Systemic effects of initial periodontal therapy with or without adjunctive systemic antibiotics

ALMAGHLOUTH, Adnan Ali

Abstract
Les sujets atteints de parodontite non traitée peuvent montrer des pics élevés pendant plusieurs marqueurs inflammatoires dans le sérum simultanément. Traitement parodontal non chirurgical avec ou sans antibiotiques réduit la plupart de ces niveaux de pointe.

Reference

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# Clinical Oral Investigations

**Effect of periodontal treatment on peak serum levels of inflammatory markers**

--Manuscript Draft--

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                     | Andrea Mombelli |
| Order of Authors Secondary Information: | |
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Effect of periodontal treatment on peak serum levels of inflammatory markers

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Running title: Periodontal treatment and inflammation

Key words: Non-surgical periodontal therapy, antibiotics, serum, inflammatory mediators

One-sentence summary:

Non-surgical periodontal treatment with or without antibiotics reduced peak levels of several inflammatory markers in serum of subjects with periodontitis.

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Abstract

**Background and Objective:** Some subjects with untreated periodontitis exhibit elevated levels of distinct inflammatory markers in serum. The aim of the study was to assess whether non-surgical periodontal therapy changes the levels of these markers and lowers these peaks.

**Methods:** 40 periodontally diseased subjects received non-surgical periodontal therapy (full-mouth scaling and root planing within 48 h) with either adjunctive systemic amoxicillin and metronidazole (n=19) or placebo (n=21). Serum samples, obtained at baseline (BL) and 3 months after treatment (M3), were evaluated for 15 cytokines and 9 acute-phase proteins using the Bio-Plex bead array multianalyte detection system. For each analyte, peak values were defined as >mean+2SD in 40 periodontally healthy control subjects. Proportions were compared using Fisher’s exact test.

**Results:** At M3, a significantly better primary clinical outcome (persisting pockets >4 mm with bleeding on probing) was obtained in patients treated with scaling and root planing plus antibiotics compared to those receiving placebo (3.3±5.1 v.s. 6.8±7.8 pockets per patient, p<0.05). The levels of cytokines and acute phase proteins of periodontitis patients were usually below the mean+2SD threshold of healthy controls. However, values above threshold were found in some individuals. 11 patients showed a peak value of one analyte, 7 patients showed two peaks. In the remaining 12 patients, between 3 and 10 analytes showed peak values. Therapy greatly reduced the number of subjects with 4 or more peaks (BL: 11 subjects, M3: 1 subject, p=0.003). With regards to the reduction of peaks, no specific benefit of adjunctive antibiotics could be seen.

**Conclusion:** Subjects with untreated periodontitis may show high peaks for several inflammatory markers in serum simultaneously. Non-surgical periodontal treatment with or without antibiotics reduced most of these peak levels.
Introduction

Periodontitis is associated with elevated serum inflammatory markers (for review see 1). This suggests that periodontitis may have an impact on systemic health and insinuates that efforts to maintain or restitute periodontal health may contribute to systemic health. Studies have indicated that biological markers of systemic conditions like atherosclerosis are more prevalent in periodontitis patients than in age and gender-matched controls (2). However, the currently available data on periodontal systemic associations are rather heterogeneous (3) and the evidence that periodontal therapy has an impact on systemic health is limited (4). A few studies have tried to elucidate whether clinically successful periodontal treatment can reduce the levels of serological markers and have yielded inhomogeneous results. A preliminary intervention study indicated that standard non-surgical therapy (scaling and root planing, SRP) might be able to change levels of IL-6, and C reactive protein (5). In a comparative study, SRP reduced levels of IL-6 and C reactive protein, and, if local antibiotics were added, also total and LDL cholesterol in serum (6). In another study, however, periodontal therapy improved clinical and microbiological parameters, but did not influence the levels of serum analytes (7). In patients with chronic periodontitis and known coronary artery disease, SRP reduced high-sensitivity C reactive proteins (hsCRP), and white blood cell counts; however, tumor necrosis factor-α (TNF-α) levels showed no statistically significant reduction (8). In patients with metabolic syndrome CRP levels decreased after non-surgical periodontal therapy, with or without adjunctive antibiotics (9). As systematic review has concluded that plasma CRP in periodontitis is elevated and modest evidence indicates an effect of periodontal therapy in lowering these levels (10).

Technological developments have made it possible to detect and quantify a range of various biological markers in relatively small fluid specimens simultaneously. A high-throughput bead-based suspension array immunoassay system can assess up to 100 analytes in a sample volume of 25 to 50 μL (11). The quantification of multiple markers in one single same sample can provide information on interactions that are inaccessible by studying single markers individually. A preliminary analysis suggested that among patients with untreated periodontitis a few might exhibit high levels of several distinct inflammatory markers at the same time (12). Studies on changes of serological inflammatory markers after periodontal therapy conducted so far focused on mean or median changes of single serological markers. The presence, and the disappearance, of multiple extreme values may however be biologically
important in affected individuals, even if this does not concern all participants of a trial. The purpose of the present investigation was to further investigate this phenomenon by measuring a range of 24 analytes in serum of periodontally diseased patients before and after non-surgical periodontal therapy. This analysis is part of a large randomized clinical trial on the adjunctive effects of antibiotics given at different time points during periodontal therapy. For the present exploratory analysis we focus on the outcomes at the 3-month re-evaluation after initial therapy, the differential effect of the antibiotics is not the primary subject here.

**Material and Methods**

**Participants and study design**

Participants were recruited among patients seeking periodontal treatment, or consulting for a routine dental check-up at the School of Dental Medicine of the University of Geneva between April 2009 and August 2011. 40 patients with untreated moderate to advanced periodontitis (presence of at least 4 teeth with a probing pocket depth (PD) >4 mm, clinical attachment loss of at least 2 mm and radiographic evidence of bone loss) participated in a single-center, randomized, placebo-controlled, parallel-group, and double-masked trial. In addition, 40 persons with no evidence for past or present periodontal disease (absence of periodontal pockets with PD >3 mm, absence of clinical attachment loss >1 mm and no radiographic evidence of bone loss) were included to provide a single venous blood sample. All participants were systemically healthy, within an age range of 25-70 years and had at least 12 scorable teeth (not including 3rd molars, teeth with orthodontic appliances, bridges, crowns or implants). Exclusion criteria were systemic illnesses (i.e. diabetes mellitus, cancer, HIV, bone metabolic diseases or disorders that compromise wound healing, radiation or immunosuppressive therapy), pregnancy or lactation, systemic antibiotics taken within the previous two months, use of non-steroid anti-inflammatory drugs, confirmed or suspected intolerance to 5-nitroimidazole-derivatives or amoxicillin, and subgingival SRP or surgical periodontal therapy in the last year.

The Ethical Committee of the University Hospitals of Geneva, Geneva, Switzerland approved the protocol. Research was conducted according to the principles outlined in the Declaration of Helsinki on human medical experimentation. Written informed consent was obtained from all participants.
Clinical protocol

Venous blood samples were drawn from all 80 participants. The 40 individuals with periodontitis were further examined and treated according to the clinical protocol outlined below. Blood samples were again obtained from the latter 12 weeks after therapy.

The clinical part of the study involved the examiner (AA), who obtained informed consent, organized the treatment sessions and recorded all data, and the operator (NC), who provided the therapy. The therapist was unaware of the recorded data, except for the periodontal pocket chart that he needed to deliver the treatment.

The operator removed supra-gingival deposits and gave oral hygiene instructions and if necessary gave reinforcement of oral hygiene during recall visits. Once the subjects had reached an appropriate level of plaque control, they were scheduled to receive subgingival treatment within one month by the operator. Immediately before treatment the examiner recorded the periodontal parameters. The operator treated the periodontally diseased teeth with thorough scaling and root planing (SRP) to the depth of the pocket under local anesthesia. He first used ultrasonic instruments, then Gracey curettes, and finally irrigated the pockets with a 0.1% aqueous solution of chlorhexidine. Subjects were instructed to rinse the mouth twice daily during the next 10 days with 0.2% chlorhexidine. SRP was completed within 48 h and usually required two sessions.

At the end of the last session each subject received a neutral package, prepared by the pharmacy of the Geneva University Hospital, containing either a test or placebo medication, and was advised to start with the drug regime in the evening of the same day. Patients in the test group received 500 mg metronidazole and 375 mg amoxicillin, to be taken three times per day during seven days, patients in the control group received similar looking placebos. The treatment was allocated using a computer-generated randomization list concealed to the patient, the clinical examiner and the therapist. The examiner called the subjects after one week, and after one month. Medical history, any concomitant medication, and any adverse events or serious adverse events were recorded. The first post-treatment visit also served as a compliance control, as subjects were asked to return any medication that remained.

Immediately before (baseline, BL), and 3 months after therapy (M3) the following clinical parameters were recorded on six sites of each tooth with a pocket >4 mm at baseline: Gingival
Index (GI)(13), PD and Recession (REC; positive if gingival margin located apical, negative if located coronal to the cemento-enamel junction), Bleeding upon Probing (BOP) or suppuration.

**Blood sampling and processing**

5 ml blood was obtained by venipuncture using tubes without anticoagulant. The specimens were immediately centrifuged for 10 minutes at 1300 g. 2 ml serum were stored at -70 °C until the day of analysis.

We used a multiplex fluorescent bead-based immunoassay and the Bio-Plex 200 suspension array system (BioRad Laboratories, Hercules, CA, USA) to assess two panels of serum markers: the first panel included the following 15 cytokines: IL-1β, IL-1ra, IL-4, IL-6, IL-8, IL-10, IL-12, IL-17, basic FGF, G-CSF, GM-CSF, IFN-γ, MIP-1β VEGF, and TNF-α (kit M5000HIXIL, BioRad Laboratories, Hercules, CA, USA). The second panel included the following 9 acute phase proteins: α2M, haptoglobin, CRP, serum amyloid P and A, ferritin, fibrinogen, tissue plasminogen activator and procalcitonin (kits 171A4009M and 171A4007M, BioRad Laboratories, Hercules, CA, USA). The assays were performed following the manufacturer’s instructions. Briefly, 96-well filter plates were pre-wetted with assay buffer and the solution was aspirated from the wells using a vacuum-filter. Microsphere beads coated with monoclonal antibodies against the 24 target analytes were added to the wells. Serum samples, controls and standards were added in separate wells and incubated 30 min for the 15-plex and 1 h for the acute phase proteins panel. The wells were washed using the vacuum-filter and a mixture of biotinylated secondary antibodies was added. After incubation for 30 min and washing of the plates, streptavidin conjugated to the fluorescent protein phycoerythrin (streptavidin-PE) was added to the wells and incubated for 10 min. After washing to remove the unbound reagents, the assay buffer was added to the wells and the beads (minimum of 100 per analyte) were analyzed in the Bio-Plex 200 suspension array system. For the 15-plex panel, the detection limit of the assay was 1 pg/ml. For the acute phase protein panel, the limit of detection was 1 ng/ml, with the exception of ferritin, procalcitonin and tissue plasminogen activator, where the detection limit was 1 pg/ml.
Statistical analysis

Because not all data were normally distributed, differences between placebo and test groups were analyzed using Wilcoxon matched-pair signed-rank test, a non parametric test. Bio-Plex Manager 3.0 (BIO-RAD, Hercules, CA, USA) was used to analyze the read-out. All analytes had a very skewed distribution, with most patients having normal values and a few patients having extreme values. Thus, we dichotomized each analyte into a normal versus extreme value. The cutoffs used to determine these peak values were defined as greater than the mean plus two standard deviations (mean+2SD) in 40 periodontally healthy control subjects. If an analyte had a detection frequency below 25% in the healthy subjects, the cutoff was set at the respective detection level. To calculate mean values, a constant (0.1) was added to remove zero values. Proportions of peaks between treatment groups were compared using Fisher’s exact tests. P values <0.05 were accepted for statistical significance. The statistical software R (version 2.15.1, The R Foundation for Statistical Computing, Vienna, Austria) was used for all analyses.

Results

The mean age of the healthy subjects was 37.2±11.0, 26 female, 14 male, 10% were smokers. The mean age of the patients was 46.8±8.5, 20 female, 20 male, 35% were smokers. In the patients, no significant differences were found between groups for the demographic variables.

The clinical results of the study before and three months after treatment are summarized in Table 1. All 40 enrolled patients with periodontitis could be followed up to M3. A total of 3612 sites (6 on a total of 602 periodontally diseased teeth with a pocket >4 mm at BL; pockets >4 mm had a mean PD of 6.5 mm), were clinically monitored at BL and at M3. At BL differences between groups were not significant. In contrast, at M3, a significantly better primary clinical outcome was obtained in patients treated with full-mouth SRP plus amoxicillin and metronidazole compared to those receiving placebo: Those treated with antibiotics had fewer persisting pockets >4 mm with BOP than those treated with placebo. In the patients of the placebo group, on average 6.8±7.8 pockets >4 mm with BOP could still be found, whereas a mere 3.3±5.1 sites in this category were still detected in the test group.
A total of 120 serum samples were collected and analyzed for the presence of 15 cytokines and 9 acute phase proteins. Table 2 shows the number of positive samples of all analytes in the 40 periodontally healthy subjects, and, if detected in at least 25% of the specimens, the mean values, standard deviations and mean+2SD. For the analytes detected in at least 25%, the mean+2SD served as threshold to define peak values in periodontitis patients. Six cytokines could not be detected in any serum sample (IL-4, IL-17, b-FGF, G-CSF, GM-CSF and IFN-γ) and three (IL-1β, IL-6 and IL-10) were detected with a frequency below 25%. For those nine cytokines, each positive reading in a patient was considered as a peak (i.e., the cutoff for inclusion was set at the respective level of detection).

Table 3 shows the incidence and range of peak values of 15 cytokines and 9 acute phase proteins in 40 periodontitis patients before and after therapy. For example, 4 patients presented peak values for IL-1β at baseline and 8 for IL-1ra. These peak values ranged between 4.8 and 22.5, and between 21.7 and 127.3, respectively. At M3, none of the patients still presented a peak for IL-1β, and only a single case still demonstrated a peak for IL-1ra.

For 5 biomarkers with high incidence of peaks of baseline, i.e., Serum Amyloid A, IL-1ra, IL-12, MIP-1β, and Procalcitonin, treatment significantly reduced the number of subjects with peaks (p<0.05). Figure 1 shows a scatter plot for serum Amyloid A, comparing the values before and after therapy for each subject. For one analyte, the number of subjects with peaks increased significantly after treatment (Haptoglobin, p=0.01).

We then calculated for each individual how many of the 24 analytes showed a peak value at baseline and how many peaks persisted after therapy, with or without antibiotics. As Figure 2a shows, none of the 24 analytes were above threshold before treatment in 10 participants. A single analyte showed a peak value in 11 patients, two peaks were found in 7 patients. In the remaining 12 patients between 3 and 10 analytes showed peak values. For individuals with 2 or more peaks, the mean values of PPD and BOP were within the range of the other patients (PPD: 4.41; BOP: 86.6).

Figure 2b shows the number of analytes with peak values per subject after therapy. Therapy did not significantly reduce the number of subjects having at least one peak (BL: 30 subjects, M3: 27 subjects, p=0.62). The number of subjects with 4 or more peaks, however, was greatly reduced (BL: 11 subjects, M3: 1 subject, p=0.003). With regards to the reduction of peaks, no specific benefit of adjunctive antibiotics could be seen. A single patient still showed 8 peaks.
after treatment. This particular subject had 8 peaks at BL also, was treated with antibiotics and responded well to therapy as far as clinical parameters are concerned (mean PPD at BL: 6.26 mm, BOP at BL: 100%, PPD after therapy: 3.07 mm, BOP after therapy: 39.9%).

Table 4 shows the mean serum biomarker levels in periodontitis patients per treatment group at baseline and M3 of those 13 analytes with a frequency of detection >75%. The levels were significantly lower at M3 than at baseline for most of these markers. Differences between groups were not significant.

There were no serious adverse events. The number of patients reporting a stomach upset was similar in both groups (3 in the placebo group and 3 in the test group). One patient treated with antibiotics, and 3 in the placebo group, reported gastro-intestinal problems, notably diarrhea. All other events were sporadic.

**Discussion**

We measured a range of 24 analytes in serum of periodontally diseased patients before and three months after non-surgical periodontal therapy and assessed to what extent levels of these analytes can be modified by treatment. A high-throughput technique was used to assess the expression profile of 15 cytokines and 9-acute phase proteins in serum. The Bio-Plex 200 Suspension Array System is a flow-based dual-laser system that detects and measures molecules bound to the surfaces of fluorescent microspheres, thus providing highly accurate analysis of serum, culture media and other biological samples. Although the ELISA technique remains the gold standard for measuring inflammatory mediators, this new technology offers a number of advantages, such as the simultaneous determination of up to 100 different analytes in a single sample, less sample volume, ability to detect different proteins across a broad range of concentrations and efficiency in terms of time and cost (14, 15). The cytokine multiplex assays were found to be comparable in sensitivity, accuracy and reproducibility to ELISAs for the same analytes (16).

Due to the distribution patterns of these markers found in preliminary analyses (7, 12), we focused on the incidence of high values of multiple analytes rather than on mean or median changes, assuming that multiple high values of several markers may be more consequential for systemic health than elevated mean levels of a single marker. The mean+2SD of each analyte, determined in 40 periodontally healthy subjects, served as a threshold to define peak
values in periodontitis patients. Among 40 patients with untreated periodontitis, 11 exhibited levels above threshold of four and up to ten inflammatory markers at the same time. After treatment, the number of subjects with four or more peaks was significantly reduced. Only a single patient remained with multiple peaks (8 analytes above threshold). The fact that this person was the only one having also 8 peaks at BL and responding well to therapy suggests that the reason for this unusual pattern was not periodontal disease. The number of subjects with peaks increased significantly after treatment for only one analyte (Haptoglobin, \( p=0.01 \)). This was probably due to random variability.

Conflicting results on the mean or median effects of periodontal therapy on systemic inflammation have been reported previously. Whereas a significant increase in plasma TNF-\( \alpha \), CRP, and IL-6 levels has been found immediately after non-surgical periodontal therapy in some trials (6, 17, 18), other studies found no changes in the serum levels of these mediators three months after therapy (19). Six months after treatment, significant reductions in serum IL-6 and CRP were found especially in subjects who responded better than average to the delivered periodontal therapy (5). However, in another study, the same group reported marked increases in the serum levels of CRP and serum amyloid A only 24 h after treatment (20).

Responses of systemic inflammatory biomarkers were highly heterogeneous following periodontal therapy in a trial of another group where a large number of analytes was assessed simultaneously (21): 1/3 of the patients showed a marked reduction, 1/4 a pronounced increase, and the remainder of the patients showed no change in systemic inflammation.

In the medical literature, IL-6 has been described as a reliable marker for systemic inflammation. Peak values of plasma IL-6 and other peripheral markers of inflammation were assessed in the first week of ischaemic stroke in one study. Significant correlations between peak plasma IL-6 in the first week of ischaemic stroke with both brain infarct volume and outcome at 3 months were demonstrated (22). In a study including patients with or without periprosthetic infection following total hip and knee arthroplasty, normal values of IL-6 were defined as those below a cut off of 10 pg/ml (23). In our study, the threshold for IL-6 was similar (14.3 pg/ml). Only one patient had a value above this threshold before, and none after therapy. However, in a large population of African children without or with malaria (24), the mean values were 54 pg/ml in healthy, and 485 pg/ml in severely diseased subjects. Very high peak values were detected in some individuals of both groups. The same holds true for three other markers assessed in the same study (IL-1\( \beta \), IL-8, IL-10), suggesting that the African
cohorts may be exposed to additional immunological challenges even in the absence of malaria. On the other hand, our threshold values for IL-12 and TNF-α corresponded well to their mean values of healthy controls.

In our study, no correlation was found between the inflammatory mediator levels and the clinical parameters either before or after periodontal treatment. This is in agreement with other studies showing poor and inconsistent correlations between changes in the levels of inflammatory markers and changes in the clinical periodontal status after therapy, reduction of periodontal pathogens and reduction of serum IgG antibody levels to periodontal microbiota (7, 21, 25). It seems that serum levels of specific cytokines or acute phase proteins do not reflect the clinical periodontal health status independently; pre-existing susceptibility for systemic inflammation may influence the individual response. However, the reduction of most of the peak values, defined as >mean+2SD in periodontally healthy subjects, can be attributed to periodontal therapy. A marked increase of CRP has been reported 24 h after instrumentation (20), indicating that patients undergoing periodontal treatment experience short-term perturbations of systemic inflammation. Due to the timing of our trial we could not corroborate the existence of short termed peaks of some of the acute phase proteins. But even if they exist it seems that they are short-lived, as they were undetectable at M3 in our study.

Systemic amoxicillin plus metronidazole significantly improved clinical outcomes of periodontal therapy, similar to previous studies (26). No significant difference on the biologic response to therapy was however found between the two groups. We assume that the mechanical removal of pathogenic bacteria by SRP was sufficient to suppress peak values of inflammatory biomarkers.

**Conclusion**

Subjects with untreated periodontitis may show isolated high peaks for one or several inflammatory markers in serum. Non-surgical periodontal treatment with or without antibiotics reduced most of these peak levels.

**Acknowledgements**

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The authors report no conflicts of interest related to this study.

References


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Figure legends

Figure 1: Scatter plot for serum Amyloid A. Each dot represents one case of 40 periodontally diseased subjects before and after therapy.

Figure 2: Number of biomarker-peaks per subject before (A) and after (B) therapy. Total number of biomarkers is 24, the number of cases is 40.
Tables

Table 1
Clinical findings at baseline and after 3 months by treatment group.
Data are means (standard deviation), n=40 participants.

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PD: probing pocket depth; REC: recession; BOP: bleeding upon probing; GI: Gingival Index.

* Difference between placebo and test, p<0.05
Table 2

Detection frequency of 15 cytokines and 9 acute phase proteins in 40 periodontally healthy persons, and, if detected in more than 10 (25%) cases, standard deviation (SD) and mean+2SD. Mean+2SD values served as threshold to define peak values in periodontitis patients. If an analyte was detected in less than 25%, the detection level was used as cut-off (*).

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<td>VEGF, pg/ml</td>
<td>40 (100.0)</td>
<td>193.4 (137.6)</td>
<td>468.7</td>
</tr>
<tr>
<td>α2M, mg/ml</td>
<td>40 (100.0)</td>
<td>1.9 (0.7)</td>
<td>3.3</td>
</tr>
<tr>
<td>CRP, μg/ml</td>
<td>40 (100.0)</td>
<td>1.9 (3.9)</td>
<td>9.8</td>
</tr>
<tr>
<td>Haptoglobin, mg/ml</td>
<td>40 (100.0)</td>
<td>1.4 (1.5)</td>
<td>4.5</td>
</tr>
<tr>
<td>Serum Amyloid P, μg/ml</td>
<td>40 (100.0)</td>
<td>44.7 (13.9)</td>
<td>72.6</td>
</tr>
<tr>
<td>Ferritin, ng/ml</td>
<td>40 (100.0)</td>
<td>58.8 (47.6)</td>
<td>154.1</td>
</tr>
<tr>
<td>Fibrinogen, ng/ml</td>
<td>40 (100.0)</td>
<td>2.5 (0.6)</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>----------------------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>Procalcitonin, ng/ml</td>
<td>36 (90.0)</td>
<td>2.2 (1.4)</td>
<td>5.0</td>
</tr>
<tr>
<td>Serum Amyloid A, µg/ml</td>
<td>40 (100.0)</td>
<td>3.4 (2.8)</td>
<td>8.9</td>
</tr>
<tr>
<td>TP Activator, ng/ml</td>
<td>17 (42.5)</td>
<td>6.3 (8.6)</td>
<td>23.5</td>
</tr>
</tbody>
</table>

IL-1β: interleukin 1 beta; IL-1ra: interleukin 1 receptor antagonist; IL-4, -6, -8, -10, -12, -17: interleukin-4, -6, -8, -10, -12, -17; b-FGF: basic fibroblast growth factor; G-CSF: granulocyte colony stimulating factor; GM-CSF: granulocyte macrophage colony stimulating factor; IFN-γ: interferon-gamma; MIP-1β: macrophage inflammatory protein-beta; TNF-α: tumor necrosis factor alpha; VEGF: vascular endothelial growth factor, α2M: alpha-2 macroglobulin; CRP: C-reactive protein; TP Activator: tissue plasminogen activator
Table 3

Incidence (number and frequency) and range of peak values (readings above mean+2SD of healthy persons) of 15 cytokines and 9 acute phase proteins in 40 periodontitis patients before and after therapy.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Baseline N (%)</th>
<th>Baseline Range</th>
<th>Month 3 N (%)</th>
<th>Month 3 Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β, pg/ml</td>
<td>4 (10.0)</td>
<td>4.8 – 226.5</td>
<td>0 (0.0)</td>
<td>-</td>
</tr>
<tr>
<td>IL-1ra, pg/ml</td>
<td>8 (20.0)</td>
<td>21.7 – 127.3</td>
<td>1 (2.5)</td>
<td>32.0</td>
</tr>
<tr>
<td>IL-4, pg/ml</td>
<td>1 (2.5)</td>
<td>2.0</td>
<td>0 (0.0)</td>
<td>-</td>
</tr>
<tr>
<td>IL-6, pg/ml</td>
<td>1 (2.5)</td>
<td>216.3</td>
<td>0 (0.0)</td>
<td>-</td>
</tr>
<tr>
<td>IL-8, pg/ml</td>
<td>1 (2.5)</td>
<td>38.3</td>
<td>1 (2.5)</td>
<td>39.6</td>
</tr>
<tr>
<td>IL-10, pg/ml</td>
<td>4 (10.0)</td>
<td>1.4 – 61.6</td>
<td>5 (12.5)</td>
<td>1.4 – 4.9</td>
</tr>
<tr>
<td>IL-12-p70, pg/ml</td>
<td>5 (12.5)</td>
<td>66.7 – 125.8</td>
<td>0 (0.0)</td>
<td>-</td>
</tr>
<tr>
<td>IL-17, pg/ml</td>
<td>1 (2.5)</td>
<td>2.3</td>
<td>0 (0.0)</td>
<td>-</td>
</tr>
<tr>
<td>basic-FGF, pg/ml</td>
<td>2 (5.0)</td>
<td>18.7 – 36.3</td>
<td>1 (2.5)</td>
<td>11.9</td>
</tr>
<tr>
<td>G-CSF, pg/ml</td>
<td>1 (2.5)</td>
<td>29.8</td>
<td>0 (0.0)</td>
<td>-</td>
</tr>
<tr>
<td>GM-CSF, pg/ml</td>
<td>2 (5.0)</td>
<td>17.2 – 162.6</td>
<td>0 (0.0)</td>
<td>-</td>
</tr>
<tr>
<td>IFN-γ, pg/ml</td>
<td>3 (7.5)</td>
<td>13.2 – 5087.0</td>
<td>4 (10.0)</td>
<td>4.2 – 49.3</td>
</tr>
<tr>
<td>MIP-1β, pg/ml</td>
<td>6 (15.0)</td>
<td>260.5 – 791.2</td>
<td>0 (0.0)</td>
<td>-</td>
</tr>
<tr>
<td>TNF-α, pg/ml</td>
<td>1 (2.5)</td>
<td>140.4</td>
<td>0 (0.0)</td>
<td>-</td>
</tr>
<tr>
<td>VEGF, pg/ml</td>
<td>5 (12.5)</td>
<td>538.6 – 2456.2</td>
<td>3 (7.5)</td>
<td>525.7 – 5518.7</td>
</tr>
<tr>
<td>α2M, mg/ml</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>CRP, μg/ml</td>
<td>4 (10.0)</td>
<td>13.4 – 52.6</td>
<td>3 (7.5)</td>
<td>14.1 – 33.4</td>
</tr>
<tr>
<td>Haptoglobin, mg/ml</td>
<td>5 (12.5)</td>
<td>5.1 – 7.9</td>
<td>16 (40.0)</td>
<td>5.8 – 9.4</td>
</tr>
<tr>
<td>Serum Amyloid P, μg/ml</td>
<td>6 (15.0)</td>
<td>73.3 – 91.7</td>
<td>10 (25.0)</td>
<td>74.6 – 138.1</td>
</tr>
<tr>
<td>Ferritin, ng/ml</td>
<td>8 (20.0)</td>
<td>212.2 – 505.8</td>
<td>9 (22.5)</td>
<td>187.6 – 418.5</td>
</tr>
<tr>
<td>Fibrinogen, ng/ml</td>
<td>1 (2.5)</td>
<td>3.9</td>
<td>0 (0.0)</td>
<td>-</td>
</tr>
<tr>
<td>Procalcitonin, ng/ml</td>
<td>6 (15.0)</td>
<td>5.4 – 6.3</td>
<td>0 (0.0)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Value</td>
<td>Normal Range</td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------------</td>
<td>-----------</td>
<td>------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum Amyloid A, µg/ml</td>
<td>14 (35.0)</td>
<td>9.7 – 20.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 (5.0)</td>
<td>13.1 – 30.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP Activator, ng/ml</td>
<td>4 (10.0)</td>
<td>23.9 – 28.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 (0.0)</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IL-1β: interleukin 1 beta; IL-1ra: interleukin 1 receptor antagonist; IL-4, -6, -8, -10, -12, -17: interleukin-4, -6, -8, -10, -12, -17; b-FGF: basic fibroblast growth factor; G-CSF: granulocyte colony stimulating factor; GM-CSF: granulocyte macrophage colony stimulating factor; IFN-γ: interferon-gamma; MIP-1β: macrophage inflammatory protein-beta; TNF-α: tumor necrosis factor alpha; VEGF: vascular endothelial growth factor; α2M: alpha-2 macroglobulin; CRP: C-reactive protein; TP Activator: tissue plasminogen activator
Table 4

Levels of 13 serum biomarkers with a detection frequency of >75% in periodontitis patients per treatment group, at baseline and M3. Data are means (standard deviation), n=40 participants.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Baseline</th>
<th>Month 3</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>Test</td>
<td>Placebo</td>
</tr>
<tr>
<td>Participants, n</td>
<td>21</td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td>IL-1ra, pg/ml</td>
<td>12.6 (29.3)</td>
<td>5.8 (8.5)</td>
<td>0.5 (1.2)</td>
</tr>
<tr>
<td>IL-8, pg/ml</td>
<td>5.7 (8.6)</td>
<td>6.7 (6.3)</td>
<td>5.6 (9.5)</td>
</tr>
<tr>
<td>IL-12-p70, pg/ml</td>
<td>25.8 (20.9)</td>
<td>30.0 (30.6)</td>
<td>5.8 (4.6)</td>
</tr>
<tr>
<td>MIP-1β, pg/ml</td>
<td>189.4 (157.9)</td>
<td>157.0 (74.4)</td>
<td>97.0 (50.1)</td>
</tr>
<tr>
<td>VEGF, pg/ml</td>
<td>237.6 (244.3)</td>
<td>354.3 (544.8)</td>
<td>148.7 (130.0)</td>
</tr>
<tr>
<td>α2M, mg/ml</td>
<td>1.3 (0.6)</td>
<td>1.6 (0.5)</td>
<td>1.7 (0.3)</td>
</tr>
<tr>
<td>CRP, µg/ml</td>
<td>5.7 (8.1)</td>
<td>6.4 (11.7)</td>
<td>4.6 (4.4)</td>
</tr>
<tr>
<td>Haptoglobin, mg/ml</td>
<td>1.0 (1.5)</td>
<td>2.3 (2.5)</td>
<td>2.8 (2.7)</td>
</tr>
<tr>
<td>Serum Amyloid P, µg/ml</td>
<td>56.2 (20.0)</td>
<td>56.5 (13.9)</td>
<td>67.1 (25.7)</td>
</tr>
<tr>
<td>Ferritin, ng/ml</td>
<td>117.0 (166.9)</td>
<td>109.4 (122.6)</td>
<td>99.0 (128.4)</td>
</tr>
<tr>
<td>Fibrinogen, ng/ml</td>
<td>2.6 (0.9)</td>
<td>2.3 (1.0)</td>
<td>1.9 (0.6)</td>
</tr>
<tr>
<td>Procalcitonin, ng/ml</td>
<td>2.5 (2.1)</td>
<td>2.3 (1.8)</td>
<td>0.5 (0.6)</td>
</tr>
<tr>
<td>Serum Amyloid A, µg/ml</td>
<td>8.9 (7.0)</td>
<td>6.5 (5.2)</td>
<td>3.6 (3.5)</td>
</tr>
</tbody>
</table>

IL-1ra: interleukin 1 receptor antagonist; IL-8, -12: interleukin-8, -12; MIP-1β: macrophage inflammatory protein-beta; VEGF: vascular endothelial growth factor; α2M: alpha-2 macroglobulin; CRP: C-reactive protein
Figure 1
Are There Specific Benefits of Amoxicillin Plus Metronidazole in *Aggregatibacter actinomycetemcomitans*-Associated Periodontitis? Double-Masked, Randomized Clinical Trial of Efficacy and Safety

Andrea Mombelli,* Norbert Cionca,* Adnan Almaghlouth,* Fabien Décailliet,* Delphine S. Courvoisier,† and Catherine Giannopoulou*

**Background:** It has been suggested that prescription of amoxicillin plus metronidazole in the context of periodontal therapy should be limited to patients with specific microbiologic profiles, especially those testing positive for *Aggregatibacter actinomycetemcomitans*. The main purpose of this analysis is to determine if patients positive for *A. actinomycetemcomitans* with moderate to advanced periodontitis benefit specifically from amoxicillin plus metronidazole given as an adjunct to full-mouth scaling and root planing.

**Methods:** This is a double-masked, placebo-controlled, randomized longitudinal study including 41 participants who were positive for *A. actinomycetemcomitans* and 41 participants who were negative for *A. actinomycetemcomitans*. All 82 patients received full-mouth periodontal debridement performed within 48 hours. Patients then received either systemic antibiotics (375 mg amoxicillin and 500 mg metronidazole, three times daily) or placebo for 7 days. The primary outcome variable was persistence of sites with a probing depth (PD) >4 mm and bleeding on probing (BOP) at the 3-month reevaluation. Using multilevel logistic regression, the effect of the antibiotics was analyzed according to the following factors (interaction effect): *A. actinomycetemcomitans*-positive or -negative at baseline, sex, age, smoking, tooth being a molar, and interdental location.

**Results:** At reevaluation, participants in the test group had significantly fewer sites with a persisting PD >4 mm and BOP than control patients (*P* < 0.01). Being *A. actinomycetemcomitans*-positive or -negative did not change the effect of the antibiotics. Patients benefited from the antibiotics irrespective of sex, age, or smoking status. Molars benefited significantly more from the antibiotics than non-molars (*P* for interaction effect = 0.03).

**Conclusions:** Patients who were positive for *A. actinomycetemcomitans* had no specific benefit from amoxicillin plus metronidazole. Sites on molars benefited significantly more from the antibiotics than non-molar sites. *J Periodontol* 2013;84:715-724.

**KEY WORDS**

*Aggregatibacter actinomycetemcomitans*; amoxicillin; metronidazole; periodontitis; root planing.

* Department of Periodontology, University of Geneva, Geneva, Switzerland.
† Department of Psychology, Harvard University, Cambridge, MA.
The benefit of systemically administered amoxicillin plus metronidazole, used as an adjunct to non-surgical, mechanical-periodontal treatment (scaling and root planing [SRP]), is well documented. During the last 10 years, 23 articles concerning 18 randomized clinical trials, have consistently corroborated the findings of two earlier systematic reviews showing significantly better mean attachment level changes in patients treated with these antibiotics than in patients treated with placebo. Comparative studies could not demonstrate better clinical results for any other antimicrobial regimen. Nevertheless, it remains a matter of controversy whether this treatment should be restricted to certain individuals, for example, those with a specific microbiologic profile. As amoxicillin plus metronidazole has a proven capacity to suppress Aggregatibacter actinomycetemcomitans from periodontitis lesions and other oral sites, and this microbe is known to resist mechanical treatment particularly well, it has been suggested to use this combination specifically for the treatment of advanced A. actinomycetemcomitans-associated periodontitis. The problem with recommending that therefore microbiologic testing should be performed to identify suitable patients is the lack of evidence that individuals testing negative for this, or any other marker microorganism, have no benefit from these antibiotics. Randomized clinical trials that assessed microbiologic data in addition to clinical data so far included too few participants who were positive for A. actinomycetemcomitans to draw conclusions regarding differential outcomes in individuals who are positive or negative for A. actinomycetemcomitans. The authors of this study therefore decided to conduct a study in a sample with equal numbers of participants who were positive and negative for A. actinomycetemcomitans in the test and control groups.

The purpose of this analysis is to evaluate the contribution of patient, tooth, and site-associated variables on the clinical outcome of full-mouth SRP with or without amoxicillin and metronidazole. In particular, the authors were interested to know if the benefit from these antibiotics differs among patients who are positive or negative for A. actinomycetemcomitans with moderate-to-advanced periodontitis. The 3-month outcomes are the focus of this analysis, as it is common practice to carry out periodontal therapy in two phases, whereby the results of the first non-surgical treatment phase are assessed after about 3 months to decide on further, frequently surgical, interventions.

**MATERIALS AND METHODS**

This is a single-center, randomized, placebo-controlled, parallel-group, and double-masked trial. The Ethical Committee of the University Hospitals of Geneva, Geneva, Switzerland, approved the protocol. The study was authorized by the Swiss Agency for Therapeutic Products (Swissmedic), Bern, Switzerland. Research was conducted according to the principles outlined in the Declaration of Helsinki, as revised in 2000, on human medical experimentation. Written informed consent was obtained from all participants.

**Patients**

Participants were recruited from individuals seeking treatment at the School of Dental Medicine, University of Geneva. A total of 82 systemically healthy patients with untreated moderate-to-advanced periodontitis were included on the basis of the following criteria: 1) aged 25 to 70 years; 2) presence of ≥12 scorable teeth (not including third molars and teeth with orthodontic appliances, bridges, crowns, or implants); 3) diagnosis of periodontitis with presence of ≥4 teeth with a probing depth (PD) >4 mm, clinical attachment loss of ≥2 mm; and 4) radiographic evidence of bone loss.

Exclusion criteria were: 1) systemic illnesses (i.e., diabetes mellitus, cancer, human immunodeficiency virus, bone metabolic diseases or disorders that compromise wound healing, radiation, or immunosuppressive therapy); 2) pregnancy or lactation; 3) systemic antibiotics taken in the previous 2 months; 4) use of non-steroid anti-inflammatory drugs; 5) confirmed or suspected intolerance to 5-nitroimidazole derivatives or amoxicillin; and 6) subgingival SRP or surgical periodontal therapy in the last year. Smoking history was recorded, but smoking was not an exclusion criterion.

The first 51 participants were recruited from November 2006 to December 2007. Forty-one of these individuals tested negative for the presence of A. actinomycetemcomitans in a pooled sample, taken in the deepest pocket of each quadrant. Treatment allocation up to that time was independent of microbiologic status, resulting in 19 and 22 participants negative for A. actinomycetemcomitans in the placebo and antibiotics groups, respectively. A second set of 31 patients was recruited from June 2008 to December 2009 with the additional inclusion criterion that a microbiologic test revealed the presence of A. actinomycetemcomitans in a pooled sample from the deepest pocket of each quadrant. These participants were randomly allocated to the treatments. Recruitment was stopped when the number of participants who were positive and negative for A. actinomycetemcomitans was equilibrated in each group.

**Test Products and Randomization**

On completion of mechanical treatment, each participant received a neutral package containing the...
test or placebo medication, identical in appearance and only marked with the patient number. The test group received 375 mg amoxicillin and 500 mg metronidazole (three times daily for 7 days). The control group received similar-looking placebos. The daily dose of metronidazole corresponded to 20 mg/kg for a patient weighing 75 kg, the amount estimated necessary to reach an effective concentration in body fluids. The pharmacy of the Geneva University Hospital prepared the capsules and allocated the participants to one of two treatment groups using a computer-generated randomization list. The treatment group was concealed to the patient, the clinical examiner (AA), and the therapist (NC). The therapist was not involved in data gathering and was unaware of the recorded data, except for the periodontal pocket chart that he needed to deliver the treatment.

**Clinical Protocol**

The clinical examiner recorded the medical history, obtained informed consent, removed supragingival deposits, and gave oral hygiene instructions. The participants were recalled to assure that proper oral hygiene was established. Once an appropriate level of plaque control was reached, they were scheduled to receive subgingival treatment within 1 month by the therapist. Immediately before treatment, the examiner recorded the periodontal parameters. The operator then treated the periodontally diseased teeth with thorough SRP to the depth of the pocket under local anesthesia. He first used ultrasonic instruments and then periodontal Gracey curets and finally irrigated the pockets with a 0.1% aqueous solution of chlorhexidine. Participants were instructed to rinse the mouth twice daily during the next 10 days with 0.2% chlorhexidine. SRP was completed within 48 hours and usually required two sessions. At the end of the last session, each patient received the medication and was advised to start the drug regimen the evening of the same day. One week after treatment the patients were seen by the examiner. Any concomitant medication and all adverse events were recorded using a structured questionnaire. This visit also served as a compliance control because patients were asked to return any medication that remained.

Immediately before and 3 months after therapy the following clinical parameters were recorded on six sites of each tooth with a pocket >4 mm at baseline: 1) gingival index (GI); 2) PD; 3) recession (REC; positive if gingival margin located apical and negative if located coronal to the cemento-enamel junction); 4) bleeding on probing (BOP); and 5) suppuration. In addition, presence or absence of plaque was recorded on six sites of all teeth by running a probe across the site (plaque score [PS]).

**Statistical Analyses**

Data were entered into a database and were checked for entry errors by two persons (AA and Céline Ippolito, dental hygienist, University of Geneva, Geneva, Switzerland). Average scores were calculated for clinical parameters for each patient at each examination by summing the scores and dividing by the number of sites graded for that patient. The absolute number of sites per patient with a PD >4 mm and BOP 3 months after therapy was the primary clinical outcome measure of the study (patients with persisting pockets >4 mm and BOP 3 months after initial therapy are commonly perceived as needing further treatment in clinical practice). Secondary clinical outcomes included differences between groups for changes in PD, BOP, and REC, as well as types and frequencies of adverse events.

The effect of treatment group on patient characteristics at baseline and at the 3-month follow-up was estimated using the χ² test when the variable was categorical and linear regression when the variable was continuous. Prespecified subgroup analyses according to patients being *A. actinomycetemcomitans*–positive or –negative at baseline, sex, age, and smoking were performed. With respect to site-level outcome, multilevel regressions were used, considering sites as nested within teeth, themselves nested within patients. Finally, using multilevel logistic regression, the effect of antibiotics on PD >4 mm and BOP at month 3 was analyzed according to the following moderating factors: *A. actinomycetemcomitans*–positive or –negative at baseline, sex, age, smoking, tooth being a molar, and interdental location. Each moderation effect was estimated by including treatment (placebo versus test), the respective factor, and their interaction in the model. The P value of the interaction indicated whether the effect of antibiotics differed significantly across the level of the moderating factor.

One statistical software program was used for all analyses. P values <0.05 were accepted for statistical significance.

**RESULTS**

Figure 1 shows the flow diagram for the different phases of the study. All 82 enrolled patients could be followed up to month 3. Table 1 displays the baseline characteristics of the participants by treatment group and presence (Aa+) or absence (Aa–) of *A. actinomycetemcomitans*. A total of 6,462 sites (six on a total of 1,077 periodontally diseased teeth with a pocket >4 mm at baseline; pockets >4 mm had a mean PD of 5.97 mm) were clinically monitored at baseline and at 3 months. Patients in the placebo group had an overall mean PD of 4.5 ± 1.6 mm; 69.7% ± 46.0%
of all sites had BOP. The patients had, on average, 31.6 ± 21.5 sites with a PD >4 mm and BOP. The patients of the test group had a mean PD of 4.5 ± 1.8; 74.6% ± 43.5% of all sites had BOP; 32.3 ± 26.5 sites had a PD >4 mm and BOP. Differences between groups were not significant. Patients who were Aa+ had a higher number of pockets >4 mm and with BOP than patients who were Aa−, but this difference was not significant (Aa+: 34.3 ± 29.0 versus Aa−: 29.7 ± 17.8; P = 0.39).

Table 2 shows the clinical results at the 3-month follow-up by treatment group. A significantly better primary clinical outcome was obtained in patients treated with full-mouth SRP plus amoxicillin and metronidazole compared with those receiving placebo. Those treated with antibiotics had fewer persisting pockets >4 mm with BOP than those treated with placebo. In the patients of the placebo group, on average, 5.7 ± 7.6 pockets >4 mm with BOP could still be found, whereas a mere 2.1 ± 3.8 sites in this category were still detected in the test group.

The primary outcomes of therapy were determined specifically in patients who were Aa+ or Aa−. Figure 2 shows box plots for the number of persisting sites per patient with PD >4 mm and BOP at 3 months by treatment group (placebo, antibiotics) and presence or absence of A. actinomycetemcomitans. Patients who were Aa+ and treated with antibiotics had, on average, 4.1 fewer sites persisting with PD >4 mm and BOP than patients who were Aa+ and treated with placebo (6.7 to 2.6 sites; see Table 2). Patients who were Aa− had 3.1 fewer such sites if treated with antibiotics than if treated with placebo (4.7 to 1.6 sites; Table 2). The benefit of the antibiotics in terms of fewer

![Figure 1.](image)

\[\text{Different phases of the study: CHX = chlorhexidine.}\]
sites persisting with PD > 4 mm and BOP was not significantly different between patients who were Aa+ and patients who were Aa− (P of odds ratio [OR] difference; see Table 3). Furthermore, the effect of treatment with antibiotics was highly significant in patients who were Aa+ as well as patients who were Aa− (OR Aa+ = 0.25; OR Aa− = 0.34).

If an efficacy endpoint in non-surgical periodontal therapy was set at a 10-fold reduction of diseased sites, 35 patients in the antibiotics group and 16 in the placebo group would have met this endpoint, irrespective of A. actinomycetemcomitans carrier status at baseline (risk difference: 37.4; 95% confidence interval [CI]: 16.5 to 55.5). If an efficacy endpoint was set at a 10-fold reduction of diseased sites at all treated teeth after 3 months, 37 patients of the antibiotics group and 23 patients in the placebo group would have achieved this endpoint, again irrespective of A. actinomycetemcomitans carrier status at baseline (risk difference: 23.6; 95% CI: 4.3 to 42.1). At baseline, patients who were Aa+ had significantly more BOP+ sites than patients who were Aa−. This difference in percentage of BOP+ sites remained present and was of the same magnitude after 3 months as tested by the interaction effect (Table 3).

The other subgroup analyses revealed that sites on molar teeth benefited significantly more from the antibiotics than sites on other teeth (P = 0.03; Table 3). If molars were treated with placebo, 3 months later 10.3% sites remained with PD > 4 mm and BOP. If these teeth were treated with antibiotics, only 3.9% such sites remained. If non-molars were treated with placebo or antibiotics, the respective figures were 4.5% and 1.9%. Treatment effects were not significantly moderated by the other analyzed characteristics on the level of the person or the site. The effect of the antibiotics, for example, was independent of PD at baseline, meaning that the benefit of the antibiotics was not limited to very deep sites only.

Table 4 shows the different events occurring during the active period of medication, as assessed 1 week after treatment. There were no serious adverse events. The number of patients reporting stomach upset was similar in both groups (six in the placebo group and eight in the test group). Nine patients in the test group reported gastrointestinal problems, notably diarrhea, versus three in the placebo group. Cramps were noted in five patients of the test group, versus one in the placebo group. All other events were sporadic.

**DISCUSSION**

The primary aim of the present analysis is to determine if microbiologic testing for presence or absence of A. actinomycetemcomitans before therapy identified patients with moderate-to-advanced periodontitis who would specifically benefit from adjunctive
amoxicillin plus metronidazole, when given in the context of full-mouth SRP within 48 hours. With respect to the persistence of pockets that are considered to be in need of further therapy according to common practice, that is, still >4 mm and BOP at reevaluation, the answer is no. The only differential advantage of the antibiotics could be identified with regard to tooth type because molars benefited more from the drugs than non-molar teeth. The definition of efficacy endpoints in periodontal therapy is a subject of debate. Changes in clinical attachment level (PD + REC) and bone height have been used as endpoint measures for clinical trials; however, none of them has been shown to be predictive of long-term success. Furthermore, bony changes cannot yet be evaluated at 3 months, and the patients would need to be exposed to repeated radiographs. Therefore, clinical practice evaluation criteria rather should focus on PD reduction <5 mm, absence of BOP, and gingival inflammation. Pocket reduction <5 mm at the 3-month reexamination has been used previously as outcome in a multilevel analysis of two non-surgical treatment protocols without antibiotics in 41 patients. A logistic model revealed significant impact of smoking, presence of plaque at the site, and location of the pocket at a multirooted tooth, but not one-session full-mouth debridement versus quadrant-by-quadrant treatment over four sessions.

Table 2. Characteristics at 3-Month Follow-Up by Treatment Group (N = 82)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Placebo</th>
<th>Test</th>
<th>P Value†</th>
<th>P Value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aa+</td>
<td>Aa–</td>
<td>Aa+</td>
<td>Aa–</td>
</tr>
<tr>
<td>n</td>
<td>19</td>
<td>19</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>PD (mm)*</td>
<td>3.2 ± 1.2</td>
<td>3.3 ± 1.0</td>
<td>2.7 ± 0.9</td>
<td>3.1 ± 0.7</td>
</tr>
<tr>
<td>REC (mm)*</td>
<td>1.3 ± 1.3</td>
<td>1.3 ± 1.2</td>
<td>1.0 ± 1.0</td>
<td>1.7 ± 1.4</td>
</tr>
<tr>
<td>BOP (%)*</td>
<td>35.5 ± 47.9</td>
<td>26.4 ± 44.1</td>
<td>26.6 ± 44.2</td>
<td>19.9 ± 39.9</td>
</tr>
<tr>
<td>GI score*</td>
<td>0.2 ± 0.4</td>
<td>0.3 ± 0.4</td>
<td>0.2 ± 0.4</td>
<td>0.3 ± 0.5</td>
</tr>
<tr>
<td>PS (%)*</td>
<td>34.6 ± 47.6</td>
<td>26.1 ± 43.9</td>
<td>38.5 ± 48.7</td>
<td>31.2 ± 46.3</td>
</tr>
<tr>
<td>PD &gt;4 and BOP+ (number of sites)*</td>
<td>6.7 ± 8.7</td>
<td>4.7 ± 6.6</td>
<td>2.6 ± 4.7</td>
<td>1.6 ± 2.5</td>
</tr>
<tr>
<td>PD &gt;4 and BOP+ (% sites at diseased teeth)*</td>
<td>8.9 ± 28.5</td>
<td>5.7 ± 23.2</td>
<td>3.2 ± 17.7</td>
<td>2.0 ± 14.1</td>
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<tr>
<td>Achieved 10-fold reduction of PD &gt;4 and BOP+ (n) (%)</td>
<td>6 (31.6)</td>
<td>10 (52.6)</td>
<td>17 (77.3)</td>
<td>18 (81.8)</td>
</tr>
<tr>
<td>Achieved ≤5% PD &gt;4 and BOP+ at diseased teeth (n) (%)</td>
<td>10 (52.6)</td>
<td>13 (68.4)</td>
<td>18 (81.8)</td>
<td>19 (86.4)</td>
</tr>
</tbody>
</table>

* Data are mean ± SD values.
† Difference between placebo and test groups irrespective of microbiologic status.
‡ Difference between Aa+ and Aa– irrespective of treatment group.
By using an efficacy endpoint for initial non-surgical periodontal therapy after 3 months of either a 10-fold reduction of sites >4 mm and BOP, or persistence of <5% such sites at treated teeth, the advantage of prescribing amoxicillin plus metronidazole is quite evident. However, no direct conclusions can be drawn from the data presented here with regard to long-term stability, with or without further therapy in a second treatment phase. Still, the first 51 patients of this trial were monitored up to 6 months before further treatments were provided. The comparison of the 3- with the 6-month outcomes showed that the results remained stable in both groups.

Studies monitoring patients over longer periods after various treatments have pointed to three crucial factors for long-term stability: the level of self-performed oral hygiene, the inclusion in a maintenance program, and not smoking. Long-term monitoring of limited numbers of patients indicated more pocket reduction on non-molars than molars. The sample size for the initial study was determined to examine the main effect of antibiotics on periodontitis and thus may be insufficient to test the hypothesis of this study and detect the specific benefit of antibiotics to patients who are negative for A. actinomycetemcomitans compared with patients who are positive. However, the findings strongly suggest that giving antibiotics to patients who are negative for A. actinomycetemcomitans is beneficial because the effect of antibiotics is highly significant, even in the group that is negative for A. actinomycetemcomitans. Given the ongoing controversy about the utility and biologic founding of the current disease classification, recruitment of participants followed practical rules that are used for screening in daily practice. The question we address in this study is not whether patients with a specific diagnosis according to a particular classification system benefit from amoxicillin and metronidazole, but rather, if testing patients for being A. actinomycetemcomitans–positive or –negative makes a difference. In a previous systematic review, we have addressed the question of whether the presence or absence of periodontal pathogens, notably A. actinomycetemcomitans, distinguishes between patients with chronic and aggressive periodontitis, and the answer was no.

Of course, one cannot exclude the possibility that A. actinomycetemcomitans could be present in other sites in the mouth or elsewhere in the body where samples were not taken, as much as one cannot exclude that the organism was present below detection levels in sampled sites. However, it has been suggested that prescription of systemic antibiotics should be based on information derived from such testing. The sampling strategy has proven to represent the status of the person reasonably well.

Except for gastrointestinal disturbances, all adverse events were sporadic and without a particular association with one group. Gastrointestinal problems, notably diarrhea, have been noted to a similar

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>OR of Antibiotics</th>
<th>P Value for OR of Antibiotics</th>
<th>P Value of OR Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Person level</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>0.30</td>
<td>0.02</td>
<td>0.77</td>
</tr>
<tr>
<td>Female</td>
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</tr>
<tr>
<td>Age</td>
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</tr>
<tr>
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<td></td>
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<td>0.03</td>
<td>0.81</td>
</tr>
<tr>
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<td>0.30</td>
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<td></td>
</tr>
<tr>
<td>Aa at baseline</td>
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<td></td>
</tr>
<tr>
<td>Positive</td>
<td>0.25</td>
<td>&lt;0.01</td>
<td>0.68</td>
</tr>
<tr>
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<td>0.34</td>
<td>0.047</td>
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</tr>
<tr>
<td><strong>Site level</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PS at 3 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0.34</td>
<td>&lt;0.01</td>
<td>0.34</td>
</tr>
<tr>
<td>No</td>
<td>0.25</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>PD at baseline</td>
<td>0.22</td>
<td>&lt;0.001</td>
<td>0.94</td>
</tr>
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<td></td>
<td></td>
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<tr>
<td>Yes</td>
<td>0.31</td>
<td>&lt;0.01</td>
<td>0.42</td>
</tr>
<tr>
<td>No</td>
<td>0.22</td>
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<tr>
<td>Molar</td>
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<tr>
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<td>0.23</td>
<td>&lt;0.001</td>
<td>0.03</td>
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<tr>
<td>No</td>
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<td>0.04</td>
<td></td>
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<td>Interdental</td>
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<tr>
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<td>0.30</td>
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<tr>
<td>No</td>
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<td>&lt;0.01</td>
<td></td>
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</tbody>
</table>

Table 3. **Generalized Multilevel Analysis of Effect of Treatment by Subgroup: Moderation of Treatment Effect by Person and Site Level Characteristics**
extent in previous studies on amoxicillin and metronidazole. Meta-analysis of adverse events showed no significant risk difference for any investigated event between SRP with or without adjunctive amoxicillin plus metronidazole. In consideration of the findings of adverse events in this and previous studies, it is concluded that the treatment was safe.

The authors emphasize that these results do not provide evidence for the indiscriminate use of any antibiotic in any periodontal patient. Even if in this study the effect of the antibiotics was independent of PD at baseline, meaning that the benefit of the antibiotics was not limited to very deep sites only, it is still recommended not to routinely prescribe antibiotics for mild-to-moderate periodontitis that according to current evidence can be treated successfully most of the time with non-surgical mechanical debridement alone.

CONCLUSIONS

In patients with moderate-to-advanced periodontitis, systemic amoxicillin plus metronidazole significantly enhanced the effects of full-mouth SRP, thereby reducing the need for further therapy, which according to current treatment concepts would frequently be surgical in nature. Patients who were positive for A. actinomycetemcomitans did not benefit more from amoxicillin plus metronidazole. Sites on molars benefited significantly more from the antibiotics than non-molar sites.

ACKNOWLEDGMENTS

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Ich bedanke mich bei den unten aufgeführten Kolleginnen und Kollegen für ihre wertvolle Mitarbeit, die sie im vergangenen Jahr geleistet haben.

Adrian Lussi
Microbial profiles of patients seeking treatment for periodontitis

Influence of origin, smoking and age?

Key words: periodontitis, microbial profile, epidemiology, A. actinomycetemcomitans, P. gingivalis

Summary

Purpose: We assessed the potential influence of the origin, the smoking status and the age on subgingival microbial profiles of subjects seeking periodontal care in Switzerland today.

Material and Methods: Subgingival samples were obtained from 182 subjects originating from 44 countries (56 native Swiss, 64 other European, 43 African, 19 others), seeking periodontal treatment at the School of Dental Medicine at the University of Geneva. Four periodontal microorganisms were quantified by direct hybridization with specific RNA probes.

Results: Tannerella forsythia and Treponema denticola were ubiquitous (95.6%, 93.9%), and Porphyromonas gingivalis was frequently detected (89%). Counts correlated with the size of the microbial sample (total load). Aggregatibacter actinomycetemcomitans was detected in only 70 (38.4%) subjects. Counts were highly variable and unrelated to total load. Subjects less than 46.8 years old (median age) had a higher risk to be positive than older subjects. Detection frequencies and counts of all four organisms were unrelated to the origin or the smoking status.

Conclusions: Based on a clinical diagnosis of untreated periodontitis, positive outcomes of tests for T. forsythia, T. denticola and P. gingivalis could be predicted with high confidence irrespective of a patient’s origin, smoking status or age. Detection of A. actinomycetemcomitans was less frequent and depended on the age of the subject.

Introduction

Treatment of periodontal diseases targets a highly diverse microbiota residing on subgingival tooth surfaces (Kroes et al. 1999, Paster et al. 2001). A few Gram-negative bacterial species are suggested among the numerous recognized taxa to play a significant role in the disease process (Socransky & Haffajee 1991). Microbiological tests are available to dentists to determine the presence of these organisms. There is an ongoing debate on the utility of such testing in clinical practice. To determine the quality of being of practical use, detailed information is needed on how a test or diagnostic algorithm works in a specific setting and what the consequences of a positive or negative test might be. In previous papers our group addressed the questions of whether the presence or absence of periodontal pathogens can distinguish between subjects with chronic or aggressive periodontitis (Mombelli et al. 2002), and whether microbial testing could indicate success or failure of non-surgical therapy earlier than a clinical assessment at six months (Brochut et al. 2005). The present report addresses another issue of diagnostic tests: Assays are often judged primarily with respect to sensitivity and specificity to identify a
phenomenon under investigation – high sensitivity is desired in order not to miss any positive cases, whereas high specificity is sought to avoid false positives (YERUSHALMY 1947) – underestimating the importance of the predictive value. The predictive value depends on the prevalence of the condition of interest within the tested population. Thus, the utility of a test to identify a microbial phenomenon (perhaps indicating an advantage for applying a specific antimicrobial therapy) depends on the prevalence of the target microbiota, and may vary in clinically distinct populations.

Similar to other European countries, Switzerland has been subject to important demographic changes in recent years (SWISS FEDERAL DEPARTMENT OF HOME AFFAIRS 2010). Microbial profiles may differ in immigrants and the Swiss population. This is true for medical pathogens, for example Mycobacterium tuberculosis (BODENMANN ET AL. 2009), and may be the case for oral microorganisms as well. Somewhat conflicting data on the prevalence of periodontal microorganisms have been reported from different regions of the world, possibly reflecting an influence of local or ethnic risk factors for colonization with putative pathogens. A high prevalence of suspected periodontal pathogens has been reported from certain populations, particularly from developing countries (AL-YAHFOUI ET AL. 1994, DAHLÉN ET AL. 1989, EISENMANN ET AL. 1983, GMÜR & GUGGENHEIM 1994, MOMBELL ET AL. 1998). In a study on refugees arriving from non-industrialized countries, even samples from sites with minimal periodontal disease showed a relatively high detection frequency of Porphyromonas gingivalis and Prevotella intermedia (MCNABB ET AL. 1992).

Detection frequencies may also vary with the smoking status and age. It has been suggested that cigarette smoking increases the risk for subgingival infection with periodontal pathogens (ZAMBO ET AL. 1996). Higher counts of putative periodontal pathogens have been reported from smokers than never-smokers (GOMES ET AL. 2005). Environmental conditions influencing the attachment, growth and metabolism of microorganisms in the oral ecosystem may change with age (MOMBELL ET AL. 1998). P. gingivalis seems to assume greater importance with increasing age (SAVITT & KENT 1991, TANAKA ET AL. 2002, UMEDA ET AL. 1998). However, these differences may in part be attributed to differences in ecological conditions prevailing in shallow or deep pockets, the latter being more prevalent in the elderly and in smokers. In contrast, A. actinomyctecomitans seems to be related to young subjects with decreasing prevalence and concentration levels in older age groups (SAVITT & KENT 1991, VAN DER WEIDEN ET AL. 1994).

Classical studies on the effect of various periodontal therapies were conducted to a large part in Caucasians who smoked (…by periodontists who smoked). Today in Europe, an increasing proportion of patients needing periodontal care are non-smokers and come from populations with diverse ethnic backgrounds.

The aim of this analysis was to assess in subjects with untreated periodontitis who seek periodontal treatment at the School of Dental Medicine at the University of Geneva the influence of origin, smoking status and age on the detection of four periodontal microorganisms by direct hybridization with specific RNA probes.

Materials and methods

This paper reports cross-sectional data of subjects investigated in a recruitment campaign for participation in a randomized clinical trial carried out by our service. The protocol was approved by the appropriate medical ethical committee (Commission Centrale d’Ethique, Hôpitaux Universitaires de Genève) and was authorized by the Swiss Agency for Therapeutic Products (Swissmedic). Research was conducted according to the principles outlined in the Declaration of Helsinki on experimentation involving human subjects.

Subjects

From June 2008 to June 2010, subjects seeking periodontal treatment at the School of Dental Medicine at the University of Geneva were offered to be screened for eligibility to participate in a randomized, placebo-controlled study evaluating the benefit of an adjunctive antibiotic regime. The screening procedure included questions regarding medical and dental health, smoking status (never-smoker, former smoker, < 10 or ≥ 10 cigarettes per day) and country of origin, a brief oral clinical exam and sampling of the subgingival microbiota. An informed consent was obtained for all the patients included in this study.

The inclusion criteria were: Age at least 18 years, presence of 12 scorable teeth, not counting the third molars, a clinical diagnosis of chronic or aggressive periodontitis with presence of at least 4 teeth with a probing depth ≥ 5 mm, clinical attachment loss ≥ 2 mm and radiographic evidence of bone loss. Exclusion criteria were systemic illnesses (i.e. diabetes mellitus, cancer, HIV, bone metabolic diseases or disorders that compromise wound healing, radiotherapy or immunosuppressive therapy), pregnancy or lactation, systemic antibiotics taken within the previous 2 months, use of non-steroid anti-inflammatory drugs, and subgingival scaling and root planning or surgical periodontal therapy in the last year.

Microbiological examination

One subgingival microbial sample was obtained in each quadrant from either the deepest pocket or the mesial aspect of the first molar using the paper point method (MOMBELL ET AL. 1994, MOMBELL ET AL. 1991). The tooth to be sampled was first isolated with cotton rolls. Supragingival plaque was removed using a cotton pellet. Subgingival samples were then collected with a medium-sized endodontic paper point inserted to the bottom of the pocket and left for 10 s. The samples from the four quadrants were pooled in a transport vial containing 4 M guanidinium thiocyanate 2-mercaptoethanol and sent to the laboratory for analysis.

The samples were analyzed using oligonucleotide probe technology according to standard procedures (DIX ET AL. 1990). They were hybridized to a specific probe for the ssrRNA of A. actinomyctecomitans, P. gingivalis, Tannerella forsythia, Treponema denticola, and to a universal bacterial probe (Institut für ange wandte Immunologie, Zuchwil, Switzerland). Bacterial counts were calculated by comparison with homologous reference standards and expressed as count × 10^6.

Statistical analysis

Data were entered into a database and were checked for entry errors. Subjects were grouped according to country of origin as follows: Switzerland, other European country, Africa, other. Backward stepwise logistic regression was used for prediction of the probability of detecting the target microorganisms by the predictor variables origin, smoking status, age and bacteriologic sample size (expressed as log total bacterial load determined with the universal probe). The association between the detection and non-detection of different target microorganisms in the same bacteriologic specimen was analyzed with
Fisher’s exact Test. Linear regression was used for modeling the relationship between log-transformed counts of the target organisms in positive samples and origin, smoking status, age and log-transformed total loads.

One statistical program package (PASW Statistics 18 for Mac OS X, IBM Corporation, Somers, NY USA) was used for all statistical analyses.

Results

We identified 206 persons with untreated periodontitis that met the inclusion criteria. 182 of these also fulfilled none of the exclusion criteria and accepted to be examined microbiologically. The 182 subjects originated from 44 countries: 56 (31%) persons were native Swiss, 64 (35%) came from another European country, 43 (24%) originated from Africa, and 19 (10%) came from elsewhere. The mean age was 47.9 years (range 18–75 years). 76 (42%) subjects were female, 77 (42%) were smokers (54% of the native Swiss, 49% of the other Europeans, 30% of the Africans, 16% of the others). Figure 1 displays the age distribution in the four categories of origin. Figure 2 shows the age distribution in the four smoking categories.

Table I shows the frequency of detection of A. actinomyctemcomitans, P. gingivalis, T. forsythia and T. denticola by origin. Table II shows the frequency of detection in subjects that never smoked in comparison to those that currently or previously smoked. Table III shows these frequencies in the younger and the older half of the sample (age > median of 46.8 years). As can be seen clearly, T. forsythia and T. denticola were ubiquitous, with detection frequencies approaching 100%. P. gingivalis was detected in 89% of the cases. In contrast, A. actinomyctemcomitans was detected in only 38.4% of the subjects.

Backward stepwise logistic regression was used for prediction of the probability of detecting A. actinomyctemcomitans or...
P. gingivalis using the predictor variables origin, smoking status, age and bacteriologic sample size. The age in years (p<0.009) and the log total bacterial load (p<0.001) were predictors for the detection of A. actinomycetemcomitans. Subjects with an age below the median of 46.8 years had a higher risk to be positive than older subjects (odds ratio 2.0, p<0.032). However, the presence or absence of A. actinomycetemcomitans was independent of the smoking status (current smoker or past/never-smoker) or the origin (Switzerland, other European country, Africa, other). For detection or non-detection of P. gingivalis, backward stepwise logistic regression analysis retained solely the log total bacterial load (p<0.003) as a variable with significant impact. Thus, in the subjects of this study, the detection risk for A. actinomycetemcomitans, P. gingivalis, T. forsythia and T. denticola was independent of their origin and smoking status; older subjects were less likely to be A. actinomycetemcomitans-positive. The age did not affect the risk of testing positive for the other three target organisms.

Table IV addresses the issue of concurrent detection of A. actinomycetemcomitans and P. gingivalis. The association between the two tests was significant (Fischer’s exact Test p<0.017). In fact, 67 out of 70 samples positive for A. actinomycetemcomitans were also positive for P. gingivalis. With one exception, all A. actinomycetemcomitans-positive persons were also positive for T. forsythus and T. denticola (a man originating from Haiti, smoking ≥10 cigarettes per day, aged 28 years, tested A. actinomycetemcomitans-positive but negative for the other three target organisms).

The relationship between the counts of target organisms and total load are shown in graphical form in Figures 3 (A. actinomycetemcomitans), 4 (P. gingivalis) and 5 (T. forsythus plus T. denticola). Counts of P. gingivalis (p<0.001), and of T. forsythus plus T. denticola (p<0.001), were significantly correlated in positive samples to the total load. In contrast, the contribution of A. actinomycetemcomitans to the total count was highly variable and unrelated to the total load. The counts of all four organisms

<table>
<thead>
<tr>
<th>Age group</th>
<th>N</th>
<th>A. actinomycetemcomitans</th>
<th>P. gingivalis</th>
<th>T. forsythia</th>
<th>T. denticola</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤46.8 years</td>
<td>91</td>
<td>41 (45.1%)</td>
<td>39 (42.9%)</td>
<td>85 (93.4%)</td>
<td>89 (97.8%)</td>
</tr>
<tr>
<td>&gt;46.8 years</td>
<td>91</td>
<td>29 (31.9%)</td>
<td>28 (30.8%)</td>
<td>89 (97.8%)</td>
<td>82 (90.1%)</td>
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<tr>
<td>Total</td>
<td>182</td>
<td>70 (38.4%)</td>
<td>162 (89%)</td>
<td>174 (95.6%)</td>
<td>171 (93.9%)</td>
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<table>
<thead>
<tr>
<th>A. actinomycetemcomitans</th>
<th>Negative</th>
<th>Positive</th>
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</thead>
<tbody>
<tr>
<td>17</td>
<td>95</td>
<td>112</td>
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</tr>
<tr>
<td>3</td>
<td>67</td>
<td>70</td>
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</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>162</td>
<td>182</td>
</tr>
</tbody>
</table>

**Fig. 3** Relationship between log count of A. actinomycetemcomitans and log total load (n.s.)

**Fig. 4** Relationship between log count of P. gingivalis and log total load (p<0.001)
Discussion

This study was conducted in the Canton of Geneva, Switzerland, with a population of 458,000 inhabitants, of which 39% do not have Swiss citizenship (Office cantonal de la statistique 2010). The high proportion of immigrants made it possible to study the influence of origin on subgingival microbial profiles. In 182 subjects with a clinical diagnosis of periodontal disease and seeking periodontal treatment, the detection frequencies and counts in positive samples of four periodontal microorganisms were independent of the origin of the person. In a similar study with a comparable sample size (n=199), carried out in the city of Los Angeles, CA, USA (Umeda et al. 1998), “Hispanics” and “Asian-Americans” with moderate or advanced periodontitis had a higher prevalence of P. gingivalis than “Caucasians” and “African-Americans”. The first two ethnic groups were not represented in sufficiently high numbers for specific statistical analysis in our cohort (7 individuals from South America, 4 from China).

The detection frequencies of A. actinomycetemcomitans and P. gingivalis (38% and 89% respectively) were slightly higher than those calculated in a systematic review, where 28% subjects with a clinical diagnosis of chronic periodontitis were A. actinomycetemcomitans-positive, and 71% were P. gingivalis-positive (Mombelli et al. 2002). In the present study we did not select subjects with a clinical diagnosis of chronic periodontitis specifically. Our analysis rather concentrated on microbial patterns in subjects aged at least 18 years, with presence of at least 4 teeth with a probing depth > 5mm, clinical attachment loss > 2mm and radiographic evidence of bone loss. To what extent current classification systems truly discriminate distinct forms of disease is the matter of an ongoing debate (Armitage et al. 2010) that was not the focus of our project. A previous study, carried out in 51 patients with a similar clinical diagno-
sis, and recruited under the same circumstances in our institution, yielded 25% A. actinomycetemcomitans- and 83% P. gingivalis-positive subjects (Cionca et al. 2010).

As can be seen in Table 1, the frequencies of detection of T. forsythia and T. denticola were very high in all subjects. This reflects results reported in prior literature (for review see Socransky & Haffajee 2008), indicating that a clinical diagnosis of untreated periodontitis implies the presence of T. forsythia and T. denticola with high confidence. As there is currently no evidence that T. forsythia- or T. denticola-negative patients may be at a particular risk, or may benefit exceptionally from a specific form of therapy, testing patients with untreated periodontitis for these two organisms has no practical utility. At best, a positive test may be taken as a confirmation that an appropriate bacteriologic sample was obtained.

67 out of 70 samples positive for A. actinomycetemcomitans were also P. gingivalis-positive. In other words, if a patient was tested to be A. actinomycetemcomitans-positive, one could assume that most probably he was also P. gingivalis-positive. We have studied the concurrent detection of A. actinomycetemcomitans and P. gingivalis previously in 60 young migrant workers in the Province of Guangzhou, People’s Republic of China (Mombelli et al. 1998). 21 of these subjects were positive for both organisms, whereas 16 were positive for A. actinomycetemcomitans but not for P. gingivalis. This may suggest that the high predictive value of an A. actinomycetemcomitans-positive test to indicate concomitant presence of P. gingivalis, T. forsythia, and T. denticola, may be noted only in subjects diagnosed clinically with periodontitis, reinforcing the need to differentiate the utility of tests in different populations.

In conclusion, based on a clinical diagnosis of untreated periodontitis, positive outcomes of tests for T. forsythia, T. denticola and P. gingivalis could be predicted with high confidence irrespective of a patient’s origin, smoking status or age. Their counts correlated with the size of the microbial sample. Detection of A. actinomycetemcomitans, however, was less frequent and was influenced by the age of the subject. Its quantitative contribution to the total microbiota was highly variable.

Acknowledgements

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Résumé

But: De manière similaire aux autres pays européens, notre pays a été sujet à d’importants changements démographiques ces dernières années. Il a été montré que le profil microbiologique peut différer entre les immigrants et la population résidente. De plus, les fréquences de détection pourraient également varier avec l’âge et la consommation de tabac. Le but de cette étude a été d’évaluer l’influence de l’origine, du tabagisme et de l’âge sur le profil microbiologique sous-gingival de sujets avec une parodontite non traitée et consultant de nos jours à Genève.

Matériel et méthode: La détection de quatre pathogènes parasites a été effectuée entre juin 2008 et juin 2010 chez 182 sujets souffrant d’une parodontite non traitée et consultant à l’école de Médecine dentaire de Genève. Des échantillons microbiologiques sous-gingivaux ont été prélevés dans les quatre poches les plus profondes ou les faces maxillaires des premières...
molaires de chaque quadrant. Quatre micro-organismes parodontaux (Aggregatibacter actinomyctecomitans, Porphyromonas gingivalis, Tannerella forsythia et Treponema denticola) ont été quantifiés par hybridation directe avec des sondes spécifiques à l’ARN ribosomal bactérien. Les données épidémiologiques concernant l’âge, les habitudes de tabagisme et l’origine des sujets étaient collectées à l’aide d’un formulaire.

Résultats: Les 182 sujets inclus étaient originaires de 44 pays différents (56 [31%] Suisses, 64 [35%] en provenance d’autres pays européens, 43 [24%] Africains et 19 [10%] autres). L’âge moyen était de 47,9 ans (entre 18 et 75 ans). 76 (42%) étaient des femmes, et 77 (42%) patients étaient des fumeurs. Concernant la microbiologie, T. forsythia et T. denticola étaient détectés dans la quasi totalité des échantillons (95,6% et 93,9%). La détection de P. gingivalis était moins fréquente (89%). La quantité de ces trois bactéries étaient en corrélation avec la taille totale de l’échantillon microbiologique. A. actinomyctecomitans n’a été détecté que chez 70 (38,4%) sujets. Les quantités de ce micro-organisme dans les échantillons étaient très variables et ne montraient pas de relation avec la charge bactérienne totale. Les sujets âgés de moins de 46,8 ans (médiane) avait un risque plus important d’être positif pour A. actinomyctecomitans que les sujets plus âgés. Les fréquences de détection et les quantités de ces quatre pathogènes parodontaux n’étaient pas liées à l’origine ou aux habitudes de tabagisme des sujets examinés.


Zusammenfassung


Resultate: Die 182 Personen stammten aus 44 verschiedenen Ländern: 56 (31%) waren Schweizer, 64 (35%) stammten aus anderen europäischen Ländern, 43 (24%) waren afrikanischer Herkunft und 19 (10%) kamen aus anderen Ländern. Das Durchschnittsalter betrug 47,9 Jahre (minimal 18, maximal 75). 76 (42%) Personen waren Frauen und 77 (42%) Raucher. T. forsythia (95,6%) und T. denticola (93,9%) waren die zwei am häufigsten nachgewiesenen Keime. Ihre jeweilige Quantität war proportional zur Gesamtkeimzahl der mikrobiologischen Probe. A. actinomyctecomitans wurde nur bei 70 (38,4%) Personen detektiert. Die Quantität dieses Keimes war sehr variabel und zeigte keine Beziehung zur Gesamtkeimzahl einer Probe. Personen mit einem Alter unter dem Medianwert (46,8 Jahre) hatten ein höheres Risiko, positiv auf A. actinomyctecomitans getestet zu werden, als ältere Personen. Es bestand kein Zusammenhang zwischen der Herkunft oder dem Tabakkonsum der Probanden und dem Nachweis oder der Quantität der vier untersuchten Keime.

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Does adjunctive antimicrobial therapy reduce the perceived need for periodontal surgery?

Andrea Mombelli, Norbert Cionca & Adnan Almaghlouth

Periodontal diseases are the result of an unsuccessful relationship between bacteria colonizing the surfaces of teeth and the tissues anchoring the teeth in the bone. Contemporary periodontal treatment generally aims at the removal of bacterial deposits (plaque and calculus) through a mechanical cleaning method termed ‘scaling and root planing’. Scaling and root planing has limitations, particularly if the disease has led to the formation of pockets deeper than 5 mm around the affected teeth (5, 12, 73). To facilitate scaling and root planing, and to allow direct visual control in deep pockets, the soft tissues can be lifted back surgically for better access. In clinical practice, periodontal therapy is usually performed in two stages. An attempt to remove a maximum of bacterial deposits is made first without flap elevation. After 3–6 months, the case is re-evaluated, and, if deemed necessary, further root surface instrumentation follows, this time in the context of a local surgical intervention (Fig. 1, top). Two systematic reviews of numerous short- and long-term clinical trials have concluded that this way of dealing with periodontal diseases is efficacious (41, 89). However, it requires a considerable amount of active involvement of qualified personnel, extends over a long period of time, may induce notable loss of tooth substance, particularly if instrumentation is repeated several times, and may provoke gingival recession, leaving sensitive root surfaces exposed.

It would be unreasonable to suppose that mechanical cleaning of root surfaces alone can completely eliminate bacteria involved in the disease process. Bacteria are beyond the reach of mechanical instruments in dentin tubules, lacunae and concavities, and are inaccessible if they invade soft tissues. One frequent member of the subgingival microbiota, Aggregatibacter actinomycetemcomitans (formerly known as Actinobacillus actinomycetemcomitans) (65), resists mechanical treatment particularly well (60, 63, 78). Longitudinal and retrospective studies have correlated persistence after therapy of A. actinomycetemcomitans, and a few other species, with continuing tissue destruction. Clinical results were better and more stable if such organisms were no longer detectable (10, 13, 14, 20, 31, 34, 36, 77, 98). Incomplete removal of pathogenic microorganisms should therefore be taken into account as a possible reason for an unsatisfactory treatment outcome.

Given the limitations of mechanical debridement, treatment protocols including antibacterial agents (antibiotics and antiseptics) may be more efficient than mechanical cleaning alone. Oral antibiotics are the most common approach for treating bacterial infections, and are the primary focus of this review. Local infections can also be treated with topically administered antimicrobials. Agents that are too toxic to be delivered by the systemic route, i.e. antiseptics, may be applied locally.

The literature on antimicrobial periodontal therapy has been thoroughly reviewed previously (23, 30, 86, 92). The aim of this review is to critically re-assess the strategies for using antimicrobial agents in periodontics, taking into account the literature summarized previously as well as the latest findings. In recent years, systematic reviews have become the preferred method of analyzing the available evidence because they search the literature using explicit procedures, appraise individual reports using pre-defined criteria, and combine the results of valid studies using appropriate statistical techniques. Where such systematic reviews are available, we do not cite individual trials in detail.
General benefits of systemic antibiotics

A large number of reports have indicated beneficial effects of systemic antibiotics for patients with periodontal diseases in various clinical situations. Drugs investigated include amoxicillin (with or without clavulanic acid), azithromycin, clindamycin, doxycycline, metronidazole, spiramycin, tetracycline, and certain combinations thereof (85). In the context of consensus conferences issuing recommendations for periodontal care, two systematic reviews have been completed that assessed the benefit of adjunctive systemically administered antibiotics (35, 43). Herrera et al. (43) included 25 controlled clinical trials of at least 6 months duration, in which systemically healthy subjects with chronic or aggressive forms of periodontitis had been treated with or without adjunctive systemic antibiotics. Patients treated with antibiotics generally showed better results than patients treated without. In deep pockets, a specific benefit of change in the clinical periodontal attachment level was found for the combination of amoxicillin and metronidazole. Haffajee et al. (35) included 27 controlled clinical trials, with a follow-up period of more than 1 month, that used mean attachment level change as primary outcome. Within the broad range of therapeutic protocols, metronidazole, alone or in combination with amoxicillin, was the most frequently used drug. In all studies, the antibiotic groups showed significantly greater changes in mean attachment level than the control groups. Trials allowing a more detailed analysis indicated that antibiotics had the greatest effect in sites with deep pockets (49, 50, 67, 68, 75, 81, 101, 102). However, specifically useful antibiotic regimes for distinct clinical or microbiological conditions could not be clearly identified based on the available evidence. Definite conclusions could also not be made concerning the optimal dosage and duration of antibiotic therapy, the long-term benefits, and to what extent the antibiotics induced resistance or other changes in the oral microbiota.

Systemic antibiotics: early or late?

Although the benefit of systemic antibiotics is clear today in general, the specific relationship between benefit and risk in various clinical situations remains a subject of debate, especially with regard to individual prescription and combination with other procedures. Concerns have been raised that an extensive use of antibiotics in periodontics, particularly when administered to counterbalance incomplete mechanical debridement or poor oral hygiene, could contribute substantially to the development of bacterial antimicrobial resistance (3, 25, 33, 58, 80, 91, 95, 97). To limit the increase of antibiotic resistance and avoid unwanted systemic effects, it seems reasonable to adopt a precautionary, restrictive attitude to using antibiotics.

The optimal timing of antimicrobial drug administration is one subject for discussion, as it remains controversial whether adjunctive systemic antibiotics should preferably be administered during the initial non-surgical phase, or during a subsequent surgical treatment phase. A landmark study, published in 1992 by Loesche et al. (51), sparked this controversy by showing that systemic metronidazole, when given as an adjunct to scaling and root planing, reduced the need for surgical therapy in periodontitis patients with elevated levels of spirochetes in subgingival samples, thereby reducing the costs and the inconvenience for the patient. These findings were contrary to the opinion that mechanical therapy should be exploited to its limits before a decision is made to administer an antibiotic. Postponing antibiotic therapy to the surgical treatment phase may be defended for two reasons. First, it is known that scaling and root planing alone are able to resolve a considerable amount of periodontal pathology on their own (41, 89), and this strategy may help to keep the prescription of antibiotics to a minimum. Second, given the restricted effects of antibiotics on intact biofilm (84), and the known limitations of scaling and root planing (11, 73), surgical intervention may be necessary for complete biofilm disruption on all contaminated surfaces. Although this reasoning seems to make sense, it is – perhaps surprisingly – not supported by data from specifically designed controlled trials. As most available studies tested systemic antibiotics in
the context of non-surgical debridement, a systematic review that tried to assess the relative benefit of prescribing antibiotics either during the non-surgical or the surgical phase of therapy was inconclusive (42). One study (45), which was not included in that review, found that administration of amoxicillin and metronidazole immediately after initial scaling and root planing provided better clinical outcomes in deep sites than late administration in the context of rescaling after 3 months, corroborating the views expressed in 1992 by Loesche et al. (51).

Amoxicillin and metronidazole as adjunct to full-mouth scaling and root planing

Amoxicillin (recommended international non-proprietary name, sometimes previously referred to as 'amoxycillin') is a moderate-spectrum, bacteriolytic β-lactam antibiotic. Metronidazole is a nitroimidazole that is particularly active against anaerobic bacteria and protozoa. The combination of these two drugs has become a favorite choice for adjunctive antibiotic therapy in periodontics in the last two decades. Due to its proven ability to suppress *A. actinomycetemcomitans* from periodontitis lesions and other oral sites (7, 21, 27, 29, 62, 71, 72, 93, 94), the combination of amoxicillin and metronidazole initially appeared well suited especially to treat advanced *A. actinomycetemcomitans*-associated periodontitis. However, clinical trials have indicated that amoxicillin and metronidazole may also be very efficient in other situations (4, 7, 24, 32, 44, 52–54, 56, 57, 59, 79, 81, 88, 90, 102). Better clinical outcomes were reported if patients were treated with amoxicillin and metronidazole than if metronidazole alone was administered (56, 81). So far no comparative randomized clinical trial has demonstrated the superiority of any other regime over amoxicillin and metronidazole in any clinically or microbiologically defined variant of periodontal disease.

We have recently reported the results of a single-centre, double-blind, placebo-controlled, randomized longitudinal study of 6 months duration involving 51 patients with chronic periodontitis (15, 16). The aim of the study was to specifically evaluate the clinical benefit of amoxicillin and metronidazole when administered immediately after full-mouth periodontal debridement completed in 48 h. Twenty-five subjects received 375 mg amoxicillin and 500 mg metronidazole three times a day for 7 days and 26 received a placebo. The absolute number of sites per subject with a pocket probing depth > 4 mm and bleeding upon probing was the primary clinical outcome measure, as persisting pockets > 4 mm that bleed upon probing are commonly perceived as an indication for further treatment. All subjects were followed up for up to 3 months, and 47 were followed for up to 6 months. Significant and clinically relevant improvements were observed in all subjects. An additional significant treatment effect was demonstrated in subjects treated with the antibiotics (Fig. 2). In the placebo group, a mean of 3.0 pockets > 4 mm that bled on probing were found per subject after 6 months, but only 0.4 sites that fall into this category were detected in the test group after 6 months, and that difference was statistically significant (*P* = 0.005). The clearest advantage of antibiotics over placebo was noted in pockets that were initially deeper than 6 mm: in the test group, the mean pocket probing depth decreased from 7.3 ± 0.3 to 3.7 ± 0.6 mm, and that in the control group decreased from 7.2 ± 0.7 to 4.9 ± 1.4 mm (*P* = 0.003 for difference between groups). Using backward stepwise logistic regression, the impact of various variables on the persistence of more than one pocket > 4 mm per subject with bleeding on probing at 6 months was evaluated. The protective risk of the antibiotics for further periodontal therapy amounted to 8.85, meaning that every subject receiving amoxicillin and metronidazole was protected by a factor of 8.85 from further periodontal therapy.

In this trial, antibiotics were not tested as an alternative, but rather as a supplement, to thorough debridement and proper oral hygiene. Guerrereo et al. (32) used a similar protocol to evaluate the
adjunctive benefit of the same antibiotic regime in 41 patients with generalized aggressive periodontitis. The data from this trial demonstrate that the observations made in subjects with chronic periodontitis can be extended to subjects with aggressive forms of the disease. Shortly after our data were published, another group reported significantly better clinical results for full-mouth debridement using an ultrasonic scaler and limited to 45 min, if supplemented with adjunctive amoxicillin plus metronidazole (79).

Two systematic reviews (22, 48) concluded that the clinical outcomes for scaling and root planing without antibiotics differed little if treatments extended over several weeks (quadrant-by-quadrant treatment) or were completed within a few hours (full-mouth treatment). Nevertheless, if a short, intensive treatment phase is acceptable for the patient, and not contraindicated for medical or other reasons, it seems irrational to delay completion by fractionating and extending therapy.

Microbial profiling and antibiotic therapy

The results of these recent studies challenge the current prevailing opinion that the use of systemic antibiotics should be restricted to specific groups of periodontal patients, for example those with highly active disease or a specific microbiological profile (43). In a commentary (95) on our first paper presenting the clinical findings of this trial (15), it was stated that treatment of Porphyromonas gingivalis-negative patients with antibiotics may be considered over-treatment. This statement was based on results from a study by another group (26). A study by another group (26) contradicts their statement as the results were interpreted to show that amoxicillin and metronidazole adversely affect the clinical outcome of patients harboring P. gingivalis but not A. actinomyctemcomitans.

Even though patient selection and treatment allocation were independent of microbiology, microbiological samples were in fact obtained from every participant in our study before and after treatment: pooled subgingival plaque was collected using paper points inserted into the deepest pocket in each quadrant (60, 61). Six periodontal marker organisms were detected and quantified using a commercially available real-time polymerase chain reaction-based method (Meridol® Perio Diagnostics; GABA International, Therwil, Switzerland). This gave us the opportunity of assessing whether patients with a particular microbial profile specifically benefited from adjunctive antibiotics (16). Confirming the remarkable capacity of amoxicillin and metronidazole to suppress A. actinomycetemcomitans already recognized previously, A. actinomyctemcomitans was no longer detected in any subject in the test group after antibiotic treatment. However, 38 of the 47 subjects completing the trial (18 in the placebo and 20 in the test group) were already negative for A. actinomyctemcomitans at baseline. None of these subjects tested positive at months 3 or 6 either. Although these subjects were thus A. actinomyctemcomitans-negative throughout the study, they had a significantly better primary clinical outcome if they received the antibiotics than if they were treated with placebo. It was therefore concluded that, even though amoxicillin and metronidazole very efficiently suppressed A. actinomyctemcomitans, testing for A. actinomyctemcomitans among subjects with a clinical diagnosis of chronic periodontitis did not identify subjects who will specifically benefit from antibiotics (16). A. actinomyctemcomitans displays a broad genetic and phenotypic diversity and is heterogeneously distributed in various populations and cohorts worldwide (46). A large prospective study (40) has indicated that only one sub-population of A. actinomyctemcomitans (the ‘JP2 clone’) (87) displays the properties of a true pathogen. One may therefore suggest that patients should be tested specifically for presence of clone JP2. However, until an interventional study identifies a specific advantage of a treatment other than amoxicillin and metronidazole in A. actinomyctemcomitans JP2-positive cases, the utility of such a test remains hypothetical.

The same holds true in principle for any other specific test of particular properties of members of the subgingival flora and any other form of periodontitis. In our study, patients treated with amoxicillin and metronidazole tended to show better results irrespective of whether they tested positive or
negative for *P. gingivalis* before treatment. Treatment of all negative cases in the test group was clinically successful, whereas, of the four *P. gingivalis*-negative subjects in the control group, one still had five pockets needing further therapy 6 months later. These results are in agreement with a previous placebo-controlled study (81). Patients treated with amoxicillin and metronidazole showed significantly better clinical outcomes for non-surgical therapy than those treated with placebo, despite the fact that only 27% of the subjects were *P. gingivalis*-positive and only 11% were *A. actinomycetemcomitans*-positive.

**Risks vs. benefits of systemic antibiotics**

Amoxicillin and metronidazole have been prescribed widely for over three decades, and their effects and side effects are therefore well documented (1, 2). The most frequent adverse reactions to amoxicillin are sensitivity phenomena. These are more likely to occur in individuals who have previously demonstrated hypersensitivity to penicillins and in those with a history of allergies in general. Most reactions are mild, and limited to a rash or skin lesion in the head or neck region. More severe reactions may induce swelling and tenderness of joints. In highly sensitized patients, a life-threatening anaphylactic reaction may develop (2). Potential side-effects of metronidazole include nausea, headaches, loss of appetite, diarrhea, a metallic taste, and sometimes a rash. Alcohol consumption enhances these symptoms, because imidazoles affect the activity of liver enzymes. Peripheral neuropathies, characterized mainly by numbness or paresthesia of an extremity, have been reported in isolated cases. Metronidazole should be discontinued immediately if abnormal neurological signs appear (1). There is evidence of carcinogenic activity of metronidazole in studies involving chronic oral administration in mice and rats, but not in other species tested. Due to inadequate evidence, metronidazole is not considered a risk factor for cancer in humans (6). Previously unrecognized candidiasis may become clinically manifest during antibiotic therapy.

To put things into perspective, the frequency and potential negative effects of antibiotics have to be balanced against the potential health consequences of not rapidly suppressing a periodontal infection, and the inconvenience, discomfort and financial consequences of further therapy. The traditional approach sometimes expands treatment over several months, while scaling and root planing plus amoxicillin and metronidazole may be able to resolve the infection within a few days. Scaling and root planing plus amoxicillin and metronidazole have been shown to decrease clinical signs of inflammation and inflammatory biomarkers in gingival crevice fluid stronger than scaling and root planing alone (28). Although not directly confirmed so far by a clinical trial, it seems preferable, from a general health point of view, to let patients benefit early from the positive systemic effects of successful periodontal therapy (18, 19, 64). Although the number of subjects complaining about gastrointestinal problems, notably diarrhea, was higher in the test group than in the placebo group in our study (15), tooth loss and suppurative despite therapy were exclusively noted in the control group. Furthermore, one-third of subjects who mentioned diarrhea upon questioning were actually treated with placebo.

With regard to the development of bacterial resistance in general, it should be remembered that systemic antibiotics are taken in thousands of subjects with untreated periodontitis worldwide every day without subgingival debridement. This happens whenever physicians prescribe antibiotics to persons with untreated periodontal disease for medical reasons. As an example, in a cohort of over 12,000 persons with destructive periodontal disease, more than 70% received at least one course of antibiotics over a 3-year period (17). The true contribution to the problem of resistance by a dentist treating once in the life of a patient a periodontal infection with adjunctive amoxicillin and metronidazole, in a controlled situation following thorough mechanical debridement, and by administering at the same time two drugs with different antimicrobial action, is unknown and warrants future research. This contribution may be minor compared to the effects of the frequent prescription of antibiotics by dentists and other healthcare providers for other therapeutic and prophylactic purposes.

**Local antibiotics and antiseptics**

A variety of methods to deliver antimicrobial agents into periodontal pockets have been devised and subjected to numerous experiments. They include pocket irrigation, application of drug-containing ointments and gels, and devices for sustained drug release.
A systematic review (38) that analyzed the literature in an effort to determine the effect of subgingival irrigation concluded that irrigation with chlorhexidine provided no additional benefit to conventional mechanical debridement. Another systematic review found no significant benefit of supplementing full-mouth debridement with chlorhexidine irrigation (48). A recent systematic review specifically addressed rinsing with povidone-iodine during nonsurgical periodontal therapy (82). A small but statistically significant beneficial effect of pocket rinsing was found in terms of reduction of probing depth.

The efficacy of several commercially available local-delivery systems as adjuncts to scaling and root planing was compared in two trials (47, 74, 83), including patients with persistent periodontal lesions. One systematic review attempted to evaluate the combined literature-based evidence to determine the relative effect of local controlled-release anti-infective drug therapy in patients with chronic periodontitis (39). A meta-analysis that included 19 studies comparing scaling and root planing plus local sustained-release agents with scaling and root planing alone confirmed the clinical advantages of minocycline gel, microencapsulated minocycline, doxycycline gel and chlorhexidine chips over scaling and root planing alone. However, due to the heterogeneity of the material, the authors could not make any firm statements regarding the superiority of one system. A further systematic review looked at the relative adjunctive benefits of various locally applied agents (9). A statistically significant mean advantage was found for four agents in terms of additional attachment gain, and this advantage was greatest for minocycline, followed by tetracycline, chlorhexidine and metronidazole. The findings reported in this review must be interpreted with caution as data were combined from studies that explored various modes of local treatment, including irrigation, impregnated strips and pastes. Differences in the outcomes may reflect the dissimilarity in modes of application and study populations, rather than the potency of the agents.

Over the recent years, the practical value of local antibiotic therapy has decreased, as a majority of adequately tested formulations have been withdrawn from the market for economic reasons (not enough opportunity to make a profit), due to administrative obstacles (obligation to re-register products as drugs rather than as medical devices), or a combination of both (too expensive to fulfill the requirements for continued approval). Although local antibiotics may have the potential to reduce the perceived need for periodontal surgery, discussion of this possibility will be limited to two specific examples. In the first example, the effect of a two-syringe mixing system for controlled release of doxycycline (Atridox®; Tolmar Inc., Fort Collins, CO, USA), applied after no more than 45 min of debridement without analgesia, was compared to 4 h of thorough scaling and root planing in a study involving 105 patients with moderately advanced chronic periodontitis in three centers. Interestingly, clinical parameters indicated a better result for the pharmaco-mechanical treatment approach after 3 months, although considerably less time had been invested than for conventional mechanical therapy (99). In the second example, the efficacy and safety of locally administered microencapsulated minocycline (Arestin®; OraPharma, Warminster, PA, USA) were shown in a multicenter trial that included 748 patients with moderate to advanced periodontitis. Minocycline microspheres plus scaling and root planing provided a substantially greater reduction in probing depth than either scaling and root planing alone or scaling and root planing plus the drug. The probability for reducing probing depths from ≥6 mm to <5 mm was nearly three times greater for scaling and root planing plus local antibiotics than scaling and root planing alone (70, 100). Few studies have addressed the issue of selection of a local or systemic route when use of antibiotics
Table 1. Protocol for the treatment of chronic or generalized aggressive periodontitis. Adapted from the protocols of two randomized controlled clinical trials (15, 32) demonstrating efficacy. Not validated for subjects below age 16 years or above age 70 years, with systemic illnesses, or pregnant or lactating women

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Action</th>
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<tbody>
<tr>
<td><strong>Preparatory procedures</strong></td>
<td></td>
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<tr>
<td>Clinical assessment</td>
<td>Full-mouth plaque score (presence or absence of plaque) (four surfaces per tooth)</td>
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<tr>
<td></td>
<td>Pocket probing depth, recession and bleeding on probing (six sites per tooth)</td>
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<td></td>
<td>Pulp vitality test</td>
</tr>
<tr>
<td>Imaging</td>
<td>Take radiographs of teeth with clinical signs of disease or unclear status (e.g. negative pulp vitality test)</td>
</tr>
<tr>
<td>Case presentation and motivation</td>
<td>Explain disease status and describe the planned therapy to the patient</td>
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<td></td>
<td>Motivate patient for self-performed oral hygiene</td>
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<td></td>
<td>Obtain written informed consent</td>
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<tr>
<td>Supragingival debridement</td>
<td>Remove supragingival calculus and plaque (ultrasonic and/or hand instruments)</td>
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<td></td>
<td>Eliminate obstacles for mechanical plaque control and remove plaque-retentive elements</td>
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<td></td>
<td>Extract teeth that are unreasonable to treat</td>
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<tr>
<td>Oral hygiene instructions</td>
<td>Instruct and train in oral hygiene procedures (toothbrush, interdental cleaning device, other)</td>
</tr>
<tr>
<td>Oral hygiene controls</td>
<td>Record plaque score within 10 days after instructions</td>
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<td></td>
<td>If plaque score &gt; 20%, provide additional instructions</td>
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<td></td>
<td>Repeat this step until plaque score &lt; 20%</td>
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<tr>
<td><strong>Subgingival debridement (within 1 month)</strong></td>
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<tr>
<td>Scaling and root planing</td>
<td>Thorough scaling and root planing of all teeth with pocket probing depth &gt; 3 mm to the depth of the pocket with ultrasonic instruments and hand curettes (carried out under local anesthesia in one or two sessions within 48 h)</td>
</tr>
<tr>
<td>Chemical plaque control</td>
<td>Instruct patient to rinse the mouth twice daily with 0.2% chlorhexidine for 10 days</td>
</tr>
<tr>
<td><strong>Adjunctive antibiotics (immediately after subgingival debridement)</strong></td>
<td>500 mg metronidazole and 375 mg amoxicillin, 3 times per day, for 7 days (corresponds to 20 mg/kg metronidazole and 15 mg/kg amoxicillin, per day, for a subject weighing 75 kg)</td>
</tr>
<tr>
<td>Standard protocol (amoxicillin and metronidazole)</td>
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<tr>
<td>Alternative protocols</td>
<td>Monotherapy with azithromycin (37, 55), or metronidazole combined with cefuroximoxetil, or ciprofloxacin (76, 96), depending on type of intolerance. The equivalence of these protocols to amoxicillin and metronidazole has not been established</td>
</tr>
<tr>
<td><strong>Post-treatment controls (at months 1 and 3)</strong></td>
<td></td>
</tr>
<tr>
<td>Oral hygiene control</td>
<td>Record plaque score</td>
</tr>
<tr>
<td></td>
<td>If plaque score &gt; 20%, review and reinforce oral hygiene</td>
</tr>
</tbody>
</table>
seems indicated. In one investigation, carried out in patients with rapidly progressing periodontitis (8), no significant differences were noted between scaling and root planing supplemented with either systemic amoxicillin/clavulanic acid or application of tetracycline using a local-delivery device. For patients with adult periodontitis, two studies reported better results for scaling and root planing supplemented with locally applied metronidazole than with adjunctive systemic metronidazole (66, 69). So far, no non-antibiotic antimicrobial alternative has proven to be equally efficient or better than systemic amoxicillin and metronidazole. One of the few studies that really evaluated this possibility concluded that adjunctive placement of chlorhexidine chips was less efficacious in the treatment of aggressive periodontitis than amoxicillin and metronidazole (44).

### Conclusions and recommendations

Adjunctive antibiotics can enhance the clinical outcome of non-surgical mechanic debridement, thereby reducing the need for further therapy, which is frequently surgical in nature (Fig. 3). Full-mouth scaling and root planing combined with amoxicillin and metronidazole has the potential to resolve the periodontal infection within a few days. However, the available evidence does not justify the indiscriminate use of just any antibiotic in any patient, and should not be interpreted as a recommendation for prescription of antibiotics to all periodontal patients.

A treatment protocol implementing the recent evidence is shown in Table 1. In summary, mild cases can, and should, be treated non-surgically and without antibiotics. In deep pockets, non-surgical, non-antibiotic treatments alone resolve the bacterial infection less predictably than open flap debridement (41). It is suggested that cases with multiple deep pockets should first be treated by thorough scaling and root planing and adjunctive amoxicillin and metronidazole. The initial treatment plan should be reviewed and adapted after 3–6 months on the basis of a clinical re-examination. Surgical interventions may be necessary to further reduce persisting pockets, for the treatment of furcations, or to regenerate periodontal tissues. Should such therapy be indicated, this sequence allows invasive interventions to be performed only in tissues with minimal persisting pathology, and without antibiotic coverage.

In its strictest sense, the statement that ‘systemic antibiotic therapy in periodontics aims to eliminate or markedly suppress specific microorganisms with the potential of causing breakdown of periodontal attachment in susceptible patients’ (92) is no longer tenable. Although it is known that some antibiotic regimes are able to specifically suppress certain organisms, it has not been proven that selective suppression of specific members of the subgingival microbial complex is the key element for success. Bacteriological efficacy (suppression of a target organism) is not proof of clinical efficacy, and vice versa. Given the large diversity of the microbiota associated with all forms of periodontitis and the multiple synergistic and antagonistic interactions among the members of the flora, the concept of specifically identifying and eradicating a particular pathogen may be illusionary. The combination of amoxicillin and metronidazole appears to have an impact on factors beyond those that have been studied using bacterial culture and currently available tests.

### Table 1. (Continued)

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Debridement</td>
<td>Remove supragingival calculus, if detected</td>
</tr>
<tr>
<td>Re-evaluation and further therapy (after 3–6 months)</td>
<td></td>
</tr>
<tr>
<td>Clinical assessment</td>
<td>Record plaque score, pocket probing depth, bleeding on probing, suppuration, furcation involvement</td>
</tr>
<tr>
<td>Further therapy</td>
<td>Plan and carry out additional therapy as needed (e.g. open flap debridement, furcation therapy, tissue regeneration, implant therapy)</td>
</tr>
</tbody>
</table>

*Adjunctive antibiotics are indicated for subjects fulfilling the following conditions: (i) presence of at least four teeth with a pocket probing depth > 4 mm, clinical attachment loss of at least 2 mm, and radiographic evidence of bone loss, (ii) no confirmed or suspected intolerance to 5-nitroimidazole derivatives or penicillins, and (iii) willingness to strictly follow prescription and to abstain from alcohol consumption during treatment.
References


63. Mombelli A, Schmid B, Rutar A, Lang NP. Persistence patterns of Porphyromonas gingivalis, Prevotella intermedia / nigrescens, and Actinobacillus actinomycetemcomitans


