

Effect of smoking cessation on non-surgical periodontal therapy: results after 24 months

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Abstract

Aim: The aim of this 24-month prospective study was to assess the effect of smoking cessation on non-surgical periodontal therapy (NSPT) in adult subjects with chronic periodontitis.

Materials and Methods: Relative to a previous 12-month follow-up study, recruitment and follow-up period were extended, resulting in 116 eligible among the 286 screened subjects. They received NSPT and concurrent smoking cessation interventions. Periodontal maintenance was performed every 3 months. A calibrated examiner, blinded to smoking status, performed full-mouth periodontal examination in six sites per tooth at baseline, 3, 12 and 24 months of follow-up. Expired air carbon monoxide concentration measurements and interviews were performed to gather demographic and behavioural information.

Results: From the 116 enrolled subjects, 61 remained up to 24 months of follow-up. Of these, 18 quit smoking (Q), 32 continued smoking (NQ) and 11 oscillated (O) at 24 months of follow-up. Thereby, Q showed significantly higher mean CAL gain in diseased sites and higher reduction in the proportion of sites with $CAL \geq 3$ mm, when compared to NQ. In addition, Q presented significantly higher mean probing depth reduction relative to NQ ($p \leq 0.05$).

Conclusion: Smoking cessation promoted additional benefits on NSPT in chronic periodontitis subjects.

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According to Hill's considerations for inferences of causation (Hill 1965, Gelskey 1999), there is accumulating evidence for the role of smoking in the aetiology of peri-

odontitis. Thereby, association between smoking and periodontal diseases was moderate to strong in cross-sectional and case-control studies (Bergström 1989, Tomar & Asma 2000, Bergström 2003, Corraini et al. 2008, Hyman & Reid 2003, Susin et al. 2004, Natto et al. 2005, Albandar et al. 2000). Temporality was evidenced by cohort studies, in which smokers presented higher clinical attachment loss and greater radiographic bone loss than

non-smokers (Bergström et al. 2000, Jansson & Lavstedt 2002, Paulander et al. 2004, Baljoon et al. 2005, Thomson et al. 2007, Gätke et al. 2012, Buchwald et al. 2013). Associations have been consistent in different populations (Bergström & Floderus-Myrhed 1983, Tomar & Asma 2000, Susin et al. 2004, Natto et al. 2005, Torrungruang et al. 2005). The biologic gradient was such that heavy smokers presented with higher attachment loss than

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light smokers (Tomar & Asma 2000, Susin et al. 2004).

Differences in biofilm composition and host immune response between smokers and non-smokers (Johnson & Guthmiller 2007) are among the most biological plausible explanations for the associations found. These associations were coherent with the current knowledge of the natural history of periodontal diseases and analogously, adverse effects of smoking were also evidenced on general health (WHO 2011).

Experimentation is considered the strongest criterion for a causal relationship. Thereby, the performance of interventional studies is necessary, preferably using a randomized controlled trial design. However, ethical limitations inherent in human experiments hamper its application. Two systematic reviews (Chambrone et al. 2013, Fiorini et al. 2014) have assessed two interventional studies, both with 12 months of follow-up, on the effect of smoking cessation in periodontal conditions (Preshaw et al. 2005, Rosa et al. 2011). Based on these studies it was suggested that smoking cessation promotes a modest additional effect on probing depth (PD) reduction, among individuals receiving non-surgical periodontal therapy (NSPT). However, no conclusive statements could be drawn due to limited number and inherent limitations of existent studies, such as small sample size, short follow-up period, high loss to follow-up and low smoking cessation rate (Fiorini et al. 2014). Regarding the follow-up period of the intervention studies, as the latency period is unknown, i.e. how long the periodontal tissues respond to the smoking cessation intervention in terms of tangible clinical outcomes, an alternative for further intervention studies is to increase follow-up period. Therefore, the aim of this prospective study was to assess the effect of smoking cessation on the NSPT response in adult periodontitis subjects, up to 24 months of follow-up.

Material and Methods

A previous paper described the effects of smoking cessation on the periodontal treatment of 93 subjects included between May 2007 and

May 2009 (Rosa et al. 2011). On the present prospective study, recruitment was extended until October 2010, and the follow-up was increased from 12 to 24 months. The study protocol was approved by the University of São Paulo Ethics Committee. Eligible subjects were willing to stop smoking and attended the Smoking Cessation Clinic at the University of São Paulo Hospital (UH), São Paulo, Brazil. Initially, eligible subjects received four lectures. Following the first lecture, they were invited to participate in the study and if so, they received a screening examination. Included subjects were smokers ≥ 18 years old, presented ≥ 10 teeth and destructive periodontal disease, defined as 30% or more of their teeth with proximal CAL ≥ 5 mm (Tonetti & Claffey 2005). Exclusion criteria were systemic conditions considered as risk factors for periodontal disease, periodontal therapy in the last 6 months prior to enrolment, or continuous systemic use of anti-inflammatory or steroidal drugs.

Included subjects answered a structured questionnaire, comprising questions about smoking habits, dental hygiene habits and demographic data. The same interviewer performed measurements of exhaled carbon monoxide (CO) using a CO monitor (Micro Medical Ltd, Kent, UK). A threshold of 8 p.p.m. discriminated smokers from non-smokers (SRNT 2002). A trained and calibrated examiner (EFR), blinded to smoking status (Rosa et al. 2011), carried out full-mouth clinical periodontal examinations, excluding third molars, at baseline and 3, 6, 12 and 24 months of follow-up. Gingival recession (GR), PD and bleeding on probing (BoP) were measured at six sites per tooth, using a manual probe (Trinity[®], São Paulo, Brazil). CAL was calculated as the sum of GR and PD measurements. Moreover, the presence or absence of visible plaque (VP) and supragingival calculus was recorded at two sites per tooth (mid-buccal and mid-lingual).

Non-surgical periodontal therapy was performed concomitantly to smoking cessation therapy by a team of periodontal specialists. It consisted of full-mouth supra and subgingival scaling using manual (Trinity[®], São Paulo, Brazil) and

ultrasonic (MiniPiezon[®], EMS, Switzerland) instruments, oral hygiene instructions (OHI) and removal of intra-oral biofilm retentive factors. After the last NSPT appointment, study participants entered into a trimonthly interval maintenance programme. Smoking cessation therapy was performed during four consecutive weeks by a multidisciplinary team comprising physicians, nurses, a psychologist and dentists. It was tailored according to the individual patient needs and consisted of four 1-h counselling lectures, psychologist-assisted cognitive behavioural therapy, nicotine replacement therapy and pharmacotherapy (bupropion or varenicline). Smoking cessation counselling and motivation was reinforced by the dental team during maintenance appointments using motivational interviewing techniques (Miller & Rollnick 2002).

Statistical analyses

All interview and clinical written data were converted electronically using data entry software (Epidata 3.1, Odense, Denmark).

Frequency distributions of CAL, PD, % sites with VP and BoP were given at baseline, 3, 12 and 24 months. At follow-up, subjects were classified as: quitters (Q) – subjects who stopped smoking up to 6 months after NSPT; non-quitters (NQ) – did not stop smoking at any time during the study and oscillators (O) – stopped smoking more than once during the study or started smoking again. The groups were dynamic, i.e. the sample size and the smoking status of the subjects varied from each follow-up examination. Initially, the three groups at 24 months of follow-up were compared across the study periods regarding mean CO, pack/years, VP and BoP using repeated measures ANOVA. Multiple comparisons were conducted with the *post hoc* Newman–Keuls test. A significance level of $\alpha = 0.05$ was used in all statistical tests. Periodontal missing data were imputed in STATA (Stata version 10.1 for Windows, Stata Corporation, College Station, TX, USA) through the last value carried forward method for 2 missing periodontal charts at 3 months of follow-up, and 1 missing periodontal

chart at 24 months of follow-up (Twisk & de Vente 2002).

Multilevel linear analyses using the STATA procedure xtmixed (Rabe-Hesketh & Skrondal 2011) were performed to assess the subject-level effect of smoking cessation on a variety of CAL and PD outcomes that could be used to define NSPT success. This analysis considered the dynamic pattern of the study groups. Initially, six variance component models, i.e. empty models with no covariates, were performed to investigate the site-, tooth- and subject-level variation on CAL and PD change at 3, 12 and 24 months of follow-up. In all cases, the baseline value was subtracted from the follow-up CAL or PD value (CAL_{dif} and PD_{dif}). Subsequently, a number of random-intercept models were performed. The CAL and PD outcomes considered were: CAL_{dif} and PD_{dif}; CAL_{dif} and PD_{dif} in initially diseased sites (CAL_{dif}PD ≥ 4 mm and PD_{dif}PD ≥ 4 mm); the difference in the proportion of sites with CAL ≥ 3 mm (% CAL_{≥3} mm) and with PD ≥ 5 mm (% PD_{≥5} mm); and the proportion of sites with CAL gain ≥ 2 mm (% CAL_{gain} ≥ 2 mm) and ≥ 3 mm (% CAL_{gain} ≥ 3 mm), and with PD reduction ≥ 2 mm (% PD_{red} ≥ 2 mm) and ≥ 3 mm (% PD_{red} ≥ 3 mm). The subject-level group covariate, i.e. the smoking cessation status, was defined as: 1 = NQ, 2 = O and 3 = Q. The models assessing CAL and PD change at 3-months of follow-up presented the smoking cessation status covariate with only two categories, i.e. 1 = NQ and 2 = Q, owing to the absence of an oscillator assessment. Possible baseline differences in CAL and PD values among the different smoking cessation covariate categories, owing to the non-randomized design of the study, were handled using alternative random-coefficient models (Rabe-Hesketh & Skrondal 2011). They were performed as previous analyses, but adjusting for the baseline value of CAL or PD, or the baseline % CAL_{≥3} mm or % PD_{≥5} mm, as appropriate.

Results

From the 1,144 subjects enrolled in the Smoking Cessation Clinic from

May 2007 until October 2010, 286 were screened and 116 were included in the present study. Approximately 47% (*N* = 55) of the enrolled subjects were lost to follow-up (Fig. 1).

The mean age of the enrolled study participants at baseline was 48.2 ± 8.4 years (range: 25–66 years). Subjects lost at 3, 12 and 24 months of follow-up presented higher baseline PD mean, in comparison with the subjects that remained in the study (*p* ≤ 0.01). In addition, subjects lost at 12 and 24 months of follow-up were younger (*p* ≤ 0.01), and presented higher baseline % of sites with BoP (*p* ≤ 0.01), in comparison with their counterparts.

From the subjects who remained in the study in each experimental period, 35%, 31% and 30% were Q, and 65%, 53% and 52% were NQ at 3, 12 and 24 months of follow-up respectively. At 12 and 24 months of follow-up, the O group consisted of 16% and 18% of the study participants (Table 1). One patient who ceased smoking until 12 months of follow-up relapsed at 24 months, and therefore was moved to the O group. There were no divergences between self-reported smoking status

and exhaled CO reading, i.e. no Q presented CO measurements >8 p.p.m. Self-reported smoking status and CO measurements correlated at 3 (*r* = 0.31; *p* = 0.02), 12 (*r* = 0.40; *p* = 0.002) and 24 months of follow-up (*r* = 0.42; *p* = 0.001).

The mean number of present teeth of the study participants at baseline was 19.8 at 3 months and 20.3 at 12 and 24 months of follow-up. Table 1 describes the baseline demographical, behavioural and clinical features of the dynamic Q, O and NQ groups at each follow-up period. In comparison with NQ, O at 12 and 24 months of follow-up presented significantly lower baseline mean % of sites with VP and supragingival calculus. Mean baseline PD ranged from 2.8 to 3.1 mm, and mean baseline CAL ranged from 3.7 to 4.3 mm. There were no statistically significant differences in the mean CAL and PD among all dynamic groups at baseline.

CAL differences between baseline and each experimental period is given in Figure 2, using CAL_{dif}, CAL_{dif}PD ≥ 4 mm, % CAL_{≥3} mm and % CAL_{gain} ≥ 2 mm as outcomes (Panels A–D). When comparing the experimental groups at 24 months of follow-up, Q showed a higher trend for mean CAL gain than O and NQ using all outcomes of interest. O and NQ showed similar CAL change trends. For the outcomes CAL_{dif} and % CAL_{≥3} mm, there was a trend for increasing CAL in NQ from 3 to 24 months of follow-up (Panels A and C respectively), which could not be observed using CAL_{dif}PD ≥ 4 mm and % CAL_{gain} ≥ 2 mm as outcomes (Panels B and D respectively).

All groups showed a significant reduction in mean pack/years. The reduction in NQ was significantly lower in comparison with the other groups at 3, 12 and 24 months. Only Q showed significant reduction in the mean exhaled CO measurements at 12 and 24 months. NQ presented significantly higher mean CO, relative to Q at 3, 12 and 24 months. There was no difference between groups regarding BoP in all periods of observation. However, only Q presented statistically significant BoP reduction over time. There were no differences in % VP between NQ and Q throughout the study (Table 2).

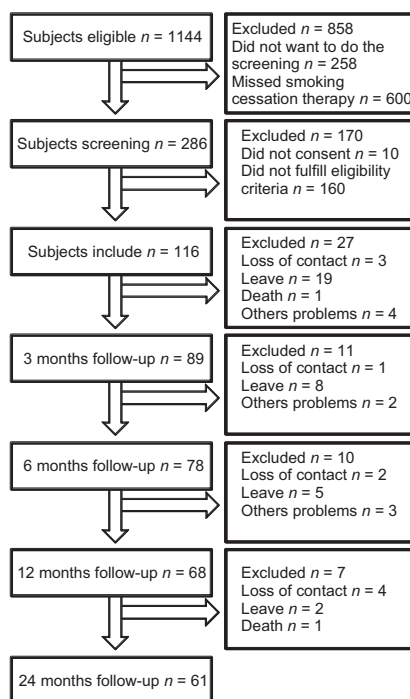


Fig. 1. Flow chart of study participants.

Table 1. Baseline demographical, behavioural and oral characteristics of the subjects that compose the dynamic Q, NQ and O groups at each one of the follow-up periods: 3 months, 12 months and 24 months

Parameters	3 months		12 months			24 months		
	Q (N = 31)	NQ (N = 58)	Q (N = 21)	NQ (N = 36)	O (N = 11)	Q (N = 18)	NQ (N = 32)	O (N = 11)
Mean (SD) age (years)	48.9 (7.7)	48.7 (8.3)	49.7 (7.0)	50.4 (6.8)	48.7 (9.6)	49.4 (7.0)	50.0 (6.7)	48.8 (9.7)
Gender, % male	34.5	32.3	42.9	38.9	18.2	50.0	37.5	18.2
N (%) subjects ≤ 10 years education	15 (48.4)	25 (45.5) [§]	10 (47.6)	14 (41.2) [‡]	5 (45.5)	8 (44.4)	11 (36.7) [‡]	5 (45.5)
N (%) monthly income ≤ 1000 BZR	12 (40.0) [†]	21 (42.9) ^{§§}	7 (33.3)	12 (41.4) ^{††}	4 (40.0) [†]	6 (33.3)	10 (38.5) ^{††}	4 (40.0) [†]
Smoking, Mean N (SD) pack/years	31.2 (20.6) [†]	32.9 (21.5)	31.8 (19.4) [†]	34.4 (20.0)	23.9 (15.8)	31.9 (17.9) [†]	35.1 (21.1)	28.6 (20.4)
Mean (SD) exhaled CO reading (p.p.m.)	13.9 (11.6)	18.7 (11.9) [†]	12.0 (12.2)	18.3 (12.2)	15.8 (8.4)	12.8 (12.7)	17.4 (11.9)	14.6 (9.4)
Mean (SD) N of teeth present	19.8 (4.6)	19.8 (5.0)	20.5 (4.5)	20.0 (5.2)	20.6 (4.4)	20.4 (4.7)	20.2 (5.2)	20.3 (4.6)
Mean (SD) % sites supragingival calculus	71.5 (27.2)	76.7 (20.8) [†]	75.0 (25.2)	79.4 (17.0)	58.5 (33.6)	75.4 (26.1)	78.7 (17.2)	54.4 (31.4)
Mean (SD) % sites visible plaque	84.1 (20.7)	84.5 (19.9)	87.3 (21.3)	87.9 (12.9)	71.2 (28.7)	91.8 (7.6)	88.0 (12.9)	69.8 (27.5)
Mean (SD) CAL (mm)	4.0 (0.8)	4.2 (1.2)	4.0 (0.9)	4.3 (1.4)	3.8 (0.9)	4.0 (0.8)	4.2 (1.2)	3.7 (0.9)
Mean (SD) PD (mm)	3.0 (0.6)	3.1 (0.7)	3.0 (0.6)	3.0 (0.7)	2.8 (0.5)	2.9 (0.6)	2.9 (0.7)	2.8 (0.5)
Mean (SD) % sites BoP	27.1 (17.6)	25.6 (18.9)	27.4 (19.4)	21.8 (14.9)	19.6 (9.6)	28.1 (20.9)	21.1 (13.2)	20.6 (10.0)

[†]1 missing value; [‡]2 missing values; [§]3 missing values; ^{††}6 missing values; ^{†††}7 missing values; ^{§§}9 missing values.

Bold: Q and O estimates are significantly different from the NQ group column estimates at a $p \leq 0.05$.

SD, standard deviation; BoP, Bleeding on probing; BZR, Brazilian reais; CO, carbon monoxide; CAL, Clinical attachment level; N, number; NQ, non-quitters; PD, Probing depth.

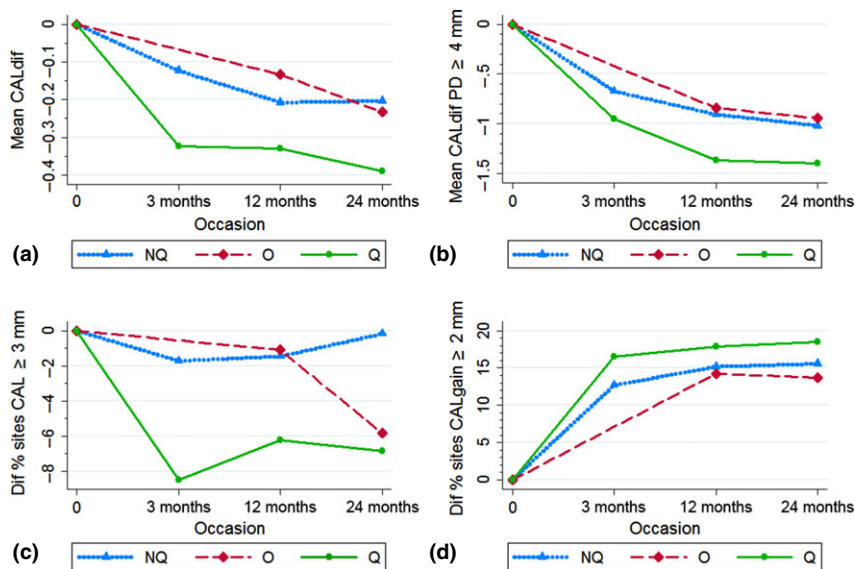


Fig. 2. CAL difference between the experimental groups according to follow-up period. (a) mean CAL difference (CALdif); (b) mean CAL difference in sites with probing depth (PD) ≥ 4 mm at baseline (CALdif PD ≥ 4 mm); (c) difference in the proportion of sites with CAL ≥ 3 mm (Dif % sites CAL ≥ 3 mm); (d) difference in the proportion of sites with CAL gain ≥ 2 mm (Dif % sites CALgain ≥ 2 mm) mos = months.

Multilevel analyses

Using variance component analyses, a significant 0.2 mm mean CAL gain was observed at 3-months examina-

tion, which remained stable over the study period. Moreover, the estimated 0.3 mm mean PD_{diff} reduction at 3-months follow-up remained sta-

ble over the study period (Table 3). For all CAL and PD outcomes of interest, variances were higher at the site-level, followed by tooth- and subject-levels. All 3-level variances were significantly different from zero using any of the CAL and PD outcomes ($p < 0.0001$).

Table 4 describes the multilevel analyses of the effect of smoking cessation on CAL change at 3, 12 and 24 months of follow-up. At 3 months of follow-up, Q presented a significantly higher CAL_{diff} gain of 0.2 mm and 0.3 mm in comparison with NQ, using random intercept and coefficient analyses respectively. Q presented an approximate 7% and 6% higher reduction in sites with CAL_{≥3} mm, in comparison with NQ, at 3 and 24 months of follow-up, respectively, using both analysis approaches. In sites with initial PD ≥ 4 mm, Q presented significantly higher CAL_{diff}PD ≥ 4 mm gain of 0.4 mm at 12 and 24 months of follow-up, using both random-intercept and random-coefficient analyses. Only using baseline adjustment, Q presented a significantly higher 3–4% CAL_{gain} ≥ 2 mm at 3 and 24 months of follow-up, respectively,

Table 2. Repeated measurement ANOVA for mean values of CO (p.p.m.), mean proportion of sites with VP, proportion of sites with BOP and pack/years, over time, according to the following groups at the end of 24 months: Quitters (Q), Oscillators (O) and Non-quitters (NQ)

Parameters	Groups (n)	Mean (SD)			
		Baseline	3 months	12 months	24 months
All sites					
CO	Q (18)	12.8 (12.7)	5.8 (5.3)*	2.9 (2.2) *[§]	3.8 (3.8)*
	O (11)	14.6 (9.4)	8.8 (9.5) [§]	16.0 (10.4)*	9.7 (5.3)
	NQ (32)	17.4 (11.9)	20.8 (15.1)* [§]	20.8 (15.1) [§]	16.5 (7.8)*
% BoP	Q (18)	28.1 (20.9)	20.6 (11.7)	19.9 (12.1)	19.5 (16.2)
	O (11)	20.6 (10.0)	18.3 (9.8)	22.3 (9.3)	15.7 (13.2)
	NQ (32)	21.1 (13.2)	18.5 (10.7)	17.1 (11.1)	17.7 (11.8)
%VP	Q (18)	91.8 (7.6)*	71.4 (28.1)*	64.0 (23.0)	60.1 (20.4)
	O (11)	69.8 (27.5)*	46.2 (32.5)*[§]	61.6 (27.6)	63.2 (20.5)
	NQ (32)	88.0 (12.9)	70.3 (22.7)[§]	68.3 (19.7)	67.9 (25.1)
Pack/years	Q (18)	31.9 (17.9) [†]	1.0 (2.8)[§]	0.0 (0.0)[§]	0.0 (0.0)^{§∞}
	O (11)	28.6 (20.4)	0.0 (0.0)*	4.6 (6.4)*	11.6 (11.7)*[∞]
	NQ (32)	35.1 (21.1)	24.0 (15.5)*[§]	22.8 (13.0)*[§]	22.1 (15.0)*[§]

CO, carbon monoxide; BOP, bleeding on probing; VP, visible plaque.

Bold: significant difference in relation to baseline at $p \leq 0.05$ and *[§]significant difference between the groups ($p \leq 0.05$); [†]one missing value.

in comparison with NQ. Accordingly, O presented a significant higher mean CAL_{diff} gain and % CAL_{gain} ≥ 2 mm, in comparison with NQ at 24 months of follow-up (Table 4). Table 5 describes the group covariate regression coefficients on PD change at 3, 12 and 24 months of follow-up. At 24 months, Q presented a significantly higher mean PD_{diff} reduction of 0.2 mm to 0.3 mm in comparison with NQ, whether baseline PD adjustment was performed or not. The difference increased to 0.4 mm when only sites with baseline PD ≥ 4 mm were considered, using both analysis approaches. Using random-coefficient models adjusting for the baseline PD value, O presented a significantly higher mean PD_{diff} reduction and a significantly higher % PD_{red} ≥ 2 mm at 24 months of follow-up (Table 5).

Discussion

The present study has shown beneficial effects of smoking cessation on CAL and PD outcomes of NSPT. To assess the effect of the smoking cessation intervention on the response to NSPT, 3 follow-up time points, i.e. 3, 12 and 24 months were chosen. At 3 months of follow-up, Q presented a significantly higher mean CAL_{diff} gain in comparison with NQ. Hence, Q presented a greater reduction in %CAL _{≥ 3 mm}, which is a more clinical meaningful outcome.

However, at 12-months of follow-up, the effect of smoking cessation in CAL_{diff} was observed only in initially diseased sites. As expected, both interventions are dynamic, i.e. the composition of the group covariate and the response to NSPT vary over time. Therefore, result comparisons for each one of the time-period analyses are not straightforward. Clearly, there was a loss of statistical power at the 12 months of follow-up analyses, in comparison with the 3 months analyses, which could have diminished the chance to detect significant differences between groups. This was due to higher loss to follow-up at 12 months, relative to 3 months. Moreover, the presence of a group covariate of 3 categories (Q, O and NQ) at 12 months of follow-up, instead of two categories (Q and NQ) at 3 months of follow-up, resulted in a further loss of power at the 12-month analyses due to the inclusion of one dummy group covariate and a reduced number of subjects in the Q group.

On the other hand, it should not be disregarded that results observed at 3 months of follow-up may be more prone to influences of differential measurement error. Evidence suggests that Q present increased gingival bleeding from 3 days up to 8 weeks after smoking cessation (Morozumi et al. 2004), which could in turn result in higher frequency of measurement errors in Q, relative to NQ at 3 months of follow-up

Table 3. Variance components models for the outcome parameters change in CAL and PD. It describes at 3, 12 and 24 months of follow-up the mean outcome and the variation across each of the 3 levels (subject-, tooth- and site-levels)

	CAL difference (follow-up – baseline)						PD difference (follow-up – baseline)					
	3 months		12 months		24 months		3 months		12 months		24 months	
	95% CI		95% CI		95% CI		95% CI		95% CI		95% CI	
	β_0		β_0		β_0		β_0		β_0		β_0	
Mean estimate	-0.2	-0.27; -0.09	-0.2	-0.30; -0.07	-0.2	-0.32; -0.08	-0.3	-0.39; -0.23	-0.3	-0.41; -0.21	-0.3	-0.42; -0.21
Variances												
Subject-level	0.1	0.10; 0.21	0.2	0.13; 0.28	0.2	0.13; 0.30	0.1	0.10; 0.19	0.2	0.12; 0.24	0.2	0.11; 0.24
Tooth-level	0.2	0.21; 0.28	0.3	0.30; 0.40	0.3	0.26; 0.36	0.1	0.09; 0.13	0.2	0.15; 0.21	0.2	0.15; 0.21
Site-level	1.6	1.58; 1.67	1.9	1.86; 1.99	1.9	1.79; 1.92	1.1	1.06; 1.12	1.2	1.14; 1.22	1.1	1.04; 1.12

CI, confidence interval; Bold intercept estimates are significant different from 0 at $p \leq 0.05$; All variance estimates were significantly different from 0 at $p \leq 0.00001$, ensuring the need to account for the hierarchical 3-level structure.

Table 4. Regression coefficients for the effect of smoking cessation at 3, 12 and 24 months of follow-up and CAL using five 3-level random-intercept models, according to the given CAL outcome. The analyses were performed with or without adjustment for baseline value of CAL

Outcome	Baseline adjustment	3 months				12 months				24 months			
		Quitters*				Quitters*				Quitters*			
		β_I	95% CI	β_I	95% CI	β_I	95% CI	β_I	95% CI	β_I	95% CI	β_I	95% CI
CAL _{diff} (mm)	No	-0.2	-0.38; -0.03	-0.2	-0.42; 0.08	0.1	-0.27; 0.36	-0.2	-0.50; 0.04	-0.1	-0.38; 0.26	-0.1	-0.38; 0.26
	Yes	-0.3	-0.49; -0.04	-0.2	-0.48; 0.02	-0.2	-0.48; 0.14	-0.3	-0.56; 0.00	-0.3	-0.66; -0.00	-0.3	-0.66; -0.00
CAL _{diff} PD ≥ 4 mm	No	-0.3	-0.53; -0.01	-0.4	-0.71; -0.06	0.1	-0.34; 0.49	-0.4	-0.68; -0.02	0.1	-0.37; 0.41	0.1	-0.37; 0.41
	Yes	-0.3	-0.61; 0.01	-0.4	-0.76; -0.01	-0.2	-0.64; 0.31	-0.4	-0.72; -0.01	-0.2	-0.66; 0.19	-0.2	-0.66; 0.19
% CAL _{≥ 3} mm	No	-6.5	-10.6; -2.4	-4.4	-9.32; 0.52	0.8	-5.37; 6.99	-6.4	-12.31; -0.57	-5.3	-12.25; 1.70	-5.3	-12.25; 1.70
	Yes	-6.5	-10.5; -2.5	-4.3	-8.71; 0.03	0.1	-5.48; 5.54	-6.2	-11.94; -0.52	-5.7	-12.60; 1.12	-5.7	-12.60; 1.12
% CAL _{gain} ≥ 2 mm	No	3.5	-0.39; 7.35	2.6	-2.71; 7.94	-1.2	-7.87; 5.50	3.2	-2.68; 9.21	-1.7	-8.77; 5.34	-1.7	-8.77; 5.34
	Yes	3.0	0.41; 5.51	1.7	-0.86; 4.19	2.2	-0.90; 5.35	2.2	-0.74; 5.22	4.0	0.53; 7.51	4.0	0.53; 7.51
% CAL _{gain} ≥ 3 mm	No	0.5	-1.40; 2.33	1.0	-1.87; 3.98	-1.7	-5.39; 1.95	1.0	-1.90; 3.86	-1.1	-4.56; 2.28	-1.1	-4.56; 2.28
	Yes	0.3	-0.63; 1.31	2.0	-0.62; 4.65	0.4	-2.87; 3.76	1.2	-0.98; 3.34	0.9	-1.66; 3.48	0.9	-1.66; 3.48

*Ref category = Non-quitters; CI, confidence interval.

Bold estimates are significant at $p \leq 0.05$; CAL_{diff}(mm), Clinical attachment level difference in mm (follow-up value - baseline value); CAL_{diff} PD ≥ 4 mm, CAL difference in sites with baseline PD ≥ 4 mm; % CAL _{≥ 3} mm, difference (follow-up value - baseline value) in the percentage of sites with CAL ≥ 3 mm; % CAL_{gain} ≥ 2 mm, percentage of sites with CAL gain ≥ 2 mm; % CAL_{gain} ≥ 3 mm, percentage of sites with CAL gain ≥ 3 mm; PD, Probing depth.

(Biddle et al. 2001). However, as measurement error is highly dependent on the characteristics of the study sample (Corraini et al. 2013), and a significantly bleeding decrease was observed both in Q and NQ, using % of sites with BoP as surrogate variable, this differential measurement error is, therefore, unlikely in the present study.

At 24 months of follow-up, the benefits of smoking cessation on CAL were evident, using mean CAL_{diff} in diseased sites and the % CAL _{≥ 3} mm as outcomes of interest. In addition, differences between Q and NQ were observed in mean PD_{diff} reduction. Since this was the follow-up period with higher drop-out rates, more stable NSPT response, and therefore expected similar measurement error among groups, we speculate that these differences between Q and NQ could be better explained by the smoking cessation intervention. Therefore, the period of the effect of smoking cessation on the periodontal tissues appears to be around 24 months for CAL and PD outcomes. Even though in clinical practice the magnitude of the difference in CAL and PD between Q and NQ after NSPT seems discrete at 24 months, it must be regarded as sufficiently optimistic. It represents the beginning of a slow process of improvement that can also encourage the patient to a lifestyle change towards better oral and general health care. Smoking cessation can make Q more self-conscious about their oral health (Krall et al. 1997), which can lead to lifestyle change (Rimer et al. 1990).

The analytical approach used, i.e. multilevel analysis, was probably more appropriated than the previous traditional linear regression analysis approaches. This was confirmed due to variances at all three levels of the hierarchy attaining statistical significance. As multilevel analysis accommodated the natural hierarchical structure of the periodontal data, in which sites are clustered into teeth that are clustered into subjects, it was less prone to biased estimates of the subject-level covariate coefficient in the association with the clinical periodontal outcomes investigated (Mueller 2007, Peugh 2010).

Using baseline adjustments, O showed significant differences in

Table 5. Regression coefficients for the effect of smoking cessation at 3, 12 and 24 months of follow-up and PD using five 3-level random-intercept models, according to the given PD outcome. The analyses were performed with or without adjustment for baseline value of PD

Outcome	Baseline adjustment	3 months		12 months				24 months			
		Quitters*		Quitters*		Oscillators*		Quitters*		Oscillators*	
		β_1	95% CI	β_1	95% CI	β_1	95% CI	β_1	95% CI	β_1	95% CI
PD _{dif} (mm)	No	-0.2	-0.32;0.01	-0.1	-0.34;0.12	0.1	-0.19;0.38	-0.3	-0.50;-0.03	-0.2	-0.46;0.09
	Yes	-0.2	-0.32;-0.05	-0.1	-0.26;0.07	0.0	-0.20;0.22	-0.2	-0.41;-0.07	-0.2	-0.42;-0.02
PD _{dif} PD ≥ 4 mm	No	-0.2	-0.45;0.09	-0.2	-0.57;0.08	0.1	-0.30;0.52	-0.4	-0.70;-0.08	-0.2	-0.53;0.21
	Yes	-0.2	-0.44;0.04	-0.2	-0.51;0.07	0.1	-0.31;0.43	-0.4	-0.66;-0.06	-0.2	-0.58;0.13
% PD _{≥ 5} mm	No	0.6	-2.63;3.88	-0.8	-5.64;3.96	1.5	-4.54;7.52	-2.7	-7.86;2.35	-0.8	-6.75;5.15
	Yes	-1.2	-4.18;1.86	-1.1	-3.81;1.52	-1.1	-4.42;2.26	-1.9	-4.71;0.98	-2.8	-6.14;0.48
% PD _{red} ≥ 2 mm	No	1.7	-2.65;6.08	-0.1	-6.03;5.83	-2.3	-9.77;5.13	2.7	-3.31;8.75	3.2	-3.93;10.38
	Yes	1.8	0.10;3.52	-0.4	-2.32;1.56	0.1	-2.24;2.54	1.2	-0.76;3.24	3.4	1.05;5.74
% PD _{red} ≥ 3 mm	No	0.7	-1.25;2.55	0.4	-2.66;3.38	-1.2	-5.04;2.55	1.1	-1.85;4.01	0.9	-2.56;4.40
	Yes	1.2	-0.33;2.78	0.7	-1.63;3.02	-0.1	-2.96;2.88	1.0	-1.01;3.07	1.4	-1.00;3.84

*Ref category, Non-quitters; CI, confidence interval.

Bold estimates are significant at $p \leq 0.05$; PD_{dif}(mm) = Probing depth difference in mm (follow-up value – baseline value); PD_{dif}PD ≥ 4 mm, PD difference in sites with baseline PD ≥ 4 mm; % CAL _{≥ 3} mm, difference (follow-up value – baseline value) in the percentage of sites with CAL ≥ 3 mm; % CAL_{gain ≥ 2} mm, percentage of sites with CAL gain ≥ 2 mm; % CAL_{gain ≥ 3} mm, percentage of sites with CAL gain ≥ 3 mm; PD, Probing depth.

CAL_{dif}, %CAL_{gain ≥ 2} mm, PD_{dif} and %PD_{red ≥ 2} mm when compared to NQ. Although O and NQ showed no significant differences in mean CAL and PD at baseline, this could be probably masked due to the smaller sample size of the O group, relative to the NQ group. In Table 1, it was clear that O presented a healthier periodontal profile at baseline than NQ and Q. This underlying heterogeneity between O and NQ at baseline resulting from the non-randomized design, often referred to as confounding by indication (MacMahon 2003), could have hampered group comparisons over time using random-intercept analysis. On the other hand, it might have led to biased estimates in the random-coefficient analyses due to over adjustment, which may explain the change of direction of mean O estimates when comparing both analyses approaches. Even though both analyses are valid, we based our conclusions only when random-coefficient analysis was confirmed by the more parsimonious random-intercept analysis. As the magnitude of the potential for bias in random-coefficient analyses would vary in different study samples, further studies are necessary to investigate whether oscillators might benefit or not from smoking cessation interventions during NSPT.

Similarly to previous intervention studies of smoking cessation involv-

ing medical (Andrews et al. 1999, Binnie et al. 2007) and dental (Preshaw et al. 2005) teams, the loss to follow-up was high, up to 47%, which may reflect psychological disorders caused by smoking abstinence syndrome (Thorndike et al. 2008, Berlin et al. 2009). However, the dropout rate has not changed significantly from 12 to 24 months to follow-up, as evidenced by the additional 6% dropout in this period. Regarding the present study smoking cessation rates, moderate to strong correlation between self-reported smoking status and exhaled CO measurements was found. Furthermore, no self-reported Q diverged from exhaled CO measurement status. This may be due to participant's prior knowledge about the validation of their smoking self-report status using exhaled CO measurements. It corroborates results from other investigations (Ripoll et al. 2012, Chen et al. 2013) which, in addition, pointed out that exhaled CO measurements may positively influence the success of the smoking cessation through a motivational effect, i.e. by showing immediately to the patient the harmful consequences of exposure to cigarette smoke. These might have contributed to the 30–35% quit rates at follow-up, which were higher than rates reported by other studies involving dental teams (Binnie et al. 2007, Ebbert et al. 2007, Gordon et al.

2010). This high smoking cessation proportion maybe further attributed to the present study multidisciplinary approach and re-motivation performed every 3 months. Interestingly, most individuals who have managed to quit for 1 year were free of addiction for the remaining period of observation, as evidenced by only one patient relapsing cessation after 12 months of follow-up. Furthermore, all patients, including those continuing smoking, significantly decreased the amount of cigarettes smoked over 24 months. Therefore, we encourage a multidisciplinary approach and the use of exhaled CO measurements to validate tobacco exposure in future studies involving smoking cessation interventions.

In conclusion, after 24 months of follow-up periodontitis patients who successfully quit smoking showed a better response to NSPT than continuing smokers.

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Clinical Relevance

Scientific rationale for the study: Few studies have suggested that smoking cessation promotes additional effect among individuals receiving non-surgical periodontal therapy. However, no conclusive statements could be drawn, mostly as result of short follow-up period.

Principal findings: After 2 years of follow-up, quitters presented significantly higher clinical attachment gain, reduction in the proportion of sites with clinical attachment level ≥ 3 mm and probing depth reduction, in comparison with non-quitters.

Practical implications: Smoking cessation therapy should be integrated

into NSPT in chronic periodontitis subjects. In addition, patients may benefit about information on the effect of smoking cessation on periodontal treatment response.