

# Adjunctive effect of chlorhexidine in ultrasonic instrumentation of aggressive periodontitis patients: a pilot study

Maria Elena Guarnelli,  
Giovanni Franceschetti, Roberta  
Manfrini and Leonardo Trombelli

Research Center for the Study of Periodontal  
Diseases, University of Ferrara, Ferrara, Italy

Guarnelli ME, Franceschetti G, Manfrini R, Trombelli L. Adjunctive effect of chlorhexidine in ultrasonic instrumentation of aggressive periodontitis patients: a pilot study. J Clin Periodontol 2008; 35: 333–341. doi: 10.1111/j.1600-051X.2008.01199.x.

## Abstract

**Aim:** The aim of the present pilot randomized clinical trial was to evaluate the effects of ultrasonic mechanical instrumentation (UMI) associated with the professional use of chlorhexidine (CHX) formulations compared with UMI alone during periodontal supportive therapy in patients with generalized aggressive periodontitis (G-AgP).

**Material and Methods:** Nine patients (test group) received a single session of UMI associated with subgingival irrigation under cavitation with CHX 0.02%. A 0.2% CHX solution was used for professional tongue brushing and mouthrinsing. Ten patients (control group) received a similar session of UMI associated with subgingival irrigation and professional tongue brushing and mouthrinsing with a control formulation. Clinical and microbiological parameters were assessed pre-treatment at 3, 6 and 12 weeks post-treatment.

**Results:** UMI either with or without additional CHX use determined a significant reduction of supragingival plaque and gingival inflammation as well as a significant reduction of subgingival bacterial pathogens. The additional use of CHX did not result in any additional clinical and microbiological benefit with respect to mere UMI.

**Conclusions:** The adjunctive professional use of CHX formulations to UMI seems to produce no additional effects over UMI alone during supportive therapy in G-AgP patients.

Key words: aggressive periodontitis; amine fluoride/stannous fluoride; chlorhexidine; supportive periodontal therapy; ultrasonic instrumentation

Accepted for publication 8 December 2007

Evidence suggests that ultrasonic mechanical instrumentation (UMI) is an effective method for removing supra- and subgingival bacterial biofilm adherent to tooth surfaces (Thornton & Garnick 1982, Breining et al. 1987), as well as reducing probing depths and

bleeding on probing scores (Greenstein 2000, Drisko 2001, Hallmon and Rees 2003). Microbial studies evaluating the effects of UMI on dental plaque have shown that subgingival debridement to determine substantial changes in the microbial composition of dental biofilm, in particular, UMI, results in reduced total bacterial count (TBC) while establishing a subgingival microflora consistent with periodontal health (Oosterwaal et al. 1987, Baehni et al. 1992).

Unfortunately, the microbial shift after periodontal debridement may be transient, and bacterial re-colonization of the root surface by pathogenic bacteria, which frequently occurs after treatment,

may lead to disease recurrence (Mousqués et al. 1980, Magnusson et al. 1984, Van Winkelhoff et al. 1988). The presence of various bacterial reservoirs in the oral cavity, such as the tongue, tonsils and other mucous membranes, periodontal sulcus/pockets and exposed roots, seems to increase the likelihood of re-infection following treatment (van der Velden et al. 1986, Adriaens et al. 1988).

In this respect, combined treatment with manual and/or mechanical instrumentation associated with local application of antimicrobial agents has been investigated widely. Recently, a systematic review suggested that the

## Conflict of interest and source of funding statement

The authors declare that they have no conflict of interests.

This study was supported by the Research Center for the Study of Periodontal Diseases, University of Ferrara, and GABA International AG, Münchenstein, Switzerland.

adjunctive subgingival irrigation with chlorhexidine (CHX), hydrogen peroxide or saline, in conjunction with periodontal debridement, offered limited advantages when compared with periodontal debridement alone (Hallmon & Rees 2003). In contrast, when professional supra- and subgingival instrumentation is associated with a more comprehensive antimicrobial regimen, based on extensive professional and home-based use of antimicrobial agents according to the "full-mouth disinfection" protocol, additional benefits on a positive shift of subgingival microbiota may be expected (Bollen et al. 1996). Data seem to indicate that one-stage, full-dentition periodontal debridement with the adjunctive use of antimicrobials may be a key factor for the observed clinical and microbiological benefits over a classical stepwise periodontal debridement, at least for non-surgical treatment of periodontally affected patients (Quirynen et al. 1995, 2000, De Soete et al. 2001, Wennström et al. 2005). However, in recent studies, a similar "full-mouth disinfection" approach, based on a combination of mechanical instrumentation as well as professional and home-care use of antimicrobials, showed limited clinical and microbiological effects compared with the conventional "staged" approach without antimicrobial supplements (Apatzidou and Kinane 2004, Apatzidou et al. 2004, Koshy et al. 2004, Kinane 2005, Jervøe-Storm et al. 2007).

The aim of the present randomized, double-blind, controlled pilot study was to evaluate the clinical and microbiological effects of UMI associated with the professional use of CHX-containing formulations compared with UMI alone during a recall session of periodontal supportive therapy in patients with generalized aggressive periodontitis (G-AgP).

## Material and Methods

### Study population

Twenty-one systemically healthy G-AgP patients were selected for study among those undergoing periodontal supportive therapy at the Research Center for the Study of Periodontal Diseases, University of Ferrara.

To be enrolled in the study, patients had to fulfil the following inclusion criteria: a clinical diagnosis of G-AgP at the initial visit according to the pre-

viously described criteria (Tonetti & Mombelli 1999); manual or mechanical instrumentation at least 3 months before the experimental phase; and patients able and willing to provide informed consent and to ensure compliance throughout the study. All patients were clinically healthy, except for the presence of periodontitis; presented rapid bone loss, as evaluated at two distinct radiographic exams; and presented a generalized attachment loss affecting at least three permanent teeth other than the first molars and incisors.

Patients were excluded from the study if they met any of the following exclusion criteria: pregnancy or lactation; physical or mental handicap that could interfere with adequate oral hygiene performance; systemic and/or topical steroidal and non-steroidal anti-inflammatory drugs and local and/or topical antimicrobials during the last 6 weeks before the study; systemic and/or topical antibiotics during the last 3 months; fixed or removable orthodontic devices; oral soft tissue pathology, excluding G-AgP, based on visual examination; reported significant adverse effects or documented allergy following use of oral hygiene products such as CHX and amine fluoride/stannous fluoride (AmF/SnF<sub>2</sub>) mouthrinse or toothpaste; and conditions requiring prophylactic antibiotic coverage before invasive dental procedures.

Before entering the experimental phase, patients were given oral and written information on the study design/aim and effects of antimicrobial agents in order to have signed consensus. The study design was approved by the local ethical committee and was found to conform to the requirements of the "Declaration of Helsinki" as adopted by the 18th World Medical Assembly in 1964 and subsequently revised ([www.wma.net/e/policy/b3.html](http://www.wma.net/e/policy/b3.html)).

### Antimicrobial formulations

In this study, we used a test formulation, which was a 0.2% CHX solution (GABA International AG, Münchenstein, Switzerland), and a control formulation (GABA International AG). Patients receiving the test formulations comprised the *test group*; those receiving the control formulation comprised the *control group*.

Test and control formulations were identical, except for CHX content, and were provided in identical coded bottles

so that neither the patient nor the investigator was aware of which treatment had been assigned (double-blinding, allocation concealment).

### Treatment regimens

At baseline, the test group received a single session of full-mouth supra- and subgingival mechanical instrumentation by means of a piezoelectric ultrasonic device and specific tips (Piezosteril 5<sup>®</sup>, Castellini s.p.a., Castel Maggiore, BO, Italy), followed by supragingival polishing with a non-fluoridated prophylaxis paste. The ultrasonic instrumentation was associated with subgingival irrigation under cavitation with 0.2% CHX formulation diluted 1:10 with saline (i.e. 0.02% CHX). Mechanical debridement was supplemented by an anti-infective regimen based on

- professional brushing of the dorsum of the tongue for 60 s using a standard toothbrush and a 0.2% CHX solution and
- two rinses with a 0.2% CHX solution of 1 min. each. The patient was asked to gargle, thereby trying to reach the tonsils, during the last 10 s.

The control group received a similar session of mechanical debridement associated with subgingival irrigation under cavitation with a 1:10 saline dilution of the control formulation. Tongue brushing and mouthrinsing were performed using the undiluted control formulation.

Working time during the instrumentation session (i.e. the time during which the ultrasonic device was actively used on the patient) was standardized (45 ± 5 min.). The amount of either the test or the control solution used as an irrigating agent was approximately 1500 ml (± 100 ml) for each patient.

Both the test and the control groups were prescribed an AmF/SnF<sub>2</sub>-containing mouthrinse and toothpaste (meridol<sup>®</sup> mouthrinse/toothpaste, GABA International AG) for 12 weeks. The mouthrinse was prescribed at 10 ml twice daily (after morning and evening toothbrushing) for 60 s. The toothpaste was prescribed three times a day (morning, afternoon and night). AmF/SnF<sub>2</sub> mouthrinse and toothpaste were provided in anonymous coded bottles and tubes.

### Experimental design

The present study was a randomized, double-blind, controlled clinical trial. The study design is summarized in Fig. 1. Four to 6 weeks before entering the experimental phase, each patient received an initial contact appointment to verify his/her eligibility for study recruitment and sign the informed consent.

At week 0 (baseline), the patients were assigned to either the test or the control formulation according to a central computer-generated randomization list. Examiners were kept unaware of the randomization sequence (allocation concealment). Clinical recordings and subgingival plaque samples were collected. Verbal oral hygiene instructions (OHI) on mechanical plaque control were given, and toothbrush, inter-proximal cleaning devices, toothpaste and mouthrinse were provided.

At week 3, 6 and 12 following the session, clinical parameters and subgingival plaque samples were recorded anew. OHI were reinforced. At week 12, periodontal debridement and prophylaxis were provided as needed for plaque/calculus/stain elimination.

### Clinical recordings

At weeks 0, 3, 6 and 12, the following periodontal parameters were recorded:

- Gingival index (GI), according to Löe & Silness (1963).
- Presence of supragingival plaque according to Plaque index (PII; Turesky et al. 1970). Plaque was visualized by means of a disclosing agent (Red Cote<sup>®</sup>, Butler, Montvale, NJ, USA).

PII and GI were recorded at six sites (mesio-buccal, buccal, disto-buccal, mesio-lingual, lingual and disto-lingual) on the following selected teeth: #1.6, #1.1, #2.4, #3.6, #3.1 and #4.4. If one of these teeth was missing, the available adjacent tooth was examined.

### Microbiological samples

At weeks 0 (immediately before instrumentation), 3, 6 and 12, subgingival plaque samples were collected at four sites, one for each quadrant, which presented with probing pocket depth (PPD)  $\geq 5$  mm. The selection of the sampling sites was made during the initial contact appointment, and the sites were kept consistent for the analysis throughout the study. Microbiological samples were collected according to a standardized procedure (meridol<sup>®</sup> Perio Diagnostics, GABA International AG) by the same investigator (M. E. G.) who was blinded as to the treatment regimen. First, sites to be sampled were isolated with cotton rolls and carefully dried with air to minimize saliva contamination. A sterile paper point was inserted subgingivally into each site and left in place for 20 s. Then, the samples were pooled, immediately transferred to a sterile transport tube and sent to a specialized laboratory (Carpegen GmbH, Münster, Germany).

Real-time PCR was used for the detection and quantification of TBC, as well as periopathogenic bacteria such as *Actinobacillus actinomycetemcomitans* (A.a.), *Porphyromonas gingivalis* (P.g.), *Tannerella forsythia* (T.f.), *Treponema denticola* (T.d.), *Fusobacterium nucleatum* (F.n.) and *Prevotella intermedia* (P.i.). The level of detection was set at  $10^2$  bacteria/plaque sample. The microbiological analysis was performed

blinded as to the study design and treatment regimen.

PPD was measured at four sampled sites at each observation interval by means of a standard periodontal probe (UNC 15, Hu Friedy, Chicago, IL, USA) with a manual pressure of approximately 25 g.

### Statistical analysis

The patient was regarded as the statistical unit; therefore, the clinical recordings (GI, PII) assessed in the 36 representative sites for each patient were averaged to obtain patient-based values. The four pooled paper points, as collected at each observation interval, were processed together to give patient-based bacterial counts. Before the analysis, bacterial counts were transformed to logarithms (base 10). In each patient, total pathogens (TP) were calculated by adding the counts of A.a., P.g., T.f., T.d., F.n. and P.i. while "red complex species" (RC) were calculated by adding the counts of P.g., T.f. and T.d.

Data from clinical parameters and microbiological analysis were expressed as the median and inter-quartile range (IR). To test the effect of "time" and "treatment" on response variables, Friedman's test was used. Post hoc multiple comparisons were performed to explore intra- and inter-treatment differences (Newman-Keuls-Student's test). Mann-Whitney's test was performed to explore inter-treatment differences.

The level of significance was set at 5%. The minimum benefit, in terms of reduction of the baseline value with respect to 12 weeks, that would be needed (with 80% statistical power and  $\alpha = 0.05$ ) for groups to show a statistical difference was calculated: 0.3 for PII

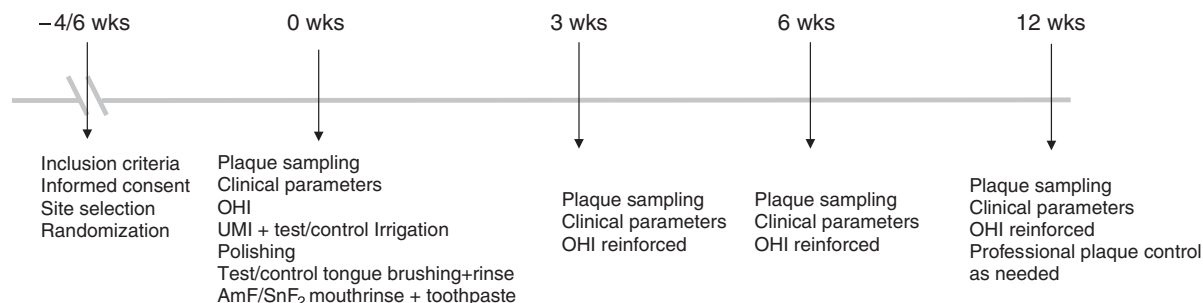


Fig. 1. Experimental design and procedures. UMI, ultrasonic mechanical instrumentation; OHI, oral hygiene instructions; AmF/SnF<sub>2</sub>, amine fluoride/stannous fluoride.

reduction; 0.2 for GI reduction; and 0.5 (log base 10) for both TBC and TP.

## Results

### Study population

Among the 21 G-AgP patients who had been enrolled, two patients (in test group) dropped out of the study because of antibiotics assumption during the observation interval. Therefore, only data from 19 fully complying patients were included for analysis.

Nine patients, three males and six females, aged 28–41 (men age: 35.1 years), and two smokers received the test treatment. Ten patients, two males and eight females, aged 30–41 (mean age: 38 years), and four smokers received the control treatment. The age, gender and smokers were similar in the test and control groups.

### Supragingival plaque accumulation

Table 1 shows the PII scores as recorded in the test and control groups at each observation interval. In both the test and the control groups, a statistically significant decrease in PII was observed during the experimental phase ( $p = 0.002$  for the test group and  $p = 0.001$  for the control group). Baseline values were significantly higher compared with the 3-, 6- and 12-week values ( $p < 0.05$ ).

No significant differences in PII were observed between the test and control groups at any observation intervals.

### Gingival inflammation

Descriptive statistics for GI, as assessed for the test and control groups, are summarized in Table 2. GI significantly decreased in the test and control groups ( $p = 0.02$  for the test group,  $p = 0.00$  for the control group). GI baseline values were significantly higher compared with the 3-, 6- and 12-week values ( $p < 0.05$ ).

No significant differences in GI were observed between the test and control groups at any observation interval.

### PPD

Descriptive statistics of PPD are summarized in Table 3. PPD significantly decreased over time in the test ( $p = 0.005$ ) and control groups ( $p < 0.001$ ). In both groups, PPD was significantly reduced from baseline to 3 weeks and remained stable thereafter.

Table 1. Plaque index (PII) in control ( $n = 10$ ) and test ( $n = 9$ ) group assessed at six selected teeth [median; inter-quartile range (IR)]

	Control group		Test group		<i>p</i> -value*
	PII		PII		
	median	IR	median	IR	
Baseline	2.25	1.86–2.56	1.89	1.81–2.08	0.102
3 weeks	0.87	0.53–1.47	0.42	0.17–0.81	0.066
6 weeks	1.04	0.72–1.33	0.38	0.28–0.83	0.131
12 weeks	1.36	1.06–1.56	1.06	0.72–1.50	0.220

\*Mann–Whitney test *p*-value refers to comparison between test and control groups at each observation interval.

Table 2. Gingival index (GI) in control ( $n = 10$ ) and test ( $n = 9$ ) group assessed at six selected teeth [median; inter-quartile range (IR)]

	Control group		Test group		<i>p</i> -value*
	GI		GI		
	median	IR	median	IR	
Baseline	1.13	0.94–1.33	0.97	0.67–1.11	0.086
3 weeks	0.44	0.19–0.5	0.14	0.11–0.17	0.093
6 weeks	0.28	0.06–0.44	0.06	0.03–0.14	0.188
12 weeks	0.33	0.17–0.75	0.17	0.14–0.28	0.190

\*Mann–Whitney test *p*-value refers to comparison between test and control groups at each observation interval.

Table 3. Probing pocket depth (PPD, in mm) measured at four microbiologically sampled sites in control ( $n = 10$ ) and test ( $n = 9$ ) group [median; inter-quartile range (IR)]

	Control group		Test group		<i>p</i> -value*
	PPD		PPD		
	median	IR	median	IR	
Baseline	5.75	5.25–6.5	5.5	5–6	0.266
3 weeks	5.37	4.75–6	4.5	4–5.25	0.175
6 weeks	4.62	4–5.25	4.25	4–4.25	0.377
12 weeks	4.62	3.75–5.25	4.25	4–5	0.935

\*Mann–Whitney test *p*-value refers to comparison between test and control groups at each observation interval.

No significant differences in PPD were observed between the test and control groups at any observation interval.

### TBC

TBC significantly decreased over time in the control group ( $p = 0.002$ ). In the test group, TBC showed a marked reduction over time; however, this change did not reach statistical significance ( $p = 0.07$ ). TBC significantly decreased from baseline to 3 weeks and remained similarly low thereafter in both groups.

No significant differences in TBC were observed between the test and

control groups at any observation interval (Fig. 2).

### TP

In both the test and the control group, treatment resulted in a significant reduction of TP over time ( $p = 0.005$  and  $p < 0.000$ , respectively) (Fig. 3). TP was significantly reduced from baseline to 3 weeks, and remained similarly low at 6 and 12 weeks for both groups.

No significant differences in TP were found between the test and control groups at any observation intervals.

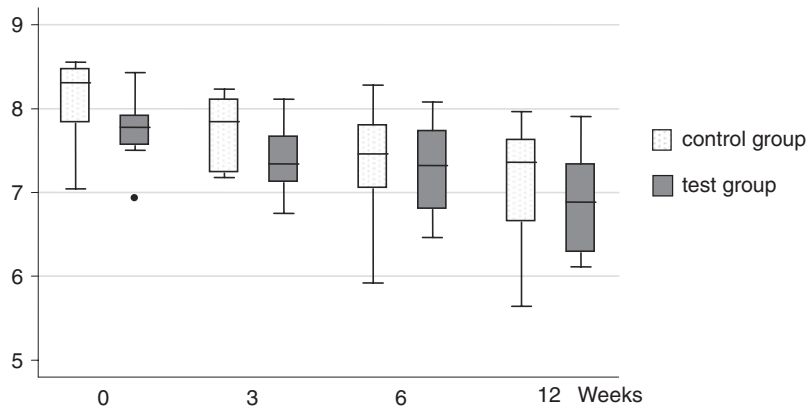


Fig. 2. Total bacteria count (TBC; logarithms base 10) in the control ( $n = 10$ ) and test ( $n = 9$ ) groups assessed at four sampled sites  $\geq 5$  mm (Box-Whisker plot).

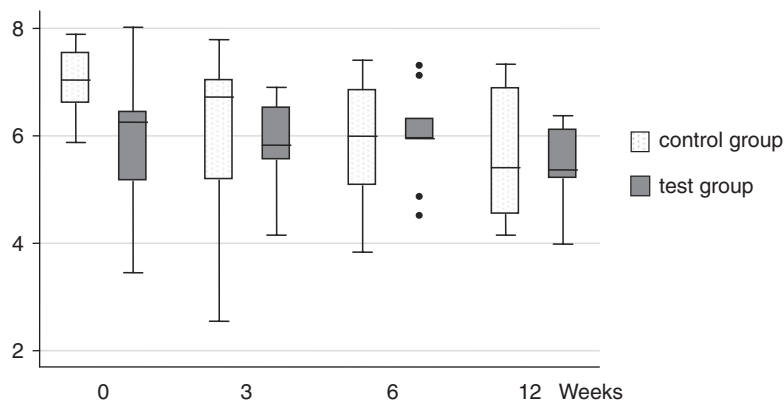


Fig. 3. Total pathogens (TP; logarithms base 10) (counts for *A.a.*, *P.g.*, *T.f.*, *T.d.*, *F.n.* and *P.i.*) in the control ( $n = 10$ ) and test groups ( $n = 9$ ) assessed at four sampled sites  $\geq 5$  mm (Box-Whisker plot). *A.a.*, *Actinobacillus actinomycetemcomitans*; *P.g.*, *Porphyromonas gingivalis*; *T.f.*, *Tannerella forsythia*; *T.d.*, *Treponema denticola*; *F.n.*, *Fusobacterium nucleatum*; *P.i.*, *Prevotella intermedia*.

#### RC species

Treatment resulted in a significant reduction of RC species in the test ( $p = 0.025$ ) and control groups ( $p < 0.000$ ) (Fig. 4). Post hoc comparisons showed that RC was significantly reduced from baseline to 3 weeks, and remained similarly low at 6 and 12 weeks for both groups.

No significant differences in RC were found between the test and control groups at any observation intervals.

#### A.a.

*A.a.* was only detected in a small number of patients at each observation interval. Low prevalence prevented any statistical comparison both within and between groups.

#### Adverse events

No adverse events that could be related to the experimental (test and control)

formulations used during the UMI session were observed. A mild desquamation of the gingival epithelium was observed at 3 and 12 weeks in one patient for the test group, and at 12 weeks in one patient for the control group.

#### Discussion

In the present study, we evaluated the clinical and microbiological effects of a single session of UMI, combined with the professional use of CHX formulations compared with UMI alone during periodontal supportive therapy in G-AgP patients. The clinical parameters and microbiological samples were assessed at 3, 6 and 12 weeks following the UMI session. The results indicate that UMI either with or without additional CHX use showed a significant reduction of supragingival plaque and gingival inflammation. Consistently,

a substantial change in the total subgingival bacterial load and a significant reduction of subgingival bacterial pathogens, including RC species, was observed in periodontal pockets (PPD  $\geq 5$  mm) for both experimental (test and control) groups. The additional use of CHX to UMI did not result in any additional clinical and microbiological benefit with respect to mere UMI.

The rationale to use G-AgP patients as the study population is based on the high susceptibility of these diseased individuals to the development and progression of periodontal lesions due to bacterial challenge, with severe destruction of the supporting apparatus of teeth in relatively young subjects. The use of specific plaque-control strategies (adopted during professional maintenance sessions as well as self-performed oral care) supplemented by antimicrobial agents in addition to conventional plaque removal procedures may be of particular benefit in highly susceptible patients, such as aggressive periodontitis patients (Moreira & Feres-Filho 2007).

In our protocol, supra- and subgingival periodontal debridement was performed by means of a piezoelectric ultrasonic device, followed by supragingival polishing with a non-fluoridated prophylaxis paste. In the test group, we additionally used a 0.02% CHX solution as an irrigating agent during the mechanical instrumentation associated with tongue brushing and oral rinsing with a 0.2% CHX solution. The control group received non CHX-containing formulations for irrigation and rinsing. The assumption was that the cleaning efficacy of ultrasonic scaling in removing plaque and calculus would have been implemented by the antimicrobial effect of CHX. In turn, plaque removal and bacterial cell disruption through the vibrating chipping action of the tip, the cavitation activity and the acoustic microstreaming due to the ultrasonic scaler would have improved the bactericidal effect of the CHX, leading to a greater reduction of subgingival microflora (Walmsley et al. 1984, 1988). Moreover, CHX for mouthrinse and tongue brushing aimed to enhance the suppression of periopathogens from all their oro-pharyngeal habitats, thus potentially delaying the re-colonization (by intra-oral translocation) of the treated pockets by bacteria from untreated sites (Faveri et al. 2006, Quirynen et al. 2006). During the 12-week observation

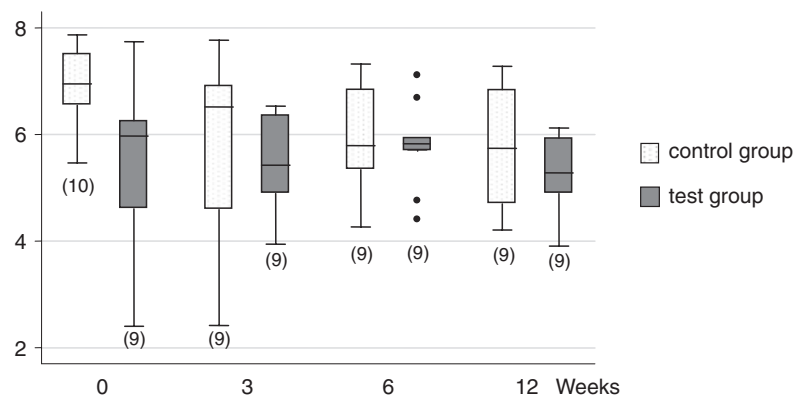


Fig. 4. "Red complex species" (RC; logarithms base 10) (counts for *P.g.*, *T.f.* and *T.d.*) in the control and test groups assessed at four sampled sites  $\geq 5$  mm (Box-Whisker plot). The number in parenthesis represents the number of patients who were positive for at least one of the three bacterial species. One patient in the control group showed a negative result for RC at 3, 6 and 12 weeks. *P.g.*, *Porphyromonas gingivalis*; *T.f.*, *Tannerella forsythia*; *T.d.*, *Treponema denticola*.

interval, both the test and the control treatment resulted in a marked reduction of approximately one log TBC/plaque sample with respect to the baseline values (median log reduction: test group: 0.89, control group: 0.95). However, no differences in any microbiological parameters (TBC, TP and RC) were observed between groups following treatment.

The evaluation of the therapeutic efficacy of a subgingival irrigation as an adjunct to mechanical debridement is largely equivocal due to differences among studies with respect to the therapeutic regimen, including the concentration/dose of CHX delivered subgingivally, frequency of irrigation and type of irrigating device (Watts & Newman 1986, Taggart et al. 1990, Chapple et al. 1992, Reynolds et al. 1992, Bollen & Quirynen 1996). The lack of effects of adjunctive CHX observed in our study may be partly due to the low concentration of the antimicrobial agents used for irrigation (0.02%). Consistently, previous studies where identical CHX concentrations were used in combination with ultrasonic scaling were unable to detect any significant difference in both clinical and microbiological parameters (Taggart et al. 1990). However, even when higher concentrations of CHX (0.12–0.2%) were used as a coolant during ultrasonic scaling, the effect on clinical parameters and subgingival microflora appeared to be limited, if any (Chapple et al. 1992, Reynolds et al. 1992). Therefore, it is possible to assume that the extent of plaque

removal exerted by the ultrasonic device may overcome the antimicrobial action of the chemical agent.

In both the test and the control group, PII was substantially diminished from baseline to week 3, and was maintained constantly below the baseline values during the 12-week interval. Similarly, GI was reduced following the instrumentation session, and remained persistently low from week 3 to week 12. These changes may be attributed to the stringent anti-plaque regimen based on repeated OHI (Emmler et al. 1980, Söderholm et al. 1982) and additional use of an antimicrobial (AmF/SnF<sub>2</sub>) mouthrinse and toothpaste (Madlena et al. 2004, Auschill et al. 2005). The use of an AmF/SnF<sub>2</sub> mouthrinse associated with conventional oral hygiene procedures has been shown to be more effective for supragingival plaque control with respect to conventional oral hygiene procedures alone (Brex et al. 1990, Brex et al. 1992, Zimmermann et al. 1993, Mengel et al. 1996, Hoffmann et al. 2001). Consistently, we have demonstrated recently that the use of an AmF/SnF<sub>2</sub>-containing mouthrinse as an adjunct to conventional mechanical oral hygiene procedures is effective in controlling the amount of supragingival plaque deposits and related gingival inflammation during periodontal supportive therapy in patients affected by G-AgP (Guarnelli et al. 2004). It can be speculated that the effect of the daily use of AmF/SnF<sub>2</sub> may have masked the potential antimicrobial effect of a single application of CHX formulations during the UMI session.

TBC and subgingival pathogens showed a marked reduction at 3 weeks following treatment and did not revert to the original values at 12 weeks in both the test and the control group. Parallel to the microbial shift, a significant reduction in PPD from baseline values ( $\geq 5$  mm) was observed at microbiologically sampled sites. A positive association has been reported between a low proportion of periodontal pathogens and a reduction in pocket depth and gain in attachment level following periodontal treatment (Van Winkelhoff et al. 1988, Renvert et al. 1996, Haffajee et al. 1997). It is reasonable to assume that post-treatment pocket reduction may have changed the ecological conditions of the pathogenic microflora, therefore leading to delayed subgingival re-colonization by periopathogens at least at 3 months.

Recolonization of the subgingival area may also have been affected by the strict plaque control regimen adopted by the patients. It has been shown that the formation of subgingival dental biofilm is closely related to the accumulation of supragingival plaque deposits (Haffajee et al. 2003, Rhemrev et al. 2006, Tezal et al. 2006), and professional removal of supragingival plaque, combined with careful self-performed plaque control, may induce marked qualitative and quantitative alterations of the subgingival microbiota in subjects with periodontitis and moderately deep pockets (Tabita et al. 1981, Smulow et al. 1983, Dahlén et al. 1992, McNabb et al. 1992, Hellström et al. 1996, Ximénez-Fyvie et al. 2000a,b). The adjunctive daily use of an antimicrobial agent to self-performed mechanical plaque control may also have reduced the contribution of bacteria present in other oral ecological niches to subgingival re-colonization (Quirynen et al. 1995, Bollen et al. 1998, Quirynen et al. 2000).

The timing of subgingival re-colonization by periodontal pathogens may influence the frequency of recalls during supportive therapy, i.e. the more persistent the suppression of pathogens in the subgingival dental biofilm, the less frequent the professional session aimed at plaque removal. Reduction in the numbers of yearly recalls during supportive therapy seems to be of paramount importance due to the poor patient compliance to the recall system as observed in long-term studies (Wilson et al. 1987a,b, Checchi et al.



2002). Therefore, the need for a more drastic and long-lasting reduction of the bacterial load in the oral cavity, in general, and in the subgingival environment, in particular, with an attempt to reduce the risk for a precocious re-colonization of the periodontal sulcus/pockets, calls for the adoption of therapeutical regimens where the conventional mechanical therapy is supplemented by the use of additional antimicrobial agents (Taggart et al. 1990, Chapple et al. 1992, Reynolds et al. 1992, Bollen and Quirynen 1996, Bollen et al. 1996, Quirynen et al. 2000, 2006, Ehmke et al. 2005). Our results support the use of stringent oral hygiene protocols supplemented by the use of antimicrobial agents to control early subgingival re-colonization by periodontal pathogens, particularly in patients with high susceptibility to destructive periodontal disease.

In conclusion, the results of the present pilot study seem to indicate that the adjunctive professional use of CHX formulations to UMI showed no additional clinical and microbiological benefits over UMI alone during supportive therapy in aggressive periodontitis patients. However, due to the limited sample size and the specificity of the study population, further randomized-controlled trials need to be conducted to confirm these preliminary findings in a broader population affected by different forms of periodontal diseases.

## Acknowledgements

This study was partly supported by the Research Center for the Study of Periodontal Diseases, University of Ferrara, Italy, and GABA International AG.

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## Address:

Prof. Leonardo Trombelli  
Research Center for the Study of Periodontal Diseases  
University of Ferrara  
Corso Giovecca 203  
44100 Ferrara  
Italy  
E-mail: l.trombelli@unife.it



**Clinical Relevance**

*Scientific rationale for the study:* Microbial shift after UMI may be transient, and bacterial re-colonization of the root surface by pathogenic bacteria after treatment may lead to disease recurrence. Therefore, com-

bined treatment with UMI associated with local application of antimicrobial agents, including CHX, has been proposed.

*Principal findings:* UMI produced clinical and microbiological benefi-

cial effects irrespective of the professional use of CHX formulations.

*Practical implications:* Our preliminary results do not seem to support the additional use of CHX formulations during a UMI session in periodontal supportive therapy.

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