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Flapless application of enamel matrix derivative in periodontal retreatment: A multicenter randomized feasibility trial.

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Authors´s Contribution

SJ designed the study and analyzed the data with RF; HJ, MR, AP and AK contributed to the clinical phases of the study and collected the data; SJ and HJ finalized the manuscript.

Abstract

Aim: To investigate the potential benefit of enamel matrix derivative (EMD) as adjunct to re-instrumentation of residual pockets persisting after steps 1 and 2 of periodontal therapy.

Material & Methods: 44 adult patients participated in a multicenter feasibility randomized clinical trial with split-mouth design. They had presented at re-evaluation after initial non-surgical periodontal therapy (steps 1 and 2 of periodontal therapy) for generalized periodontitis with at least 2 teeth with residual probing pocket depths (PPD) ≥ 5 and ≤ 8 mm, with bleeding on probing

(BOP). Two teeth with similar PPD were randomized to receive re-instrumentation either with (test) or without (control) adjunctive flapless administration of EMD. Differences in the changes of PPD and BOP from baseline to 6 and 12 months were analyzed, and the frequencies of pocket closure (PPD ≤ 4 mm and no BOP) compared.

Results: For the primary outcome "change of mean PPD after 6 months" a significant additional benefit of 0.79 ± 1.3 mm ($p < 0.0001$) could be observed for the test group. At 12 months, this difference could be maintained (0.85 ± 1.1 mm; $p < 0.0001$). The frequency of pocket closure in the test group was 69% at 6 and 80% at 12 months and significantly higher than in the control group with 34% and 42%, respectively ($p < 0.01$).

Conclusion: The results of the present feasibility study indicate a benefit of adjunctive EMD during non-surgical retreatment (step 3 of periodontal therapy) of residual deep pockets.

Clinical Relevance

Scientific rationale for the study: The selection of an efficient method for retreatment of residual periodontal pockets after non-surgical periodontal therapy presents a challenge to the clinician. Various adjunctive measures to non-surgical retreatment have been evaluated, however the potential benefit of enamel matrix derivative (EMD) is unclear.

Principal findings: The results of this multicenter randomized feasibility study indicate that adjunctive EMD application during subgingival re-instrumentation of selected sites with residual deep probing depths resulted in enhanced treatment outcomes compared to re-instrumentation alone.

Practical implications: Flapless use of EMD during periodontal retreatment should be further explored as it may reduce the need for additional periodontal surgery in step 3 of periodontal therapy.

Introduction

Subgingival instrumentation is an effective approach during initial periodontal therapy directed at the suppression/elimination of the subgingival bacterial load. Clinical endpoints of treatment that are usually assessed at re-evaluation after this first phase of therapy have been subject of debate (Claffey & Egelberg 1995, Lang & Tonetti 2003, Tomasi et al. 2017, Matuliene et al. 2008). In recent years there is consensus that the use of "no bleeding following pocket probing" and "a probing pocket depth of ≤ 4 mm" (pocket closure) can be considered as meaningful clinical endpoints of treatment success (Tomasi & Wennström 2017, Loos & Needleman 2020). A stable periodontitis patient after completion of periodontal therapy has been defined by gingival health on a reduced periodontium, characterized by shallow probing depths of ≤ 4 mm and no 4 mm sites with bleeding on probing (Chapple et al. 2018).

However, many factors are known to compromise the healing response to initial non-surgical treatment (D´Aiuto et al. 2005, Tomasi et al. 2007, Jepsen et al. 2011) and it is well known that these endpoints cannot always be achieved and therefore, further treatment needs to be implemented. At this point the clinician has to make a choice between non-surgical re-instrumentation (with or without adjunctive measures) or periodontal flap surgery/surgical access (Graziani et al. 2018).

The adjunctive application of enamel matrix derivative (EMD) on the surgically exposed root surface is well established in regenerative periodontal therapy for the treatment of deep residual sites associated with intrabony and furcation defects and has been shown to promote significant additional gain in clinical attachment levels and enhanced reduction of probing pocket depths (Cortellini & Tonetti 2015, Miron et al. 2016, Nibali et al. 2020, Jepsen et al. 2020, Trombelli et al. 2020). Positive effects were also observed in supra-alveolar periodontal defects (Jentsch & Purschwitz 2008, Di Tullio et al., 2013, Graziani et al. 2014).

In contrast, the non-surgical ("flapless") application of EMD as an adjunctive measure during the initial phase of periodontal therapy by root instrumentation has led to conflicting results. While several studies did not observe an added benefit of EMD (Gutierrez et al. 2003, Mombelli et al. 2005, Giannopoulou et al. 2006, Wyganowska-Świątkowska et al. 2013) other studies reported positive effects (Wennström & Lindhe 2002, Mellonig et al. 2009, Aimetti et al. 2017, Graziani et al. 2019).

However, to the best of our knowledge no study has been performed to evaluate whether a selective re-instrumentation of residual pockets after active initial periodontal therapy could benefit from the adjunctive non-surgical application of EMD.

Therefore, it was the aim of the present randomized feasibility trial to test the hypothesis that an adjunctive flapless application of EMD could lead to superior clinical outcomes compared to re-instrumentation of residual periodontal pockets alone.

Material and Methods

Study design and patient selection

This study was designed as a multicenter feasibility randomized split-mouth trial of 12 months duration. The study protocol was approved by the ethical committees of the authors´ institutions in Leipzig, Turin, Rome, Mainz and Bonn, and was registered at the ISRCTN (http://www.isrctn.com/ISRCTN14654696). The study was conducted in accordance with the declaration of Helsinki and all patients gave their informed consent. The study was performed between September 2015 and August 2018 at four different specialist clinics for periodontology: Centre for Periodontology at the Department for Cariology, Endodontology and Periodontology, University Hospital of Leipzig, Leipzig, Germany; Private Practice Turin, Italy; Sapienza, University of Rome, Italy; Department of Periodontology and Conservative Dentistry, University of Mainz, Germany. Study participants were consecutively screened for inclusion by the four centers. Patients with moderate to advanced generalized periodontitis (in retrospect fulfilling the criteria for Stage III periodontitis (Papapanou et al. 2018)) were eligible for inclusion if they presented for re-evaluation 3 – 6 months after active initial non-surgical periodontal treatment. Initial treatment had been performed according to operator discretion within each center but consisted of the usual measures of step 1 and 2 therapy, including subgingival instrumentation with hand and/or power driven instruments under local anesthesia. Patients had to have at least two residual pockets with probing pocket depth (PPD) ≥ 5 mm and ≤ 8 mm, bleeding on probing (BOP), mobility ≤ degree 1 and without furcation involvement. Experimental teeth with similar PPD had to be located in different quadrants, or at least 3 teeth apart from each other. Individuals were excluded from the study for the following reasons: (a) full mouth plaque score (modified O´Leary et al. 1972) > 20%, (b) uncontrolled systemic disease, requiring high dose steroid therapy, radiation or other immune-suppressive therapy and history of malignant disease in the oral cavity or previous radiotherapy in the head or neck area, (c) pregnant or lactating females, (d) drug and alcohol abuse, (e) smoking > 10 cigarettes per day and (f) inadequate restorative therapy or malocclusion.

Sample size and randomization

The sample size calculation was based on earlier reports on periodontal retreatment (Tomasi et al. 2008). The primary outcome "change of PPD after 6 months" was used to determine the sample size. With an alpha error defined as 0.05, the power calculation based on the detection of a 0.5 mm difference in mean PPD reduction between treatment groups (considering a standard deviation of 0.5 mm) revealed that 32 patients were required in each treatment group to have a power of 80%. To account for possible drop-outs each of the 4 centers aimed at enrolling at least 10 patients for the study.

Immediately following completion of periodontal re-instrumentation a person otherwise not involved in the study randomly assigned the 2 experimental teeth in each patient to either test or control group by toss of a coin.

Periodontal retreatment

The treatment protocol had been discussed in detail and agreed on during an investigator meeting. Following the application of a local anesthetic with vasoconstrictor experimental sites were carefully debrided by a combined use of mini curets (Hu-Friedy, Chicago, IL) and ultrasonic instruments with thin and delicate tips (PS (Perio Slim), EMS, Nyon, Switzerland).

Caution was taken to avoid soft tissue trauma. In the test sites the root surfaces were conditioned for 2 min with 24 % ethylenediaminetetraacetate EDTA (PrefGel®, Institut Straumann AG, Basel, Switzerland). The sites were then copiously rinsed with saline solution and thoroughly dried with air. Blood if present was removed using either absorbent paper points, pointed tips, gauze swabs, and/or sponge pellets followed by repeated irrigation and air-drying until complete bleeding control. Then EMD (Emdogain®, Institut Straumann AG, Basel, Switzerland) was gently applied with a blunt tipped sterile syringe until overflowing from the gingival margin. By means of sterile wetting gauzes the gingival margin was gently compressed until pocket marginal closure was obtained. Re-instrumentation procedures and the application of EMD were performed with magnification systems according to the individual needs of the operator.

Patients were advised to rinse with 0.2% chlorhexidine digluconate mouth rinse (Chlorhexamed forte 0.2%, GlaxoSmithKline Healthcare, Bühl, Germany) for one minute twice daily for 4 weeks. They were instructed to avoid tooth brushing including flossing and interproximal brushes in the treated area for 2 weeks. After that they were allowed to use a soft tooth brush. After 4 weeks subjects resumed their normal oral hygiene practices with manual or powered tooth brushes and

interdental cleaning devices. Recall appointments were scheduled weekly during the first postoperative month and every 3 months during the 12 months study period for reinforcement of oral hygiene and supragingival plaque removal. No additional subgingival instrumentation was performed. Complications possibly related to the study procedure or study device were collected, from the treatment visit until the final study visit. To this end, at each visit the investigator observed the clinical situation and inquired the patient if any complication occurred since the last visit. Findings were recorded in the patient file. In addition, the status of adverse events recorded in the patient file was evaluated by the investigator throughout the study.

Clinical measurements

The calibrated and blinded clinical examiner in each center was different from the clinician who provided the treatment. No inter-examiner calibration was conducted, however, the examiner in each of the centers underwent an intra-examiner calibration exercise (Hasturk & Cugini 2014). This consisted of PPD assessments in 3 patients and a repeat exam 3 days later. Agreement level for PPD within 1 mm (± 1 mm) was set at 97%. At baseline the clinical parameters PPD and BOP were recorded at 6 sites per tooth with a manual 1-mm graduated periodontal probe (PCP-UNC 15, Hu-Friedy Manufacturing Co., Chicago, IL, USA) and the target sites were defined. Measurements were repeated at 6 and 12 months after retreatment of the residual pockets.

Data analysis

Statistical analysis of the clinical and laboratory data was performed by an independent biostatistician (RF) using the software SAS (SAS Institute Inc., Cary, NC, USA). Unit of analysis in all statistical tests was the individual. The difference in PPD-change from baseline between test and control was analyzed with a one-sample t-test at a level of 5%. For further intra- and inter-group comparisons of quantitative variables the paired t-test was used. Changes in frequencies of PPD ≤ 4 mm, of BOP and of pocket closure (no BOP and PPD ≤ 4 mm) were analyzed by 2 x 2 tables and the McNemar-test. The difference in PPD-change from baseline between test and control was compared between the four centers by one factorial analysis of variance to check for center effects.

Results

A total of 51 individuals were screened and 44 patients (age range: 31 -74 years; 23 female, 21 male; 11 smokers (≤ 10 cigarettes/day)) were finally included in the study (center 1: 13 patients,

center 2: 10 patients, center 3: 10 patients, center 4: 11 patients). Tooth types (incisors/ premolars/ molars) of experimental teeth were evenly distributed among both groups: 19/14/10 for test and 19/13/11 for control, respectively. The 2 experimental teeth were located in different quadrants, except in 4 patients where they were at least 3 teeth apart from each other. A small portion of sites (8 out of 88) showed radiographically slight angular bone loss (≤ 2 mm). Due to drop-outs 43 patients were available for the 6 months examination and 40 patients could be followed up for 12 months. Figure 1 presents the study flow chart according to CONSORT. Patients maintained a good level of oral hygiene throughout the study. No complications or device-related adverse events were observed or reported by the patients.

For the primary outcome "change of mean PPD after 6 months" a significant additional benefit of 0.79 ± 1.3 mm (95% Confidence Interval (CI): 0.39 - 1.18 mm) could be observed ($p < 0.0001$) for the test group, and thus, the hypothesis tested could be confirmed. At 12 months, this difference could be maintained (0.85 ± 1.1 mm; 95% CI: 0.47 - 1.22 mm; $p < 0.0001$). No evidence for a center effect could be observed ($p = 0.246$ and 0.219 at 6 and 12 months, respectively).

Periodontal retreatment led to clinical improvements in both control and test groups, showing significant reductions of mean PPD and of BOP at test and control sites after 6 and 12 months ($p < 0.0001$). Results are presented in Table 1. Baseline mean PPD in test sites was significantly reduced from 6.0 ± 0.9 mm to 3.9 ± 1.2 mm after 6 months and to 3.9 ± 1.2 mm after 12 months. Corresponding values for control sites at baseline, 6 and 12 months were: 5.9 ± 0.9 mm, 4.6 ± 1.2 mm, and 4.6 ± 1.1 mm. At 6 months 9.3 of test sites and 27.9% of control sites showed BOP, and after 12 months 5.0% vs. 22.5% ($p < 0.01$).

With regard to a conversion of residual deep sites to sites with shallow probing depth (PPD ≤ 4 mm), the frequency of conversion amounted to 76% at 6 months and 80% at 12 months for the test sites compared to 46% and 45%, respectively, for the control sites ($p < 0.001$) (Table 2). The frequency distribution of sites according to their PPD at baseline, 6 and 12 months, respectively, for both groups is displayed in Figure 2 and the supplementary Table 1.

Pocket closure, as defined by PPD ≤ 4 mm and absence of BOP, was attained in 69% and 80% of test sites at 6 and 12 months, respectively. These frequencies were superior to the 34% and 42% of sites, respectively, with pocket closure observed in the control group ($p < 0.01$) (Table 3).

Discussion

The results of the present randomized feasibility trial could indicate that adjunctive EMD application during subgingival re-instrumentation of selected sites with residual deep probing depths following initial non-surgical therapy resulted in enhanced treatment outcomes compared to re-instrumentation alone. For the primary outcome "change of PPD after 6 months" a significant effect could be demonstrated in favor of the test group. Thus, the hypothesis of the study could be confirmed. Moreover, adjunctive non-surgical EMD application resulted in a significantly higher number of sites reaching the endpoints of PPD ≤ 4 mm and "pocket closure".

The study question is of high clinical relevance. In the recently published S3-level clinical guideline for the treatment of periodontitis (Sanz et al. 2020) the decision making for retreatment after step 2 therapy (initial non-surgical phase including subgingival instrumentation) has been addressed. Based on a systematic review (Sanz-Sanchez et al. 2020) it was recommended to re-instrument residual pockets with a PPD of 5 mm by a non-surgical approach, whereas residual pockets of ≥ 6 mm should be reduced by periodontal flap surgery in order to reach the endpoint of active therapy (PPD ≤ 4 mm, no BOP of 4 mm sites; Tomasi & Wennström 2017, Chapple et al. 2018, Loos & Needleman 2020, Sanz et al. 2020). Thus, it appears attractive to enhance the outcomes of subgingival re-instrumentation by adjunctive measures in order to avoid the need for additional surgery even for residual sites of ≥ 5 mm. Therefore, in the present trial residual deep pockets with a PPD of 5-8 mm were included to explore this possibility.

The present study has obviously strengths and limitations. The trial had a multinational multicenter design and included experienced clinicians and blinded calibrated examiners. The number of patients were evenly distributed among the centers. The fact, that the study was conducted in different settings (2 private practices and 2 university clinics) and no center-effect could be observed adds to the credibility and also generalizability of the observed results. On the other hand, limitations have to be addressed as well. Since the initial non-surgical phase of therapy (step 1 and 2) was not part of the prospective study protocol, the possibility cannot be ruled out that differences with regard to the quality of the initial instrumentation among patients and between centers could have existed. Due to the feasibility character of this study, the hypothesis was tested in relatively few selected target sites only and no placebo-group was included. Furthermore, due to the split-mouth design a possible spill-over effect of the active ingredient (EMD) may be discussed. However, test and control sites were located in different quadrants, except in 4 patients where they were at least 3 teeth apart from each other, and thus such an effect appears to be very unlikely. No efforts were made in this feasibility study to collect reliable clinical attachment level data across the centers, what can also be seen as a limitation. It has to be emphasized, however, that it was not our assumption that we would be able to

stimulate new connective tissue attachment by non-surgical EMD application, but rather to enhance pocket reduction and pocket closure as stated in our hypothesis due to the reported anti-inflammatory and antibacterial properties of EMD (Brett et al. 2002, Arweiler et al. 2002, Newman et al. 2003, Miron et al. 2014).

Previous studies have explored the effect of adjunctive topical measures to enhance the outcomes of non-surgical subgingival re-instrumentation of residual pockets after initial therapy. For example, in a well-designed and thoroughly reported study that included the initial instrumentation (step 1 and 2) as part of the prospective protocol, Tomasi et al. (2008) tested locally delivered doxycycline as an adjunct to subgingival re-instrumentation. After 9 months both test and control groups showed a mean PPD reduction of 1.1 mm. These outcomes are in agreement with the mean PPD reduction of 1.3 mm (at 6 and 12 months) in the control group of the present study. Other studies reported mean PPD reductions between 0.7 and 1.9 mm after non-surgical re-instrumentation (Kinane & Radvar 1999, Aimetti et al. 2004, Cappuyns et al. 2012). Significantly higher PPD reductions were observed when tetracycline-loaded fibers were applied as adjunctive measure compared to instrumentation alone (Kinane & Radvar 1999, Aimetti et al. 2004), however this treatment modality is no longer available. Tomasi et al. (2008) reported that the probability of pocket closure was not improved by the adjunctive topical doxycycline therapy. The observed percentages of pocket closure (in their study defined as PPD ≤ 4mm, regardless of BOP) amounted to 45% (at 3 months) and 53% (at 9 months) correspond well to the 46% and 45% at 6 and 12 months, respectively, observed in the control group of the present trial.

The rationale for the selection of EMD as an adjunct in the present trial was based on an earlier publication by Wennström & Lindhe (2002), reporting in a comparative study more favorable clinical soft tissue healing outcomes during the first 3 weeks when EMD had been applied topically in instrumented pockets and based on a case series by Mellonig et al. (2009) who observed substantial reduction in PPD at 6 months after the application of EMD as an adjunct to scaling and root planing. Mellonig et al. (2009) also provided some histological evidence for periodontal regeneration in 3 out of 4 teeth. Indeed, encouraging results for the flapless application of EMD in conjunction with the objective of a regenerative treatment of deep intrabony defects were also published by Aimetti et al. (2016, 2017). In a prospective case series the flapless application of EMD was applied to 11 residual deep intrabony defects in 11 patients and significant clinical and radiographic improvements were obtained 12 and 24 months post-operatively (Aimetti et al. 2016). In a 24-months RCT including 30 patients (Aimetti et al. 2017) EMD was applied for regenerative treatment of intrabony defects either in a flapless fashion or

using a minimally invasive flap procedure. Both modalities led to comparable CAL gain and PPD reduction, but the flapless approach required less chair time.

More recently, adjunctive flapless EMD application was tested in 2 randomized clinical trials in comparison to subgingival debridement only in the initial (step 2) phase of periodontal therapy. Graziani et al. (2019) in a 3 months parallel group study with 38 patients evaluated this approach in initially deep sites (≥ 6 mm). Even though the focus of their study was on the systemic acute-phase response and on medium term inflammation, PPD reduction and pocket closure were analyzed as secondary outcomes. Interestingly, at 3 months there was a significantly higher mean PPD reduction and number of sites with pocket closure in the test group. These findings are in agreement with the results of our study, although time point of EMD application and follow-up was quite different.

This concept of adjunctive flapless EMD was further investigated in a multi-center split-mouth study by Schallhorn et al. (2020). They tested the effects of a repeated application of EMD in conjunction with initial non-surgical therapy as compared to scaling and root planing alone in sites with baseline PPD of 5-8 mm over a period of 12 months. In contrast to the study by Graziani et al. (2019) no significant differences for CAL gain and PPD reduction were found. Even though the study design by Schallhorn et al. (2020) differs from the present study with regard to mode of application (repeated vs. single) and timing in the overall treatment sequence (adjunctive to initial instrumentation (step 2) vs. adjunctive to re-instrumentation (step 3)) it is of interest to note that the investigators observed a similar benefit of EMD with regard to the percentage of sites converted to a PPD ≤ 4 mm after 12 months (79.8% in their study vs. 80% in the present study). Furthermore, they reported significantly more BOP reduction in test compared to control sites.

In view of the results derived from the studies described above and the present trial, there appears to be some potential for flapless EMD application in various phases of periodontal therapy. At the same time data obtained so far are somewhat contradictory and there is a definite need for further well-designed investigations. Such studies should ideally include step 1 and 2 therapy as part of their prospective protocol followed by re-evaluation and then follow-up with step 3 therapy by either nonsurgical re-instrumentation of residual deep pockets with the goal of pocket reduction/closure (Wennström et al. 2001) or in case of presence of deep intrabony defects a regenerative approach with the goal of CAL and bone level gain.

Only such studies with appropriate follow-up, which must also include cost-benefit analyses will be able to help us to determine if and when, where and how adjunctive flapless EMD can be

reasonably embedded in the overall context of the incremental stepwise approach to the therapy of stage III periodontitis.

In conclusion, the results of the present feasibility study indicate a potential benefit of adjunctive EMD during non-surgical retreatment of residual deep pockets. Flapless use of EMD during periodontal retreatment should be further explored as it may reduce the need for additional periodontal surgery.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Legends

Figure 1: CONSORT Flow chart of patient enrollment and follow-up exams

Figure 2: Frequency distribution of test and control sites according to their PPD (mm) at baseline, 6 and 12 months

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Table 1. Mean probing pocket depth (PPD) ± standard deviation (mm), percent bleeding on probing (%BOP) at baseline, 6 months and 12 months at test and control sites

	Baseline n = 44		**6 months** n = 43		**12 months** n = 40
PPD (Test)	6.0±0.9		3.9±1.2*		3.9±1.2*
PPD (Control)	5.9±0.9		4.6±1.2*		4.6±1.1*
Δ PPD Change (Test - Control)		0.79±1.3** (0 vs. 6 mo)		0.85±1.1** (0 vs. 12 mo)	
BOP (Test)	100		9.3***		5.0***
BOP (Control)	100		27.9		22.5

**significantly different from baseline (p < 0.0001)*

***significantly different between test and control (p < 0.0001)*

**** significantly different between test and control (p < 0.01)*

PPD = Probing pocket depth, BOP = Bleeding on probing

Table 2. Proportion of sites with PPD ≤ 4 mm (irrespective of BOP)

	Test	**Control**	**p-value***
Baseline	0/44	0/44	
6 months	33/43 – 76%	20/43 – 46%	0.0029
12 months	32/40 – 80%	18/40 – 45%	0.0010

**McNemar-Test*

PPD = Probing pocket depth, BOP = Bleeding on probing

Table 3. Proportion of sites with “pocket closure” (PPD ≤ 4 mm and no BOP)

	Test	Control	p-value*
Baseline	0/44	0/44	
6 months	30/43 – 69%	15/43 – 34%	0.0011
12 months	32/40 – 80%	17/40 – 42%	0.0003

**McNemar-Test*

PPD = Probing pocket depth, BOP = Bleeding on probing

Figure 1: CONSORT Flow chart of patient enrollment and follow-up exams

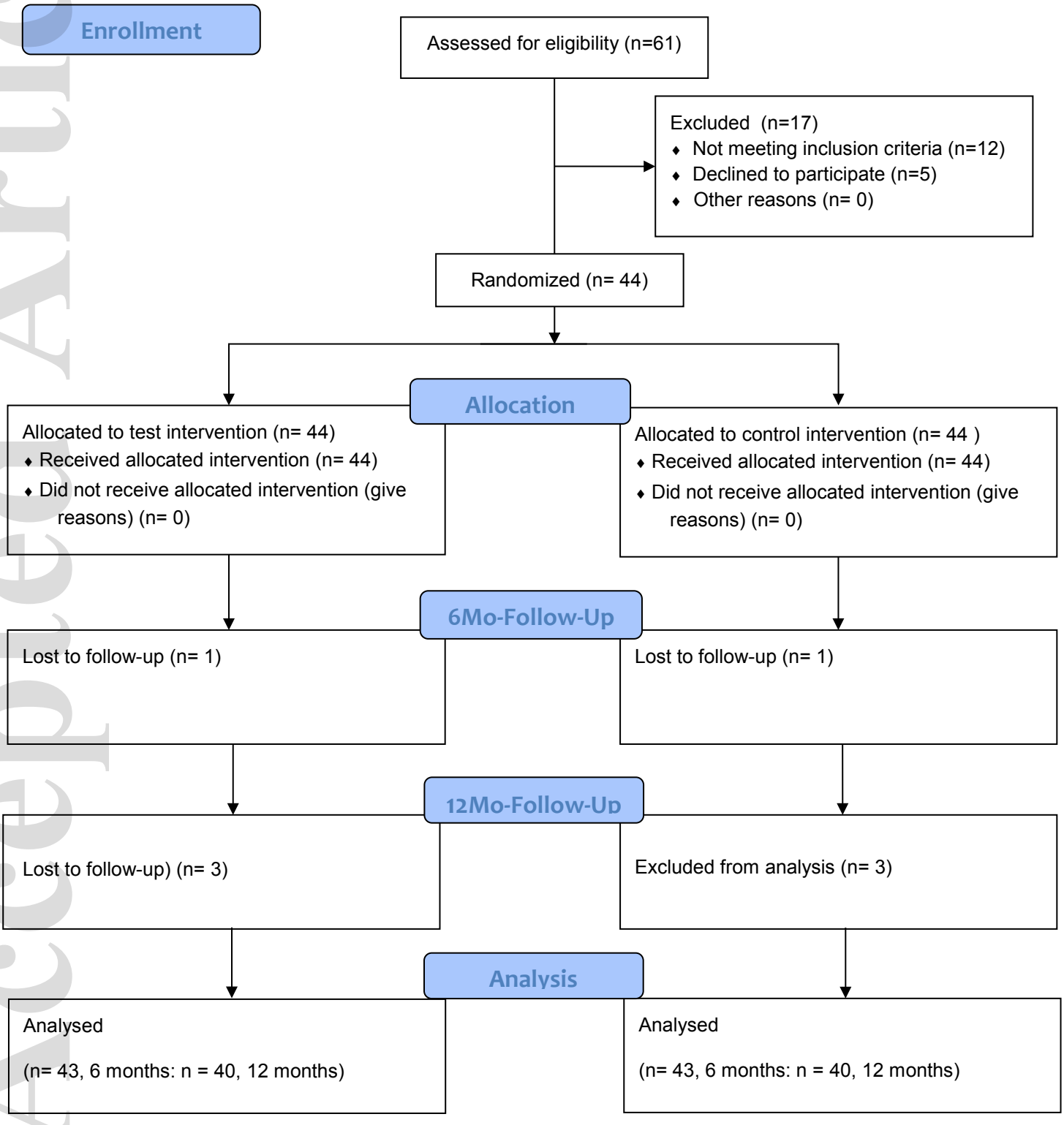

Figure 2: Frequency distribution of test and control sites according to their PPD (mm) at baseline, 6 and 12 months (PPD = Pocket probing depth)