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TESI DI DOTTORATO

TIPOLOGIA DI DIFETTO PARODONTALE ED EFFICACIA DELLA TERAPIA PARODONTALE NON CHIRURGICA: UN'ANALISI MULTILIVELLO

TUTOR/RELATORE

Chiar.mo Prof. Gianrico Spagnuolo

CANDIDATO/A

Dott. Emanuele Vaia

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PH.D. THESIS

PERIODONTAL DEFECT TYPE AND EFFECTIVENESS OF NON-SURGICAL PERIODONTAL THERAPY: A MULTILEVEL ANALYSIS

TUTOR

Prof. Gianrico Spagnuolo

PH.D. STUDENT

Dr. Emanuele Vaia

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ABSTRACT

AIM: To investigate the role of periodontal defect types on pocket healing

MATHERIALS AND METHODS: Forty patients treated in a specialist and controlled environment were retrospectively selected. Pockets with probing depth (PD) > 4 mm were included and divided into infra- and supra- bony defect types according to clinical attachment level differences. The impact of defect type and other covariates on the probability of pocket closure (PD ≤ 4 mm) 3 (T1) and 6 months (T2) after non-surgical periodontal therapy was explored using a logistic multilevel model.

RESULTS: The logistic model at T1 revealed a significant impact of smoking ($p<0.001$), heavy smokers (OR=0.22; $p<0.001$), FMPS (OR=0.98; p=0.017) multi-rooted teeth (OR=0.42; $p<0.001$), PD (OR=0.44; <0.001) negatively affected closure rate, while suprabony (OR=1.89 p=0.006) was a positive condition. The same analysis revealed female patients (OR=2.48; p=0.021), suprabony defects at T1 (OR=2.22; p=0.003), low levels of PD at T1 (OR=0.34; p=0.001) to increase closure rate at T2. Opposite to that, multi-rooted teeth affected negatively the success at T2 (OR=0.50; $p<0.036$).

CONCLUSION: Infrabony defects significantly reduced closure rate 3 and 6 months after NSPT, together with female gender, heavy smoking, high FMPS at baseline, multi-rooted teeth, high initial PD and presence of plaque at the site.

INTRODUCTION

Periodontitis is a chronic multifactorial inflammatory disease associated with dysbiotic plaque biofilms and characterized by progressive destruction of the tooth-supporting apparatus [1]. If left untreated, it could not only lead to severe tooth loss worsening patient's quality of life, but also increase the risk of systemic diseases and their sequelae [2-4].

The initial phases of periodontal treatment aim at the control of etiologic and risk factors by inducing behavioural changes in patient's life and reduce the amount of biofilm accumulation both with professional sessions of subgingival instrumentation and oral hygiene instructions, thus being commonly identified as "non-surgical periodontal therapy" (NSPT) [5]. Despite a proper treatment could also be in need of surgical corrections of disease sequelae, the first steps (i.e. Step I, II) of therapy are a key factor for a successful treatment and can substantially increase patients' quality of life [6].

When pockets with probing depth (PD) ≥ 4 mm associated with bleeding on probing (BOP) are still present after NSPT, further steps of therapy will be needed [5]. However, the choice of non-surgically retreating residual pockets over performing corrective surgeries is weighting on clinicians. Thus, proper comprehension of positive and negative factors affecting the results of NSPT and the retreatment is needed to avoid surgery abuses as well as non-surgical overtreatments.

Classic manuscripts studying the effects of NSPT were aiming at describing the mean values of periodontal parameters changes such as pocket depth (PD), recession (REC) and clinical attachment loss (CAL) [7-10]. Although this represents the foundations of scientific research, the lack of differentiation among various clinical conditions led to a flattening of real values bringing to the common understanding that the process of pocket healing following NSPT was very limited in time and effectiveness [9].

However, the common clinical belief that healing is dependent on specific features in the short and long-term, suggested the need to differentiate among clinical conditions. As opposite to underestimation, the study of NSPT effects on specific pockets would have brought to the risk of an overestimation due to the presence of various parameters working on different levels that could add their potential indistinguishable influence on the results.

To overcome these limitations, already in 1992 Albandar and Goldstein built a statistical model with patient, tooth and site-levels analysis to study periodontal disease progression [11]. Thereafter, this model has been used in periodontal research to avoid the underestimation due to mean values as well as the overestimation risk deriving from single level analysis, leading to a better understanding of the influencing factors [12, 13].

Thanks to the multilevel model studies, an increasing knowledge on factors influencing pocket healing after NSPT has given indications on the results expected for each pocket, adding information leading to different treatment options such as an eventual non-surgical retreatment of residual pockets.

Patient related factors such as smoking and genetic conditions, tooth features like multi-rooted teeth and tooth mobility contribute to negatively influence pocket healing. Anyway, site level seems to be the greatest responsible for the healing variability with initial PD, furcation involvement, plaque presence and interdental defects playing a bigger role in reducing the percentage of pocket closure [13-20].

However, while intrabony defects and their morphology have been proven to have an influence on surgical results, not many efforts have been made to investigate their effect on NSPT healing [21].

While comprehensive data coming from systematic reviews show that subgingival retreatment is pushing NSPT to close 74% of the pockets, the proportion of regenerative procedures to treat residual pockets is increasing [22]. Thus, despite the role of intrabony defects is still unclear, a critical analysis of these considerations could suggest a possible role in the healing following NSPT [15, 16].

Therefore, the primary outcome of this study is to investigate the role of defect types (infra- and supra- bony defecs) on pocket healing after NSPT:

- trying to find a threshold for ICs that could better identify supra- and infra-bony defects basing on the probability to reach pocket closure;
- investigating the influence of the infrabony component on pocket closure (PD ≤ 4 mm) 3 and 6 months after NSPT;

The secondary outcome is to investigate the influence of different factors on the probability to obtain pocket closure 3 and 6 months after NSPT in infra- and supra-bony pockets by means of a multilevel analysis.

MATERIALS AND METHODS

ETHICAL APPROVAL

This study was conducted at the University of Bern, department of Periodontology. The study protocol was submitted to and approved by the Ethical Committee of the Canton of Bern (KEK), Switzerland (Nr.: 2018-01877). The investigation was conducted according to the revised principles of the Helsinki Declaration (2013), and signed informed consent was obtained from each patient before entering the study.

STUDY DESIGN AND POPULATION

The database of the department of Periodontology, University of Bern, was screened to select patients treated for periodontitis between 2014 and 2021 by graduate students under senior staff supervision as part of their educational training in periodontology. Among them, only cases presented at the European Federation of Periodontology as part of post-graduate students' final exam were selected to set up the population for this retrospective study. Every patient was treated following the guidelines for periodontitis treatment Stages I to III with NSPT by means of a combination of hand and power-driven instruments at baseline (T0) [5]. A non-surgical retreatment 3 months after therapy was performed in all the patients presenting residual pockets at the re-evaluation (T1). Thus, it was possible to study NSPT until 6 months (T2) after treatment. Periodontal charts were analysed to detect interproximal pockets with a PD > 4 mm, which were considered the statistical units of the study. Thereafter, parameters and measurements were registered at 3 timepoints; baseline (T0), after 3 months (T1) and 6 months (T2)

INCLUSION AND EXCLUSION CRITERIA

Patients under the age of 18 y.o. or with systemic diseases and conditions (including diabetes) were excluded. No limits were set up to the number of NSPT sessions at baseline, likewise cases treated with antibiotic administration were included.

Pockets facing an edentulous site at baseline (T0) or at the second timepoint (T1) were excluded due to the inability of collecting all the measurements needed. Third molars and teeth extracted at T1 were not included in the study. To avoid any bias, patients presenting with a FMPS > 25% after baseline were also excluded from the study.

Independently on the reason for a site or patient exclusion at T2, data were even so analysed at T0 and T1 if criteria were fulfilled.

MEASUREMENTS

The following parameters and measurements were registered at baseline (T0), after 3 months (T1) and 6 months (T2) and used for a multilevel analysis according to Axtelius [12];

- Patient level
    - Gender:
    - Age at the time of the treatment:
    - Smoking status: never smokers, previous smokers (quitting at least 10 years before the treatment), light smokers (less than 15 cigarettes per day or previous smokers quitting less than 10 years before the treatment), heavy smokers (more than 15 cigarettes per day):
    - Antibiotics administration:
    - NSPT sessions number:

- Full Mouth Plaque Score (FPMS):
- Full Mouth Bleeding Score (FMBS).

- Tooth Level
  - Tooth type: multi rooted including molars and first upper premolars, and single rooted including the rest of the teeth):
  - Tooth mobility according to Miller's classification [23].

- Site Level
  - Pocket position 1: mesial or distal:
  - Pocket position 2: buccal or oral:
  - PD: distance between the gingival margin and the bottom of the pocket
  - REC: distance between the gingival margin and the cemento-enamel junction (CEJ)
    - Gingival recessions were described by negative values
    - Pseudo-pockets were described by positive values
  - Clinical Attachment Loss: distance between the CEJ and the bottom of the pocket, calculated by subtracting REC on PD:
  - Infraosseous component (IC) measured with 2 different methods:
    - Clinical Infraosseous Component (CIC) of the pocket: difference in mm between the CAL of the site and the corresponding one facing it on the adjacent tooth (i.e., IOC of the mesio-buccal pocket of a canine is equivalent to its CAL minus the disto-buccal lateral incisor CAL).
    - Radiographic Infraosseous Component (RIC) of the pocket: vertical distance in mm between the bony peaks by means of a standardized periapical x-ray. This value will be attributed to the site with the highest CAL.
  - Interproximal furcation according to Hamp 1975 [24]:
  - Plaque visible at the site:
  - Bleeding on probing (BOP).

With the exception of REC, ICs and tooth mobility at T2 and RIC at T1, the other measurements and parameters were registered at every timepoint.

Measurements were used to build two more constructed variables.

PD ≤ 4 mm at T1 and T2 was defined “pocket closure” and used to set the success of NSPT.

Pockets were divided into two types according to ICs values: infrabony (IB) or suprabony (SB). According to classic parameters, ICs ≤ 2 mm (including negative values) identified SB pockets, while ICs > 2 mm were IB. CIC and RIC measurements were considered separately giving a clinical and radiographic definition of pocket type. Before further analysis could be produced, a threshold analysis was used to confirm or refuse this definition and choose the best threshold for the study.

Since ICs are a key parameter in this study, pockets facing edentulous spaces at T0 or T1 were excluded from due to the inability of collecting measures.

STATISTICAL ANALYSIS

This is a multilevel data structure (patient, tooth and tooth site) where different variables were measured at different levels. The main endpoint of NSPT treatment was pocket closure (PD ≤ 4 mm) at T1 and T2.

One of the most important independent variables was the IC that was used to define the type of defect according to the conventional threshold 2 mm. ROC curve was estimated in order to explore an optimal cut-off point of the IC between closure and non-closure sites. Area under curve (AUC) and 95% confidence interval was obtained.

Statistical analysis consists of a description of categorical variables (absolute and relative frequencies) and continuous variables (mean, standard deviation, range, median and IQR).

Multi-level simple binary logistic regression based on using generalized estimation equations (GEE) was conducted to assess the association between each independent variable and Pocket closure (yes/no). Non-adjusted odds ratio (OR) and 95% confidence intervals were obtained from the Wald´s Chi2 statistic. Then, a multiple model was estimated according to the relevant factors and covariates detected in the simple models.

Multi-level simple linear regressions were estimated to compare changes at clinical variables between types of defects. Beta coefficients and 95%CI were also estimated.

Significance level used in analysis has been 5% (α=0.05).

All the statistical analyses shown in this study were performed using IBM SPSS Statistics for Windows, version 26.0.0.0.§.

RESULT

A number of 112 patients has been included in this study. Unfortunately, measures collection is still ongoing, thus the presented results are only a part of the final dataset. RIC was excluded by the analysis due to the limited measures collected at the time of the thesis production.

The sample included 40 patients, 17 males (42.5%) and 23 females (57.5%), averaging 46.8 ± 11.2 years through a range from 21 to 69. Patients provided for a total of 643 teeth, involving 1,452 pockets or sites at baseline. No exclusion regarding FMPS were set, however 28 sites (1.9% of the sample) were excluded because of extractions or surgeries that made impossible to record measures. Sample characteristics are described in table 1 (Tab.1)

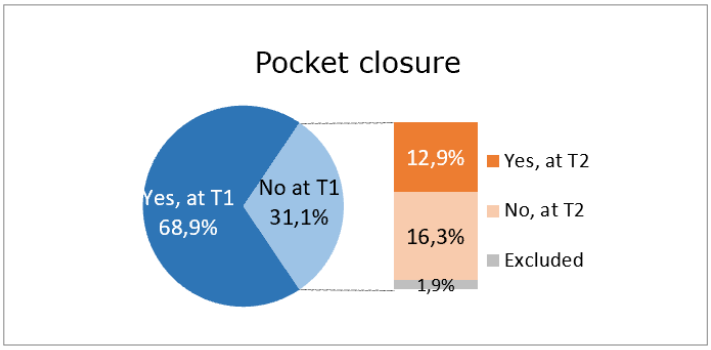
PATIENT LEVEL		
	Gender (male/female)	17/23
	Age [mean(SD)/percentile25/median/percentile75]	46,8(11,2)/38,0/49,0/55,5
	Smoking (never/previous/light/heavy)	15/4/15/6
	Antibiotics (yes/no)	29/11
	Sessions number (1/2)	6/34
	FMPS [mean(SD)/percentile25/median/percentile75]	54,3(21,2)/37,5/54,0/71,0
	FMBS [mean(SD)/percentile25/median/percentile75]	62,9(18,5)/48,5/62,0/74,0
TOOTH LEVEL		
	Tooth type (single/multi rooted)	425/218
	Mobility (grade 0/1/2/3)	543/89/11/0
SITE LEVEL		
	Mesio-Distal position (M/D)	744/708
	Bucco-Oral position (B/O)	717/735
	PD [mean(SD)]	6,27(1,22)
	REC [mean(SD)]	-0,28(1,07)
	CAL [mean(SD)]	6,56(1,64)
	Defect Type (infrabony/suprabony)	309/1143
	Furcation (grade 1/2-3)	35/27
	Plaque (yes/no)	922/497
	BOP (yes/no/suppuration)	1124/192/136

Tab.1: Sample description

CLOSURE AT T1: SIMPLE BINARY LOGISTIC REGRESSION

Pocket closure was reached in 1001 sites at T1, assessing closure rate at 68.9% (95%CI: 66.5-71.3%) 3 months (T1) following NSPT (Tab. 4).

		N	%
CLOSURE T1	Total	1452	100.0
	no	451	31.1
	yes	1001	68.9
CLOSURE T2	Total	1375	100.0
	no	236	17.2
	yes	187	13.6
	closed at T1	952	69.2



Tab. 4: Pocket closure at T1 and T2

A simple binary logistic regression (odds ratio OR and 95%CI), using GEE model for probability of closure, was first performed to investigate the effect of single independent variables on the dichotomous dependent variable pocket closure (Tab. 5).

		OR	95% CI	p-value
PATIENT LEVEL	**AGE (years)**	0.99	0.95 - 1.02	0.425
	GENDER			
	Male	1		
	Female	0.75	0.36 - 1.58	0.450
	SMOKING			**0.005****
	Never	1		
	Previous	1.96	0.86 - 4.47	0.111
	Current light	1.56	0.73 - 3.35	0.255
	Current heavy	0.43	0.19 - 0.95	**0.038***
	ANTIBIOTIC			
	No	1		
	Yes	0.85	0.34 – 2.11	0.728
	FMPS (%) T0	0.98	0.97 – 0.99	**0.027***
	FMBS (%) T0	0.99	0.97 – 1.01	0.461
	FMPS (%) T1	0.98	0.93 – 1.04	0.636
	FMBS (%) T1	0.96	0.93 – 0.99	**0.006****
TOOTH LEVEL	**TOOTH TYPE**			
	Single rooted	1		
	Multi-rooted	0.44	0.31-0.62	**<0.001*****
	MOBILITY T0			0.653
	No	1		
	Grade 1	0.81	0.50 – 1.32	0.401
	Grade 2	1.02	0.35 – 2.99	0.976
	FURCATION T0			0.336
	No	1		
	Grade 1	0.56	0.22 – 1.43	0.222
	Grade 2-3	0.54	0.21 – 1.38	0.200
SITE LEVEL	**MD SITE**			
	Mesial	1		
	Distal	1.28	0.95-1.72	0.105
	BO SITE			
	Buccal	1		
	Oral	1.07	0.92 - 1.24	0.366
	INFRABONY COMPONENT	0.74	0.67 – 0.80	**<0.001*****
	DEFECT TYPE			
	Infabrony (>2mm)	1		
	Suprabony (<=2mm)	3.82	2.50 – 5.83	**<0.001*****
	PD T0	0.43	0.33 – 0.55	**<0.001*****
	BOP T0			
	No	1		
	Yes	0.67	0.44 – 1.02	0.062
	BOP T1			
	No	1		
	Yes	0.32	0.23 – 0.44	**<0.001*****
	SUPPURATION T0			
	No	1		
	Yes	0.87	0.45 – 1.68	0.677
	SUPPURATION T1			
	No	1		
	Yes	0.14	0.05 – 0.35	**<0.001*****

Tab. 5: Pocket closure at T1 and multi-level variables: Results of simple binary logistic regression using GEE model for probability of closure.

OR>1 protector condition and OR<1 negative condition

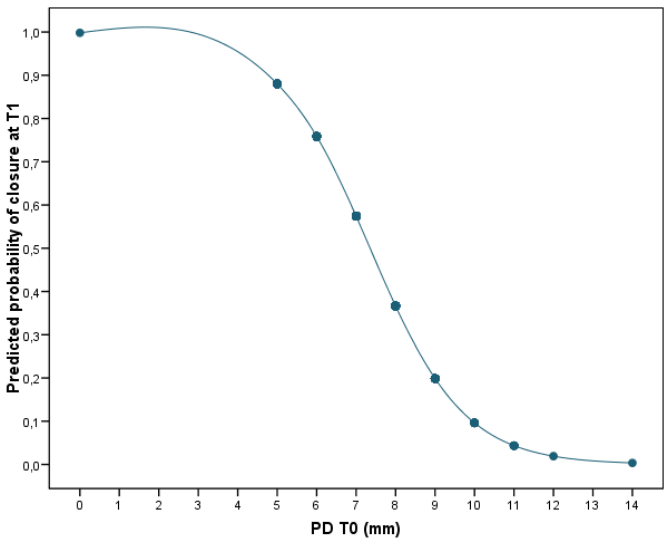
Regarding factors at patient-level, smoking showed a significant effect on the probability of closure (p=0.005). Current heavy smokers reduced risk of closure at 57% (OR=0.43; p=0.038) compared to reference non-smokers.

FMPS at baseline was detected as a significant covariate influencing on the outcome (OR=0.98; p=0.027). One additional unit involved a reduction of odds of closure at 2%. Consequently, the higher plaque level was in the patient, the lower the probability of pocket closure at T1.

FMBS at baseline did not influence on the ulterior probability of closure. However, there was found a relationship involving this score at T1 (OR=0.96; p=0.006). One additional unit involved a reduction of odds of closure at 4%.

At tooth-level, a strong significance was estimated involving the tooth type (OR=0.44; p<0.001). Multi-rooted teeth decreased the probability of closure at 56% compared to single rooted ones.

At site level, a relevant predictor at T0 was the PD measurement (OR=0.43; p<0.001). One additional 1 mm involved a reduction of odds of closure at 57%. If PD was larger than 7.5 mm, probability was estimated lower than 50% (Tab. 6).

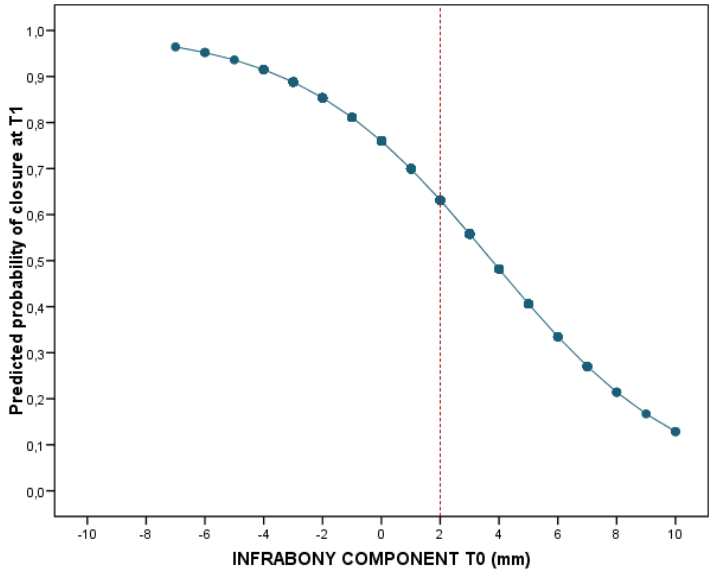


CLOSURE T1 BY PD T0 (mm)

	PD T0																					
	Total		0		5		6		7		8		9		10		11		12		14	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Total	1452	100	1	100	410	100	575	100	229	100	161	100	55	100	13	100	6	100	1	100	1	100
no	451	31.1	0	.0	30	7.3	157	27.3	115	50.2	90	55.9	39	70.9	12	92.3	6	100	1	100	1	100
yes	1001	68.9	1	100	380	92.7	418	72.7	114	49.8	71	44.1	16	29.1	1	7.7	0	.0	0	.0	0	.0

Tab.6: predicted probability of closure at T1 against PD values at T0 by model at Tab.5 and by PD.

BOP at T0 showed a strong tendency (OR=0.67; p=0.062). BOP at T0 must be interpreted as a bad-prognosis factor. Note that bop and suppuration are usual events with closure at T1 (p<0.001 both). Finally, the infrabony component measurement showed a significant association with the outcome (OR=0.74; p<0.001). One additional 1 mm involved a reduction of odds of closure at 26% (Tab. 7). Observe that predicted probability was 0.65 at the threshold 2 mm separating infra and suprabony defects. As expected, differences at closure rate between both type of defects were significant (OR=3.82; p<0.001). Suprabony defects showed a higher success rate than infrabony ones.



Tab. 7 predicted probability of closure at T1 against IC values at T0 by model at Tab. 5

CLOSURE AT T1: MULTIPLE BINARY LOGISTIC REGRESSION

The independent variables affecting pocket closure at T1 according to the simple binary logistic regression, were considered in the following multiple model (Tab. 8).

FMPS, FMBS, BOP and suppuration at T1 are not included because they are not strictly predictor variables, provided that they are recorded at the same time point of T1 closure.

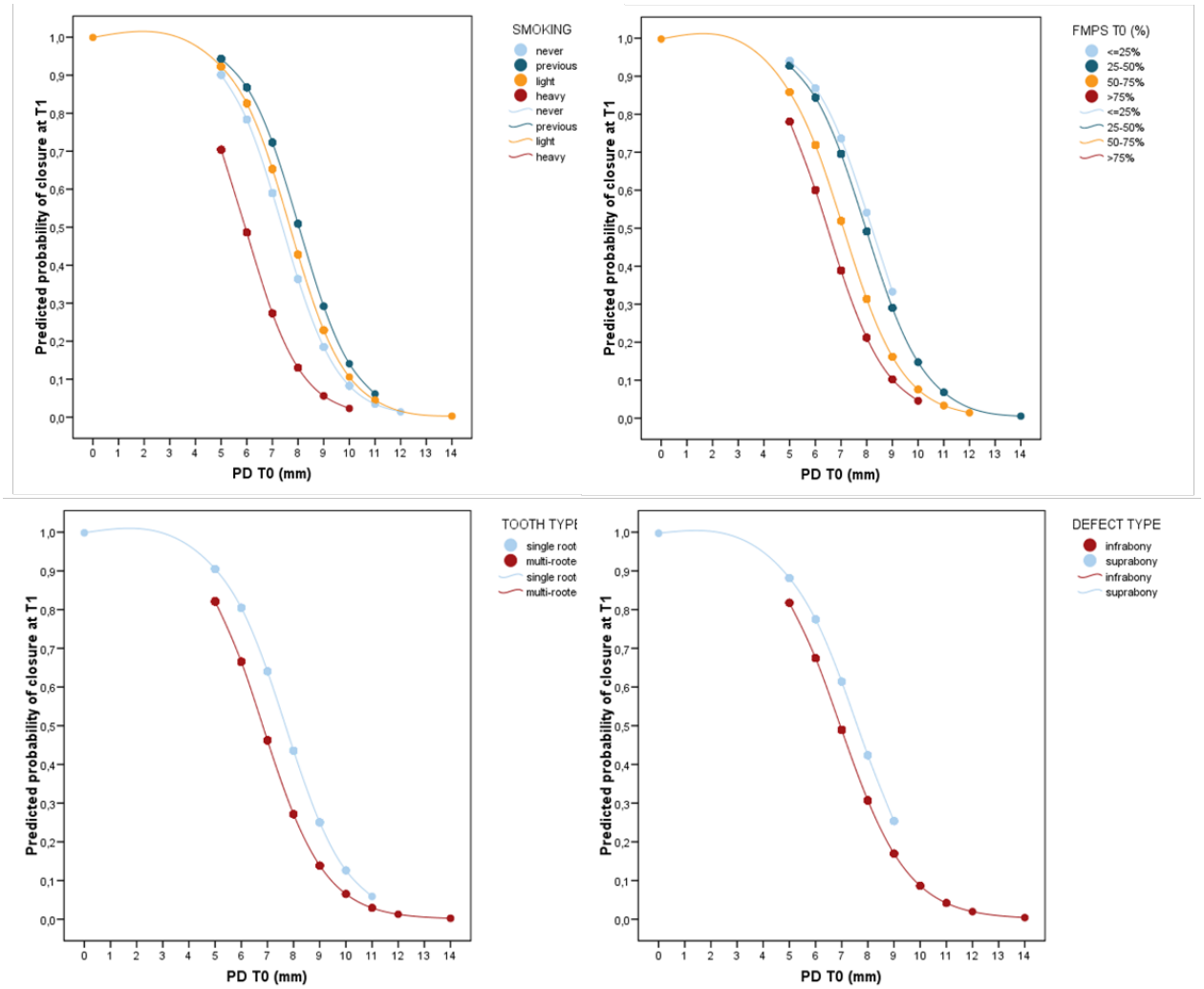
		OR	95% CI	p-value
PATIENT LEVEL	**SMOKING**			**<0.001*** **
	Never	1		
	Previous	1.70	0.71 - 4.11	0.235
	Current light	1.15	0.44 - 3.03	0.779
	Current heavy	0.22	0.13 - 0.39	**<0.001*** **
	FMPS (%) T0	0.98	0.97 – 0.99	**0.017***
TOOTH LEVEL	**TOOTH TYPE**			
	Single rooted	1		
	Multi-rooted	0.42	0.30 - 0.59	**<0.001*** **
SITE LEVEL	**DEFECT TYPE**			
	Infabrony (>2mm)	1		
	Suprabony (<=2mm)	1.89	1.20 – 2.96	**0.006****
	PD T0	0.44	0.33 – 0.60	**<0.001*** **
	BOP T0			
	No	1		
	Yes	0.84	0.57 – 1.25	0.383

Tab. 8: Pocket closure at T1 and multi-level relevant variables. Results of multiple binary logistic regression using GEE model for probability of closure. OR>1 protector condition and OR<1 negative condition

All the previous significant factors and covariates remained as significant in this adjusted model; smoking (p<0.001), heavy smokers (OR=0.22; p<0.001), FMPS (OR=0.98; p=0.017) multi-rooted teeth (OR=0.42; p<0.001), PD (OR=0.44; <0.001) negatively affected closure rate, while suprabony (OR=1.89 p=0.006) was a positive condition.

Observe that relevance of BOP at T0 is now weaker than before, not showing a tendency under the presence of the rest of variables.

Tab.9 describes the probability of pocket closure at tooth sites according to initial PD and each of the significant factors



Tab. 9: predicted probability of closure at T1 according to PD at T0 and different significant factors

CLOSURE AT T2: SIMPLE BINARY LOGISTIC REGRESSION

Additional pocket closure at T2 was reached in 187 sites out of 423, pushing pocket closure from 68.9% (95%CI: 66.5-71.3%) at 3 months (T1) to a final closure rate of 82.8% (95%CI: 80.8-84.8%) 6 months (T2) following NSPT (Tab. 4).

A simple binary logistic regression (odds ratio OR and 95%CI), using GEE model for probability of closure, was first performed to investigate the effect of single independent variables on the dichotomous dependent variable pocket closure (Tab. 11).

Regarding factors at patient-level, gender showed a significant effect on the probability of closure (p=0.002). Pockets hosted in female patients increased risk of closure (OR=3.07; p=0.002) compared to male. FMPS at T1 was detected as a significant covariate influencing on the outcome (OR=0.95; p=0.048). One additional unit involved a reduction of odds of closure at 5%. FMBS at T1 at was detected as a significant covariate influencing on the outcome (OR=0.96; p=0.047). One additional unit involved a reduction of odds of closure at 4%. Note that levels of these scores at T2 are correlated (FMPS remarkably and FMBS significantly) with the final status of the pocket.

At tooth-level, significance was shown by tooth type (OR=0.48; p=0.010). Multi-rooted teeth decreased the probability of closure at 52% compared to single rooted ones. At T1, presence of mobility was a bad prognosis factor according to the tendency observed (OR=0.55; p=0.092). The same explanation regarding grade 1 of furcation at T1 compared to absence (OR=0.44; p=0.080).

At site-level, oral sites showed less probability of success (OR=0.80; p=0.067). Other relevant predictor was the PD measures at T0 (OR=0.68; p=0.003) and at T1 (OR=0.26; p<0.001). One additional 1 mm involved a reduction of odds of closure at 32% and 74% respectively.
BOP at T1 and T2 were significantly associated to the outcome. The same conclusion was taken regarding suppuration, but at T2 only a tendency was observed.
Finally, the infrabony component measurement at T0 and T1 showed a significant association with the outcome (OR=0.75 at T0 and OR=0.72 at T1; p<0.001). One additional 1 mm involved a reduction of odds of closure at 25% roughly.

		OR	95% CI	p-value
PATIENT LEVEL	**AGE (years)**	1.01	0.97 - 1.06	0.577
	GENDER			
	Male	1		
	Female	3.07	1.53 - 6.15	**0.002****
	SMOKING			0.777
	Never	1		
	Previous	1.20	0.33 - 4.38	0.789
	Current light	1.24	0.57 - 2.68	0.593
	Current heavy	1.95	0.52 - 7.37	0.325
	ANTIBIOTIC			
	No	1		
	Yes	2.40	0.94 – 6.12	0.066
	FMPS (%) T1	0.95	0.90 – 0.99	**0.048***
	FMBS (%) T1	0.96	0.93 – 0.99	**0.047***
	FMPS (%) T2	0.95	0.89 – 1.01	0.092
	FMBS (%) T2	0.91	0.86 – 0.97	**0.004****
TOOTH LEVEL	**TOOTH TYPE**			
	Single rooted	1		
	Multi-rooted	0.48	0.27 - 0.84	**0.010***
	MOBILITY T0			0.385
	No	1		
	Grade 1	0.68	0.35 – 1.34	0.268
	Grade 2	0.58	0.09 – 3.59	0.561
	FURCATION T0			0.873
	No	1		
	Grade 1	0.88	0.26 – 2.95	0.836
	Grade 2-3	0.65	0.13 – 3.32	0.603
	MOBILITY T1			
	No	1		
	Grade 1	0.55	0.28 – 1.10	0.092
	FURCATION T1			0.191
	No	1		
	Grade 1	0.44	0.18 – 1.10	0.080
	Grade 2-3	0.64	0.12 – 3.46	0.601
SITE LEVEL	**MD SITE**			
	Mesial	1		
	Distal	1.41	0.87 - 2.67	0.162
	BO SITE			
	Buccal	1		
	Oral	0.80	0.62 - 1.02	0.067
	INFRABONY COMPONENT T0	0.75	0.65 – 0.86	**<0.001*****
	DEFECT TYPE T0			
	Infabrony (>2mm)	1		
	Suprabony (<=2mm)	2.90	1.64 – 5.11	**<0.001*****
	INFRABONY COMPONENT T1	0.72	0.61 – 0.85	**<0.001*****
	DEFECT TYPE T1			
	Infabrony (>2mm)	1		
	Suprabony (<=2mm)	4.28	2.50 – 7.31	**<0.001*****
	PD T0	0.68	0.53 – 0.88	**0.003****
	PD T1	0.26	0.16 – 0.45	**<0.001*****
	DIFF.ABS. PD T1-T0	0.90	0.76 – 1.07	0.233
	DIFF.PCT. PD T1-T0	0.98	0.97 – 0.99	**0.003****
	PL T1			
	No	1		
	Yes	0.86	0.45 – 1.64	0.641
	PL T2			
	No	1		
	Yes	0.78	0.34 – 1.76	0.543
	BOP T1			
	No	1		
	Yes	0.46	0.26 – 0.84	**0.012***
	BOP T2			
	No	1		
	Yes	0.30	0.17 – 0.54	**<0.001*****
	SUPPURATION T1			
	No	1		
	Yes	0.14	0.05 – 0.35	**<0.001*****
	SUPPURATION T2			
	No	1		
	Yes	0.35	0.10 – 1.18	0.090

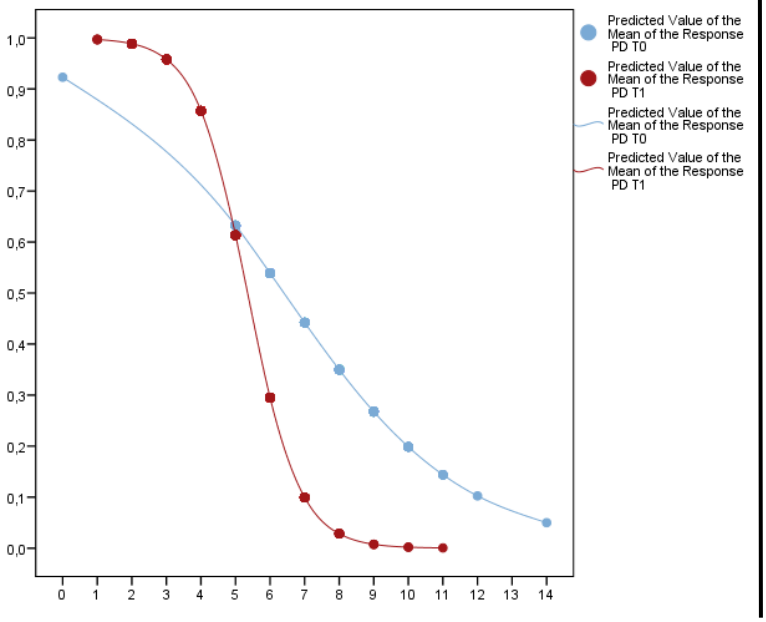
*p<0.05; **p<0.01; ***p<0.001

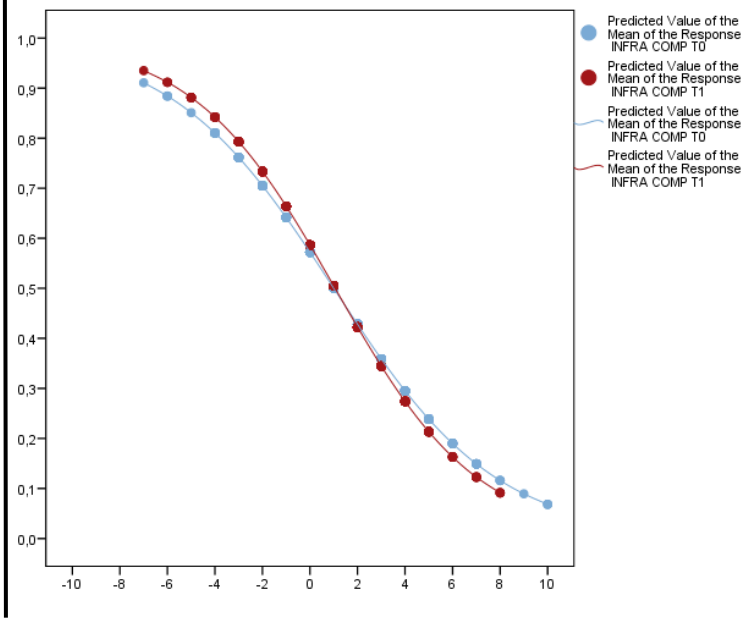
Tab. 11: Association between Pocket closure at T2 and variables at patient, tooth and site-level: Results of simple binary logistic regression (odds ratio OR and 95%CI) using GEE model for probability of closure.

OR>1 protector condition and OR<1 negative condition

The following tables (Tab 12, Tab 13) represent the predicted probability of closure by the model against PD values at T0 and T1 (Tab. 12) and against defect type (Tab. 13)

Tab. 12 Predicted probability of pocket closure against PD at T0 and T1.





Tab. 13 Predicted probability of pocket closure against IC at T0 and T1.

Tables 14 and 15 show closure rates at T2 relatively to the initial PD at T0 and T1.

CLOSURE AT T2: MULTIPLE BINARY LOGISTIC REGRESSION

The independent variables affecting pocket closure at T1 according to the simple binary logistic regression, were considered in the following multiple model (Tab. 14).

FMPS, FMBS, and BOP at T2 are not included because they are not strictly predictor variables, provided that they are recorded at the same time point of T1 closure.

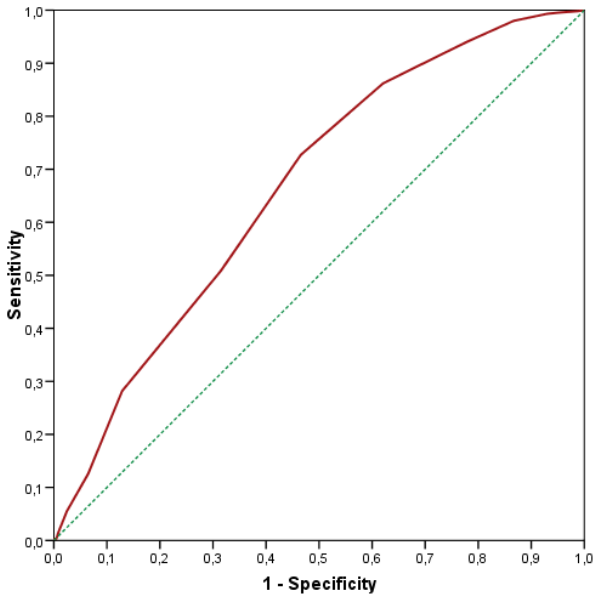
		OR	95% CI	p-value
PATIENT LEVEL	**GENDER**			
	Male	1		
	Female	2.48	1.14 – 5.36	**0.021***
	FMPS (%) T1	1.02	0.94 – 1.11	0.568
	FMBS (%) T1	0.97	0.93 – 1.02	0.244
TOOTH LEVEL	**TOOTH TYPE**			
	Single rooted	1		
	Multi-rooted	0.50	0.26 - 0.96	**0.036***
	MOBILITY T1			
	No	1		
	Grade 1	0.67	0.27 – 1.66	0.390
SITE LEVEL	**BO SITE**			
	Buccal	1		
	Oral	0.73	0.51 - 1.05	0.090
	DEFECT TYPE T1			
	Infabrony (>2mm)	1		
	Suprabony (<=2mm)	2.22	1.31 – 3.76	**0.003****
	PD T1	0.34	0.18 – 0.63	**0.001****
	DIFF.PCT. PD T1-T0	1.01	0.99 – 1.03	0.449
	BOP T1			
	No	1		
	Yes	1.00	0.56 – 1.81	0.993
	SUPPURATION T1			
	No	1		
	Yes	1.94	0.58 – 6.48	0.281

Tab. 14: Association between Pocket closure at T2 and variables at patient, tooth and site-level: Results of multiple binary logistic regression (odds ratio OR and 95%CI) using GEE model for probability of closure. OR>1 protector condition and OR<1 negative condition

In this adjusted model the following conditions increased the probability of closure at T2: female patients (OR=2.48; p=0.021), suprabony defects at T1 (OR=2.22; p=0.003), low levels of PD at T1 (OR=0.34; p=0.001). Opposite to that, multi-rooted teeth affected negatively closure rate at T2 (OR=0.50; p<0.036). The other factors and covariates are not relevant in presence of these 4 variables.

INFRAOSSEOUS COMPONENT TRESHOLD ANALYSIS

Apart from estimating a multiple model, the predictive value of the IC threshold of 2 mm to define infrabony and suprabony defects was compared to the other values. ROC curve estimation to predict closure at T1 according the infrabony measurement is smooth, not showing any relevant vortex that could be proposed as a remarkable cut-off (Tab. 2a).



Tab. 2a: ROC Curve

Table 2b describes the validity of the model for predicting closure outcome using different cutoff for infrabony component.

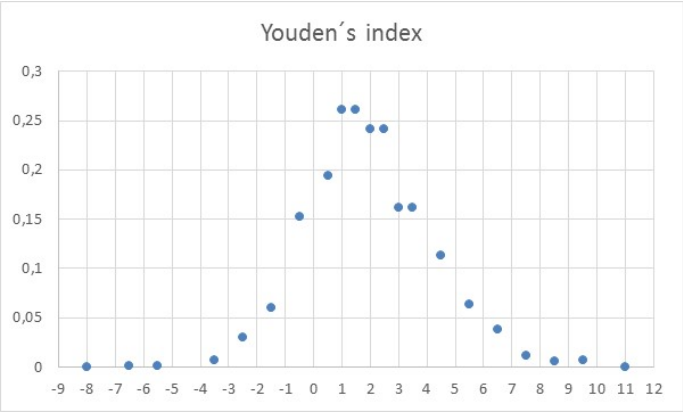
	Cutoff = 1 mm	Cutoff = 2 mm	Cutoff = 3 mm
Sensitivity	72.7%	86.2%	94.0%
Specificity	53.4%	37.9%	22.2%
PPV	77.6%	75.5%	72.8%
NPV	46.9%	55.3%	62.5%
Total corrected	66.7%	71.2%	71.7%
AUC		0.67 (0.64-0.70); **p<0.001*** **	

Tab. 2b: Validity of the model for closure outcome prediction. Positive predictive value (PPV), negative predictive value (NPV), AUC (area under curve from ROC analysis).
Sensitivity is interpreted as the ability of the IC measurement to detect closures at T1. On the other hand, specificity is the ability to detect non-closures.

The choice was then based on the Youden's Index (Tab. 2). The highest values were reached in the range 1.0-2.5 with slight differences between 1 and 2 mm:

- The threshold 2 mm should be accepted if we are interested in a higher sensitivity to predict closures assuming that specificity is very poor. The total rate of corrected classified sites is higher than cut-off 1 mm.
- The threshold 1 mm should be accepted if we are interested in a lower sensitivity to predict closures assuming specificity is poor. The total rate of corrected classified sites is lower than cut-off 2 mm.

Therefore, there were not found strong reasons to reject the conventional threshold 2 mm. On this basis, the intra- an -supra- bony definition of this study and their analysis were confirmed.



Tab. 3: Youden's Index

DISCUSSION

Primary outcomes of this study were investigating the effect of IC and infrabony defects on the healing of NSPT.

The results demonstrated that the anatomic distinction in supra- and infra- bony defects cannot be refused by clinical findings. Thus, closure rate confirms a difference in the probability of closing a pocket using a cut-off of 2 mm.

This study could also demonstrate a significant effect of the IC on closure rates 3 and 6 months after NSPT, with infrabony defects closing significantly less that suprabony defects.

In addition, heavy smoking, higher FMPS, multi-rooted teeth, infrabony defects, high baseline PD, presence of plaque and BOP at sites were significant factors for an inferior outcome of NSPT. Similarly, male patients, multi-rooted teeth, infrabony defects and low levels of PD at T1 significantly reduced the probability to obtain pocket closure.

The decision to use surrogate variables such as PD and CAL to evaluate the clinical outcome of NSPT is a common approach, since the true outcome variable to be assessed (tooth loss) fails to describe changes in short-term follow-ups [25]. In this study, the main clinical outcome used to assess NSPT success was pocket closure, defined as PD $\leq$ 4 mm. This value is based on the modern clinical guidelines indicating the need of treatment to pockets with PD > 4 mm [5]. Furthermore, classic studies demonstrated that pocket reduction positively changes subgingival microbiota and that pockets with PD $\geq$ 5 mm have a significant higher risk of tooth loss [26, 27].

Patients treated in a severely controlled environment by postgraduate students under strict supervision of expert specialists certified by the European Federation of Periodontology (EFP) were selected for this retrospective study. All the cases were chosen among the ones presented to the EFP commission and were previously discussed by a panel of international experts. Therefore, despite

the cases were treated by different clinicians, all the treatments are considered to be standardized at a high level of therapy and followed actual guidelines [5].

To avoid any possible bias related to the instruments used and different treatment approaches due to the retrospective design of the study, only data from patients treated after 2014 were screened. Closure rates presented in this study are 68.9% (95%CI: 66.5-71.3%) 3 months after NSPT and 82.8% (95%CI: 80.8-84.8%) 6 months after NSPT and 3 months after retreatment. If compared to Suvan et al., rates seem to be in accordance and slightly above [22]. This confirms the high standard of care provided for the selected cases. Likewise, the level of treatments together with the selection of cases can explain the slight positive difference in closure rates.

The study was designed with the primary outcome of better understanding the role of periodontal defect types (infra- and supra- bony) on pocket healing after NSPT. The results of the multiple binary linear regressions assuming pocket closure as the dependent variable showed infrabony pockets to have a significant negative effect on NSPT both at after a 3 months and 6 months follow-up period. Thus, according to our study both the first treatment and the second retreatment are reducing the odds to obtain a closure. These results are in conflict with similar studies using multilevel analysis showing slight or no effects of this independent variable [15, 16].

The difference could be possibly explained by the method used to assess infra- and supra- bony defects.

Previous studies by other research groups used RIC to estimate IC of periodontal pockets in non-surgical research projects [15, 16]. Although studies focusing on NSPT cannot use the intra-surgical measurements considered to be the gold standard for defect type evaluation, we believe that striving to the radiographic approach has 2 limitations. First, some studies describe the lack of precision of 2D intraoral radiographs. Among them, Tonetti demonstrated that intraoral standardized radiographs correctly estimated bone levels within 1 mm (± 0.5 mm) in 25% of cases; underestimated the bone loss in 55% and overestimated it in 20% of defects at pre-treatment. A

significant better result was instead given by the use of CAL measures, correctly estimating bone levels within 1 mm in 85% of sites [28]. On this basis, we decided to use CAL measures to evaluate IC (CIC) and divide pockets in supra- and infra-bony.

In addition to a better precision, CIC can be measured for every single site meaning that each interproximal space could possibly provide for 4 CIC values, while RIC can provide a maximum of one site per interproximal space because of 2D radiographic limits. This could be responsible of an underestimation of infrabony defects in previous studies. While in our research CIC allowed us to analyse 1452 sites divided in 309 infrabony and 1143 suprabony pockets, Tomasi could detect only 34 intrabony pockets out of 1413 sites [15].

Other variables seemed to have a negative impact on pocket closure. However female gender, heavy smoking, higher FMPS at baseline, multi-rooted teeth, infrabony defects, high initial PD, presence of plaque at sites are frequently attributed the role of negative factors [13-16, 19, 20, 29].

In discordance with other studies, tooth mobility doesn't have a significant effect according to our results [13, 14, 16, 19, 20, 29]. In our study, teeth being assigned a grade II or III mobility were often splinted without any standardized approach. These teeth represent the category usually showing a decrease in closure rate, thus splinting could be a possible reason for discordance.

Furcation involvement is another point that must be further analysed since discordance is frequently seen among similar studies. While Tomasi found furcation involvement to significantly reduce pocket closure, Tonetti did not show any differences[13, 16]. However, Tonetti assigned furcation involvement to tooth level giving its value to all the sites of the tooth involved, while Tomasi attributed it to sites. The present study assigned furcation involvement to the site level similarly to what was done by Tomasi in 2008, thus the discordance cannot be explained by a different interpretation of the variable. However, it must not be forgotten that the present study aimed at evaluating defect type effect on NSPT. Thus, interproximal spaces only were included in the study, and so interproximal furcation involvements only could be recorded. In addition, interproximal

defects can have various morphologies and furcations can be placed differently in the interproximal space, with conditions such as tooth rotation that can radically change their position. As a logic consequence, we decided to attribute the eventual grade of furcation involvement to both the buccal and oral pockets. Reasonably, an underestimation of the effect of furcations can be present in this study due to the assignment of furcation involvement to some pockets not suffering from this condition.

A key analysis of the study is represented by the definition of a proper value of IC basing on its ability to predict a change in closure rates. Anatomic classifications have always directly and indirectly considered an intrinsic difference in CAL of about 2 mm being the responsible to place the bottom of the pocket over or above the bony peak. However, anatomic classifications are not always mirrored in the clinics. In fact, previous studies using multilevel analysis struggled or failed to find an influence of defect type on pocket closure after NSPT [15, 16]. On the contrary, the definition of defect types with CIC helped this study to find a strong effect and so on to study discuss the classic IC cut-off basing on clinical performances. Although a marked difference could not be found, the cut-off of 2 mm could not be refused. Thus clinical performances of infra- and supra- bony defects can be adequately assessed by a 2 mm threshold.

After data collection, a post-hoc power analysis was also obtained. A sample size of 1,452 independent sites provides 99.9% power at confidence 95% to detect closure rates at 50% and 80% as significantly different in two groups (infrabony and suprabony defects) using a logistic regression model and assuming confidence 95%. However, sites were not independent and this power must be corrected because of the three-level structure of data. Each patient provided at average 36.3 sites and within-subject correlation CCI=0.5 (moderate) was assumed, leading to a correcting coefficient D=18.5.

Therefore, 1,452 dependent sites provide the same power than 78 independent ones, providing power at 80% under the same previous conditions.

CONCLUSION

Within the limits of a retrospective study, it can be stated that the anatomic threshold of 2 mm used to define infra- and supra- bony defects is reflected by a difference in closure rates.

Infrabony defects significantly reduced closure rate 3 months after both NSPT and the retreatment of residual pockets, together with female gender, heavy smoking, high FMPS at baseline, multi-rooted teeth, high initial PD, presence of plaque.

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