UNIVERSITÀ DEGLI STUDI DI TORINO

Journal of Clinical Periodontology, 39, 3, 2012, doi: 10.1111/j.1600-051X.2011.01795.x.

FULL-MOUTH DISINFECTION AND SYSTEMIC ANTIMICROBIAL THERAPY IN GENERALIZED AGGRESSIVE PERIODONTITIS: A RANDOMIZED, PLACEBO-CONTROLLED TRIAL

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Running title: OSFMD and antibiotics in G-AgP patients

Key words: antibiotics; generalized aggressive periodontitis; microbiology; periodontitis/microbiology; periodontitis/therapy, randomized-controlled clinical trial.

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Conflict of Interest and Sources of Funding Statement: The authors declare that they have no conflict of interest. The study was supported by a research grant from Regione Piemonte, Turin, Italy.

ABSTRACT

Aim: The present investigation aimed to analyze clinical and microbiological effects of systemic administration of metronidazole and amoxicillin combined with the One-Stage-Full-Mouth-Disinfection protocol (OSFMD) in generalized aggressive periodontitis patients (G-AgP).

Materials and Methods: Thirty-nine systemically healthy patients with G-AgP were consecutively included. The test group (n= 19) received amoxicillin-metronidazole combination (500 mg of each, three times a day for 7 days) and the OSFMD, the control group (n= 20) received the OSFMD and a placebo. In addition to clinical parameters subgingival plaque samples from moderate (4-5 mm) and deep (≥ 6 mm) pocket sites were analysed for the presence of *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia*, *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola* using polymerase chain reaction.

Results: Both therapies led to a statistically significant decrease in clinical and microbiological parameters compared to baseline ($p<0.001$). The most beneficial changes were observed in the test group which showed significantly greater improvements in probing depth and clinical attachment level and a lower prevalence of *Aggregatibacter actinomycetemcomitans, Treponema denticola,* and *Tannerella forsythia* compared to the control one ($p< 0.05$).

Conclusions: Systemic administration of metronidazole and amoxicillin as an adjunct to OSFMD therapy significantly improved clinical and microbiological outcomes in patients with G-AgP over a 6-month period.

Clinical relevance

Scientific rationale of the study: Adjunctive systemic antibiotics may benefit aggressive periodontits patients during non-surgical therapy. No information is available regarding the efficacy of systemic antibiotics as an adjunct to OSFMD.

Principal findings: The administration of systemic amoxicillin/metronidazole in combination with OSFMD enhances the efficacy of OSFMD in reducing PD, improving CAL, and in decreasing the prevalence of periodontal pathogens especially in initially deep pocket sites.

Practical implications: These data support the administration of systemic antibiotics during the OSFMD protocol in the treatment of generalized aggressive periodontitis patients.

INTRODUCTION

Generalized aggressive periodontitis (G-AgP) is a rapidly progressive disease that affects otherwise healthy individuals and results in rapid loss of attachment and bone destruction, which may lead to edentulism early in life (Armitage 2004). Therefore, this disease, despite its relatively low prevalence in developed countries, has important social implications (Susin Albandar & Tinoco 2002, Demmer & Papapanou 2010). The treatment of this condition has always represented a challenge for clinicians, because there are no established protocols and guidelines for efficiently controlling the disease (Xajigeorgiou et al. 2006). Treatment plans have traditionally centred on quadrant scaling and root planing combined with effective supragingival plaque control (AAP 2000, Deas & Mealey 2010). However despite this therapy some patients may experience ongoing periodontal attachment loss, probably due to the persistence of periodontal pathogens and the occurrence of recontamination by pathogens residing in other intra- and extradental sites (Danser et al. 1996). Several studies suggested the existence of a translocation of periodontal pathogens from untreated pockets as well as from other intra-oral reservoirs such as the tongue dorsum, the mucosa, the saliva and the tonsils to treated periodontal sites (Danser et al. 1996, Koshy et al. 2004). In this context, two approaches have been introduced to improve the outcomes of non-surgical periodontal therapy: the instrumentation of all pockets within a 24-h period in combination with a disinfection of intra-oral niches by means of chlorhexidine (CHX) applications (one-stage full-mouth disinfection, OSFMD) and the use of adjunctive antibiotics. Despite recent studies, performed in chronic periodontitis patients, questioned the advantage of the full-mouth disinfection over quadrant scaling (Eberhard et al. 2008, Lang et al. 2008; Teughels et al. 2009), aggressive periodontitis patients may benefit of this non-surgical approach (Bollen et al. 1998, Mongardini et al. 1999, Quirynen et al. 1999, De Soete et al. 2001). Key-pathogens

such as *Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia*, *Treponema denticola*, *Prevotella intermedia* and *Porphyromonas gingivalis* were found to colonize nearly all the above-mentioned intraoral reservoirs (Beikler et al. 2004). The relative importance of microbial intra-oral translocation in the development and maintenance of periodontal infection in G-AgP patients is still undetermined (Koshy et al. 2004).

Adjunctive antimicrobial therapy with systemic antibiotics kills bacteria out of the range of root surface instrumentation and affects periodontal pathogens residing in non-periodontal mucosal surfaces (Müller et al. 1998). The recent reports of the European Federation of Periodontology and the American Academy of Periodontology suggest that patients with G-AgP appear to benefit from their adjunctive use (Herrera et al. 2002, Haffajee et al. 2003, American Academy of Periodontology 2004, Herrera et al. 2008). Among the possible regimens the combination of amoxicillin and metronidazole may be a more effective therapy because of the synergistic effect of this combination, and its wide spectrum of activity (Pavicic et al. 1994, Guerrero et al. 2005, Xajigeorgiou et al. 2006, Herrera et al. 2008, Mestnik et al. 2010). This is of particular importance in G-AgP patients who yielded a high prevalence of *Aggregatibacter actinomycetemcomitans* and anaerobic pathogens in subgingival microbiota (Ishikawa et al. 2002, Lee et al. 2003, Takeuchi et al. 2003, Kamma et al. 2004, Faveri et al. 2008). Previous investigations demonstrated that suppression of these bacteria below the detection level results in remarkable improvement in clinical parameters (Haffajee et al. 1997, Winkel et al. 1998).

To the best of our knowledge a limited number of randomized controlled clinical trials focused on the additional effects of adjunctive amoxicillin-metronidazole in the non-surgical treatment of G-AgP patients (Guerrero et al. 2005, Xajiegeorgiou et al. 2006, Kaner et al. 2007a, Yek et al. 2010, Mestnik et al. 2010) and no information is available regarding the added benefits to the OSFMD. Thus, the aim of the present investigation was to evaluate the

adjunctive clinical and microbiological effects of the administration of amoxicillin and metronidazole in the OSFMD protocol in G-AgP patients.

MATERIALS AND METHODS

Study design

The present study was a randomized, double-blind, placebo-controlled, single centre, 6-month clinical trial. Research was conducted according to the principles outlined in the Declaration of Helsinki on experimentation involving human subjects. The protocol was approved by the Ethics Committee of the University of Turin, Italy. All patients were informed individually about the nature, potential risks and benefits of the proposed treatment and signed informed consent forms.

Population screening

Consecutive patients from those referred for treatment to the Division of Periodontology, Dental School, University of Turin, were recruited after a screening examination that included a full medical and dental history, intraoral examination, full-mouth periodontal probing and radiographs. Subjects who were invited to participate met the inclusion criteria of G-AgP described by Armitage (1999), as follows: 1) good general health; 2) generalized interproximal attachment loss involving at least 3 teeth apart from molars and first incisors; 3) amount of microbial deposits inconsistent with the severity of periodontal tissue destruction; 4) familial aggregation (during the anamneses patients were asked whether at least one member of the family presented with a history of periodontal disease).

In addition all patients had to present with a minimum of 20 teeth excluding teeth indicated for extraction and ≥ two sites around at least 12 teeth with clinical attachment level (CAL) and probing depth ≥ 6 mm. Exclusion criteria included: smoking habits, medical disorders that require prophylactic antibiotic coverage or that could influence the progression or treatment of periodontitis (i.e. diabetes mellitus, autoimmune dysfunctions, bone metabolic

diseases); consumption of drugs known to affect periodontal status (anticonvulsants, immunosuppressant, calcium channel blockers); antibiotic therapy within the last 6 months; long-term administration of anti-inflammatory medications; allergy to penicillin and/or metronidazole; periodontal treatment in the previous 6 months; pregnancy and lactation. Each accepted participant received a patient number in ascending order.

Sample size calculation

According to a systematic review (Herrera et al. 2002) adjunctive systemic antibiotics may result in additional reduction of approximately 0.5 mm for mean full-mouth PD when compared to scaling and root planning (SRP) alone. Thus, a difference of 0.5 mm between groups for mean full-mouth PD reduction after 6 months was considered to be clinically relevant. The sample size calculation determined that 17 subjects per treatment group would provide 80% power to detect a true difference of 0.5 mm between test and placebo, assuming 0.5 mm as the common standard deviation. To compensate for possible drop-outs 21 patients were recruited per treatment group.

Randomization and allocation concealment

Before the first session of the OSFMD, subjects were randomly assigned by a computer-generated list to receive one of the two treatments (OSMFD and systemic antimicrobials or OSFMD and placebo). A balance random permuted block approach was used to prepare the randomization tables in order to avoid unequal balance between the two treatment groups. Allocation was implemented by a person not involved in the study. Using this list, treatment assignments were distributed to numbered opaque envelopes. The plastic bags containing two identical opaque bottles each (19 bottles with amoxicillin 500 mg, 19 with metronidazole 500 mg, 20 with amoxicillin placebo and 20 with metronidazole placebo) were sent to the clinician responsible for the research (M.A.). He opened the sealed envelope and marked the subject number on the neutral bottles containing the test or the placebo medications. The

plastic bags were given by a study assistant to each patient. The treatment codes of the study were not available to the treating investigator and to the examiner until the data were analysed by the statistician.

Clinical measurements

Clinical recordings were performed by one masked and calibrated examiner (F.R.) using a standardized periodontal probe with 1-mm markings (PCP-UNC 15, Hu-Friedy, Chicago, IL, USA), and rounded up to the nearest mm. The following parameters were assessed at six sites around all present teeth: presence/absence of plaque (IP), presence/absence of bleeding on probing (BOP), probing depth (PD), recession of the gingival margin (REC), and CAL, defined as the algebraic sum of PD and REC. The percentages of total surfaces which revealed the presence of plaque or BOP within each subject were expressed as full mouth plaque score (FMPS) and full mouth bleeding score (FMBS).

Examiner calibration

A total of 6 non-study patients with G-AgP were recruited for the calibration exercise. The single designated examiner (F.R.) recorded full-mouth PD and REC with an interval of 24 h between the first and the second recording. The intra-examiner repeatability for CAL was measured. The K coefficient (± 1 mm) was 0.93.

Microbiological examination

Subgingival plaque samples were collected at 4 sites, 2 with moderate PD (4-5 mm) and the other 2 with severe PD (≥ 6 mm), with no endodontic or furcation involvement. Periodontal sites for bacterial sampling were randomly selected. After isolating with cotton rolls, drying, and removal of supragingival plaque, subgingival samples were taken by inserting two sterile paper points into the apical extent of each selected pocket, placed in a sterile Eppendorf tube containing 1 ml of a prereduced transport medium (Ringer solution) and sent to the laboratory of the Department of Microbiology. After centrifugation for 1 min at 12,000 *g* the supernatant

was removed and the pellets were stored at –20°C until processing and analysis by polymerase chain reaction (PCR). The microbiologist who performed analysis of subgingival samples was not aware of the treatment that the patient had received (coded samples). The PCR procedures were described in a previous report (Aimetti et al. 2007). Primers for *Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia*, *Prevotella intermedia*, *Treponema denticola*, and *Porphyromonas gingivalis* were designed on the basis of 16S rRNA sequence by Ashimoto et al. (1996). PCR amplification products were analyzed by 2% agarose gel electrophoresis in Tris-borate EDTA buffer. The gel was stained with ethidium bromide (0.5μg/ml), and photographed under a UV light transilluminator (Becton, Dickinson, Franklin Lakes, NJ, USA). Species-specific primers were previously tested for cross-reactivity with other closely related species by Ashimoto et al. (1996). In addition, all primer sequences were compared with the GenBank to further ensure their specificity. The PCR detection limit was 10 genome equivalents.

Non-surgical treatment

After an initial screening visit for recruitment, all patients underwent a session of supragingival scaling and polishing and received instructions in proper self-performed plaque control measures, including special instructions in the Bass technique and interproximal cleaning with dental floss and interdental brushes. They were also motivated to brush the tongue dorsum once a day. At the end of the session, baseline clinical measurements were recorded except for both plaque index and bleeding index that were assessed immediately prior to scaling. One week later, patients were subjected to OSFMD according the protocol by Quirynen et al. (1995) modified by Bollen et al. (1998). Full-mouth subgingival SRP were performed in two sessions within 24 h by a single experienced therapist (G. N.) using hand instruments (Gracey curettes, Hu-Friedy) and ultrasonic scalers (Cavitron Select, Dentsply, York, PA, USA). The therapist was blinded to the treatment assignment. Subgingival

instrumentation was performed under local anesthesia without a time limit until the root surface felt smooth and clean to an explorer tip. Mechanical debridement was supplemented by the disinfection of intra-oral niches. Immediately after each instrumentation session the dorsum of the tongue was brushed by the patient with a 1% CHX gel for 1 min (Corsodyl® gel, GlaxoSmithKline Consumer Healthcare, Baranzate di Bollate (MI), Italy), the mouth was rinsed twice with a 0.2% CHX solution for 1 min (Corsodyl® mouthrinse), the farynx was sprayed (4x tonsil) with a 0.2% CHX spray (Corsodyl® spray), and all pockets were irrigated (3 times within 10 min) with a 1% CHX gel. This subgingival application was repeated 8 days after. Test and control subjects were instructed to use a 0.2% CHX rinse twice a day for 1 min and to spray the tonsils twice daily with a 0.2% CHX for 2 months post-treatment. All patients were provided with both 0.2% CHX solution and spray to use during the experimental period.

The CHX rinses and the antibiotic and placebo therapies started immediately after the first session of OSFMD. Test subjects received systemic antibiotics (amoxicillin 500 mg and metronidazole 500 mg), while control subjects received the placebo medications. All subjects were advised to use placebo or antibiotic three times a day for 7 days (Guerrero et al. 2005, Yek et al. 2010).

Evaluation of adverse events and compliance

The study assistant called each subject every two days by telephone to remind him to take the placebo or antimicrobial medications. Adverse effects and compliance with medication intake were recorded by the same study assistant not involved in the randomization process. Subjects were asked to return the bottles of medications the day after the last capsule had been taken and the missing capsules were registered. At the same time subjects were asked to answer a questionnaire about any side effect that could be associated with the medication/placebo intake. Compliance with the use of CHX was evaluated at each visit scheduled during the first

2 months post-operatively. The study assistant recorded the presence of CHX staining on both teeth and tongue dorsum according to the criteria of the stain index described by Lobene (1968). CHX staining was eliminated by polishing at each session.

Post-treatment controls

During the post-treatment appointments, the patients' oral hygiene standards were reviewed and oral hygiene procedures were reinforced. Moreover, all patients received full-mouth supragingival debridement and professional tooth cleaning. No subgingival instrumentation was performed until the end of the study in order to not interfere with subgingival microbiota. The appointments were on a 2-week interval during the first 6 weeks post-operatively and every 2 months up to the 6-month evaluation.

Re-assessment examinations

Full-mouth clinical examination was performed at 3 and 6 months after the completion of the second session of OSFMD. During these appointments the clinical periodontal parameters assessed at baseline were recorded again. Bacterial sampling was repeated at 15 days, 3 months and 6 months.

Statistical analysis

The primary outcome measure of the study was reduction in mean full-mouth PD values. Secondary outcomes included reduction in mean CAL, decrease of FMPS and FMBS values, reduction in PD values at different initial PD categories, decrease of the proportion of sites with PD ≥ 5 mm, change of proportions of moderate and deep sites harbouring periodontal pathogens.

For clinical parameters a mean value per patient and per follow-up moment was calculated in order to maintain the patient as the statistical unit. The balancing of experimental groups by demographic and clinical parameters was tested by Student's *t*-test (age, PD, CAL, number of sites with PD ≥5 mm) and the Mann-Whitney *U* test (FMPS, FMBS). The groups were

compared with respect to categorical variable using the chi-square analysis (gender, ethnicity). To test the effect of time and treatment on response variables within each group the repeated measures of analysis of variance (PD, CAL, number of sites with PD ≥5 mm) and the Friedman's test (FMPS, FMBS) were used. Multiple comparisons were conducted with the post-hoc tests (Newman-Keuls test and Dunn test). The proportions of sites with PD ≥ 5 mm were analysed by the chi-square test.

Subsequently the Student's *t*-test for independent samples and the Mann-Whitney *U* test were used for pairwise comparisons between the groups. For categorical data the chi-square test was applied. The Bonferroni correction was used to confirm any significant values arising from multiple comparisons.

As further analysis the changes in PD and CAL in initially moderate (4-5 mm) and deep (≥ 6 mm) pocket sites were calculated and tested to explore intra- and inter-treatment differences by parametric methods (ANOVA, Newman-Keuls post hoc test and Bonferroni-corrected *t*-test for independent samples).

Finally, microbiological data were analyzed with the subject and the site as the observational unit. The number of subjects positive for the investigated species were calculated at baseline, 15 days, 3 and 6 months after treatment. The patient was scored positive for a microorganism if at least one out of the four sampled sites harbored this microorganism. The prevalence of the five pathogens was analyzed separately in sites with moderate and severe PD. When the PCR did not detect bacteria, the value for the statistical evaluation was zero. Microbiological data were analyzed by applying the chi-square test except when expected counts were less than five where the Fisher's exact test was used. An experimental level of significance (alpha) was set at 0.05.

Results

The flow chart of the experimental design is presented in Fig. 1. Sixty-six subjects were

assessed for their eligibility from January 2009 to February 2010. Of these, 24 were excluded: 20 did not meet the inclusion criteria, while the other 4 refused to participate. Forty-two subjects were enrolled in the study. Three patients had to be excluded after enrolment. Two subjects did not return to the pre-treatment session of supragingival scaling and polishing for unknown reasons and one subject dropped out of the study at the same appointment due to antibiotic intake for unrelated systemic illness. Random assignment resulted in 39 patients (20 in the placebo group and 19 in the test group).

Table 1 summarizes the demographic characteristics and Tables 2 and 3 present the mean full-mouth values for the clinical parameters at baseline, 3 and 6 months after therapy. There were no relevant differences between test and placebo group at baseline relative to gender, age and ethnic distribution. There were also no statistically significant differences in clinical parameters ($p>0.05$).

Clinical results

Regarding compliance with medication intake, all participants returned the bottles and a 100% compliance was achieved. No serious adverse effects were observed or reported from antibiotic intake other than a mild gastrointestinal discomfort in three subjects. None had to stop the intake of medications. All test and placebo patients had similar amount of CHX staining at the end of each scoring visit. Dental stain of light to moderate intensity was observed on the buccal surfaces of incisive, canine and premolar teeth and covered one-third to two-thirds of the surface area. Similar findings were seen when considering tongue stain.

Full mouth plaque score and FMBS decreased significantly in both groups at the end of 3 and 6 months compared to baseline values ($p<0.001$, Table 2). Plaque scores remained below 15% during the experimental phase in both groups ($p < 0.001$). Between-group analysis at each time point did not indicate relevant differences. When analysing FMBS, the improvement was greater in the test group. At 3 months after therapy 9.3% and 16.6% of the sites in the test

and control groups, respectively, exhibited BOP ($p< 0.001$). The percentages remained fairly unchanged at 6 months.

As reported in Table 3, both therapies led to a statistically significant decrease in overall mean PD and CAL at 3 and 6 months compared to baseline ($p< 0.001$). The greatest reduction in the mean PD occurred during the first 3 months after treatment, whereas no further significant changes were observed within the treatment groups between 3 and 6 months ($p>0.05$). After 6 months the mean PD reduction and the mean clinical attachment gain amounted to 1.6 ± 0.5 mm and 1.4 ± 0.6 mm, respectively, in the test group and to 1.2 ±0.3 and 1.0 ± 0.3 mm, respectively, in the placebo group. The differences between treatment modalities were statistically significant at months 3 (PD, $p=0.015$) and 6 (PD, $p= 0.01$ and CAL, $p= 0.01$) favouring the test group. An adjunctive benefit of 0.4± 0.1 mm for PD reduction was observed in the amoxicillin-metronidazole group at 3- and 6- month examinations compared to baseline ($p_{0-3}=0.009$, $p_{0-6}=0.004$) and of 0.4 mm ± 0.2 for CAL changes at 6 months ($p_{0-6}=0.01$).

A separate analysis of initially moderate (4-5 mm) and deep (≥ 6 mm) pocket sites was performed. Within the treatment groups significant PD and CAL changes were found over the course of the study in both moderate and deep pocket sites ($p<0.001$). At moderate pockets, differences between treatment groups reached statistical significance only for clinical attachment gain at 6-month evaluation ($p=0.009$). At pockets initially ≥ 6 mm PD reduction and clinical attachment gain were significantly greater in test subjects at both 3- and 6-month examination. At 3 months the difference between test and placebo treatment was of 0.6 mm for PD reduction ($p=0.004$) and of 0.8 mm for CAL changes ($p<0.001$). These values were maintained at 6 months.

Regarding both the number and the proportion of sites with PD ≥ 5 mm, a statistically significant greater decrease was observed in the amoxicillin-metronidazole group in

comparison with the placebo group over the course of the study ($p<0.001$). At baseline subjects in the test group had an average of 92.2 ± 25.1 sites with PD ≥ 5 mm and subjects in the placebo group had 89.3 ± 22.4 sites in this category. At 3 months the average number of such sites was 29.7 ± 8.1 per patient in the test group compared to 48.3 ± 14.7 in the control one. These values were fairly unchanged at 6 months (28.3 ± 7.7 in the test group *versus* 46.2 ± 13.4 in the placebo group). When considering the proportion of sites with PD ≥ 5 mm, at baseline 56.6% of the test sites had PD ≥ 5 mm and 54.9 % of the control sites did. At 3-month evaluation residual PDs ≥ 5 mm were observed in 18.6% of the test sites compared to 29.7% of the control sites. These values were maintained at 6 months (17.7% in the test group *versus* 28.4% in the control one).

Microbiological observations

Change in the prevalence of bacterial species

Table 4 presents the frequency of subjects colonized by the five target periodontal pathogens over the 6-month period in the two treatment groups. Even though no differences in the detection frequencies of all bacterial species were observed between the two groups at baseline, the test group showed a significantly greater decrease in the percentage of patients colonized by both *A. actinomycetemcomitans* and the red complex species in comparison with the control group. No difference was observed between the two treatment groups for *P. intermedia* at any time point ($p>0.05$). The greatest decrease was observed for *A. actinomycetemcomitans.* At 6 months after treatment three subjects in the test group tested positive for this species compared to 13 subjects in the control group ($p=0.005$).

Figure 2 and Figure 3 summarize the number of moderate and deep sampled pockets that harboured the target species at each time point. During the experimental period the combination OSFMD and antibiotic intake was more effective than OSFMD and placebo in

decreasing the number of sites positive for all the monitored species. The difference between test and placebo groups was statistically significant at 6-month evaluation for moderate pockets and at 3 and 6 months for deep pockets sites. With regard to 4-5 mm sampled pockets 25 (65.8%) test sites compared to 15 (37.5%) control sites were free of pathogens at 6 months ($p< 0.001$). Among pockets initially ≥ 6 mm 26 (68.4%) and 22 (57.9%) test sites were free of pathogens at 3 and 6-month examination compared to 14 (35%) and 6 (15%) control sites, respectively ($p= 0.01$ at 3 months and $p < 0.001$ at 6 months).

When analyzing the pattern of each bacterial species, the administration of antimicrobials resulted in the elimination of *A. actinomycetemcomitans* at both moderate and deep sites 15 days after the second session of the OSFMD. No significant differences were observed between treatments in the prevalence of *P. intermedia, P. gingivalis, T. forsythia* and *T. denticola* at this time point neither in moderate nor in deep sites ($p >0.05$). Six months from baseline a recolonization or regrowth of all the investigated species was noted. At moderate sites (Figure 2) no differences were detected between groups ($p>0.05$) with the exception of *A. actinomycetemcomitans* that was isolated in 5.3% of test sites compared to 35% of the control ones ($p=0.02$). At deep sites (Figure 3) the combined therapy was more efficient than OSFMD and placebo to achieve a lower prevalence of *A. actinomycetemcomitans* and *T. denticola* at 3 ($p= 0.03$ and $p= 0.04$, respectively) and 6 months ($p= 0.02$ and $p= 0.03$, respectively), and of *T. forsythia* at 6-month examination ($p= 0.04$). The proportions of *P. gingivalis* and *P. intermedia* were not significantly lower in the test sites than in the placebo sites at any time point ($p>0.05$).

Discussion

The OSFMD has been proposed as a therapeutic approach particularly indicated for patients who experienced advanced and rapidly progressing periodontal diseases (Quirynen et al. 2006, Teughels et al. 2009). Its benefits could become more significant with the adjunctive

administration of systemic antibiotics. The unavailability of guidelines for the use of systemically administered antimicrobials in G-AgP patients have led to conflicting decisions on the selection of different therapeutic approaches (Herrera et al. 2008, Shaddox & Walker 2009).

In the current study we evaluated the 6-month clinical and microbiological effects of systemic amoxicillin-metronidazole or placebos combined with the OSFMD protocol (Quirynen et al. 1995, Bollen et al. 1998). Our data indicated that the adjunctive antimicrobial therapy achieved significantly greater improvement in clinical parameters than OSFMD and placebo. At 6 months patients who took systemic antibiotics presented a lower percentage of bleeding sites, a greater reduction in full-mouth PD, and fewer sites with PD $\geq$ 5 mm in comparison with the placebo group. It is important to point out that plaque levels were maintained at a low level (< 15%) through the study period in both treatment groups, indicating both good oral hygiene performance of all patients and successful re-motivation during post-treatment controls. Recall appointments for reinforcement of oral hygiene measures and supragingival professional debridement were scheduled every two weeks during the first 6 weeks and at 2-month interval up to the 6-month evaluation. This protocol might be relevant to the reduction of plaque because full-mouth instrumentation is performed in two visits and thus the therapist has fewer opportunities to deliver, check, and reinforce the necessary oral hygiene instructions (Teughels et al. 2009). Moreover, in according to the full-mouth disinfection approach (Quirynen et al. 1995), patients had not to reach an appropriate level of plaque control before receiving the first session of OSFMD.

The results obtained in the control group were within the range expected from the OSFMD in aggressive periodontitis patients (Mongardini et al. 1999, Aimetti et al. 2011). Six months after this non-surgical approach the mean full-mouth PD reduction was 1.2 mm and the mean full-mouth clinical attachment gain amounted to 1.0 mm. At this time the mean PD reduction

was 1.4 mm and 2.4 mm for initially moderate and deep pockets, respectively. These results are better or comparable to those reported after conventional SRP in G-AgP patients with similar baseline mean PD. Hughes et al. (2006) reported a mean full-mouth PD reduction of 0.4 mm at 10 weeks, whereas Rosalem et al. (2011) and Xajigeorgiou et al. (2006) of 0.69 mm at 3 and 6 months, respectively. Sigush et al. (2001) showed that in pockets initially >6 mm a 2.3 mm PD reduction was achieved 6 months after treatment.

The clearest advantage of antibiotics over placebo was noted on inflammatory values and in pockets initially ≥ 6 mm. In the test group the FMBS remained below 10%, whereas in the control group the percentage ranged between 12% and 20% over all the experimental period. In deep pocket sites the test treatment resulted in additional 0.7 mm in mean PD reduction and 1.00 mm in clinical attachment gain, while not statistically significant differences were observed in moderate periodontal sites. At 6 months post-therapy the proportion of sites with PD ≥ 5 mm was 17.7% in the test group compared to 28.4% in the placebo group. As previously demonstrated the effect of the antibiotics was particularly evident at deeper pockets where mechanical debridement was less effective (Guerrero et al. 2005).

Only a few randomized controlled clinical trials focused on the additional effects of adjunctive amoxicillin-metronidazole in the therapy for well-defined G-AgP in patients with severe disease (Guerrero et al. 2005, Xajiegeorgiou et al. 2006, Kaner et al. 2007a, Moreira & Flores-Filho 2007, Mestnik et al. 2010, Yek et al. 2010, Griffiths et al. 2011). In these reports the combination of antimicrobials with quadrant or full-mouth SRP resulted in average values of 1.2-1.6 mm for PD reduction and of 0.5-1.3 mm for CAL gain over a 3- or 6-month period. A direct comparison of these measures of clinical outcomes is unfeasible because of discrepancy among study designs, especially regarding the schedule for debridement, time of clinical measurements, dosage and timing of drug administration. The variation between studies concerning the time of antimicrobials administration is noticeable, as medication was

started simultaneously with SRP (Guerrero et al. 2005, Yek et al. 2010, Mestnik et al. 2010), 1 week (Kaner et al. 2007a), 6 weeks after SRP had been completed (Xajiegeorgiou et al. 2006) and during re-treatment (Griffiths et al. 2011). Recent investigations report greater clinical improvements when the adjunctive agent is used concomitantly with non-surgical treatment (Kaner at al. 2007b, Griffiths et al. 2011). In the present investigation the administration of antibiotics started on the same day of full-mouth periodontal instrumentation, signifying that all quadrants were equally benefited by the antimicrobial therapy. This eliminates the problem associated with the timing of drug administration during quadrant-wise SRP (Killoy 2002).

In the present study we prescribed 500 mg of both antibiotics three times a day for 7 days. This dosage had been reported in recent randomized controlled clinical trials (Guerrero et al. 2005, Xajiegeorgiou et al. 2006) to reach and maintain effective antimicrobial concentration in body fluids (Winkel et al. 1998). Previous studies which used a moderate dose of metronidazole (250 mg) combined with 500 mg of amoxicillin reported lack of significant adjunctive benefits over SRP alone (Heller et al. 2011).

No serious adverse effects were reported from antibiotic intake other than a mild gastrointestinal discomfort in three subjects (16%). This was in agreement with the results of previous investigations (Xajiegeorgiou et al. 2006, Mestnik et al. 2010).

The clinical outcomes were supported by the microbiological findings. The baseline profile was comparable in both treatment groups and was consistent with data reported in the literature (Mullally et al. 2000, Albandar et al. 1997, Kamma et al. 2004, Xajigeorgiou et al. 2006), except for the lower prevalence by Kamma et al. (2004) for *A. actinomycetemcomitans* in G-AgP patients of Greek origin. Several papers have suggested varying species dominating the microbiota of G-AgP patients, according to the ethnicity (Rylen & Kilian 2008). The systemic administration of amoxicillin-metronidazole was more effective in decreasing the

number of sites positive for all the monitored species than OSFMD alone. In the test group all the target bacteria were suppressed below the detection level in 65.8% and 57.9% of moderate and deep sites, respectively, at 6-month evaluation. While, the percentage of moderate and deep sites free of pathogens amounted to 37.5% and 15% in the control group at the same time point ($p<0.001$).

When individual species were analysed the most significant difference between the combined therapy and the OSFMD alone was the pronounced effect of the antibiotic combination on *A. actinomycetemcomitans*. Previous investigations on the effects of SRP and OSFMD on the subgingival microflora reported conflicting results about the ability to eradicate or suppress important periodontal pathogens. *T. forsythia* and especially *A. actinomycetemcomitans* have been shown to remain in periodontal pockets after non-surgical therapy (Takamatsu et al. 1999, Mombelli et al. 2000, Koshy et al. 2005). Therefore, the combination of amoxicillin and metronidazole was originally proposed for the elimination of *A. actinomycetemcomitans* (van Winkelhoff et al. 1989). In the present investigation, *A. actinomycetemcomitans* was eliminated from 10 subjects in the test group and from 2 subjects in the control group at 6 months post-therapy. When bacterial samples were analysed according to initial PD categories, the test group showed complete suppression of *A. actinomycetemcomitans* 15 days following treatment and recolonization by this species in 5.3% and 7.9% of initially moderate and deep periodontal pockets, respectively, at 6 months. Few studies have evaluated the added effects of high dosage of amoxicillin-metronidazole on the subgingival microbiota of G-AgP patients. They reported complete suppression of *A.actinomycetemcomitans* in four subjects at 3 months post-therapy (Mestnik et al. 2010) or in one subject at 6-month evaluation (Xajiegeorgiou et al. 2006). The variations in the complexity of the subgingival microbiota (Haffajee et al. 2005) as well as in the antibiotic susceptibility of periodontal pathogens in distinct geographic locations (van Winkelhoff et al 2005) might in part explain

the differences in the effectiveness of systemic antimicrobials.

In contrast to the findings of Yek et al. (2010) who reported eradication of *T. forsythia* in the test group after SRP and a 7-days course of systemic metronidazole (500 mg, tid) and amoxicillin (500 mg, tid), in the current investigation the combined therapy was unable to completely eliminate *T. forsythia* in both moderate and deep sites. Nevertheless, it prevented its tendency to increase over the 6- month period in deep sites when compared to the control group. No differences between the two treatment groups were observed at pockets initially 4-5 mm deep. A similar pattern was observed for *T. denticola.*

The prevalence of sampled sites colonized by *P. gingivalis* and *P. intermedia* was not significantly lower in the test group than in the OSFMD group neither at 3- nor at 6-month examination. Our data are in agreement with the findings by Yek et al. (2010) and Heller et al. (2011) who reported a reduction in the level of *P. gingivalis* over a 6-month period but they did not detect any difference between the treatment groups. It is interesting to point out that when a per-patient analysis was performed a significant difference was detected in the proportion of subjects positive for *P. gingivalis* between the two treatment groups. At 6-month examination 6 subjects in the test group in comparison with 14 subjects in the placebo group were colonized by this periodontal pathogen. In addition test subjects who tested positive for *P. gingivalis* presented this species in 2 (four subjects) and 3 (two subjects) sampled sites. Differences in host response, in bacterial susceptibility to antibiotic agents and in bacterial clones could partly account for variations of treatment efficacy.

A shortcoming of the present investigation was the evaluation of the adherence to antibiotic/placebo medications. Patients were asked to return the bottles of medications the day after the intake of the last capsule and the remaining pills were counted (Guerrero et al. 2005, Heller et al. 2011, Griffiths et al. 2011). Guerrero et al. (2007) reported that this way of monitoring patients compliance may underestimate non-adherence or partial adherence. Thus,

we called patients every two days to remind them to take the placebo or antimicrobial medications (Haas et al. 2008, Mestnik et al. 2010).

Another aspect to be considered is the lack of microbiological entry criteria. In agreement with previous investigations the antibiotic regimen was applied empirically (Mombelli 2005, Heller et al. 2011, Mestnik et al. 2010). It is interesting to point out that as previously reported by Yek et al. (2010) in the present investigations G-AgP patients presented at baseline comparable frequency of detection of the five periodontal pathogens. In addition, the percentage of patients positive for *A. actinomycetemcomitans* was high. In such patients, as previously reported by Xajigeorgiou et al. (2006), the addition of amoxicillin enhances clinical as well as microbiological effects of metronidazole alone.

In conclusion, the OSFMD represents a viable approach to deal with G-AgP patients. When combined with systemic amoxicillin plus metronidazole it resulted in statistically significantly greater improvement in FMBS, reduction in full-mouth PD and decrease in the number of sites with PD $\geq$ 5 mm. These observations were valid for both 3- and 6-month evaluations after the completion of active treatment. Clinical results are supported by microbiological findings.

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Table 1. Demographic characteristics at baseline (mean ± SD)

Parameter	Test group (n=19)	Placebo group (n=20)	Difference between groups P-value
Age (years)	36.3 ± 3.2	35.7 ± 2.8	NS*
Females (%)	58	50	NS**
Caucasians (%)	79	85	NS**

NS=difference between groups is not statistically significant (P>0.05)
* *T*-test for independent samples **X^2- test

Table 2. Changes of FMPS and FMBS at baseline, 3 and 6 months

Clinical parameter	Group	Baseline	3 months	6 months
FMPS (%)	**Test**	59.7 ± 19.0*	12.8 ± 2.1**	13.0 ± 1.6**
	Placebo	60.4 ± 27.1*	12.6 ± 2.4**	12.1 ± 2.1**
Differences between groups		NS†	NS‡	NS‡
FMBS (%)	**Test**	61.5 ± 17.7*	9.3 ± 0.8**	9.4 ± 0.6**
	Placebo	56.2± 18.2*	16.6 ± 5.0**	15.5 ± 3.8**
Differences between groups		NS†	P<0.001‡	P<0.001‡

FMPS full-mouth plaque score; FMBS full-mouth bleeding score
NS=difference between groups is not statistically significant (P>0.05)
*P<0 .001; p-values represent changes among the three time points (Friedman's test)
**P<0.001 p-values represent longitudinal changes from baseline (Dunn test)
†Mann-Whitney U test; ‡Bonferroni-corrected Mann-Whitney U test.

Table 3. Changes (mean ± SD) of probing depth and clinical attachment level

Variable	Group	Baseline	3 months	$\Delta_{0\text{-}3\ months}$	6 months	$\Delta_{0\text{-}6\ months}$
Full-mouth PD (mm)	**Test**	4.3 ± 1.1*	2.8 ± 0.6	1.5 ± 0.5**	2.7 ± 0.6	1.6 ± 0.5**
	Placebo	4.5 ± 1.1*	3.4 ± 0.8	1.1 ± 0.4**	3.3 ± 0.8	1.2 ± 0.3**
Difference between groups		NS	P=0.015§		P=0.01§	
PD (mm) at sites with initial pockets 4-5 mm	**Test**	4.7 ± 0.2*	3.0 ± 0.3	1.7 ± 0.3**	2.9 ± 0.5	1.8 ± 0.3**
	Placebo	4.4 ± 0.1*	2.9 ± 0.3	1.5 ± 0.3**	3.0 ± 0.4	1.4 ± 0.3**
Difference between groups		NS	NS§		NS§	
PD (mm) at sites with initial pockets ≥ 6 mm	**Test**	6.9 ± 0.7*	3.8 ± 0.4	3.1 ± 0.7**	3.8 ± 0.8	3.1 ± 0.6**
	Placebo	7.1 ± 0.6*	4.6 ± 0.7	2.5 ± 0.5**	4.7 ± 0.8	2.4 ± 0.7**
Difference between groups		NS	P=0.004§		P=0.003§	
Full-mouth CAL (mm)	**Test**	4.7 ± 1.1*	3.4 ± 0.8	1.3 ± 0.5**	3.3 ± 0.6	1.4 ± 0.6**
	Placebo	5.0 ± 1.2*	4.0 ± 1.1	1.0 ± 0.3**	4.0 ± 1.0	1.0 ± 0.3**
Difference between groups		NS	NS§		P=0.01§	
CAL (mm) at sites with initial pockets 4-5 mm	**Test**	5.0 ± 0.4*	3.5 ± 0.4	1.5 ± 0.3**	3.4 ± 0.4	1.6 ± 0.3**
	Placebo	4.8 ± 0.5*	3.4 ± 0.4	1.4 ± 0.4**	3.8 ± 0.5	1.0 ± 0.4**
Difference between groups		NS	NS§		P=0.009§	
CAL (mm) at sites with initial pockets ≥ 6 mm	**Test**	7.7 ± 0.7*	4.7 ± 0.6	3.0 ± 0.7**	4.7 ± 0.8**	3.0 ± 0.7**
	Placebo	7.8 ± 0.6*	5.6 ± 0.8	2.2 ± 0.7**	5.8 ± 1.0**	2.0 ± 0.8**
Difference between groups		NS	P<0.001§		P<0.001§	

PD probing depth; CAL clinical attachment level
NS=difference between groups is not statistically significant (P>0.05)
*P<0 .001; p-values represent changes among the three time points (ANOVA)
**P<0.001 p-values represent longitudinal changes from baseline (Newman-Keuls test)
§Bonferroni-corrected *t*-test

Table 4. Number (percentage) of patients positive for the five periodontal pathogens at various time points (X^2 –test).

	Group	Baseline	15 days after OSFMF	3 months	6 months
A. actinomycetemcomitans	Test	13 (68.4)	0*	2 (10.5)*	3 (15.8)**
	Placebo	15 (75.0)	7 (35.0)§	8 (40.0)#	13 (65.0)#
Differences between groups		NS	P=0.008	P=0.04	P=0.005
P. intermedia	Test	13 (68.4)	3 (15.8)**	4 (21.1)**	5 (26.3)§
		12 (60.0)	4 (20.0)§	5 (25.0) §	7 (35.0)#
Differences between groups		NS	NS	NS	NS
P. gingivalis	Test	14 (73.7)	3 (15.8)**	4 (21.1)**	6 (31.6)*
	Placebo	16 (80.0)	5 (25.0)**	6 (30.0)**	14 (70.0)#
Differences between groups		NS	NS	NS	P=0.03
T. forsythia	Test	16 (84.2)	4 (21.1)*	6 (31.6)**	6 (31.6)**
	Placebo	17 (85.0)	5 (25.0)*	8 (40.0)**	14 (70.0)#
Differences between groups		NS	NS	NS	P=0.04
T. denticola	Test	18 (94.7)	5 (26.3)*	7 (36.8)*	7 (36.8)*
	Placebo	18 (90.0)	5 (25.0)*	11 (55.0)§	15 (75.0)#
Differences between groups		NS	NS	NS	P=0.03

NS=difference between groups is not statistically significant (P>0.05)
*P<0 .0001; p-values represent longitudinal changes from baseline
**P<0.005 p-values represent longitudinal changes from baseline
§P<0.05; p-values represent longitudinal changes from baseline
#P>0.05; p-values represent longitudinal changes from baseline

Fig. 1 Flow chart of the study design.

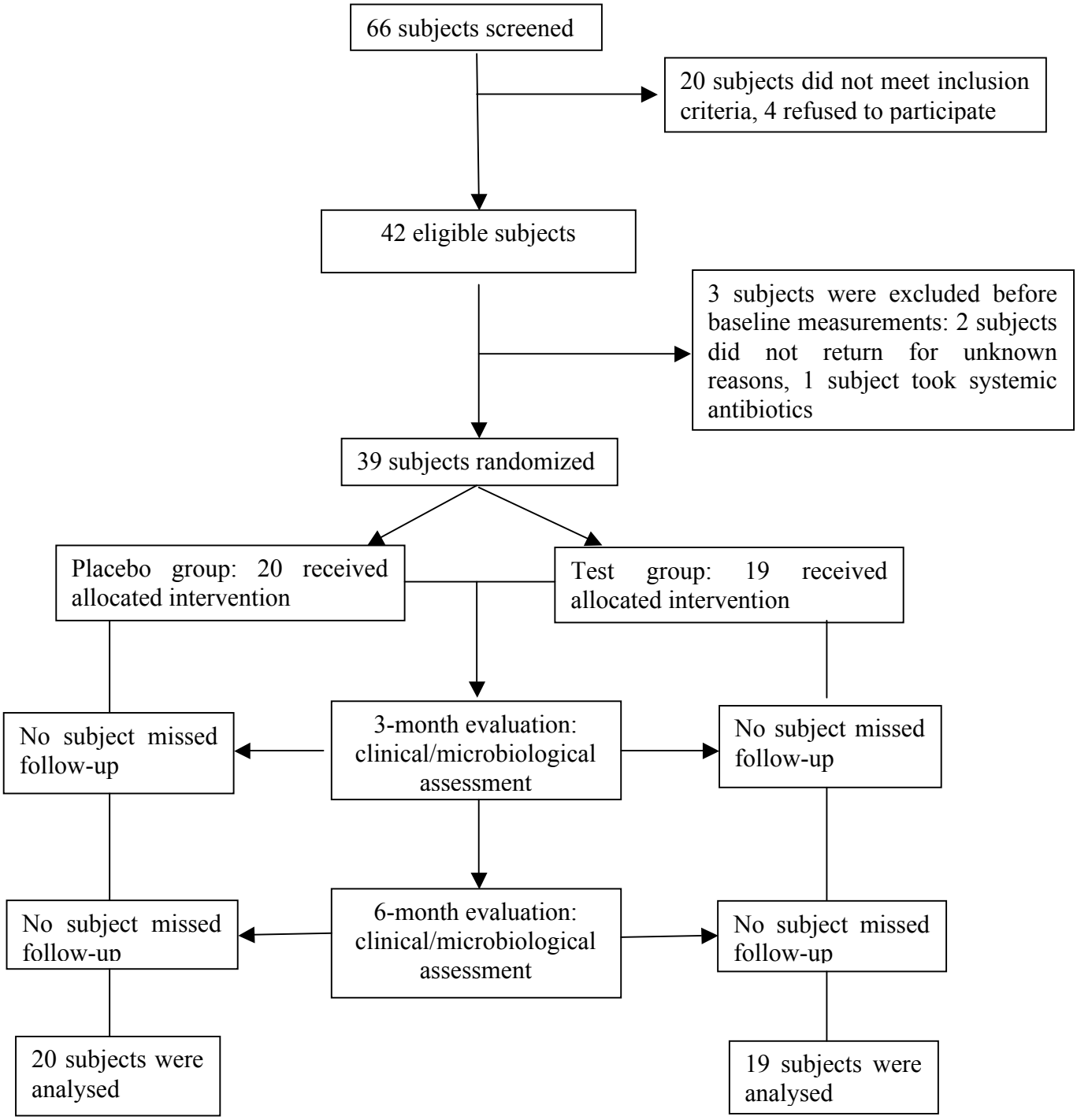

Figure 2. Prevalence of single species in moderate periodontal pockets in test and placebo group at various time points (number of sites evaluated)

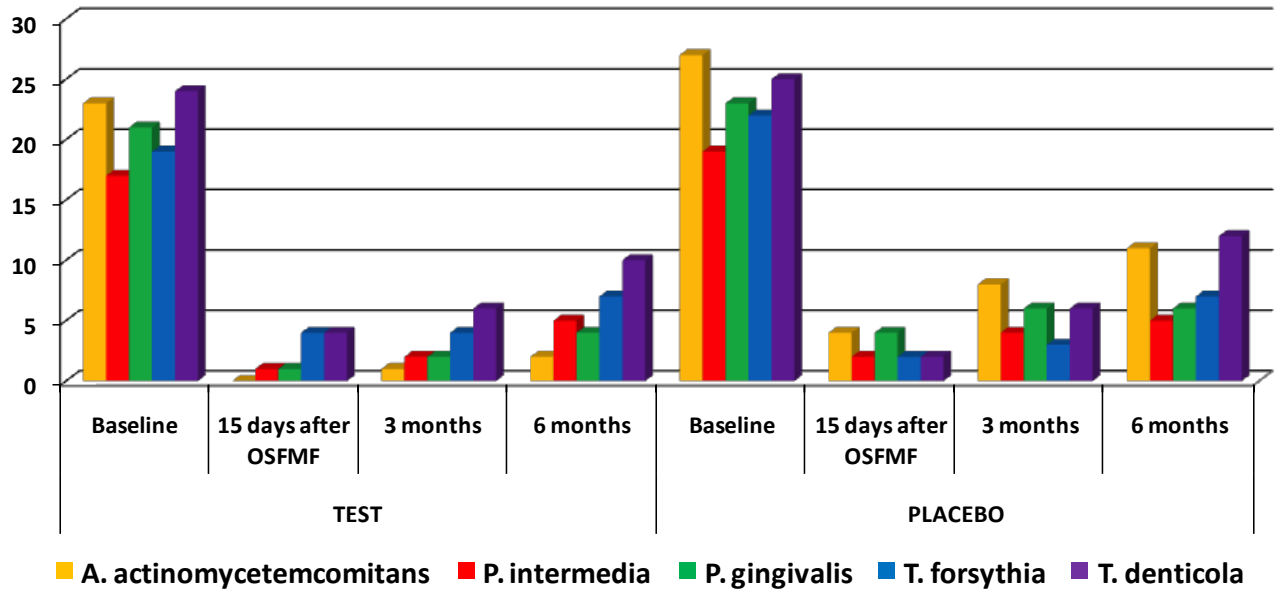

Figure 3. Prevalence of single species in deep periodontal pockets in test and placebo group at various time points (number of sites evaluated)