randomized placebo-controlled clinical trial

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Abstract

Background: The objective of this study was to assess the adjunctive clinical effect of the administration of systemic amoxicillin and metronidazole in the non-surgical treatment of generalized aggressive periodontitis (GAP).

Methods: Forty-one systemically healthy subjects with GAP were included in this 6-month double-blind, placebo-controlled, randomized clinical trial. Patients received a course of full-mouth non-surgical periodontal treatment delivered over a 24 h period using machine-driven and hand instruments. Test subjects received an adjunctive course of systemic antibiotic consisting of 500 mg amoxicillin and 500 mg metronidazole three times a day for 7 days. Clinical parameters were collected at baseline, and at 2 and 6 months post-treatment.

Results: In both the test and the placebo groups, all clinical parameters improved at 2 and 6 months. In deep pockets ($\geqslant$7 mm), the test treatment resulted in an additional 1.4 mm (95% confidence interval 0.8, 2.0 mm) in full-mouth probing pocket depth (PPD) reduction and 1 mm (0.7, 1.3 mm) of life cumulative attachment loss (LCAL) gain at 6 months. In moderate pockets (4–6 mm), the adjunctive benefit was smaller in magnitude: PPD reduction was 0.4 mm (0.1, 0.7 mm) and LCAL gain was 0.5 mm (0.2, 0.8 mm). In addition, the 6-month data showed LCAL gains $\geqslant$2 mm at 25% of sites in test patients compared with 16% in placebo ($p = 0.028$). Similarly, PPD reductions of 2 mm or more were observed in 30% of sites in test and 21% of sites in placebo patients. Seventy-four percent of pockets with PPD $\geqslant$5 mm at baseline were 4 mm or shallower at 6 months in the test group. This compared with 54% in the placebo group ($p = 0.008$). Disease progression at 6 months was observed at 1.5% of test and 3.3% of sites in test and placebo, respectively ($p = 0.072$).

Conclusions: These data indicate that a 7-day adjunctive course of systemic metronidazole and amoxicillin significantly improved the short-term clinical outcomes of full-mouth non-surgical periodontal debridement in subjects with GAP.

Key words: antibiotics; generalized aggressive periodontitis; human; randomized-controlled clinical trial; treatment

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Generalized aggressive periodontitis (GAP) affects a minority of periodontal patients but is highly significant because it is characterized by severe destruction of the supporting apparatus of the teeth, which may lead to edentulism early in life. Because of its relatively rare occurrence, few studies have evaluated how to treat this condition. The current notion is that, as with most forms of periodontitis, the first step in the treatment of GAP is a cause-related treatment phase aimed at the reduction and/or elimination of the pathogenic microflora.

In recent years, two approaches have been introduced to improve the clinical outcomes of cause-related periodontal therapy in chronic periodontitis patients: the use of adjunctive antibiotics, and performing debridement within a 24 h period, the so-called ''full-mouth disinfection'' approach (Quirynen et al. 1995).

The adjunctive use of systemic antibiotics is supported by evidence published in systematic reviews of trials assessing the benefit of systemic antibiotics in cases with advanced periodontitis (Herrera et al. 2002, Haffajee et al. 2003). Among the possible regimens, the combination of amoxicillin and metronidazole has gained increasing popularity because of its wide spectrum of activity and effectiveness in terms of suppression of *Actinobacillus actinomycetemcomitans* (Van Winkelhoff et al. 1989), possibly because of a synergistic effect of the combination of amoxicillin and metronidazole against *A. actinomycetemcomitans* that has been demonstrated in vitro (Pavicic et al. 1991, 1994a, b). In addition, chronic periodontitis patients harbouring subgingival *Porphyromonas gingivalis* benefit significantly from the combined therapy (Winkel et al. 2001). Randomized-controlled clinical trials have reported clinical and microbiological improvements in chronic periodontitis patients treated with these two antibiotics (Berglundh et al. 1998, Flemmig et al. 1998, Winkel et al. 2001, Rooney et al. 2002). Furthermore, some clinical studies have reported good long-term clinical outcomes in patients with ''severe disease which was associated with *A. actinomycetemcomitans*'' (Van Winkelhoff et al. 1992, Pavicic et al. 1994a, Winkel et al. 1998) and in aggressive periodontitis (Buchmann et al. 2002) patients when periodontal treatment was completed with adjunctive use of amoxicillin and metronidazole. However, because of the lack of a control group in these studies, the benefits achieved could not be confirmed as attributable to the adjunctive antibiotic.

Recent investigations, performed in chronic periodontitis patients, have indicated that full-mouth scaling and root planing within 24 h (FSR) results in different degrees of clinical and microbiological additional benefits (Quirynen et al. 1995, Bollen et al. 1996, Vandekerckhove et al. 1996, Mongardini et al. 1999, Apatzidou & Kinane 2004). The benefits of this approach have not been systematically evaluated in aggressive periodontitis patients but initial data in chronic periodontitis patients suggest that the full-mouth scaling within 24 h may perform as well as the conventional treatment (Apatzidou & Kinane 2004, Kinane 2005, Koshy et al. 2005, Wennstrom et al. 2005). In addition, some potential benefits, such as the application of a better understanding of the infectious process, a reduced number of treatment sessions for patients, a more efficient use of treatment time and a reduced cost of therapy, may all be in favour of the FSR (Greenstein 2002).

The aim of this double-blind randomized placebo-controlled study was to test the null hypothesis of ''no difference in treatment effect of adjunctive use of systemic amoxicillin plus metronidazole during full-mouth non-surgical cause-related periodontal treatment (FSR) performed within 24 h compared with FSR alone, in patients with GAP patients at 2 and 6 months after the completion of active treatment.

Material and Methods

Experimental design

This study was a randomized placebo-controlled, parallel-design, double-blind clinical trial with 6-month follow-up. Ethical approval was obtained from the Eastman Dental Institute University College London Hospitals joint Research and Ethics Committee, and the study was conducted according to the principles outlined in the Declaration of Helsinki on experimentation involving human subjects.

Population screening

Potential subjects eligible for the study were identified from the population referred to the periodontal clinic of the Eastman Dental Hospital, London. A complete periodontal examination was performed including a full medical and dental history, an intra-oral examination and a full-mouth periodontal probing. A radiographic examination was undertaken using either periapicals or a pantomogram. A periodontal diagnosis was made, and subjects who fulfilled the study inclusion/exclusion criteria were provided with a written information sheet, related to the study protocol, and they were invited to participate in the study.

The study included subjects with (i) GAP (according to the criteria of the 1999 international classification (Armitage 1999); (ii) at least 20 teeth present; (iii) good general health and (iv) age between 16 and 35 when first diagnosed with aggressive periodontal disease. Subjects were excluded from the study if they: (i) were considered to have a diagnosis of chronic periodontitis (according to the criteria of the 1999 international classification (Armitage 1999); (ii) were pregnant or lactating females; (iii) were females of childbearing age not using a standard accepted method of birth control; (iv) required antibiotic pre-medication for the performance of periodontal examination and treatment; (v) suffered from any other systemic diseases (cardiovascular, pulmonary, liver, cerebral, diseases or diabetes); (vi) had received antibiotic treatment in the previous 3 months; (vii) were taking long-term anti-inflammatory drugs; (viii) had received a course of periodontal treatment within the last 6 months; (ix) were allergic to penicillin or metronidazole; and (x) were not able to provide consent to participate in the study, or they did not accept the proposed treatment plan. Informed consent was obtained from all the subjects to be entered in the study.

Pre-treatment

All the subjects went through motivation sessions during which oral hygiene instructions were given. The purpose of these sessions was to ensure that the subjects could maintain a proper level of oral hygiene before starting active treatment, and this was repeated until subjects showed the ability to maintain good plaque control as evidenced by pre-treatment plaque scores $<20\%$. During these sessions, a case presentation was given to the subject related to the specific features of his/her disease, and a supragingival debridement was performed.

Sample size calculation

The sample size calculation determined that 17 subjects per treatment arm would provide 80% power to detect a true difference of 1.0 mm between test and placebo using probing pocket depth (PPD) reduction in pockets $\geqslant 7$ mm as the primary outcome variable, assuming that the common standard deviation is 1.0 mm. Accordingly, a sample of 21 subjects per arm (42 in total) were to be recruited to compensate for possible drop-out during the study period.

Randomization and allocation concealment

Subject numbers were assigned in ascending order at the enrolment visit. Subjects were randomly assigned by a computer-generated table to receive one of the two treatments. A balanced random permuted block approach (4-unit block size) was used to prepare the randomization tables in order to avoid unequal balance between the two treatments. Restricted randomization (minimization) took place, stratifying for smoking habits (yes or no) and number of initial pockets $\geqslant 5$ mm (<50, 50–80, >80).

The randomization table was sent to the University College London (UCL) pharmacy, which prepared the medication for 50 subjects (25 tests and 25 controls). Fifty plastic bags containing two bottles each (amoxicillin, metronidazole or placebo tablets) were sent back to the study coordinator, who was the only person who had access to them. Consequently, the study coordinator completed the treatment assignment and matched the code of the plastic bag with the subject number. The research nurse provided the subject with the plastic bag containing the two bottles. The randomization code was not broken until all data had been collected and analysed. Thus, the treatment group was concealed to the patient, the clinical examiner, the therapist and the statistician.

Clinical parameters

Clinical parameters were assessed using a UNC-15 periodontal probe by the calibrated examiner at six sites/tooth excluding third molars. Full-mouth plaque score (FMPS) was recorded by assigning a binary score to each surface (1 for plaque present, 0 for absent) and calculating the percentage of total tooth surfaces that revealed the presence of plaque detected by the use of a periodontal probe as modified by Tonetti et al. (2002). Similarly, a full-mouth percentage bleeding score (FMBS) was calculated after assessing dichotomously the presence of bleeding on probing from the bottom of the pocket when probing with a manual probe with a force of 0.3 N (Tonetti et al. 2002). Full-mouth PPD and recession of the gingival margin (REC) were recorded at the same time, with measurements rounded to the nearest millimetre. REC was recorded as a positive value if the free gingival margin (FGM) occurred apical to the cemeno–enamel junction (CEJ), whereas it was recoded as a negative value if it was coronal to the CEJ. In the latter case, the examiner reinserted the probe angled 45° into the site in order to detect the CEJ. If the CEJ was not detectable for anatomical or restorative reasons, the examiner adopted clinical landmarks that were noted in the case report forms. Lifetime cumulative attachment loss (LCAL) was calculated as PPD plus REC.

Investigator calibration

A total of 10 non-study subjects with aggressive periodontitis were recruited and used for the calibration exercise. The single designated examiner (L. N.) measured full-mouth PPD and REC of the gingival margin for all 10 subjects. On the same day (with a minimum of 15 min separation), the examiner repeated the examination. Upon completion of all measurements, the intra-examiner repeatability for LCAL measurement was assessed. The examiner was judged to be reproducible after fulfilling the pre-determined success criteria (the percentage of agreement within ± 2 mm between repeated measurements had to be at least 98%). The examiner showed 99.7% reproducibility.

Non-surgical periodontal therapy

Periodontal therapy was initiated within 1 month of the baseline screening examination. If for any reason, the initiation of the therapy was delayed, a new full-mouth periodontal examination was performed.

A standard cycle of periodontal therapy consisting of oral hygiene instructions, supra- and sub-gingival mechanical instrumentation of the root surface (scaling and root planing) was performed by a single experienced therapist (A. G.) using a piezoelectric instrument with fine tips (EMS, Nyon, Switzerland) and hand instruments, as appropriate. Both groups received this treatment in two long appointments during the same day. Two quadrants were instrumented in a morning session, followed by the other two quadrants in an afternoon session. A 2 h session for each appointment was planned. Local anaesthesia was used as necessary. Test subjects received an adjunctive course of systemic antibiotics consisting of 500 mg of amoxicillin and 500 mg of metronidazole three times a day for 7 days, while control subjects received placebo. Subjects were asked to take the first dose of the medication before mechanical instrumentation had started at the first treatment session. All subjects used a 0.2% chlorhexidine rinse (supplied to improve complicance) twice a day for 2 weeks post–treatment, and relied on standard oral hygiene methods as instructed at the commencement of the study.

Post-treatment controls

The objectives of the post-treatment appointments were to control and reinforce the oral hygiene habits of the subject, to monitor the early healing events, report on any adverse events or additional medications taken. In addition, the 1-week post-treatment visit served as a compliance control, as subjects were asked to return any medication not taken and/or the empty bottles. The number of pills not taken by the subject was documented.

Re-assessment examinations

Re-assessment visits occurred at 2 and 6 months after the completion of the treatment. During these appointments, the examiner recorded any medical history changes, and the clinical periodontal parameters recorded at the baseline visit were repeated. At the end of the appointment, a session of supragingival debridement was performed as necessary. No attempt was made to re-instrument residual periodontal pockets.

Primary and secondary outcome measures

The primary outcome measure of the study was PPD reduction in sites with initial PPD $\geqslant 7$ mm. Secondary outcomes included differences between

groups for the (i) changes in mean full-mouth PPD and the changes in PPD and LCAL at different initial PPD categories; (ii) changes in FMPS and FMBS; (iii) percentage of sites with PPD reduction or LCAL gain of $\geqslant 2$ mm; (iv) percentage of sites that showed LCAL loss $\geqslant 2$ mm (disease progression); (v) percentage of sites with PPD changing from $\geqslant 5$ to $\leqslant 4$ mm and the percentage of sites with PPD changing from $\geqslant 4$ to $\leqslant 3$ mm (need for re-treatment); (vi) description and frequency of adverse events; and (vii) compliance with the systemic medication.

Data management and statistical analysis

Data were entered into an Excel (Microsoft office 2000) database and were proofed for entry errors. The database was subsequently locked, imported into SPSS for Windows (SPSS Inc. version 11.0) formatted and analysed. A subject-level analysis was performed by computing a subject-level variable (full-mouth or at different PPD categories) for each of the parameters. Numerical data were summarized as means and 95% confidence intervals (CIs), categorical data were summarized as frequency distribution and the percentage-based measures (e.g. FMPS) were summarized as the median of the percentage and inter-quartile range. The significance of differences between test and placebo groups in terms of numerical data was evaluated via univariate analysis using the independent samples *t*-test. Likewise, the significance of the difference within each group before and after treatment was evaluated with the paired samples *t*-test. Categorical data were analysed with the χ^2 test, and the percentage data between the two groups were compared with the Mann–Whitney test, while the within-group percentage changes were evaluated with the Wilcoxon's sign-rank test. The significance of the treatment option (test or placebo) on the dependent variables PPD reduction and LCAL gain at different initial PPD categories was estimated by analysis of covariance (ANCOVA). The models were adjusted for baseline values and controlled for smoking. The final model was then selected by including significant factors only. Model estimates included adjusted means and 95% CIs. An intention-to-treat, last observation carried forward analysis was performed (Hollis & Campbell 1999).

Results

Subject accountability

Figure 1 shows what happened to all potential subjects throughout the study from possible recruitment to completion. Fifty-one subjects were assessed for their eligibility before entering the study. Of these, 10 subjects were excluded; seven because they did not meet the inclusion criteria, while the other three refused to participate. One of the three who refused to participate did so after signing the consent form, but before treatment assignment had taken place. Thus, 41 subjects were randomly allocated to participate in the study. All participants received the allocated intervention and one patient from the placebo group was lost to follow-up between the 2-month visit and the 6-month visit because of reasons unrelated to the study. All participants were included in the analysis.

Study Schedule

Subject recruitment started in January 2003 and was completed by the end of December 2003. All the 6-month follow-up visits were completed by July 2004. Data entry of all information and statistical analysis were performed by the end of September 2004.

Subject characteristics at baseline

The baseline characteristics of the 41 participants who were treated non-surgically with the adjunctive use of the test or the placebo medication are displayed in Table 1. The mean age of the participants was 31.3 ± 5.2 years (SD) for the test group and 31.7 ± 5.1 years for the placebo group. Females accounted for 80% of the test group and 57% of the placebo group. In the test group 25% of the participants were smokers whereas 19% smoked in the placebo group. Caucasians constituted the major ethnic group in the study, accounting for 65% of the test group and 47.6% of the placebo group. None of these demographic parameters showed a statistically significant difference between groups.

Clinical characteristics

The baseline examination revealed that the two study groups showed similar

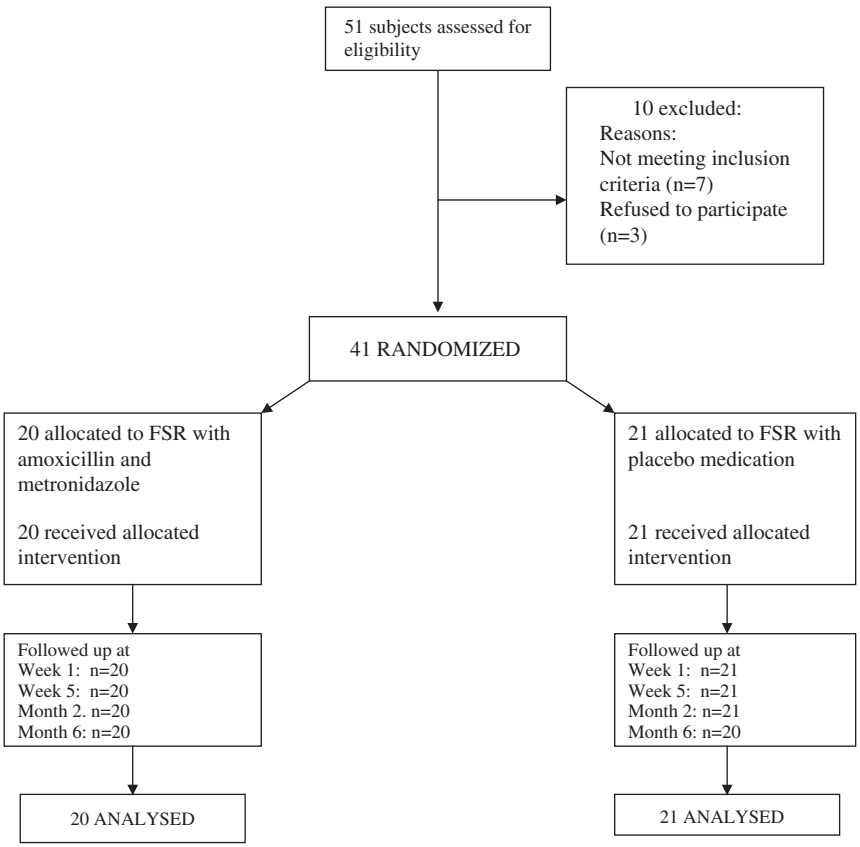

Fig. 1. Flow chart for study patients.

Table 1. Subject and clinical characteristics at baseline

Parameter	Test group ($N = 20$)	Placebo group ($N = 21$)	p-value
Age	31.3	31.7	0.779
Mean (95% CI)	(28.8, 33.7)	(29.4, 34.1)	t-test
Females (percentage)	16 (80%)	12 (57%)	0.108 χ^2
Smokers (percentage)	5 (25%)	4 (19%)	0.719 χ^2
Caucasians (percentage)	13 (65%)	10 (47.6%)	0.530 χ^2
Teeth at baseline	25.5	26.0	0.482
Mean (95% CI)	(24.5, 26.6)	(25.0, 27.0)	t-test
Number of pockets $\geqslant 5$ mm	60.8	58.4	0.795
Mean (95% CI)	(46.3, 75.3)	(46.74, 70.20)	t-test
Percentage of pockets $\geqslant 5$ mm	35.5	31.5	0.514
Median (IQ)	(26.6, 47.3)	(24.4, 48.1)	Mann–Whitney
Full-mouth plaque score	25.5	20.0	0.155
Median (IQ)	(13.3, 36.8)	(10.0, 29.0)	Mann–Whitney
Full-mouth bleeding score	61.5	55.0	0.175
Median (IQ)	(50.8, 74.8)	(35.5, 66.5)	Mann–Whitney

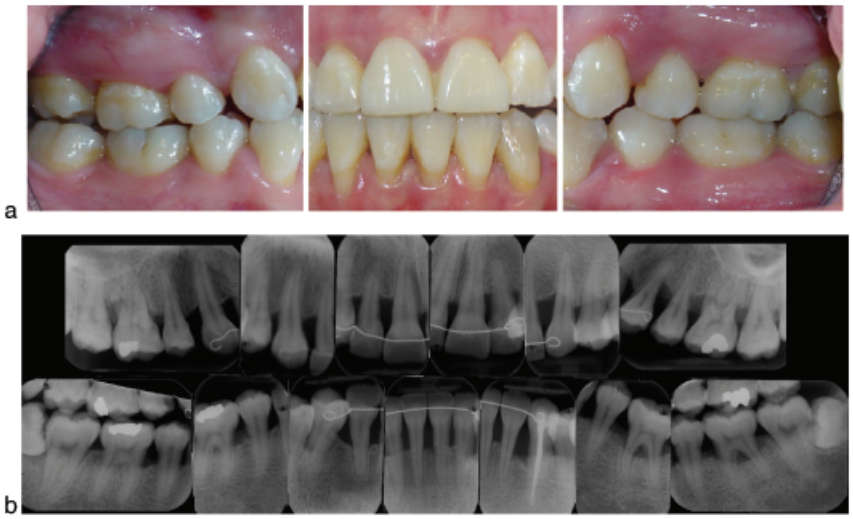

Fig. 2. Baseline clinical (a) and radiographic (b) appearance of a 21-year-old, smoking female (eight cigarettes per day). She presented with a plaque score of 24%, a bleeding score of 68% and 70 sites with pockets 5 mm or deeper.

characteristics for number of teeth present, percentage of pockets $\geqslant 5$ mm, plaque and bleeding levels, with no significant differences between the groups. The data show that the subjects had retained most of their teeth, but had approximately a third of sites exhibiting pockets requiring treatment and high levels of bleeding, but low levels of plaque (Fig. 2).

Mean values for clinical parameters

Mean full-mouth clinical outcomes and mean clinical outcomes at shallow ($\leqslant 3$ mm), moderate (4–6 mm) and deep ($\geqslant 7$ mm) pocket categories for baseline, and the differences between baseline – 2 months and baseline – 6 months are displayed in Table 2. At baseline, there were no significant differences between test and placebo. All parameters, with the exception of the mean LCAL gain at the initial shallow pockets, showed a statistically significant difference between baseline and 2 months. This was also true between baseline and 6 months except for LCAL gain and PPD reduction at shallow pockets. At 2 months, there were statistically significant differences ($p < 0.02$) between test and placebo in the mean PPD at moderate pockets (4–6 mm) and mean PPD at deep pockets ($\geqslant 7$ mm). At 6 months, statistically significant differences were detected between test and placebo groups in the mean PPD at moderate pockets ($p < 0.02$), mean PPD at deep pockets ($p < 0.001$) and LCAL at deep pockets ($p < 0.05$).

The significance of this treatment effect between the groups at 2 and 6 months (difference between the test group and the placebo group in the mean PPD reduction and the mean LCAL gain at different pocket categories) is displayed in Table 3. Multivariate models based on linear regression ANCOVA were constructed taking into account the potential sources of variability such as smoking status and baseline pocket depth. As the adjustment for baseline PPD value could constitute a problem related to mathematical coupling (Tu et al. 2004), the same analyses were undertaken with a model that did not include an adjustment for baseline values. This resulted in almost identical results (data not shown). When considering full-mouth mean LCAL, there were no statistically significant differences between test and placebo. Similarly when examining pockets $\leqslant 3$ mm, no differences were observed between test and placebo for either LCAL or PPD. For ease of presentation, these analyses have been omitted from Table 3.

There were highly significant treatment effects for full-mouth PPD reduction, PPD reduction at 4–6 mm pockets and PPD reduction at $\geqslant 7$ mm pockets at 2 and 6 months, with the outcomes favouring the test treatment. For PPD reduction in 4–6 mm pockets, the adjusted differences between test and placebo treatment were 0.5 mm at 2 months and 0.4 mm at 6 months. In the deeper pockets $\geqslant 7$ mm this difference was much larger: 0.9 mm at 2 months and 1.4 mm at 6 months.

For sites with initial PPD $\geqslant 7$, LCAL gain was also significantly better in test subjects: an adjunctive benefit of 0.6 mm at 2 months and 1.0 mm at 6 months was observed. While there was no statistically significant benefit in terms of 2-month LCAL gain ($p = 0.650$) at sites with initial PPD 4–6 mm, a highly significant difference of 0.50 mm in favour of the test group was observed at 6 months ($p = 0.001$).

In addition, the effect of smoking on the primary outcome variable (PPD

Table 2. Mean clinical outcome variables at baseline and differences between 0–2 and 0–6 months

Clinical outcomes mean (95% CI)	Group	Baseline 0 months	Difference between 0 and 2 months	Difference between 0 and 6 months	*p*-value paired *t*-test	
					difference 0–2 months	difference 0–6 months
Full-mouth mean PPD	Placebo	4.1 (3.6, 4.5)	0.8 (0.6, 1.1)	0.7 (0.4, 1.0)	<0.001	<0.001
	Test	4.1 (3.6, 4.5)	1.1 (0.9, 1.4)	1.2 (0.9, 1.4)	<0.001	<0.001
Mean PPD at pockets ⩽3 mm	Placebo	2.3 (2.2, 2.4)	0.2 (0.1, 0.3)	−0.1 (−0.2, 0.0)	0.003	0.052
	Test	2.3 (2.2, 2.3)	0.2 (0.2, 0.3)	0.0 (0.0, 0.0)	<0.001	0.931
Mean PPD at pockets 4–6 mm	Placebo	5.0 (4.9, 5.1)	1.2 (0.9, 1.5)	1.0 (0.8, 1.3)	<0.001	<0.001
	Test	5.02 (4.9, 5.1)	1.7 (1.6, 1.9)	1.5 (1.3, 1.7)	<0.001	<0.001
Mean PPD at pockets ⩾7 mm	Placebo	7.7 (7.4, 8.1)	2.1 (1.6, 2.5)	1.8 (1.3, 2.3)	<0.001	<0.001
	Test	7.7 (7.5, 7.9)	3.0 (2.6, 3.3)	3.1 (2.7, 3.5)	<0.001	<0.001
Full-mouth mean LCAL	Placebo	4.8 (4.1, 5.5)	0.5 (0.3, 0.6)	0.5 (0.2, 0.7)	<0.001	<0.001
	Test	4.7 (4.1, 5.2)	0.7 (0.5, 0.8)	0.8 (0.7, 0.9)	<0.001	<0.001
Mean LCAL at sites with initial pockets ⩽3 mm	Placebo	3.2 (2.7, 3.7)	0.0 (−0.1, 0.1)	0.0 (−0.2, 0.1)	0.683	0.704
	Test	2.9 (2.6, 3.1)	0.0 (−0.01, 01)	0.0 (−0.1, 0.1)	0.631	0.634
Mean LCAL at sites with initial pockets 4–6 mm	Placebo	5.7 (5.2, 6.2)	0.8 (0.5, 1.0)	0.8 (0.5, 1.1)	<0.001	<0.001
	Test	5.7 (5.3, 6.1)	1.1 (1.0, 1.2)	1.3 (1.2, 1.4)	<0.001	<0.001
Mean LCAL at sites with initial pockets ⩾7 mm	Placebo	8.2 (7.5, 8.9)	1.3 (1.0, 1.6)	1.3 (1.0, 1.6)	<0.001	<0.001
	Test	8.1 (7,7, 8.4)	1.8 (1.6, 2.1)	2.3 (2.0, 2.5)	<0.001	<0.001

PPD, probing pocket depth; LCAL, life cumulative attachment loss.

Table 3. Analysis of covariance for PPD reduction and LCAL gain at 2 and 6 months in different pockets categories

Multivariate ''ANCOVA'' analysis models	Parameter	Difference 0–2 months		Difference 0–6 months	
		estimate (95% CI)	*p*-value	estimate (95% CI)	*p*-value
Full-mouth mean PPD reduction	Treatment group (test-placebo)	0.3 (0.1, 0.5)	0.002	0.5 (0.2, 0.7)	0.001
	Smoking (no-yes)	0.4 (0.1, 0.7)	0.007	0.4 (0.1, 0.7)	0.040
Mean PPD reduction in pockets 4–6 mm	Treatment group (test-placebo)	0.5 (0.2, 0.8)	0.001	0.4 (0.1, 0.7)	0.005
	Smoking (no-yes)	0.4 (0.0, 0.8)	0.050	0.2 (−0.1, 0.6)	0.183
Mean PPD reduction in pockets ⩾7 mm	Treatment group (test-placebo)	0.9 (0.4, 1.5)	0.001	1.4 (0.8, 2.0)	<0.001
	Smoking (no-yes)	0.9 (0.3, 1.5)	0.007	1.0 (0.3, 1.7)	0.007
Mean LCAL gain in sites with initial PPD ⩾7 mm	Treatment group (test-placebo)	0.6 (0.2, 0.9)	0.002	1.0 (0.7, 1.3)	<0.001
	Smoking (no-yes)	0.6 (0.2, 1.0)	0.005	0.7 (0.3, 1.1)	0.001
Mean LCAL gain in sites with initial PPD 4–6 mm	Treatment group (test-placebo)	−0.2 (−0.4, 0.0)	0.650	0.5 (0.2, 0.8)	0.001
	Smoking (no-yes)	0.0 (−0.4, 0.2)	0.203	0.0 (−0.3, 0.4)	0.903

PPD, probing pocket depth; LCAL, life cumulative attachment loss.

reduction at ⩾7 mm pockets) was statistically significant ($p = 0.007$), and the difference between a non-smoker and a smoker on PPD reduction at deep pockets was 0.9 mm (95% CI 0.3, 1.5) at 2 months and 1.0 mm (0.3, 1.7) at 6 months. The corresponding value for the difference between non-smokers and smokers on PPD reduction in initial pockets of 4–6 mm was 0.4 mm (0.0, 0.8), demonstrating a borderline significance ($p = 0.050$) at 2 months and a non-significant difference of 0.2 mm (−0.1, 0.6) at 6 months ($p = 0.183$), whereas there were no statistically significant differences for LCAL.

Percentage of sites with pockets

The percentage of sites (median and inter-quartile range) with a PPD of a specific threshold at baseline and the reduction in the percentage of pockets within groups (difference between baseline – 2 months and baseline – 6 months) are reported in Table 4. The significance of the reduction in the percentage of pockets between the groups is also shown. At baseline, there were no statistically significant differences between the groups in any of the specific PPD thresholds.

Analysis within groups (Wilcoxon's Signed rank test) indicated that all PPD thresholds within each group showed a highly statistically significant difference between baseline and two months and between baseline and 6 months in both treatment groups. Furthermore, analysis of the differences between test and placebo treatments for the outcome of treatment (baseline – 2 months) showed a statistically significant difference ($p < 0.05$) for the reduction in the percentage of pockets ⩾5, ⩾6 and ⩾7 mm that favoured the test treatment. The statistical significance of the difference between baseline and 6 months was $p < 0.02$ for the reduction in the percentage of pockets ⩾5, ⩾6 mm, and was $p < 0.05$ for pockets ⩾4, ⩾7 mm.

Oral hygiene and bleeding on probing

FMPS and FMBS at baseline, and the reduction (difference baseline – 2 months and baseline – 6 months) within each group are displayed in Table 5.

Plaque scores decreased in both treatments from baseline to 2 months, and the difference was statistically significant for test and placebo groups. However, the 6-month plaque scores values were equal to baseline values. The effects of both treatments had a large impact on bleeding, and these changes were statistically significant at 2 and 6 months ($p < 0.001$). Furthermore, there was a statistically significant difference between test and placebo for the improvement in the percentage of bleeding sites at 2 and 6 months ($p < 0.02$).

Percentage of sites with clinically relevant changes

A subset analysis was carried out to test the changes of some clinically relevant parameters at 2 and 6 months (Table 6). All the median values (inter-quartile range) showed statistically significant differences favouring the test treatment for the percentage of sites with LCAL gain ⩾2 mm at 2 months ($p = 0.047$), at 6 months ($p = 0.028$), the percentage of sites with PPD reduction of ⩾2 mm at 2 months ($p = 0.029$) and at 6 months ($p = 0.021$), and the percentage of pockets that had converted from ⩾5 mm at baseline to ⩽4 mm at 2 months ($p = 0.039$) and at 6 months ($p = 0.008$). Conversely, the percentage of sites with LCAL loss ⩾2 mm was higher in the placebo group as compared with the test group at 2 months ($p = 0.041$) and at 6 months ($p = 0.072$).

The percentage of pockets that converted from ⩾4 mm at baseline to ⩽3 mm at 2 months failed to show a statistically significant difference ($p = 0.112$) while at 6 months the difference between the groups demonstrated a statistical significance ($p = 0.038$).

Adverse events, concomitant antimicrobials and compliance

At the 1-week follow-up visit, 11 subjects (55%) in the test group and four subjects (19%) in the placebo reported

Table 4. Percentage of pockets at baseline and differences between 0–2 and 0–6 months

Median of percentage (IQ)	Group	Baseline	Difference between 0 and 2 months	Difference between 0 and 6 months	*p*-value Wilcoxon's signed-ranks test	
					difference 0–2 months	difference 0–6 months
Percentage of pockets ⩾4 mm	Placebo	45.8 (33.7, 60.0)	15.3 (9.4, 24.2)	14.9 (7.6, 24.8)	<0.001	<0.001
	Test	46.3 (37.6, 57.4)	19.6 (16.1, 28.5)	21.3* (14.6, 32.0)	<0.001	<0.001
Percentage of pockets ⩾5 mm	Placebo	31.5 (24.9, 48.1)	17.3 (10.9, 24.7)	17.3 (9.2, 23.1)	<0.001	<0.001
	Test	35.5 (26.6, 47.3)	22.1* (19.5, 30.2)	24.1** (19.2, 32.8)	<0.001	<0.001
Percentage of pockets ⩾6 mm	Placebo	18.0 (13.0, 31.0)	11.9 (7.0, 18.3)	11.9 (4.1, 16.3)	<0.001	<0.001
	Test	22.1 (13.2, 28.9)	16.4* (11.5, 27.5)	18.2** (10.7, 27.7)	<0.001	<0.001
Percentage of pockets ⩾7 mm	Placebo	9.0 (5.2, 17.3)	6.0 (1.0, 11.5)	5.3 (0.7, 10.5)	<0.001	0.001
	Test	12.0 (5.8, 19.0)	10.5* (5.7, 16.6)	10.8* (5.6, 17.6)	<0.001	<0.001

*Significant difference between groups favoring test treatment ($p < 0.05$).
**Significant difference between groups favoring test treatment ($p < 0.02$).

Table 5. Analysis on FMPS and FMBS at baseline (post-oral hygiene instructions) and differences between 0–2 and 0–6 months.

Median (IQ)	Group	Baseline	Difference 0–2 months	Difference 0–6 months	*p*-value Wilcoxon' signed-ranks test	
					difference 0–2 months	difference 0–6 months
Full-mouth plaque score (%)	Placebo	20.0 (10.0, 29.0)	3.0 (−1.0, 7.0)	0.0 (−2.5, 8.0)	0.029	0.112
	Test	25.5 (13.3, 36.8)	6.0 (−3.8, 19.0)	1.0 (−5.0, 15.0)	0.038	0.170
Full-mouth bleeding score (%)	Placebo	55.0 (35.5, 66.5)	17.0 (10.0, 32.5)	21.0 (9.5, 28.5)	<0.001	0.001
	Test	61.5 (50.8, 74.8)	34.5** (27.3, 34.5)	32.0** (26.0, 39.0)	<0.001	<0.001

**Significant difference between groups favoring test treatment ($p<0.02$).
FMPS, full-mouth plaque score; FMBS, full-mouth bleeding score.

Table 6. Percentage of sites with clinically relevant changes in test and placebo groups at 2 and 6 months

Median of percentage (IQ)	0–2 months data			0–6 months data		
	test group	placebo group	*p*-value Mann–Whitney test	test group	placebo group	*p*-value Mann–Whitney test
Percentage of sites with ⩾2 mm of LCAL gain at 2 months	19.6 (15.5, 26.2)	14.3 (8.6, 21.3)	0.047	25.4 (18.5, 34.1)	16.1 (8.8, 24.9)	0.028
Percentage of sites with ⩾2 mm of PPD reduction at 2 months	29.4 (23.9, 43.8)	20.8 (10.5, 32.7)	0.029	30.2 (21.1, 46.6)	20.8 (8.6, 32.7)	0.021
Percentage of sites with LCAL loss ⩾2 mm at 2 months	1.3 (0.3, 3.5)	3.0 (1.8, 5.4)	0.041	1.5 (1.2, 2.3)	3.3 (1.5, 5.5)	0.072
Percentage of pockets converting from ⩾5 mm at baseline to ⩽4 mm after treatment	71.2 (63.3, 78.2)	56.6 (30.4, 72.7)	0.039	74.1 (63.7, 83.5)	54.2 (29.1, 75.7)	0.008
Percentage of pockets converting from ⩾4 mm at baseline to ⩽3 mm after treatment	49.0 (42.9, 60.6)	41.6 (21.1, 58.1)	0.112	55.2 (38.9, 65.2)	37.2 (18.0, 56.4)	0.038

LCAL, life cumulative attachment loss.

adverse events. At the 5-week follow-up visit only two subjects (10%) in the test group and one subject (4.8%) in the placebo group reported to have continuing adverse events. The types of adverse events at the 1- and 5-week follow-up visits are described in Table 7. Two subjects (one in the test group and one in the placebo group) had a tooth extraction each between the baseline visit and the 2-month visit. The baseline values of these two teeth were carried forward through all the analysis. One subject from the test group lost three front teeth because of an accident between the 2-month visit and the 6-month visit. The 2-month values of these three teeth were carried forward to the 6-month visit.

Concomitant antimicrobial medication during the study period was recorded. Two subjects in the test group took amoxicillin capsules for medical reasons, while no subject in the placebo group had any concomitant antimicrobials.

Compliance with the course of systemic medication and the number of pills not taken by the non-compliant patients were also documented. All subjects returned the medication bottles. Sixteen subjects (80%) in the test group and 19 subjects 90.5% in the placebo group completed the course of systemic medication as indicated. The reasons advocated by the four non-compliant subjects in the test group for not having taken all the pills were the following: two subjects reported diarrhoea, one subject reported vomiting and one subject failed to remember to take the prescribed medication. These four subjects missed 38%, 45%, 52% and 9% of the whole number of pills, respectively.

Discussion

This was the first randomized-controlled clinical study (RCT) designed to assess the adjunctive effect of the metronidazole–amoxicillin antibiotic combination, originally proposed by van Winkelhoff et al. (1989), in the treatment of GAP. The data indicated that the experimental therapy resulted in clinically significant short-term improvements in clinical parameters.

As all patients had pre-treatment sessions of oral hygiene instructions and reinforcement as necessary, FMPS were low from the beginning of the study. There was little additional benefit to plaque reduction from the therapy at 2 an 6 months, and there was no difference between the treatment regimes. Pre-treatment sessions to achieve plaque scores <20% were included in order to reduce the impact of inadequate plaque control in the success of non-surgical treatment (Magnusson et al. 1984). This was important because full-mouth instrumentation was performed in two visits, and thus the therapist had fewer opportunities to deliver, check and reinforce the necessary oral hygiene instructions.

Our experimental population consisted of subjects with the clinical characteristics of GAP according to the criteria of the 1999 international classi-

Table 7. Type of adverse events at the 1- and 5-week follow-up visit

Type of adverse event number (percentage) of subjects	Time period (week)	Test group, $N = 20$	Placebo group, $N = 21$
Stomach upset (nausea and vomiting)	1	3(15%)	0
	5	1(5%)	0
Gastrointestinal disorder (diarrhea)	1	3(15%)	0
	5	0	0
Headache	1	1(5%)	0
	5	0	0
Periodontal abscess	1	0	2 (9.5%)
	5	0	0
Tooth loss, tooth extraction	1	0	0
	5	1(5%)	1(4.8%)
Metallic taste	1	1(5%)	0
	5	0	0
Intra-oral tissue alteration	1	0	2 (9.5%)
	5	0	0
General unwellness (irritability, flu, etc.)	1	3 (15%)	0
	5	0	0

fication (Armitage 1999). Because of the recently reported difficulties encountered with this classification (Mombelli et al. 2002, Meyer et al. 2004), extra efforts were directed towards selecting ''clear cases''. Our patients were mainly Caucasians, with an average of 31 years of age, who presented with almost all teeth and with severe widespread disease (an average of 33% of the sites with PPD $\geqslant$5 mm after probing six sites around each tooth). Patients, however, were not screened or selected based upon a microbiological diagnosis. The lack of microbiological entry criteria had implications on the choice of antibiotic regimen, and indicates that our results are applicable to subjects where the antibiotic is used empirically, i.e. without specific targeting based on the microbial results (Mombelli 2005, van Winkelhoff et al. 2005). Nevertheless, microbiological outcomes of treatment will be reported in a separate paper.

In this study, we prescribed 500 mg of amoxicillin combined with 500 mg of metronidazole three times a day for 7 days. This dosage has not been reported in previous clinical trials. It aims to provide a wide spectrum of activity and to reach and maintain serum concentrations above the minimum effective concentration. The rationale for the wide spectrum has been previously discussed and is based on the reported high prevalence of *A. actinomycetemcomitans* and anaerobic pathogens in GAP patients (Sasaki et al. 1989, Kamma et al. 1994, Listgarten et al. 1995, Lopez et al. 1995, Tonetti and Mombelli 1999, Ishikawa et al. 2002, Lee et al. 2003, Takeuchi et al. 2003). The choice of dosage comes from an analysis of previous studies in chronic periodontitis subjects and the lack of significant adjunctive benefit reported in studies that have used marginally effective doses of antibiotic (Van Winkelhoff et al. 1992, Winkel et al. 1997, Palmer et al. 1999). Attention has been drawn recently to the fact that the amount of metronidazole needed for effective concentration in body fluids amounts to 20–25 mg/kg, and that an insufficient antibiotic concentration would turn into a lack of effect on clinical and microbiological parameters (Van Winkelhoff et al. 1999). This means that 1400–1750 mg/day of the medication should be taken by a 70 kg adult patient (Winkel et al. 1997). Therefore, in GAP patients with a high prevalence of anaerobic and micro-aerophilic periodontal pathogens, a moderate dose of amoxicillin (375–500 mg) will have synergistic effects with metronidazole and its hydroxymetabolite against *A. actinomycetemcomitans* (Pavicic et al. 1992), while a high dose of metronidazole (500 mg) will target the anaerobic microflora.

It is clear from other clinical trials that mean full-mouth PPD and LCAL values may not be the best way to describe the data. Shallow sites, which are not expected to change as a result of therapy, are likely to significantly dilute the changes observed at the deeper sites, which are the ones of therapeutic concern. Therefore, the primary outcome variable selected was the difference in PPD reduction between the treatment groups at deep pockets. The mean PPD reduction at 6 months was 1.8 mm (95% CI 1.3, 2.3) for the placebo group and 3.1 mm (2.7, 3.5) for the test group. A multivariate model (Table 3) determined that the additional benefit for test subjects after taking into account the potential sources of variability was 1.4 mm (0.8, 2.0). It should be noted that the results obtained in the control group were within the range expected from non-surgical periodontal treatment in chronic periodontitis patients. Cobb (1996) reviewed the most relevant clinical studies related to PPD reduction after non-surgical therapy alone, and found that at deep pockets (PPD$\geqslant$ 7 mm), the mean PPD reduction was 2.2 mm. This was in excellent agreement with the results of our placebo group, which exhibited a mean PPD reduction of 2.1 mm at 2 months and 1.8 mm at 6 months. The results are also comparable with those of the control group in a similar study in aggressive periodontitis patients treated with the adjunctive use of three different single antibiotic regimens (Sigusch et al. 2001). They showed that in pockets $>$6 mm, a 2.3 mm PPD reduction was achieved 6 months after treatment. The similarity between the outcomes of subgingival instrumentation at deep pockets of chronic and aggressive periodontitis patients is in agreement with reports indicating that aggressive periodontitis patients respond well to mechanical instrumentation alone (Wennstrom et al. 1986).

In contrast, the adjunctive benefits are more difficult to compare with other studies because of the paucity of randomized controlled clinical trial in GAP patients. Recent meta-analyses by Herrera et al. (2002) and Haffajee et al. (2003) have suggested that the adjunctive benefit expected from antibiotic usage may be greater in aggressive periodontitis patients. An additional gain in LCAL of 0.7 mm was observed in seven studies including 231 subjects receiving the antibiotic adjunctively to non-surgical or surgical root instrumentation. In the present study, the use of adjunctive antimicrobials resulted in an additional benefit of 0.5 mm in LCAL gain in moderate pockets (4–6 mm), while a 1.0 mm benefit was observed in deeper pockets ($\geqslant$7 mm) compared with non-surgical root debridement alone.

Our test subjects showed a mean PPD reduction of 3.1 mm in pockets $\geqslant$7 mm at 6 months. These results are slightly inferior but comparable with the 6-month outcomes reported by Sigusch et al. (2001) with the use of adjunctive metronidazole or clindamycin. In terms of PPD reduction at 6 months, the added benefit

that we observed was 0.4 mm for moderate pockets (4–6 mm) and 1.4 mm for the deep pockets ($\geqslant$7 mm). This gradient of effect is consistent with the notion that the benefit of the antibiotic is particularly evident at deeper pockets where mechanical debridement is less effective.

The multivariate analysis identified cigarette smoking as a significant factor ($p = 0.007$): in a non–smoker, deep pockets reduced by an average of 0.9 mm more than in a smoker, regardless of the treatment group. This result is in accordance with previous studies that have demonstrated, in chronic periodontitis patients, that less PPD reduction and less LCAL gain occur in smokers as compared with non-smokers (Preber and Bergstrom 1986, Jin et al. 2000) or even former smokers (Grossi et al. 1997).

For most clinicians, results reported as mean full-mouth values offer little insight into the clinical relevance of the findings. To illustrate tangible clinical benefits, after the analysis on the primary outcome variable (PPD reduction in pockets $\geqslant$7 mm) and the other parameters based on full-mouth mean or median values, secondary analyses were carried out. Accordingly, the test treatment significantly decreased the percentage of pockets that remained above specific thresholds at 2 and 6 months after subgingival debridement (Table 6). As in previous studies (Berglundh et al. 1998), the percentage of all sites that gained $\geqslant$2 mm in LCAL and the percentage of all sites that had a PPD reduction $\geqslant$2 mm were statistically significantly higher in the test group at 2 and 6 months. Furthermore, the percentage of sites experiencing disease progression (LCAL loss of 2 mm or more over the 6-month observation period) was significantly decreased by the antibiotic combination.

However, a critical question at re-evaluation 3–6 months after the completion of non-surgical therapy is related to what to do with residual pockets and the need for further treatment beyond maintenance care. A recent systematic review on the clinical parameters used during re-evaluation (residual pockets, bleeding on probing and presence of furcation defects) to predict further attachment loss (Renvert & Persson 2002) concluded that the presence of deep residual pockets was associated with further disease progression and hence decreased maintainability. This conclusion can be used as a rationale for providing further treatment to patients, e.g. pocket reduction surgery. As reported in other studies (Loesche et al. 1992, Smith et al. 2002), we compared the reduction in the frequency of sites in test and control patients who would require surgical intervention for PPD reduction at 2 or 6 months using 5 mm as the discriminant value. At 2 months post-therapy, we found that the subjects receiving the test treatment showed a 71% reduction in the number of sites in need of surgical intervention, while the subjects in the placebo group had a reduction of 57% ($p = 0.039$). These values were maintained at 6 months. Using an even stricter discriminant value (4 mm), at 6 months, 55% of the test sites with baseline pockets showed maintainable probing depths (3 mm or less) as compared with 37% of the placebo sites ($p = 0.038$).

Two subjects in the placebo group and four subjects in the test group did not fully comply with the medication. The fact that 20% of test subjects did not complete the full cycle of antibiotic may have led to an underestimation of the adjunctive effect in our intention-to-treat analysis. On the other hand, this fact provides the study with a higher external validity as lack of compliance is a reality in clinical practice (Grob 1992). Furthermore, given the fact that some of the observed incomplete compliance can be attributed to the onset of significant side effects, clinicians prescribing this regimen to their patients should expect less than optimal compliance. An additional analysis assessing the impact of incomplete compliance will be presented in a companion paper focused on the microbiological outcomes of the study where an ''on-drug'' analysis gives important additional information.

In conclusion, the findings of the present study have indicated that the adjunctive use of systemic amoxicillin plus metronidazole, during full-mouth non-surgical cause-related periodontal treatment (FSR) performed within 24 h, has resulted in significant additional improvements in the clinical conditions of GAP patients when compared with FSR alone. These observations are valid for both the 2- and 6-month evaluations after the completion of active treatment.

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Clinical relevance

Scientific rationale for study: Adjunctive systemic antibiotics may benefit aggressive periodontitis patients during non-surgical therapy. Insufficient data, however, exist supporting this approach.

Principal findings: Subjects receiving the adjunctive amoxicillin and metronidazole combination demonstrated statistically significant additional improvements in clinical outcomes with respect to debridement alone. The improvements were clinically significant, as demonstrated by the reduced prevalence of pockets.

Practical implications: Patients with generalized aggressive periodontitis may benefit from the adjunctive administration of amoxicillin and metronidazole during the initial phase of periodontal treatment.