REAL TIME PCR IDENTIFICATION FOR TARGET ADJUNCTIVE ANTIBIOTIC THERAPY OF SEVERE CHRONIC PERIODONTITIS. PART II - MICROBIOLOGICAL EFFECTIVENESS

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ABSTRACT:
INTRODUCTION: Antibiotic use in chronic periodontitis may result in improvement in periodontal status, although many questions regarding the indications for this therapy remain unanswered. The polymicrobial etiology of the periodontal infection hinders the choice of the proper antibiotic agent. Furthermore, the indiscriminate use of antibiotics could lead to high levels of resistance and to various adverse reactions. In the recent years, various molecular diagnostics protocols were proposed in order to facilitate the decision for adjunctive antibiotic administration.

OBJECTIVE: The aim of this study is to compare the microbiological effectiveness of adjunctive antibiotic administration with the mechanical periodontal therapy.

METHODS: 30 patients with severe chronic periodontitis were enrolled in this study and were divided in 3 groups:
- Control group – with mechanical debridement only.
- Test group 1 – with combined adjunctive antibiotic administration using Amoxicillin + Metronidazole.
- Test group 2 – with target antibiotic administration according to the results from the Real Time PCR identification.

RESULTS: The prevalence of all the isolated microorganisms (except E. nodatum and C. gingivalis) in Test Group 2 demonstrates statistically significant reduction compared with the other treatment approaches. Almost complete elimination was registered for the consensus pathogens from the red and orange complexes (above 99% and 100% for P. intermedia).

CONCLUSION: The adjunct antibiotic treatment targeted with Real-Time PCR identification demonstrates almost complete elimination of the putative periodontal pathogens in the deep periodontal pockets in patients with severe chronic periodontitis. This result suggests slower recolonisation of these habitats thus limiting the risk for progression of the periodontal destruction.

Key words: severe chronic periodontitis, adjunctive antibiotic therapy, periodontal pathogens, Real Time PCR identification.

INTRODUCTION:
The microbial etiology of inflammatory periodontal diseases provides the rationale for the use of antimicrobial medication in periodontal therapy. As evidence for bacterial specificity in periodontitis has accumulated and strengthened over the past three decades, dentists have increased their use of systemic antibiotics in periodontal therapy. This concept is based on the premise that specific microorganisms cause destructive periodontal disease and that the antibiotic agent in vivo can exceed concentrations necessary to kill or inhibit the pathogen(s).

The most effective use of antibiotics for the treatment of periodontitis presupposes knowledge of the pathogenic microbiota. At least 500 bacterial taxa have been identified within periodontal pockets. [1]

However, relatively few species have been clearly associated with progressive periodontitis (Table 1)[2]. Most putative pathogens are indigenous to the human oral cavity, but possible superinfecting organisms (enteric Gram-negative rods, pseudomonas, staphylococci, yeasts) may also inhabit periodontal pockets. Periodontitis lesions usually harbor a constellation of putative pathogens rather than a single pathogenic species. Most putative periodontal pathogens are Gram-negative anaerobic rods. However, some pathogens are Gram-positive facultative and anaerobic cocci and rods and others are Gram-negative facultative rods. Putative periodontal pathogens vary considerably in sensitivity to several antibiotics making simplistic approaches to antimicrobial chemotherapy problematic. [3]

Table 1. Association between putative periodontal pathogens and periodontitis.

<table>
<thead>
<tr>
<th>Very strong</th>
<th>Strong</th>
<th>Moderate</th>
<th>Unclear</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. actinomycetem-comitans</td>
<td>B. forsythus</td>
<td>S. intermedius</td>
<td>Selenomonas sp.</td>
</tr>
<tr>
<td>P. gingivalis</td>
<td>P. intermedia</td>
<td>P. nigrescens</td>
<td>Gr(-) intestinal MO</td>
</tr>
<tr>
<td>Spirochetes of acute necrotizing gingivitis</td>
<td>C. rectus</td>
<td>P. micros</td>
<td>Staphylococcus sp.</td>
</tr>
<tr>
<td>E. nodatum</td>
<td>Treponema sp.</td>
<td>F. nucLearturn</td>
<td>B. gracilis</td>
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</table>
In the last decades a lot of new data for periodontal microbial etiology was acquired. Teles et al. [4] propose a contemporary classification of putative periodontal pathogens (Table 2). This new scheme does not replace the consensus classification, but gives guidelines for further research.

The various microorganisms inhabiting the periodontal environment and their complex relationships were presented by Socransky and Haffajee (2005) [5] and were united in periodontal complexes (Fig.1).

Table 2. Putative periodontal pathogens

<table>
<thead>
<tr>
<th>Consensus pathogens</th>
<th>Strong association</th>
<th>Moderate association</th>
<th>Data for probable association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aggregatibacter actinomycetemcomitans</td>
<td>Eubacterium nodatum</td>
<td>Campylobacter rectus</td>
<td>Acinetobacter baumannii</td>
</tr>
<tr>
<td>Porphyromonas gingivalis</td>
<td>Fusobacterium nucleatum</td>
<td>Dialister pneumosintes</td>
<td>Bacteroides strain AU126</td>
</tr>
<tr>
<td>Tannerella forsythia</td>
<td>Prevotella intermedia</td>
<td>Eikenella corrodens</td>
<td>Strains OP11 &amp; TM7 phyla</td>
</tr>
<tr>
<td>Prevotella nigrescens</td>
<td>Filifactor alocis</td>
<td>Cryptobacterium curtum</td>
<td></td>
</tr>
<tr>
<td>Treponema denticola</td>
<td>Peptostreptococcus micros</td>
<td>Deferribacteres Strains D084 &amp; BH017</td>
<td></td>
</tr>
<tr>
<td>Selenomonas sp.</td>
<td>Enterococcus faecalis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptococcus milleri group</td>
<td>Escherichia coli</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treponema socranskii</td>
<td>Eubacterium saphenum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exiguobacterium aurantiacum</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Megasphaera strain BB166</td>
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<tr>
<td>Mogibacterium timidum</td>
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<tr>
<td>Peptostreptococcus magnus</td>
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<tr>
<td>Porphyromonas endodontalis</td>
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<tr>
<td>Prevotella corporis</td>
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<tr>
<td>Prevotella denticola</td>
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<tr>
<td>Prevotella disiens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slackia exigua</td>
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</tbody>
</table>

Fig.1. Microbial complexes in the periodontal environment. (Copied from Socransky SS, Haffajee AD. Periodontal microbial ecology. Periodontology 2000, 2005; 38: 135–187)
These complexes are used as framework for a better understanding of the periodontal ecosystem. In the dependence of the activity of the periodontal site changes of the inhabiting complexes occur. The microorganisms from the red complex are associated with the active periodontal lesions.

Patients with gingivitis or chronic periodontitis usually respond well to mechanical debridement and topical antiseptics and may not derive clinically significant additional benefit from antibiotic therapy. [6]

However, evidence exists suggesting that antibiotic use in chronic periodontitis may result in improvement in clinical attachment level, although many questions regarding the indications for this therapy remain unanswered. [7]

If antibiotics confer therapeutic advantage, should they be given to all individuals? If not, then who should receive these agents and how severe does the periodontal infection have to be in order to justify the use of an antimicrobial agent? There are no “evidence-based” guidelines for the use of systemically administered antibiotics. It is recognized that many factors impact on this decision such as the systemic well being of the patient, concomitant medical conditions, the nature of the infecting agent(s), etc. For this reason, guidelines for antibiotic use will always remain that; guidelines. They provide starting points to make complex decisions. In the treatment of periodontal infections, we do not even have this starting point, this guideline. We feel that antibiotics are useful in the treatment of aggressive forms of periodontal diseases, “refractory” periodontitis and in smokers. However, in the most common form of the disease, chronic periodontitis, which patients would benefit from systemically administered antibiotics and how would the decision to use antibiotics be made? [8]

Many protocols for antibiotic administration are proposed in the literature:

- Metronidazole [9]
- Amoxicillin [10]
- Clindamycin [11]
- Azithromycin [12]
- Doxycline [13]

Combination drug therapy may be useful in periodontitis that involves a variety of periodontopathic species with differing antimicrobial susceptibilities or to overcome the drug-protective effects of the biofilm. Also, therapeutic failure with some antibiotic regimens due to the presence or development of resistant strains may be an emerging problem in periodontal treatment. One strategy aimed at combating resistant subgingival bacteria is the use of treatment regimens that incorporate agents with complementary but different mechanisms of action. Combination therapy should include drugs that exhibit synergy or additive effects in vitro. Metronidazole-amoxicillin act synergistically against Actinobacillus actinomycetemcomitans and other major periodontal pathogens.

- **Amoxicillin + Metronidazole** [14,15]

The polymicrobial etiology of the periodontal infection hinders the choice of the proper antibiotic agent in the periodontal treatment. Furthermore the indiscriminate use of antibiotics could lead to high levels of resistance in the population and to various adverse reactions. In the recent years various molecular diagnostics protocols were proposed in order to facilitate the decision for adjunctive antibiotic administration.

**OBJECTIVE:**

The aim of this study is to compare the microbiological effectiveness of adjunctive antibiotic administration with the mechanical periodontal therapy. Two protocols of antibiotic administration were tested - combined antibiotic treatment with Amoxicillin + Metronidazole and target antibiotic administration according to the result of the Real Time PCR test.

**METHODS:**

The materials and methods are described in “Real time PCR identification for target antibiotic therapy of severe chronic periodontitis. Part I - Clinical results”.

**1. Patient selection**

70 patients were diagnosed and 30 patients were enrolled in this study

**Inclusion criteria:**

- Age – 18-75 years
- > 20 natural teeth in situ
- clinical and radiographic signs of severe chronic periodontitis (CAL-loss of 5 mm or more at least at 20 sites)
- at least 6 pockets with PPD of 5 mm
- at least 4 pockets with PPD ≥7 mm
- no professional periodontal therapy during the 6 months preceding the baseline clinical evaluation

**Exclusion criteria:**

- have known systemic diseases that may influence the periodontal conditions, in particular Down’s syndrome, known AIDS/HIV or diabetes type I or II as determined by assessment of erythrocyte HbA1c levels (more than 6.5%);
- regularly take drugs that may affect the periodontal conditions, e.g. phenytoine, nifedipine, and/or anti-inflammatory drugs;
- require antibiotic treatment for dental appointments;
- are undergoing or require extensive dental or orthodontic treatment;
- are pregnant or breastfeeding;
- have any oral or extra oral piercing in or around the oral cavity with ornaments or accessory jewelry;
- have participated in a clinical dental trial in the six months preceding the study.
- have known allergies against the antibiotics to prescribe.
- take antibiotics in the three months preceding the study.

The patients enrolled in this study were divided in three groups:

- **Control group** – with mechanical debridement only.
- **Test group 1** – with combined adjunctive antibiotic administration using Amoxicillin 500mg + Metronidazole 250mg tid – 7 days
- **Test group 2** – with target antibiotic administration according to the results from the Real Time PCR identification.
2. **Anti-infective mechanical therapy** - after oral hygiene instructions and achievement of the proper oral hygiene standard all patients received the same amount of full mouth mechanical debridement.

3. **Microbiological testing** - a Real Time PCR test - PET Test (MIP Pharma) was used in all patients. A pool sample with five sterile paper points from the pockets with PPD≥7mm were taken on the baseline and on the 8 week reevaluation.

   This test provides information for:
   A. Total Counts - MO
   - Identified periodontal microorganisms – Ident
   - Non-identifies microorganisms – No Ident
   B. Quantity and prevalence of red complex:
   - Porphyromonas gingivalis (Pg)
   - Treponema denticola (Td)
   - Tannerella forsythia (Tf)
   C. Quantity and prevalence of some pathogens from orange complex:
   - Prevotella intermedia (P.i.)
   - Peptostreptococcus (Micromonas) micros (Pm)
   - Fusobacterium nucleatum (Fn)
   D. Quantity and prevalence of some pathogens from orange associated complex:
   - Eubacterium nodatum (En)
   E. Quantity and prevalence of some pathogens from green complex:
   - Capnocytophaga gingivalis (Cg).
   F. Quantity and prevalence of Aggregatibacter actinomycetemcomitans (Aa).

   The data analysis contained:
   1. Initial levels
   2. Microbial levels at reevaluation

4. **Statistical analysis**

   The acquired data was analyzed with IBM SPSS Statistics 19.0. The chosen level of significance was p<0.05.

   The following methods were applied:
   1. Descriptive analysis
   2. Analysis of variations
   3. Graphical analysis
   4. Test χ² Shapiro-Wilk test
   5. ANOVA test
   6. Kruskal-Wallis test
   7. Student T -test
   8. Mann-Whitney test
   9. Wilcoxon test

**RESULTS:**

   The analysis of the prevalence of the identified periodontal microorganisms reveals reduction of their prevalence after therapy in all treatment groups. A significantly greater reduction of the prevalence is obtained in both test groups (84.88% test group 1; 97.96% test group 2), compared to the control group (40.84%). In test group 2 an almost complete elimination of the periodontal pathogens was achieved (Fig.2).

   In all treatment groups a slight increase of the prevalence of non-pathogen species was detected, as in the test groups demonstrate statistically more pronounced increase compared to the control group.

**Fig. 2.** Reduction of the prevalence of the detected microorganisms.
A pronounced reduction of the prevalence of the periodontal pathogens of the red complex (Fig. 3) was detected in all treatment groups. In test group 2 the greatest reduction was achieved with almost complete elimination of all pathogens (above 99.8%). In the other treatment groups the reduction of the prevalence is with significantly lower levels.

These results imply a creation of a more stable microbiological environment with diminished risk of progression of the periodontal destruction with the application of the Real Time PCR approach for the selection of the adjunctive antibiotic therapy.

**Fig. 3.** Changes of the prevalence of the microorganisms of the “red complex”.

![Graph showing relative reduction of prevalence for different groups](image)

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Control group</th>
<th>Test group 1</th>
<th>Test group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pg</td>
<td>81,99000%</td>
<td>70,50000%</td>
<td>99,83000%</td>
</tr>
<tr>
<td>Td</td>
<td>78,77000%</td>
<td>96,45000%</td>
<td>99,92000%</td>
</tr>
<tr>
<td>Tf</td>
<td>75,52000%</td>
<td>81,14000%</td>
<td>99,76000%</td>
</tr>
</tbody>
</table>

The data for the pathogens from the orange complex (Fig.4.) reveals the differences between the applied approaches. The most pronounced reduction is reported in test group 2 with elimination of P.intermedia and almost complete absence of F. nucleatum (99.99 reduction of the prevalence). In test group 1 also a reduction of the prevalence of these pathogens is reported but with statistically significant lower levels, whereas in the control group a fundamental increase the prevalence is present of F. nucleatum (150.26% increase) and P.intermedia (378.32% increase) compared with the prevalence at baseline. The pathogens of the orange complex play an important role in the formation of the complex biofilm and their higher levels of prevalence are associated with increased risk of colonization with the pathogens of the red complex and recurrence of the periodontal disease. These results suggest that the adjunctive antibiotic therapy, especially using a Real Time PCR test for the selection of the antibiotic could lead to a successful long-term result due to slower recolonisation of the periodontal habitat with pathogens.

**Fig. 4.** Changes of the prevalence of the microorganisms of the “orange complex”.

![Graph showing relative reduction of prevalence for different groups](image)

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Control group</th>
<th>Test group 1</th>
<th>Test group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fn</td>
<td>-150.26000%</td>
<td>95,32000%</td>
<td>99,98000%</td>
</tr>
<tr>
<td>Pm</td>
<td>76,20000%</td>
<td>87,13000%</td>
<td>98,32000%</td>
</tr>
<tr>
<td>Pi</td>
<td>-378,32000%</td>
<td>98,14000%</td>
<td>100,00000%</td>
</tr>
</tbody>
</table>
The data for the changes of the prevalence of the E. nodatum (yellow complex) doesn’t present a significant difference between groups, and regarding C. gingivalis (green complex), a significant greater reduction of its prevalence was reported for both test groups compared to the control group (Fig. 5).

**Fig. 5.** Changes of the prevalence of the microorganisms of the “yellow and green complexes”.

CONCLUSION:

The Real-Time PCR identification for the selection of the adjunct antibiotic treatment demonstrates almost complete elimination of the putative periodontal pathogens in the deep periodontal pockets in patients with severe chronic periodontitis. This result suggests slower recolonisation of these habitats thus limiting the risk for progression of the periodontal destruction.

Our data supports the application of the adjunctive antibiotic treatment with Real Time PCR test for the selection of the antibiotic for patients with severe chronic periodontitis and presence of pockets with PPD>7mm.

REFERENCES:


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