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

Kamen Kotsilkov, Christina Popova.
Department of Periodontology, Faculty of Dental Medicine, Medical University – Sofia, Bulgaria.

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 a 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 resuts from the Real Time PCR identification.

RESULTS: The prevalence of all the isolated microorganisms (exept. 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.intemedia).

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 inыammatory 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 speсiыc 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 identыed 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.

| Very strong	Strong	Moderate	Unclear
A.actinomycetem-comitans P.gingivalis Spirochetes of acute necrotizing gingivitis	B.forsythus P.intermedia C.rectus E.nodatum Treponema sp.	S.intermedius P. nigrescens P. micros F.nucLeaturn Eubacterium sp. E. corrodens	Selenomonas sp. Gr(-) intestinal MO Staphylococcus sp. B. gracilis

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 guves 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

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

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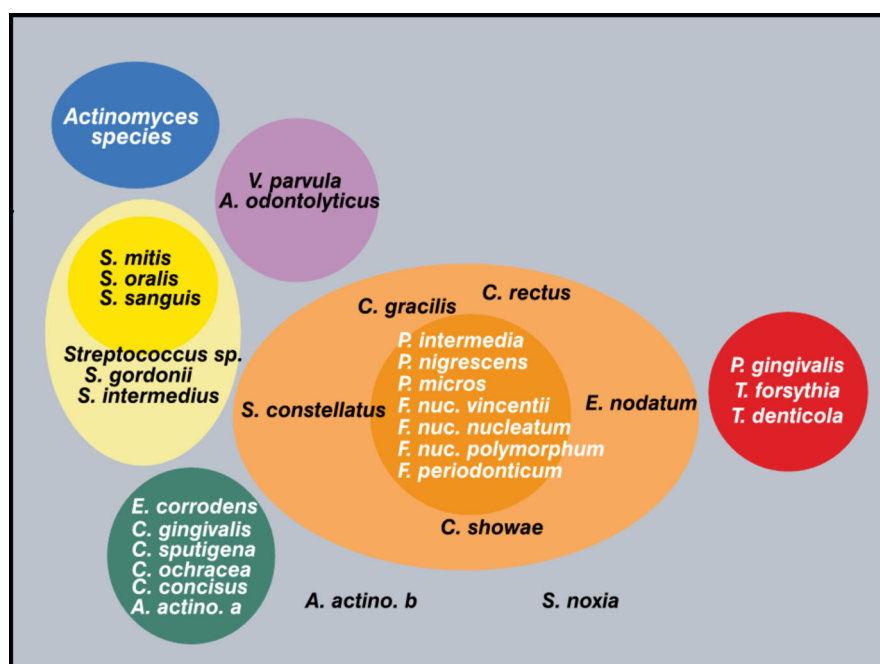
These complexes are used as framework for a better understanding of the periodontal ecosystem. In the dependence of the activity of the periodontal site changes of the inhabiting complexes occur. The microorganisns from the red complex are associated with the active periodontal lesions.

Patients with gingivitis or chronic periodontitis usually respond well to mechanical debridement and topical antiseptics and may not derive clinically signiьcant additional beneьt 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 beneьt 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]
- Doxycycline [13]

Combination drug therapy may be useful in periodontitis that involves a variety of periodontopathic species with differing antimicrobial susceptibilies or to overcome the drug-protective effects of the bioьlm. 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 additiveeffects 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 a 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 adjunctive 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 PPDe"7mm
- 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 resuts from the Real Time PCR identification.
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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)
  - Tanerella forsythia (Tf)

C. Quantity and prevalence of some pathogens from orange complex:
  - Prevotella intermedia (P.i.)
  - Peptosteptococcus (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 Aggregatibacer actinomycetemcomitans (Aa).

The data analysis contained:
1. Initial levels
2. Microbial levels at reevaluation
3. Mean reduction of the microbial levels
4. Relative reduction - represents the relative share of the changes in the microbial levels as a percentage from the initial levels, thus allowing more accurate comparison between the different approaches.

4. Statistical analysis

The acquired data was analyzed with **IBM SPSS Statistics 19.0.** The chosen level of significalce was **$p<0,05$.**
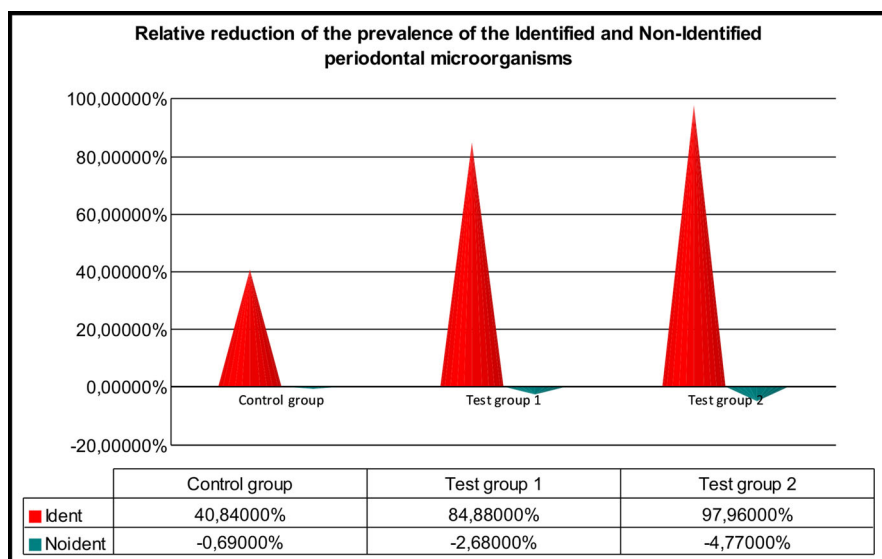
The following methods were aplied:
1. Descriptive analysis
2. Analysis of variations
3. Graphical analysis
4. Test χ2 Shapiro-Wilk test
5. ANOVA test
6. Kruskal-Wallis test
7. Student T -test
8. Mann-Whitney test
9. Wilcoxon test

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.
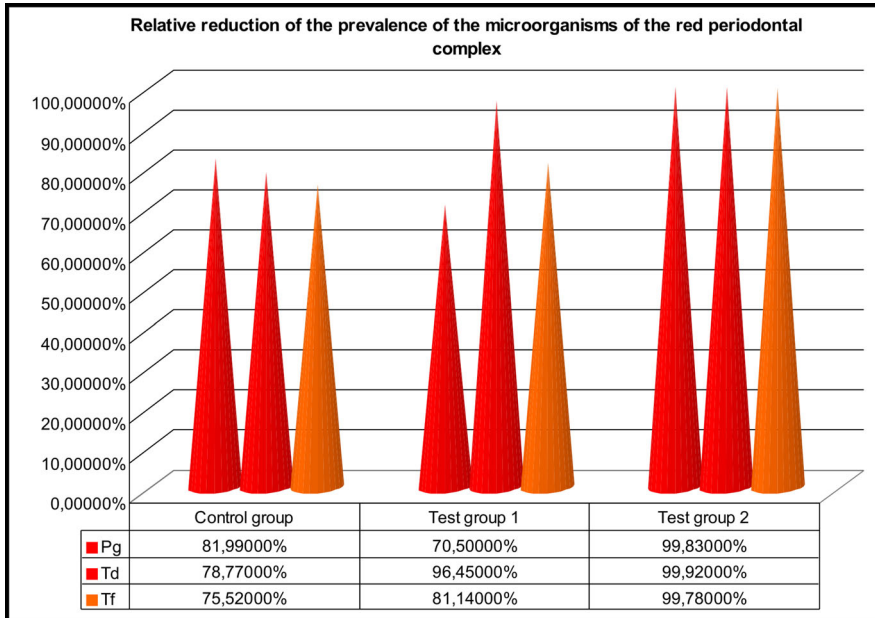
Fig. 2. Reduction of the prevalence of the detected microorganisms.

	Control group	Test group 1	Test group 2
■ Ident	40,84000%	84,88000%	97,96000%
■ Noident	-0,69000%	-2,68000%	-4,77000%

A pronounced reduction of the prevalence of the peri-odontal 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.
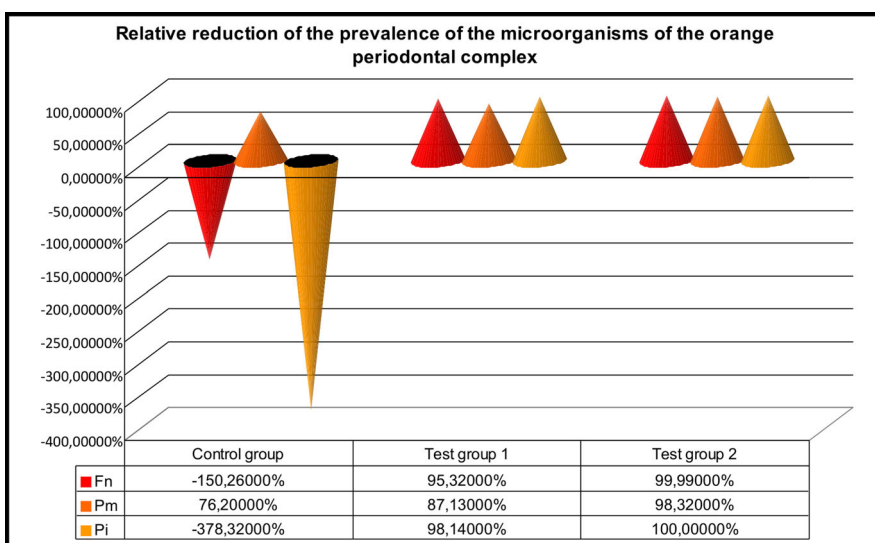
Fig. 3. Changes of the prevalence of the microorganisms of the "red complex".

Relative reduction of the prevalence of the microorganisms of the red periodontal complex

	Control group	Test group 1	Test group 2
Pg	81,99000%	70,50000%	99,83000%
Td	78,77000%	96,45000%	99,92000%
Tf	75,52000%	81,14000%	99,78000%

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.
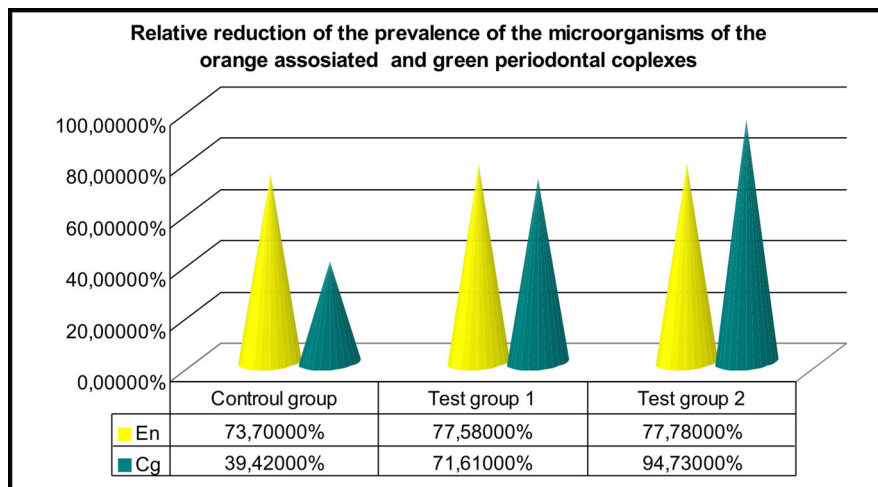
Fig. 4. Changes of the prevalence of the microorganisms of the "orange complex".

Relative reduction of the prevalence of the microorganisms of the orange periodontal complex

	Control group	Test group 1	Test group 2
Fn	-150,26000%	95,32000%	99,99000%
Pm	76,20000%	87,13000%	98,32000%
Pi	-378,32000%	98,14000%	100,00000%

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".

Relative reduction of the prevalence of the microorganisms of the orange assosiated and green periodontal coplexes

	Controul group	Test group 1	Test group 2
En	73,70000%	77,58000%	77,78000%
Cg	39,42000%	71,61000%	94,73000%

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.

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Address for correspondence:
Kamen Kotsilkov, Assistant Professor at Department of Periodontology, Faculty of Dental Medicine, Medical University - Sofia.
1, St. Georgi Sofiyski Blvd, 1431 Sofia, Bulgaria
E-mail: kamenkotsilkov@gmail.com