Strictly Confidential

Efficacy of combined Air-abrasive + Ultrasonic (FM-EPAPT) *vs.* standard root debridement in the treatment of severe generalized periodontitis. A prospective, single-masked, randomized, split-mouth, longitudinal clinical and microbiological trial.

Contents

1. STUDY SUMMARY

1.1. Introduction

Periodontitis is a widely diffused infectious disease. Over 700 million people worldwide are supposed to suffer from it, in different degrees, although only 10-15% of the population is affected from severe forms.

The tooth supporting structures may be lost with different progression rates, depending on individual susceptibility, and may lead to edentulism early in life.

If diagnosed on time, treatment is able to reduce the clinical signs and the microbiological biofilm related to the disease.

Traditionally the first step of periodontitis treatment aims to disrupt the sub-gingival biofilm and to re-establish a healthier microbiological environment. This is commonly done by a combined manual and power-driven (ultrasonic or piezo-electric) debridement of the pocket by scaling and root-planing (SRP) associated with effective supra-gingival plaque control.

Periodontal indices that are associated with periodontitis are the increase of probing pocket depth (PPD), loss of clinical attachment (CAL) and bleeding on probing (BOP). SRP is able to reduce these indices to a certain degree although changes following treatment may be less acceptable by the patients, mainly the recession of the gingival margin (REC) and the increase in dentinal hypersensitivity (SEN).

Recently an air-abrasive system for supra- and sub-gingival debridement has been introduced, for the treatment of periodontally affected teeth.

1.2 Background information on Air-Polishing and Perio-Flow EMS

In the past few years indications for the use of the air polishing technology have been expanded from supragingival use (airflow) to subgingival air polishing (perioflow) by the development of new low-abrasive glycine-based powders (GPAP) and devices with a subgingival nozzle. Several studied on the subgingival use of air polishing have been completed. During the Europerio 7 Congress in Vienna a consensus conference on mechanical biofilm management took place aiming to review the current evidence from the literature on the clinical relevance of the subgingival use of air polishing and to make practical recommendations for the clinicians. (Sculean et al. 2013).

The latest development of a specially designed nozzle for subgingival air-polishing devices led to a reduction of the working pressure along with an air jet directed perpendicularly to the root surface. Since none of the published studies reported any adverse events related to the use of subgingival air- polishing devices, it was suggested that for the removal of supra- and subgingival dental biofilms this treatment regime may be superior to hand instrumentation, particularly with respect to patient comfort, dental hard tissue loss, and time efficiency. Recently, a new low abrasive erythritol powder (EPAP) with comparable physical properties to GPAP was introduced for subgingival air polishing. Erythritol, a non-toxic, chemically neutral, and completely water- soluble polyol is widely used as an artificial sweetener and as a food additive. Due to its comparable particle size to that of glycine and its promising chemical characteristics allowing the binding of antiseptic substances, it was recently suggested to be suitable for subgingival biofilm removal. Additionally, recently published data demonstrated an inhibitory effect of erythritol to some periodontopathogenic bacteria such as Porphyromonas gingivalis. Very recent data indicate that subgingival air polishing with EPAP resulted in short-term clinical outcomes comparable to SRP. Moreover, the use of EPAP yielded superior outcomes to hand instrumentation in terms of patient comfort and time efficiency.

1.3 Rationale for clinical study of Air-Polishing and Perio-Flow for the treatment of periodontitis.

So far the first treatment of severe generalized periodontitis is based on the biofilm removal, from the dental roots in the supra- and the sub-gingival environment, with the use of power-driven and hand instrumentation. This instrumentation, although proven to be successful, leads to substantial discomfort to the patients and to unpleasant gingival recessions. It is, therefore, of utmost importance to develop methods optimized for therapy with the aim to reduce these problems and to maintain the same successful healing as with previously described instrumentation.

1.4 Study design

This will be a prospective, single-masked, randomized clinical trial.

1.5 Study period

01.01.2017-31.12.2018

1.6 Study setting

This will be a prospective, single-masked, randomized, split-mouth clinical trial.

The study will follow the Consort Statement and be registered in the Clinical Trials Registry.

Study setting will be the clinical periodontal clinic of the University of Brescia. Patient screening, inclusions and all clinical examinations will be performed by one specialist in periodontology (MM – Principal Investigator), while treatments will be performed by one DDS (ES) other than the dedicated examiner.

1.7 Patients:

32 patients diagnosed with severe generalized periodontitis will be included.

1.8 Main Inclusion criteria:

Age: 30-75 with diagnosis of severe generalized periodontitis.

Systemically healthy.

At least 5 teeth per quadrant and a minimum of 4 sites per quadrant with PPD ≥ 5 mm and loss of CAL ≥ 3mm.

Sites with PPD ≥ 10 mm will be excluded from measurement

1.9 Main Exclusion criteria:

Severe Smoking more than 10 cigarettes per day

Pregnancy or nursing.

Radiotherapy.

Chemotherapy.

BPCO, asma

Systemic long-term corticosteroid treatment.

AB treatment in the period of 3 months before the start of the study.

Non surgical therapy in the period of 3 months before the start of the study

1.10 Preoperative evaluation:

Clinical and radiographic evaluation including periodontal and dental status.

The radiographic evaluation includes intra oral radiography.

Clinical screening includes routine history and physical examination.

Admission criteria.

Signed informed consent.

1.11 Treatment procedure:

Quadrants I&IV and II&III will be randomly treated either by ultrasonic instrumentation followed by root-planing with mini curettes or by ultrasonic instrumentation and air-abrasive treatment FM-EPAPT.

Follow up every 3 months

1.12 Main outcome measures:

Baseline, 6, 12, 24, 52 weeks after initial treatment:

Change in tissue inflammation (Bleeding on probing (BoP).

Pocket probing depth (PPD).

Recession of the gingival margin (REC).

Clinical Attachment Level (CAL).

Plaque Index (PI).

Tooth Hypersensitivity Test (Schiff Index).

Changes in composition of the subgingival microbial biofilm.

1.13 Adverse event evaluation and reporting:

Adverse events by open questioning and by completing a written questionnaire. Any serious adverse event, injury or negative effect, death or life-threatening event must be reported within 24 hours to the ethical committee.

2. HYPOTHESIS AND STUDY OBJECTIVES

2.1 Null hypothesis and alternate hypothesis

2.1.1 H_0:

There will be no significant difference in reduction in parameters of periodontitis after debridement with Ultrasonic and Air-abrasive (Test treatment) instrumentation neither 6 weeks, 12 weeks and 24 weeks post therapy as compared with baseline.

There will be a significant difference in reduction in parameters of periodontitis after Test treatment at 6 weeks, 12 weeks and 24 weeks post therapy in favour of Control treatment.

2.1.1 H_A:

There will be a significant difference in reduction in parameters of periodontitis after debridement with Ultrasonic and Air-abrasive (Test treatment) instrumentation neither 6 weeks, 12 weeks and 24 weeks post therapy as compared with baseline.

There will be no significant difference in reduction in parameters of periodontitis after Test treatment Tr 6 weeks, 12 weeks and 24 weeks post therapy as compared with Control treatment.

2.2 Primary Objective

To assess the clinical efficacy of Ultrasonic and Air-abrasive (Test treatment) instrumentation used as root debridement for treatment of severe generalized periodontitis. Primary end-point is pocket closure (PPD $\leq$ 4mm).

2.3 Secondary Objectives

To assess other clinical indices measured for periodontitis (REC,CAL, BOP, PI; Schiff Index).

To assess the changes in the sub-gingival microbial biofilm after treatment.

To assess the time spent by the therapist in treatment.

To assess patient subjective appraisal of morbidity (i.e., pain) and preference of both treatments used (VAS scale).

To assess safety of treatment by evaluating the occurrence of adverse events

3 Interventions

3.1 Treatment procedure

The initial debridement is performed with local anesthesia as needed. Randomly assigned quadrants will be treated by FM-EPAPT or SRP.

Maintenance procedure will be performed at 12, 24 and 36 weeks. Each quadrant in both active treatment groups, will be randomly maintained by either a FM-EPAPT procedure or by ultrasonic debridement.

3.2 Examinations

Clinical examinations are to be made six weeks, at 12, 24, 36 and 52 weeks after initial therapy (i.e. the terminal evaluation is performed 12 months after initial (baseline) therapy and 9 months after the maintenance therapy).

PPD, REC, CAL, BOP, will be recorded at 6 sites (mesio-buccaly, buccaly, disto-buccaly, disto-palataly, palataly, mesio-palataly) around each tooth using a UNC- PCP 15 periodontal probe (Hu-Friedy), rounding up the measure at the nearest millimeter. Bleeding on probing is assessed at all teeth (6 sites/tooth) using dichotomous scoring within 30 seconds following probing of the pocket. Plaque for the full dentition, will also be assessed using dichotomous scoring. Radiographs will be taken at baseline and at 6 months after therapy.

Microbiological samples will be collected from the 2 deepest pockets in each quadrant with sterile

size 40 paper points and pooled in a transferring vial (4 samples/patient/examination). Sites to be collected will be isolated with cotton rolls. Supra-gingival plaque from the tooth surface will be removed with sterile curettes and thereafter air-dried. A n°40 sterile paper-point will be inserted in the depth of the pocket and removed after 10 sec, both from the vestibular and from the palatal side. Samples will be transferred into a sterile eppendorf containing suitable transport fluid and then transported to the microbiological laboratory for processing.

4 STUDY METHODS

4.1 INCLUSION CRITERIA

In addition to general medical condition and history, patients can be included if they meet the following conditions:

1. Age: 30-75 with diagnosis of severe generalized periodontitis.
2. At least 5 teeth per quadrant and a minimum of 6 sites per quadrant with PPD ≥ 5 mm and loss of CAL ≥ 3mm. (Sites with PPD ≥ 10 mm will be excluded from measurement but not from treatment).
3. Eligible for treatment in an outpatient dental clinic (ie, ASA I and II).
4. Signed Informed Consent obtained prior to start.
5. Psychological appropriateness.
6. Consent to complete all follow-up visits.

4.2 EXCLUSION CRITERIA

In addition to general conditions and history, patients should be excluded if they meet any of the

following conditions:

1. Was receiving medications known to induce gingival hyperplasia.
2. Had uncontrolled diabetes (HbA1c > 6.5).
3. Was receiving systemic antibiotics < 3 months prior to intervention.
4. Were pregnant or lactating.
5. Any condition or current treatment for any condition, which in the opinion of the investigator and/or consulting physician, may constitute an unwarranted therapeutical risk.
6. Presence of psychological characteristics such as inappropriate attitude or motivation which, in the opinion of the investigator, are incompatible with the risks involved with the treatment.
7. Unwillingness to undergo treatment
8. Ongoing or previous radiotherapy to the head-neck region
9. Ongoing or previous chemotherapy
10. Systemic long-term corticosteroid treatment
11. Patients taking anticoagulants
12. Heavy smokers more then 10 cigarettes per day

4.3 WITHDRAWAL CRITERIA

A patient will be discontinued from the study under the following circumstances:

1. Progression in bone loss at teeth or other complication such as pronounced inflammation more than at study start (i.e., puss or abscesses).
2. Change in status with respect inclusion/ exclusion criteria such as pregnancy or psychological illness.

3. Intake of antibiotic medications or any local antiseptics during the length of the clinical trial.

4.4 Schedule of investigational procedures

The investigational procedures are given in *Table 1*.

Table1. See attached file ERISRP CHART - Investigational procedures before, during and after therapy.

4.5 Clinical study procedures

PREOPERATIVE PROCEDURES

Routine history and physical examination will be performed and information recorded on the Preoperative Patient History Record. The therapist may use his/her customary history and physical procedures; however all data specified on the Preoperative Patient History Record must be recorded on the form. Exclusion Criteria are also included on the form. Entries checked YES will exclude patients from admission into the study.

4.6 PREOPERATIVE PROCEDURES

Treatment approach and technique will be carried out according to a standardized procedure.

Collection of clinical indices involved in the study by the principal investigator (MM) who will be always unaware of which quadrants have been treated with the test procedure.

Clinical operator (ES) opens a sealed envelope containing randomization allocation that assigns which of the group of quadrants I&IV or II&III quadrants to treat with test and control treatments.

Treatment of the quadrants will always start with the group of quadrants I&IV.

Treatment of the assigned group of quadrants with the assigned test or control treatment under local anesthesia.

Clinical operator takes note of treatment time/number of teeth

Patient is instructed again on proper personal oral hygiene procedures.

Self evaluation questionnaire is given to the patient.

DAY 2:

The clinical operator treats the remaining two quadrant with the remaining treatment (test or control).

Clinical operator takes note of treatment time/number of teeth

Patient is instructed again on proper personal oral hygiene procedures.

Self evaluation questionnaire is given to the patient.

4.7 CONTROL TREATMENT

Under local anesthesia supra- and sub-gingival scaling and root planing, of the two selected quadrants (by randomization) will be performed with the use of piezo-electric instrumentation (EMS Piezon Master) and hand instrumentation by using mini curettes. The clinical operator annotates the time spent per quadrant/number of teeth and per quadrant/sum of mm of pocket depth, but has no limitation of treatment time.

4.8 TEST TREATMENT

The two quadrants treated or remaining to be treated will be covered with a Teflon membrane to avoid a cross-over effect of the air-abrasive powder used. Disclosing plaque. Under local anesthesia

the gingiva and the oral mucosa are decontaminated with the Air-flow device with an Erythritol based powder. The supra-gingival hand-piece is used with a power of 50% and maximum irrigation. The supra-gingival biofilm is removed using the same tool and the same setting, directing the hand-piece with a 45° degree angle towards the coronal part. Each site is decontaminated for 5 seconds. The gingival crevices and the most coronal part of the pockets is then decontaminated by directing the hand-piece towards the gingival margin with a 45°angle, again for 5 seconds at each site.
For the pockets deeper than 5 mm, the Perio-flow hand-piece is used with the flexible nozzle attached in Perio modality setting with a 50% power and maximum irrigation. The nozzle is inserted in the depth of the pocket and then moved coronally and mesio-distally in order to decontaminate the root surface as much as possible. This was repeated at each of the 6 sites for 5 seconds using the same powder as for the supra-gingival part.
Thereafter, supra- and sub-gingival debridement e calculus removal is performed using a piezo-electric device[I] with a PS-P-PL3-PL4-PL5 tips. The tool is set with a power of 70% and an irrigation of 100%, and discontinued when the surface is judged as clean.
The clinical operator annotates the time spent per quadrant/number of teeth and per quadrant/sum of mm of pocket depth, but has no limitation of treatment time.

4.9 **POSTOPERATIVE FOLLOW-UP PROCEDURES**

All postoperative evaluations will be recorded on the Postoperative Report Form 6, 12, 24, 36 and 52 weeks post therapy. Furthermore radiographs will be taken after 12 months to exclude progression in bone loss.
At the 12, weeks recall Each quadrant in both active treatment groups, will be randomly maintained by either a FM-EPAPT procedure or by ultrasonic debridement.
Clinical complications such as infection, pain, etc. will be recorded during all postoperative follow-up visits, should they occur.

4.10 DATA COLLECTION STUDY REPORT FORM – COMPLETION SCHEDULE

The schedule for the completion of the various forms and report forms is provided in *Table 2.*

Table 2: Data Collection / Report Form Completion Schedule

Form Name	Completion Schedule
Preoperative Patient History Record	Prior to the study procedure
Informed Consent Form	Prior to the study procedure
Baseline report	Immediately prior to treatment
Treatment Report	Immediately following procedure
Postoperative Report	Immediately at 6 weeks , 12 and 24 weeks
Adverse Event Reporting	Immediately following event

5.13 Case Report Forms

Case Report Forms (CRFs) of a design mutually agreed upon by the Investigators. A CRF is required and should be completed for each included patient, and signed. Corrections of data should be made using a single line, leaving the corrected data clearly visible. The accurate data should be entered next to the inaccurate data. All changes should be initialled and dated by the Investigator. If

corrections are performed by another member of the staff, the Investigator has to approve the correction.

6. Adverse events

An Adverse Event (AE) is any untoward medical occurrence in a patient during a clinical study, whether or not considered to be related to the investigational therapy. An AE can therefore be any unfavorable or unexpected sign (including abnormal laboratory findings), symptom, or disease which develops or worsens in intensity during the course of the study, whether or not related to the investigational therapy.

AE also include accidents, reason for medical consultation, reason for admission to hospital, and reason for surgical operation. However, any hospital admission or surgical operation for illness or disease that existed before the patient was enrolled in the study, and did not worsen during the study, is not to be reported as AE.

Clinical Investigators must report any serious adverse reaction, injury or effect, death or life-threatening occurrence that may be reasonably suspected of being associated with the treatment to the Ethics Committee. All other complications, device related or otherwise, occurring to any patient in the course of this investigation must be recorded.

6. PATIENT INFORMATION AND CONSENT

See attached model

7. STATISTICAL ANALYSIS

Data will be analyzed both at patient level focusing on a within patient difference between two

proportions (proportion of PPD ≤ 4mm in the two treatments) as well as considering the response at site level fitting a GLMM model with binomial link function and multilevel structure matching the design.

7.1. SAMPLE SIZE

Sample size for primary outcome (proportion of PPD<4mm) was computed through simulation assuming a non-inferiority trial with 2x2 crossover design for proportions. Data were simulated (B=1000 simulations) assuming three random intercepts (subject, tooth and site) and a within subject random slope (the within subject treatment effect). Variance estimates for the random effects were computed from existing data on a similar setting. Assuming a significance level of 5%, a tolerance margin of 10% a sample size of N=32 subjects will provide a 80% power. Considering potential drop outs after phase I randomization to treatment phase (phase I) and maintenance phase (phase II) will be performed in a two step procedure. Subjects will be randomized to a AB scheme with varying sequence of treatment administration to the two split-mouth areas (or maintenance therapy). The seed used for randomization is 2640.

7.2. STATISTICAL EVALUATION