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Title:

Clinical, microbiological and immunological effects of 3- or 7-day systemic antibiotics adjunctive to subgingival instrumentation in patients with aggressive (stage III/IV grade C) periodontitis: a randomized placebo-controlled clinical trial

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ABSTRACT

Aim: To evaluate the clinical non-inferiority of a 3-day-protocol of systemic antibiotics adjunctive to subgingival instrumentation (SI) compared to a 7-day-protocol in patients with stage III/IV grade C periodontitis.

Methods: 50 systemically healthy patients (32.7±4.3 years) with aggressive periodontitis (stage III/IV grade C periodontitis) were treated by SI and adjunctive amoxicillin and metronidazole randomly assigned to group A: (n=25) 500mg antibiotics 3-times-daily for 3 days, followed by placebo 3-times-daily for 4 days, or group B: (n=25) 500mg AB 3-times-daily for 7 days. Clinical, microbial and immunological parameters were assessed at baseline, 3 and 6 months, and patient-related outcomes after 2 weeks. The primary outcome variable was the number of residual sites with PD≥6mm at 6 months.

Results: For the primary outcome variable (the number of residual sites with PD≥6mm at 6 months), the null hypothesis was rejected and demonstrated the non-inferiority of the 3d AB protocol compared to 7d AB (the upper limits of the 95%CI for ITT: [-2.572; 1.050] and PP: [-2.523; 1.318] were lower than the assumed margin of Δ=3.1). Comparable clinical improvements were obtained for all parameters with both antibiotic protocols ($p>0.05$). All investigated periodontopathogens and pro-inflammatory host-derived markers were statistically significantly reduced, without differences between the treatments ($p>0.05$).

Conclusion: These findings indicate that in patients with aggressive periodontitis (stage III/IV grade C periodontitis), a 3-day systemic administration of amoxicillin and metronidazole adjunctive to SI may lead to non-inferior clinical outcomes after 6-months with fewer adverse events compared to a 7-day-protocol.

Clinical Relevance

Scientific rationale for the study

At present, it is unclear whether a short-term systemic administration of amoxicillin and metronidazole adjunctive to subgingival instrumentation in severe forms of periodontitis with rapid progression, may provide similar clinical, microbiological and immunological benefits as traditional 7-day protocols.

Principal findings

Both 3- and 7-days antibiotic regimes resulted in statistically significant clinical, microbiological and immunological improvements at 3 and 6 months after treatment, with no statistically significant differences between the two treatment groups.

Practical implications

In patients with aggressive periodontitis (stages III/IV, grade C periodontitis), adjunctive systemic antibiotics for only 3 days can yield improvements non-inferior to a 7-day regime, with reduced frequency of adverse events.

Conflict of interest

The authors declare no conflict of interest.

INTRODUCTION

Cause-related periodontal therapy aims at reducing/eliminating the pathogenic bacteria by mechanical removal of the subgingival biofilm from the root surfaces through subgingival instrumentation (SI). There is evidence for additional clinical and microbiological benefits from the adjunctive use of systemic antimicrobials (Haffajee, Socransky, & Gunsolley, 2003; Herrera, Sanz, Jepsen, Needleman, & Roldan, 2002; Teughels et al., 2020). In this context, the combination of amoxicillin (AMX) and metronidazole (MET) has been most widely investigated and shown to result in significant additional clinical improvements and suppression of *Aggregatibacter actinomycetemcomitans* and other periodontopathogenic bacteria especially in younger patients with rapidly progressive forms of periodontitis (Aimetti, Romano, Guzzi, & Carnevale, 2012; Aral, Aral, & Kapila, 2019; Jepsen & Jepsen, 2016; Keestra, Grosjean, Coucke, Quirynen, & Teughels, 2015a; Pavicic, van Winkelhoff, Douque, Steures, & de Graaff, 1994; Rabelo et al., 2015; Teughels et al., 2020; van Winkelhoff et al., 1989). Moreover, enhanced clinical results in terms of probing pocket depth (PD) reduction and clinical-attachment-level (CAL) gain as compared to placebo were reported in several clinical studies (Aimetti et al., 2012; Aral et al., 2019; Griffiths et al., 2011; Guerrero, Echeverria, & Tonetti, 2007; Keestra et al., 2015a; Rabelo et al., 2015). Recently, the S3-level clinical practice guideline of the European Federation of Periodontology for periodontitis stages I to III gave an open recommendation for the adjunctive use of systemic antibiotics (Sanz et al., 2020; Teughels et al., 2020) for specific patient categories (e.g. stage III periodontitis in young adults). Moreover, based on an exploratory analysis from a large multicentre RCT (Harks et al., 2015), a consensus report on the adjunctive systemic administration of antibiotics during non-surgical periodontal therapy emphasized the importance of limiting the antibiotic use to younger patients with severe/rapidly progressing forms of periodontitis: aggressive periodontitis (≤36 years old) or chronic periodontitis with ≥35% of deeper sites (≥5mm) and ≤56 years old (Pretzl et al., 2019).

Nonetheless, dosage and duration of antibiotic protocols vary in the literature, ranging from 375mg AMX and 250mg MET for 7 days (Pavicic et al., 1994; van Winkelhoff et al., 1989) to 500 mg AMX and 250-500 mg MET for 7 days (Aimetti et al., 2012; Griffiths et al., 2011; Guerrero et al., 2005) or 10-14 days (Kaner et al., 2007; Mestnik et al., 2012; Silva-Senem et al., 2013). In view of the global continuous increase of antimicrobial resistance (*WHO Global Antimicrobial Resistance Surveillance System (GLASS) Report: Early Implementation 2017–2018*; WHO: Geneva, Switzerland, 2018)(Report, 2018) and in the context of antibiotic stewardship (Dyar, Huttner, Schouten, Pulcini, & Esgap, 2017) it is important to optimize the antibiotic protocols considering a minimal bactericidal concentration and a minimal duration to limit the side effects (Aslam et al., 2021; Buonavoglia et al., 2021; Vogelman & Craig, 1986).

Prolonged antibiotic regimes increase the possibility of development of microbial antibiotic resistance (Ventola, 2015) as well as other undesired side effects/adverse events (e.g. compliance regarding intake, taste disturbance, nausea, vomiting, diarrhoea, renal and liver toxicity, high costs) which strengthens the importance in limiting and optimizing antibiotic use in dentistry. Shortened antibiotic protocols have already been investigated and partly employed in several areas of general medicine (Chastre et al., 2003; Joshi, 2011; Milo et al., 2005). Notably, in a previous study on subjects with chronic periodontitis (stage III/IV grade B periodontitis), we have demonstrated the clinical, microbial and immunological efficacy up to one year of a short-term administration of 3 days of AMX and MET (Cosgarea et al., 2020; Cosgarea et al., 2017; Cosgarea et al., 2016). However, it is not known whether such an approach can also be successfully applied to patients with advanced and rapidly progressing forms of periodontitis (i.e. aggressive periodontitis -AgP, patients younger than 36 years)(Pretzl et al., 2019).

Therefore, the aim of the present study was to evaluate the clinical effects after SI and adjunctive use of AMX+MET in step 2 of periodontal therapy administered for either 3 or 7 days in patients with aggressive periodontitis (stage III/IV grade C periodontitis). Secondarily, microbiological and immunological effects at 6 months after treatment were also to be determined.

MATERIAL AND METHODS

Trial design, patient population

The hypothesis to be tested in this prospective, randomized, triple-masked, placebo-controlled clinical trial was that "the systemic use of AMX and MET administered for three days adjunctive to SI leads to non-inferior clinical results compared to the protocol for seven days".

The sample size was determined based on previous studies and calculated for a non-inferiority margin of 3.1 sites with PD≥6 mm and a standard deviation (SD) of 3.8 sites at 6-months for the 3-day related to the 7-day antibiotic protocol (Cosgarea et al., 2017; Cosgarea et al., 2016). In order to obtain a study power of 80% for an α=0.025, 25 subjects per treatment group (i.e. a total of 50 subjects) were included. All subjects were selected from the patients seeking dental treatment at the University Clinic of the Iuliu-Hatieganu University Cluj-Napoca.

The study was conducted according to the Declaration of Helsinki (1964, revision 2008) and its protocol was approved by the University Ethical Committee (Application #398/02.07.2015). The study was registered in the international registry ISRCTN (ID ID nr.

ISRCTN55637591https://www.isrctn.com/ISRCTN55637591). All eligible participants gave their informed written consent to participate in the study prior to study commencement.

Following study inclusion criteria were considered:

- Age: 18-38 years old (≤ 35 years old at the timepoint of diagnosis)
- ≥12 teeth distributed in all four quadrants
- aggressive periodontitis (Armitage, 1999): primary (familial aggregation, rapid attachment loss and bone destruction, except for periodontitis, otherwise clinically healthy) and/or secondary features (microbial deposits inconsistent with the severity of periodontal tissue destruction, generalized interproximal attachment loss affecting at least three permanent teeth other than first molars and incisors)
- full-mouth plaque scores (FMPS)≤25% (O'Leary, Drake, & Naylor, 1972)
- systemically healthy

Staging and grading, considering the new Classification of Periodontal and Peri-implant Diseases (Papapanou et al., 2018; Tonetti, Greenwell, & Kornman, 2018), were secondarily performed in all cases based on secondary evidence (i.e. quotient of relative bone loss at the worst site by age). Patients were excluded if nonsurgical periodontal therapy had been performed within the previous 12 months, had taken systemic/local use of antibiotics within the previous 6 months, were pregnant/ in lactation, smoking >10 cigarettes/day.

Treatment protocol

All patients received oral hygiene instructions and professional supragingival cleaning sessions until a FMPS≤25% (O'Leary et al., 1972) was reached. Thereafter, SI was performed within two consecutive days under local anaesthesia at all sites with PD≥4mm using Gracey curettes (Hu-Friedy, Chicago, IL, USA) and ultrasonics (EMS, Nyon, Switzerland) by one experienced periodontist (C.R.). Following SI, patients were instructed to rinse with chlorhexidine-digluconate-0.2%mouthrinse (Corsodyl®, GlaxoSmithKline, Brentford, London, UK) and to brush their teeth with 0.2% chlorhexidine-digluconate tooth paste (Elugel®, PierreFabre, Paris-France) for the following two weeks. At the end of the last SI session, patients were allocated by one blinded examiner (R.A.) to one of the two treatment groups according to a computer-generated randomisation list provided by the statistician (block-randomisation, Fig. 1.):

Group-A (n=25 patients): AMX+MET, 500mg, 3t.i.d. for 3-days,

Group-B (n=25 patients): AMX+MET, 500mg, 3t.i.d. for 7-days.

Patients received their medications by one examiner (R.A.) contained in four identical bottles provided by the university pharmacy (University Iuliu-Hatieganu Cluj, Romania). The bottles

were numbered 1-4 and all patients were instructed on their use: for the first 3 days patients took pills from bottles No.1 and 2., while bottles No.3. and 4. were used for the following four days, taking one pill/bottle every 8 hours. Both groups had in the bottles No. 1 and 2 the AMX+MET; Group A had in bottles No. 3 and 4 placebo pills, while group B had in these bottles AMX+MET. All capsules were identical in appearance and the bottles were prepared by the University pharmacy.

Two weeks after SI, patient reported outcomes (PROMS) containing information related to any adverse events, as well as compliance with the medication intake were recorded by interview (R.A.).

Clinical parameters

At baseline, at 3 and 6 months after therapy medical and smoking history, as well as clinical periodontal parameters, oral hygiene and gingival inflammation indices (FMPS)(O'Leary et al., 1972), gingival-bleeding-index-GBI (Ainamo & Bay, 1975)) were recorded by one calibrated examiner, blinded to treatment allocation (R.B.). PD, CAL and bleeding-on-probing (BOP) were determined at 6 sites/tooth with a mm scaled manual periodontal probe (PCP UNC 15, Hu Friedy, Chicago, USA). Additionally, supragingival professional tooth cleaning and oral hygiene instructions were performed. Persistent/recurrent sites (PD≥4mm, BOP+ or PD≥5mm) were not re-instrumented.

For calibration, ten patients (minimum 10 teeth with PD≥6mm), were measured twice within 48 hours. Calibration was accepted for PD- and CAL-measurements with no difference >1mm in >90% sites. The mean intra-examiner reliability (Cohen`s Kappa Analyses) was calculated as 0.85 (PD) and 0.79 (CAL).

Immunomarkers and periodontal pathogens

Gingival-crevicular-fluid (GCF) and microbial sampling were performed at the deepest site/quadrant at all timepoints as previously described (Cosgarea et al., 2020) using standard paper-strips (Periopaper, Oraflow, New York, USA) and sterile paper-cones. GCF-samples were pooled and stored at -70°C, while paper-points stored at -20°C until analyses.

The host-derived biomarkers MMP-8, IL-1β, IL-10 and IL-8 were analysed as previously described (Cosgarea et al., 2020) using a commercially available ELISA-kits (R&DSystems Europeltd, Abington-UK). For IL-1β, IL-10 and IL-8 detection levels were 2pg/sample, while for MMP-8 the detection level was 1 ng/sample.

Quantitative and qualitative microbiological analyses of the periodontopathogens *A. actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola, Prevotella intermedia, Fusobacterium nucleatum, Campylobacter rectus and*

Filifactor allocis were performed using real-time polymerase-chain reaction (rtPCR) as recently described (Cosgarea et al., 2020).

Statistical analyses

Statistical analysis was performed using the statistical software program (SPSS-statistics 21, IBM, Armonk, NY, USA) by an experienced professional statistician (C.H.), who was blinded with regard to treatment modality. Data entry was performed by one blinded examiner (I.B.A.) and checked for errors by a further blinded examiner (R.A.). All sites with initial PD ≥ 4mm were considered for statistical analyses. The statistical unit was the patient and the primary outcome variable was the number of sites/patient (N) with PD≥6mm at 6-months (Cosgarea et al., 2017; Cosgarea et al., 2016; Matuliene et al., 2008). Secondary variables were average changes and means of PD, CAL, BOP, GBI, FMPS, N PD≥4-6mm, N PD≥7mm, N sites reaching pocket closure (PD≤3mm). *P*-values < 0.05 were considered statistically significant. Considering that the present study had only one single primary outcome variable and only two treatment groups, no adjustments for multiple testing were performed. All other comparisons should be viewed as exploratory analyses only. Inter- and intragroup comparisons between the various time-points were performed using paired t-tests and Wilcoxon signed-ranks-tests. The relationship between N residual sites with PD ≥ 6mm at 6-months and the variables AB-protocol, smoking, FMPS at baseline and 6-months, baseline CAL and at 6-months and N of initially deep sites (PD≥ 6mm) was determined (Poisson-regression analysis). Counts(log10) and frequency of the above mentioned periodontopathogens were calculated and inter- and intragroup comparisons were determined (Wilcoxon-signed-rank-test, Fisher`s Exact-test, Mann-Whitney *U*-test). Immunomarker levels were determined and inter- and intragroup-comparisons were performed (Wilcoxon-signed-rank-test, Mann-Whitney *U* test).

RESULTS

Patients and adverse events

Fifty systemically-healthy patients (n=25/treatment-group as initially planned), aged 18-38 years (mean age 32.7±4.3 years, 31 females, 11 smokers) diagnosed with aggressive periodontitis (Armitage, 1999) were included in the study. Considering that a new classification had been published meanwhile, all patients were re-classified to generalised stage III or IV grade C periodontitis (Table 1).(Papapanou et al., 2018; Tonetti et al., 2018). All included patients received non-surgical periodontal therapy between September 2015 and March 2020. Nine patients (2 in group A and 7 in group B) were lost at the 6-months follow-up due to additional AB-intake (n=5) (one of these patients also informed about positive pregnancy) or

appointment-no-show (n=4) (Fig. 1). At baseline, no statistically significant intergroup-differences could be detected for gender, smoking, diagnosis stage distribution (i.e. III/IV) or the initial clinical periodontal parameters PD, CAL, N of various pocket depths (Tables 1, 2).

All patients were compliant with the medication/placebo intake reporting to have taken all given pills, mostly at the prescribed 8h-interval. No allergies were reported. Registered adverse events included gastrointestinal disorders (i.e. nausea, diarrhea), headaches, musculoskeletal-pain, taste-disorders (e.g. metallic taste), shivering, candida and vertigo (Table 2). Statistically significantly more patients in the 7-day AB-group (n=15) reported adverse-events compared to the 3-day AB-group (n=3) in both ITT ($p=0.001$, Fisher`s exact test) and PP ($p<0.0001$, Fisher`s exact test) patient collectives. Evaluating each AE separately (Fisher`s Exact Test for count data), statistically significantly more patients reported taste disorders ($p=0.032$).

Clinical findings

Table 3 shows the results for periodontal clinical parameters registered at baseline, at 3- and 6-months. At baseline, no statistically significant group-differences could be found for any of the evaluated parameters ($p>0.05$). Both treatments led to statistically significant clinical improvements of all investigated clinical parameters (Table 3, $p<0.05$).

No statistically significant intergroup-differences were found for the primary outcome variable frequency of residual sites with PD ≥ 6mm at 6-months. Considering the 95%CI (ITT: [-2.572; 1.050]; PP: [-2.523; 1.318]) and that the obtained upper limits of these confidence intervals are lower than the assumed margin of $\Delta=3.1$, our null hypothesis was rejected (H0: δ = No. PD ≥6mm in 3d AB group – No. PD≥6mm in 7d AB group ≥ $\Delta=3.1$; H1: δ = No. PD ≥6mm in 3d AB group – No. PD≥6mm in 7d AB group < $\Delta=3.1$). Thus we were able to show the non-inferiority of the 3d AB compared to the 7d AB therapy for the main outcome variable N of sites with PD≥6mm ($p=0.343$) (supplementary figure 1)(Piaggio et al., 2006).

Intergroup comparisons revealed that all other investigated clinical parameters, except for mean CAL-gain at 6-months, were comparable at both follow-ups in both ITT and PP analyses (Table 3, $p>0.05$, paired t-test and Wilcoxon-signed-rank-test). Mean CAL-gain at 6-months, was statistically significantly higher in the 7-day AB-group. This trend was also observed for CAL-gain at 6-months for very deep sites (baseline PD≥7mm) indicating in the ITT analysis a borderline statistically significantly higher CAL-gain in group B compared to group A (Table 3, $p=0.050$).

Moreover, the N treated sites (baseline PD≥4mm) reaching pocket-closure (PD≤3mm) at 3- and at 6-months was comparable between the two groups (Table 3, $p<0.05$). Furthermore, comparable findings between the groups were observed for the number of patients reaching the clinical endpoint ≤4 sites with PD≥5mm (Feres et al., 2020; Lang & Tonetti, 2003) at 6-months (Table 3, $p=1.000$). Only two patients/treatment-group remained with a high number (≥ 9) of sites with pathological depth (PD≥5mm) at 6 months (Table 3).

BOP, GBI and FMPS showed in both patient groups statistically significant decreases up to 6-months (Table 3).

The Poisson-regression-analysis showed that only mean PD and CAL-values at baseline as well as mean CAL at 6 months correlated statistically significantly with the residual N deep-sites (PD≥6mm), indicating that an increase of 1mm in mean-CAL leads to a 9.89-fold-increase in the residual N deep-sites (Table 4).

Microbiological findings

All investigated periodontal pathogens showed in the 3-day AB-group (group-A) statistically significant reductions at both follow-ups. In the 7-day AB-group, except for *A. actinomycetemcomitans*, all other bacteria were also reduced statistically significantly (Table-5). Except for *A.actinomycetemcomitans*, statistically significant less patients harbored *P. gingivalis, T.forsythia, T.denticola, P.intermedia, F.nucleatum* and *C.rectus* at the follow-ups ($p<0.05$) (Table 5).

Immunological findings

MMP-8, IL-1β and IL-8 were statistically significantly reduced at both follow-ups in both groups ($p<0.05$). MMP-8 and IL-8 showed a slight increase from 3- to 6-months in the 3-day AB-group, while these markers showed further decrease in the 7-day group (Table 6). IL-10 (non-inflammatory-marker) showed an increase in both treatment groups.

Intergroup comparisons revealed statistically significant higher levels of IL-1β in the 7-day AB-group at 3-months ($p=0.013$); however, these differences levelled at 6-months ($p=0.121$). IL-8 was present in statistically significant higher amounts at baseline in group B; while at the follow-ups the amounts were comparable in both groups (Table 6, $p>0.05$).

DISCUSSION

The results of the present RCT support the non-inferiority hypothesis of a 3-day systemic administration of AMX+MET adjunctive to subgingival instrumentation as compared to the traditional antibiotic regime of 7-days with respect to the frequency of residual deep pockets (PD≥ 6mm) in patients with aggressive periodontitis (stage III/IV grade C). The number of residual deep sites was statistically significantly reduced ($p<0.05$) and no statistically significant differences between the two groups could be detected ($p>0.05$). The choice of the primary outcome number of deep residual sites is justified also in light of the recommendations by the recent EFP S3-level clinical guideline, that consider residual sites with a PD≥6 mm at reevaluation after step 2 of therapy as targets for additional surgical treatment in step 3 therapy (Sanz et al., 2020). The findings of the present study could demonstrate that both treatment modalities led to a significant reduction in the number of such pockets.

Another important finding was the statistically significantly lower total number of adverse events recorded in patients on the shorter AB-protocol compared to the 7-day AB-regime ($p<0.05$, Table 2). Moreover, statistically significantly more patients reported taste disorders in the longer AB protocol group ($p=0.032$). This is in line with the findings by others on the occurrence of several adverse-events [nausea/vomiting(15%), diarrhea(15%), headaches(5%), metallic taste(5%) and general un-wellness(15%)] in patients receiving AB for 7-days compared to the placebo-group (Guerrero et al., 2005). In a large randomized placebo-controlled multicenter RCT with 542 patients diagnosed with chronic and aggressive periodontitis, the patients receiving the antibiotics (amoxicillin 500 mg, metronidazole 400 mg each 3 tid for 7 days) showed only slightly more serious adverse events (i.e. 43 SAEs) compared to 39 SAEs recorded in the placebo group (Harks et al., 2015). One possible explanation for the different outcomes, may be due to the fact that Harks et al. 2015 reported only serious AEs (i.e., one anaphylactic shock in the AB group) without reporting milder AEs such as metallic taste or headaches as it was the case in our study. Therefore, a larger range and consecutively a higher number of AEs was reported in our study. In line with our results, a recent systematic review confirmed that the combination of AMX+MET for 7+days was the clinically most effective adjunctive AB-protocol but also the one with most adverse events (Teughels et al., 2020). Therefore, the present findings of non-inferior clinical efficacy and more favorable patient-related outcomes – a better balance between desired effects and unwanted side effects - indicate a potentially very relevant advancement in the search for improved antibiotic protocols in the treatment of severe periodontitis with rapid progression.

Such side-effects including the risk for allergic/hypersensitivity reactions and onset of antibiotic resistance represent a serious concern and should limit as much as possible the use of systemic antibiotics. Thus, adjunctive systemic AB should be limited strictly to patients who could mostly benefit from them. Nonetheless, deciding which are these patients and the

optimal AB-protocol still represents a challenge and a hot topic for current and future research (Duarte & Spencer, 2016). A recent consensus provided evidence for additional clinically relevant benefits for systemic antibiotics in younger patients with severe/rapidly progressing forms of periodontitis like aggressive periodontitis (≤36 years old) or chronic periodontitis with ≥35% of deeper sites (≥5mm) and ≤56 years old (Pretzl et al., 2019). The authors point out the importance of determining the clinical relevance of this additional benefit individually on patient level and that not solely the diagnosis of periodontitis should be decisive for the indication of systemic antibiotics.

Both treatment protocols led to statistically significant improvements at 3- and 6-months for all evaluated clinical, microbiological and immunological parameters. In particular, findings for residual very deep (PD≥7mm) and moderately-deep pockets (PD=4-6mm) show additional statistically significant reductions between the timepoints ($p<0.05$) without intergroup-differences ($p>0.05$). These findings relate well to the increased and comparable number of sites reaching pocket closure (PD≤3mm) in both antibiotic groups ($p<0.05$). Other authors with a longer AB-protocol including AMX+MET for 14-days reported more sites (3.7±6.5) with PD≥6mm at 6-months (our study: group A: 0.74±1.32, group B: 1.50±3.50), despite comparable baseline parameters (Mestnik et al.: N sites PD≥6mm: 33.5±18.4)(Mestnik et al., 2012). These discrepancies may be explained by the higher initial plaque scores (61.3±19.8%) and shallower baseline PD-values (4.27±0.71mm) compared to those from the present study (Cosgarea et al., 2017; Herrera et al., 2002; Keestra et al., 2015a; Mestnik et al., 2012; Nibali, Koidou, Hamborg, & Donos, 2019).

Statistically significant PD-reductions and CAL-gains were observed in both groups at both follow-ups. Even though these parameters were apparently comparable between the groups, CAL-gain at 6-months seemed to be statistically significantly higher in the 7-day AB-group ($p=0.041$; group A: 1.73±0.38mm; group B: 2.01±0.47mm). This may be related to the initially deeper, even though statistically insignificant, PD- and CAL-values, to the higher N of deep and very deep sites in group B, implying poorer CAL-outcomes for these patients. Mestnik &coworkers showed similar PD-reductions and CAL-gain for moderate (PD-reduction: 2.09±0.40mm; CAL-gain: 1.72±0.41mm) and deep sites (PD-reduction: 4.27±1.34 mm, CAL-gain: 3.43±1.14mm), with slightly less full-mouth PD-reductions (1.59±0.51mm) and CAL-gain (1.23±0.41mm)(Mestnik et al., 2012). Comparable PD- and CAL-values for moderate (PD 4-6mm) and very deep sites (PD≥7mm) at 6-months were obtained by Arweiler et al. (moderate sites: PD: 2.9±0.5mm; CAL: 3.5±0.8mm; deep sites: PD: 3.7±0.9mm; CAL: 4.6±1.3mm) despite lower AB-doses (375mg AMX, 250mg MET,3 t.i.d. for 7-days)(Arweiler et al., 2014). Another study with comparable initial clinical parameters and similar 7-day AB-protocol reported slightly lower PD-reductions (full-mouth: 1.2mm; PD 4-6mm: 1.5mm; PD≥7mm:

3.1mm) and CAL-gains (full-mouth: 0.8mm; PD 4-6mm: 1.3mm; PD≥7mm: 2.3mm)(Guerrero et al., 2005). Furthermore, a meta-analysis on patients with aggressive periodontitis reported statistically significant higher PD-reductions for AMX+MET compared to placebo at 3- and 6-months (mean differences full-mouth: 3-months: 0.39±0.16mm; 6-months: 0.51±0.56mm; PD 4-6mm: 3-months: 0.43±0.22mm, 6-months: 0.50±0.21mm; PD≥7mm: 3-months: 0.88±0.27-mm; 6-months: 1.09±0.39mm). Similar outcomes were also reported for CAL-gain (Keestra et al., 2015a) as well as by other authors (Casarin et al., 2012; Sgolastra, Petrucci, Gatto, & Monaco, 2012).

Detecting the proper clinical endpoint after periodontal treatment is essential for clinical stability and arresting disease progression. Recently, Feres et al. proposed a clinical status of "≤4 sites with PD≥5mm" as a clinical endpoint for clinical trials assuring disease control during SPT(Feres et al., 2020). In our study, both treatment groups showed a comparable percentage of patients reaching the above mentioned clinical endpoint (group A: 73.9%, group B: 77.8%, p=1.000); few patients in both groups showed a moderate-risk for disease progression with 5-8 sites with PD≥5mm (group A: n=4/17.4%, group B: n=2/11.1%, p=0.678) or high-risk (≥9 sites with PD≥5mm: group A: n=2/6.7%, group B: n=2/11.1%; p=1.000). Fewer patients (26.7%) with low-risk and more patients (53.3%) with high-risk of progression were reported by Mestnik et al. despite the prolonged antibiotic regime of 14-days. On the other hand, a comparable percentage of patients (20%) showed a moderate-risk of progression (Mestnik et al., 2012). These discrepancies may be related to the elevated full-mouth biofilm levels prior treatment in Mestnik et al., which is known to have a major impact upon the outcomes of non-surgical periodontal therapy being one of the major causative factors of the disease. Tomasi and coworkers (Tomasi, Leyland, & Wennstrom, 2007) showed in a logistic multilevel analyses, that the initial presence of plaque at the individual tooth sites led to significantly less pocket closure after non-surgical periodontal treatment ($p<0.001$).

At 6-months, statistically significant reductions of all investigated microorganisms without statistically significant group-differences were obtained. These results corroborate those of other authors, who showed by checkerboard DNA-DNA hybridization a significant decrease in the proportions of *A.actinomycetemcomitans, P.gingivalis, T.forsythia* and *T.denticola* in the AB-group (Mestnik et al., 2010; Xajigeorgiou, Sakellari, Slini, Baka, & Konstantinidis, 2006). Notably, in Mestnik et al. *A.actinomycetemcomitans* was statistically significantly reduced only in initially deep sites. Similarly, Yek et al. reported by using PCR-method statistically significant reductions of *P.gingivalis*, *T.forsythia* and *T.denticola* which were at 6-months significantly higher in the AB- compared to the control group (Yek et al., 2010). Casarin et al. reported statistically significant reductions in the proportions of *A.actinomycetemcomitans, P.gingivalis*

and *T.forsythia* in the AB-group, with no differences to the patients receiving only mechanical therapy (Casarin et al., 2012).

Immunological outcomes indicate statistically significant reductions of the pro-inflamatory markers MMP-8, IL-1β and IL-8 in both treatment groups at both follow-ups with no differences between the two antibiotic protocols. The anti-inflammatory marker IL-10 showed an increase at 3-months, and a decrease at 6 months, reaching almost the baseline values in group B. In the 7-day AB-group, the decrease was slower, so that at 6-months, elevated levels of IL-10 were still detectable. Few studies on subjects with generalised aggressive periodontitis have determined immunological outcomes. Statistically significant reductions of IL-1β were observed by Casarin et al., with no statistically significant differences between AB and patients receiving solely mechanical therapy (Casarin et al. 2012).

Considering the increase in AB resistant bacterial strains as a global threat (Levy & Marshall, 2004; Ventola, 2015), the Council-for-Appropriate-and-Rational-Antibiotic-Therapy (CARAT) has indicated five criteria for proper antibiotic selection (i.e. evidence-based results, therapeutic benefits, safety, optimal drug-dose and duration, cost-effectiveness) (Slama et al., 2005). In general medicine, several areas have already implemented antimicrobial protocols with high drug doses and shorter durations (Chastre et al., 2003; Joshi, 2011; Milo et al., 2005; Schrag et al., 2001). Concomitantly, the recent EFP S3-level clinical practice guideline calls for a rather restrictive use of adjunctive systemic antibiotics (Sanz et al., 2020). For very advanced forms of periodontitis (stage IV), no recommendations for adjunctive antimicrobial protocols exist so far. Recently, periodontal pathogens collected from almost 8000 patients with advanced periodontitis were shown to be non-susceptible to at least one of the antibiotics tested in about two-thirds of the patients and the data further revealed a trend towards decreasing susceptibility profiles, however, no relevant change in susceptibility to amoxicillin and metronidazole (Jepsen et al., 2021). Future studies using advanced microbiome sequencing technology will have to evaluate metagenomics patterns associated with bacterial tolerance development against antibiotics (resistome) following different durations of antimicrobial administration (Curtis, Diaz, & Van Dyke, 2020; Joseph & Curtis, 2021; Sedghi, DiMassa, Harrington, Lynch, & Kapila, 2021).

Based on our previous studies with severe chronic periodontitis patients ~~(stages III/IV, grade B-periodontitis)~~ that evaluated clinical, microbiological and immunological effects of a short-term AB-protocol(i.e. 3-days, AMX+MET, 500mg, 3t.i.d.)(Cosgarea et al., 2020; Cosgarea et al., 2017; Cosgarea et al., 2016), we investigated in the present set of patients with aggressive periodontitis (re-classified as stages III/IV grade C periodontitis) the short-term AB regime compared to the traditional AB-protocol (7-days), whose efficiency for generalized aggressive periodontitis had been previously already been shown (Aimetti et al., 2012; Griffiths et al.,

2011; Keestra et al., 2015a; Yek et al., 2010). Since the clinical superiority of adjunctive AMX+MET compared to other antibiotics or to the mechanical treatment alone had already been reported, we have included in the present study only AB-treatment groups (Aimetti et al., 2012; Griffiths et al., 2011; Keestra et al., 2015a; McGowan, McGowan, & Ivanovski, 2018; Sgolastra et al., 2012; Yek et al., 2010).

One limitation of the present study is the fact that the power calculation has been performed based on findings of a study conducted for chronic periodontitis ~~(stageIII/IV grade B periodontitis)~~. When interpreting the present results, it is important to point out that the study was designed as a non-inferiority trial, since no previous studies have evaluated similar protocols in patients with aggressive periodontitis ~~(grade C periodontitis)~~. The lack of an additional placebo-group may also be considered a limitation. However, based on the current evidence, it is generally accepted that patients diagnosed with advanced forms of periodontitis (i.e. aggressive periodontitis, patients ≤36 years old, generalized severe chronic periodontitis in patients ≤56 years, rapidly progressive forms) benefit mostly if non-surgical therapy is performed in conjunction with systemic administration of AMX+MET (Aimetti et al., 2019; Keestra, Grosjean, Coucke, Quirynen, & Teughels, 2015b; Pretzl et al., 2019; Teughels et al., 2020). Refraining to offer the most efficient treatment approach for patients diagnosed with such advanced and rapid forms of periodontitis, may not be ethical. A further limitation of the study could be considered the higher number of dropouts observed in the 7-day AB-group, compared to the 3-day AB-group. This was however an uncontrollable study issue. Despite the high attrition over 6 months, the study shows sufficient power ≥89%: the post-hoc analysis reveals a power of 92% for the ITT collective (group A: n=23, SD=1.322, group B: n=18, SD=3.502) and a power of 89 % for the PP analysis (group A: n=21, SD=1.365, group B: n=17, SD=3.589).

Conclusion

Within their limits, the present findings suggest that in patients with stages III/IV grade C periodontitis, a 3-day systemic administration of AMX+MET adjunctive to non-surgical periodontal therapy may lead to non-inferior clinical, microbiological and immunological outcomes at 6-months as compared to the 7-day traditional protocol, and is associated with a lower frequency of adverse events, thus proving a better benefits/harms ratio.

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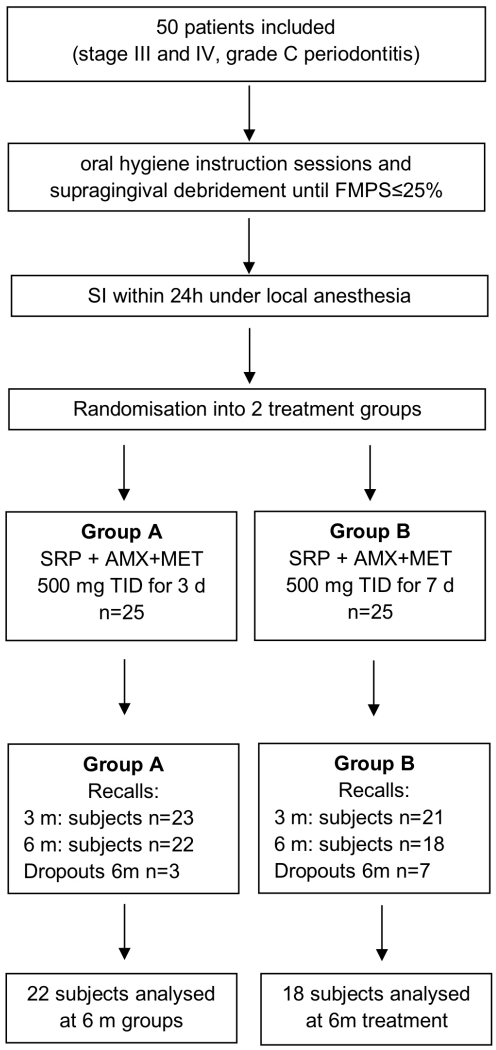
Figure 1. Flowchart of the study



Tables

Table 1. Baseline demographic characteristics (ITT and PP analysis)

Variables	ITT patient collective			PP patient collective		
	Group A (3d AB) N=25	**Group B (7d AB) N=25**	***Group comparisons p-value (T-test)***	**Group A (3d AB) N=21**	**Group B (7d AB) N=17**	***Group comparisons p-value (T-test)***
Female gender (n/%)	18/72%	13/52%	*0.145 (Chi-Quadra n Pearson)*	16/76%	7/41%	*0.046 (Fisher`s Exact Test)*
Age (years)	31.04±4.48	34.40±3.51	*0.005 (t-Test)*	31.19±4.69	34.35±3.35	*0.025 (t-Test)*
Smoker (n/%)	3/12%	8/32%	*0.088 (Chi-Quadra n Pearson)*	3/14.28%	5/29.41%	*0.426 (Fisher`s Exact Test)*
Diagnosis (n/%)						
Stage III grade C	15/ 60%	12/ 48%	*>0.05*	*13/ 62%*	*11/ 64%*	*>0.05*
Stage IV grade C	10/ 40%	13/ 52%	*>0.05*	*8/ 38%*	*6/ 35%*	*>0.05*

PD: pocket depth, CAL: clinical attachment level, BOP: bleeding on probing; FMPS: full-mouth plaque score after O'Leary (O'Leary et al., 1972).

Table.2 Adverse events (ITT and PP analysis); intergroup comparisons for each assessed adverse event (Fisher`s Exact Test for Count data)

Symptoms	ITT collective			PP collective		
	Group A 3-day AB N=25	**Group B 7-day AB N=25**	***p-values Fisher`s Exact Test***	**Group A 3-day AB N=21**	**Group B 7-day AB N=17**	***p-values Fisher`s Exact Test***
Gastrointestinal disorder	1	2	1	1	1	1
Headaches	1	2	1	1	2	0.576
Allergy	-	-	-	-	-	-
Musculoscheletal pain	-	1	1	-	1	0.447
Respiratory disorder	-	-	-	-	-	-
Taste disorders	-	5	0.050	-	4	0.032
Fever	-	-	-	-	-	-
Shivering	-	1	1	-	1	0.447
Candida	1	1	1	-	1	0.447
Vertigo	-	3	0.234	-	3	0.080
Total N AEs	3 **2**	**15** 5	**<0.0001**	**2**	**15**	**2** **<0.0001**	**15**	**<0.0001**
Group comparison (p-value)	**0.001**			**<0.0001**		

AB: antibiotics; N: number; ITT: intention to treat analysis; PP: per protocol analysis

Table 3.
Clinical outcomes: mean values and group comparisons (T-test) of PD, CAL, BoP, GBI, FMPS, number (N) of sites with PD ≥ 6mm, with PD ≥ 7mm, with PD=4 BOP+ and PD≥5 and their differences (Δ) between the follow-ups and baseline (mean±SD); number of sites with pocket closure (PD≤3mm, mean±SD) and risk of disease progression (N, %) (ITT and PP analysis)

	ITT analysis			*PP analysis*		
Variables	**Group A** (SI+AB 3d) N=25/22/23	**Group B** (SI+AB 7d) N=25/21/18	***p value A-B T Test***	**Group A** (SI+AB 3d) N=21/21/21	**Group B** (SI+AB 7d) N=17/17/11	***p value A-B T Test***
Primary outcome variable:						
No. PD ≥ 6mm						
Baseline	28.64±20.97	34.92±26.40	*0.356*	31.71±21.49	37.29±29.92	*0.508*
3 months	0.52±0.89	1.52±2.20	*0.051*	0.57±0.89	1.24±1.78	*0.148*
6 months	**0.74±1.32**	**1.50±3.50**	***0.343***	**0.81±1.36**	**1.41±3.59**	***0.482***
Δ baseline-3 month	29.39±21.19 [s]	35.24±27.68 [s]	*0.434*	31.14±21.32 [s]	36.05±29.25 [s]	*0.553*
Δ baseline-6 month	29.65±21.06 [s]	34.56±28.19 [s]	*0.527*	30.90±21.65 [s]	35.88±28.47 [s]	*0.554*
PD (mm)						
Baseline	5.35±0.45	5.56±0.55	*0.136*	5.39±0.45	5.55±0.58	*0.354*
3 months	2.83±0.32	2.97±0.39	*0.221*	2.82±0.28	2.95±0.39	*0.232*
6 months	2.81±0.37	2.88±0.44	*0.605*	2.83±0.37	2.81±0.36	*0.894*
Δ baseline- 3month	2.53±0.56 [s]	2.57±0.67 [s]	*0.858*	2.57±0.51 [s]	2.59±0.62 [s]	*0.892*
Δ baseline- 6month	2.56±0.54 [s]	2.70±0.64 [s]	*0.470*	2.56±0.56 [s]	2.73±0.63 [s]	*0.375*
CAL (mm)						
Baseline	5.19±0.91	5.47±0.85	*0.259*	5.10±0.83	5.46±0.87	*0.197*
3 months	3.50±0.78	3.61±0.83	*0.641*	3.45±0.77	3.57±0.82	*0.638*
6 months	3.42±0.81	3.44±0.76	*0.926*	3.39±0.76	3.44±0.78	*0.813*
Δ baseline- 3month	1.59±0.44 [s]	1.87±0.60 [s]	*0.093*	1.64±0.36 [s]	1.89±0.60 [s]	*0.135*
Δ baseline- 6month	1.73±0.38 [s]	2.01±0.47 [s]	*0.041* [s]	1.71±0.38 [s]	2.01±0.47 [s]	*0.036* [s]
PD 4-6 mm (mm)						
Baseline	4.88±0.19	4.95±0.21	*0.254*	4.90±0.19	4.97±0.22	*0.318*
3 months	2.71±0.33	2.78±0.36	*0.498*	2.69±0.27	2.76±0.34	*0.488*
6 months	2.65±0.31	2.72±0.39	*0.539*	2.67±0.30	2.66±0.31	*0.934*
Δ baseline- 3month	2.18±0.37[s]	2.16±0.47 [s]	*0.893*	2.20±0.32[s]	2.20±0.45 [s]	*0.994*
Δ baseline- 6month	2.23±0.37 [s]	2.24±0.49 [s]	*0.935*	2.23±0.38 [s]	2.31±0.42 [s]	*0.563*
CAL of PD 4-6 mm (mm)						
Baseline	4.84±0.89	4.94±0.72	*0.672*	4.73±0.79	4.95±0.69	*0.381*
3 months	3.35±0.79	3.35±0.77	*0.998*	3.30±0.78	3.30±0.75	*0.989*
6 months	3.24±0.79	3.20±0.67	*0.861*	3.21±0.75	3.21±0.68	*0.980*
Δ baseline-3 month	1.38±0.39 [s]	1.62±0.44 [s]	*0.070*	1.42±0.32 [s]	1.64±0.45 [s]	*0.091*
Δ baseline-6 month	1.54±0.34 [s]	1.69±0.40 [s]	*0.188*	1.52±0.33 [s]	1.73±0.38 [s]	*0.078*
PD >7 mm (mm)						
Baseline	7.45±0.42	7.61±0.56	*0.332*	8.35±0.50	8.48±0.64	*0.518*
3 months	3.37±0.64	3.64±0.81	*0.218*	4.09±1.11	4.22±1.47	*0.783*
6 months	3.47±0.82	3.41±0.63	*0.800*	4.20±1.56	3.78±1.05	*0.383*
Δ baseline-3 month	4.13±0.69 [s]	3.91±0.85 [s]	*0.356*	4.26±1.18 [s]	4.26±1.54 [s]	*0.991*
Δ baseline-6 month	4.04±0.81 [s]	4.15±0.73 [s]	*0.671*	4.15±1.36 [s]	4.70±1.15 [s]	*0.224*
CAL of PD >7 mm (mm)						
Baseline	6.80±0.81	7.16±1.22	*0.231*	7.43±1.10	7.94±1.53	*0.275*
3 months	4.23±0.97	4.47±1.20	*0.467*	5.04±1.68	5.35±1.99	*0.630*
6 months	4.22±1.05	4.15±1.03	*0.817*	5.08±1.68	4.94±1.67	*0.824*
Δ baseline-3 month	2.48±0.61 [s]	2.63±1.12 [s]	*0.605*	2.38±1.18 [s]	2.58±2.06 [s]	*0.729*
Δ baseline-6 month	2.53±0.58 [s]	3.02±0.88 [s]	*0.050*	2.35±0.96 [s]	2.99±1.52 [s]	*0.151*
BoP (%)						
Baseline	64.15±29.01	71.98±32.02	*0.369*	67.27±30.29	76.67±25.15	*0.312*
3 months	13.96±7.79	18.22±13.99	*0.229*	13.97±7.98	15.01±8.79	*0.705*
6 months	12.16±6.27	12.10±5.67	*0.976*	12.32±6.44	11.91±5.79	*0.839*
Δ baseline-3 month	52.42±26.56	57.95±28.32	*0.512*	53.29±26.88	61.66±28.62	*0.360*
Δ baseline-6 month	54.26±29.06	60.32±32.35	*0.532*	54.94±30.33	64.76±27.08	*0.305*
GBI (%)						
Baseline	7.33±19.83	13.69±17.23	*0.232*	8.17±21.50	14.08±14.41	*0.339*
3 months	8.20±10.62	8.44±11.75	*0.944*	8.06±10.86	9.82±12.64	*0.647*
6 months	7.94±9.34	7.37±9.35	*0.848*	7.50±9.65	7.80±9.45	*0.848*
FMPS (%)						
Baseline	20.94±6.50	22.18±13.99	*0.691*	21.75±6.09	22.90±16.41	*0.767*
3 months	39.40±20.41	40.64±24.65	*0.859*	38.52±20.47	38.80±26.39	*0.971*
6 months	37.28±18.87	29.70±18.38	*0.204*	37.85±19.62	30.85±18.26	*0.267*

No. PD 4-6 mm						
Baseline	50.60±19.25	54.44±18.94	*0.480*	53.90±18.83	54.88±20.38	*0.879*
3 months	10.26±7.09	16.62±16.12	*0.093*	10.57±7.29	16.06±16.40	*0.177*
6 months	9.61±7.07	13.39±14.37	*0.276*	10.10±7.22	13.00±14.71	*0.431*
Δ baseline-3 month	41.78±18.08 [s]	40.28±17.17 [s]	*0.780*	43.33±17.68 [s]	38.82±17.75 [s]	*0.440*
Δ baseline-6 month	43.30±16.93 [s]	39.94±18.08 [s]	*0.544*	43.80±17.66 [s]	41.88±16.59 [s]	*0.733*
No. PD≥5 mm						
Baseline	44.20±25.71	54.00±28.71	*0.210*	*48.24±26.02*	*56.24±32.71*	*0.407*
3 months	3.35±3.42	5.62±6.41	*0.145*	*3.48±3.50*	*5.47±6.07*	*0.213*
6 months	3.00±3.36	4.17±7.17	*0.494*	*3.29±3.38*	*3.82±7.24*	*0.764*
Δ baseline-3 month	42.74±24.78 [s]	50.95±28.18 [s]	*0.310*	*44.76±24.76*	*50.76±29.89*	*0.502*
Δ baseline-6 month	43.52±25.01 [s]	50.44±29.91 [s]	*0.425*	*44.95±25.72*	*52.41±29.61*	*0.411*
No. PD≥7 mm						
Baseline	13.40±14.82	18.36±17.78	*0.289*	14.86±15.74	19.29±19.23	*0.289*
3 months	0.30±0.70	0.33±0.66	*0.889*	0.33±0.73	0.24±0.56	*0.889*
6 months	0.35±0.65	0.22±0.65	*0.541*	0.38±0.67	0.12±0.48	*0.541*
Δ baseline-3 month	13.69±15.25 [s]	18.95±19.41 [s]	*0.327*	14.52±15.68 [s]	19.05±19.33 [s]	*0.430*
Δ baseline-6 month	13.96±15.11 [s]	18.56≥18.71 [s]	*0.402*	14.47±15.74 [s]	19.17±19.09 [s]	*0.411*
No. PD≤3 mm						
3 months	52.22±26.12	58.52±24.27	*0.666*	57.67±25.37	57.35±25.83	*0.970*
6 months	57.09±25.03	58.00±27.68	*0.914*	58.10±26.01	60.53±26.29	*0.777*
Risk of disease progression [Feres et al. 2020]						
Low risk: ≤4 sites with PD≥5mm						
3 months (n/%)	17/73.9%	12/57.1%	0.342	15/71.4%	10/58.8%	0.502
6 months (n/%)	17/73.9%	14/77.8%	1.000	15/71.4%	14/82.3%	0.318
Moderate risk: 5-8 sites with PD≥5mm						
3 months (n/%)	4/17.4%	5/23.8%	0.716	4/19.0%	4/23.5%	1.000
6 months (n/%)	4/17.4%	2/11.1%	0.678	4/19.0%	2/11.7%	0.672
High risk: ≥ 9 sites with PD≥5mm						
3 months (n/%)	2/8.7%	4/19%	0.403	2/9.5%	3/17.6%	0.640
6 months (n/%)	2/8.7%	2/11.1%	1.000	2/9.5%	1/5.9%	1.000

PD: pocket depth, CAL: clinical attachment level, BOP: bleeding on probing, GBI: gingival bleeding index (Ainamo & Bay, 1975), FMPS= full-mouth plaque score after O'Leary (O'Leary et al., 1972); N: number; AB: antibiotics; d: days; [s] statistical significant *p* values;

Table 4. Poisson regression analysis for the number of residual sites with PD ≥ 6 mm at 6 months after non-surgical periodontal therapy (values are for both ITT and PP analysis)

Variables	Exp. Coefficient	95% CI	*p* value
Group B (AB for 3 d)	0.58	0.22-1.54	0.278
Diagnosis III	2.00	0.63-6.27	0.234
Non-Smoker	0.55	0.24-1.27	0.163
FMPS baseline	0.95	0.97-1.01	0.099
FMPS 6 months	0.99	0.97-1.01	0.615
Mean PD baseline	9.18	1.91-44.176	0.006
Mean CAL baseline	0.08	0.02-0.37	0.001
Mean CAL 6 months	9.89	3.10-31.52	<0.0001
N sites with PD≥6mm at baseline	1.00	0.97-1.03	0.774

PD: pocket depth, CAL: clinical attachment level, FMPS: full-mouth plaque score after O'Leary (O'Leary et al., 1972); [s] statistically significant *p* values. Reference categories are: Group A, Male, Non-Smoker (Exp. Coefficient equals 1)

Table. 5

Quantitative and qualitative microbial analysis: mean values, quantitative intergroup comparisons (T-test) and intragroup comparisons between the timepoints (Wilcoxon test); number and detection frequency: intragroup comparisons (McNemar Test) and intergroup comparisons (Fisher`s exact test).

Microorganisms	Bacterial counts (mean±SD)			Detection frequency N/%		
	Group A (SI+AB 3d) N=24/22/22	Group B (SI+AB 7d) N=21/19/18	*p value A-B*	Group A (SI+AB 3d) N=24/22/22	Group B (SI+AB 7d) N:21/19/18	*p value A-B*
A. actinomycetemcomitans						
Baseline	2.02±2.52	1.90±2.57	*0.877*	10/41.7%	8/38.1%	*1.000*
3 months	0.44±1.14 [s]	0.71±1.26	*0.406*	3/13.6%	5/26.3%	*0.436*
6 months	0.48±1.04 [s]	0.87±1.80	*0.669*	4/18.2%	4/22.2%	*1.000*
P. gingivalis						
Baseline	5.41±2.63	5.93±2.57	*0.236*	20/83.3%	18/85.7%	*1.000*
3 months	0.27±0.90 [s]	0.27±1.20 [s]	*0.685*	2/9.1% [s]	1 /5.3%[s]	*1.000*
6 months	0.63±1.46 [s]	0.38±1.65 [s]	*0.277*	4/18.2% [s]	1 /5.6% [s]	*0.355*
T. forsythia						
Baseline	5.84±2.33	6.61±1.63	*0.124*	21/87.5%	20/95.2%	*0.611*
3 months	1.72±2.43 [s]	2.16±2.44 [s]	*0.447*	8/36.4% [s]	9/47.4% [s]	*0.537*
6 months	1.79±2.45 [s]	1.70±2.51 [s]	*0.949*	8/36.4% [s]	6/33.3% [s]	*1.000*
T. denticola						
Baseline	4.43±2.45	5.41±1.91	*0.056*	19/79.2%	19/90.5%	*0.422*
3 months	1.35±2.38 [s]	2.27±2.52 [s]	*0.214*	6/27.3% [s]	9/47.4% [s]	*0.211*
6 months	1.59±2.19 [s]	2.21±2.45 [s]	*0.415*	8/36.4% [s]	9/50.0% [s]	*0.523*
P. intermedia						
Baseline	2.79±2.94	4.58±3.03	*0.023* [s]	12/50%	15/71.4%	*0.223*
3 months	0.67±1.79 [s]	1.47±2.55 [s]	*0.290*	3/13.6% [s]	5/26.3% [s]	*0.436*
6 months	0.69±1.85 [s]	1.87±2.52 [s]	*0.083*	3/13.6% [s]	7/38.9%	*0.140*
F. nucleatum						
Baseline	7.29±0.59	7.66±0.41	*0.026* [s]	25/100%	21/100%	-
3 months	5.58±1.11 [s]	6.01±1.11 [s]	*0.467*	22/100%	19/100%	-
6 months	5.46±1.07 [s]	5.49±1.74 [s]	*0.463*	22/100%	17/94.4%	*0.450*
C. rectus						
Baseline	4.93±1.98	5.13±1.96	*0.820*	22/91.7%	19/90.5%	*0.643*
3 months	0.94±2.07 [s]	1.56±2.00 [s]	*0.200*	5/22.7% [s]	8/42.1% [s]	*0.313*
6 months	0.89±1.81 [s]	0.81±1.64 [s]	*0.941*	5/22.7% [s]	4/22.2% [s]	*1.000*
F. allocis						
Baseline	6.40±2.16	7.28±0.77	*0.092*	22/91.7%	21/100%	*0.491*
3 months	1.36±2.35 [s]	2.54±2.63 [s]	*0.102*	6/27.3% [s]	10/52.6%	*0.120*
6 months	1.86±2.59 [s]	2.07±2.72 [s]	*0.827*	8/36.4% [s]	7/38.9%	*1.000*

SD:standard deviation, N: number; %: percentage; AB: antibiotics; d: days.

[s] statistically significant *p* values compared to baseline;

Table. 6
Immunomarker analysis: intragroup comparisons between the timepoints (Wilcoxon test) and intergroup comparisons (Mann-Whitney-U test).

Variables	Group A (SI+AB 3d) N=25/22/23	Group B (SI+AB 7d) N=25/21/18	*p value A-B Mann-Whitney-U*
MMP-8 (ng/site)			
Baseline	17.79±4.00	18.59±6.35	*0.228*
3 months	10.375±4.05 [s]	12.19±5.29 [s]	*0.150*
6 months	10.448±4.71[s]	10.08±4.36 [s]	*0.807*
IL-1β (pg/site)			
Baseline	209.62±107.76	289.67±190.53	*0.076*
3 months	76.12±67.87 [s]	136.34±73.56 [s]	*0.013* [s]
6 months	58.48±57.86 [s]	88.56±70.97 [s]	*0.121*
IL-10 (pg/site)			
Baseline	0.06±0.32	0.26±0.99	*0.461*
3 months	1.12±3.12	4.55±8.01	*0.467*
6 months	0.16±0.76	2.90±9.29	*0.183*
IL-8 (pg/site)			
Baseline	215.25±142.03	351.06±267.88	*0.038* [s]
3 months	99.40±81.25 [s]	166.88±149.08 [s]	*0.180*
6 months	119.58±120.08 [s]	131.21±105.82 [s]	*0.634*

MMP: matrix metallo proteinase 8; IL interleukin; N: number; AB: antibiotics; d: days
[s] statistically significant *p* values;