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Acute-phase response following one-stage full-mouth versus quadrant non-surgical periodontal treatment in subjects with comorbid type 2 diabetes: A randomized clinical trial

By

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Data availability Statement

The data that support the findings of this study are available upon reasonable request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Authors contribution

FG contributed to study conception, to study design, to data interpretation, to manuscript drafting and critically revised the manuscript. SG and CM contributed to data analysis and interpretation, and to manuscript drafting. MP, LG and UM contributed to data interpretation, and critically revised the manuscript. MP contributed to data analysis and interpretation, manuscript drafting, and critically revised the manuscript.

Abstract

Aim: To compare the level of inflammatory markers and endothelial function 24 hours (Day1) and 90 days (Day90) after conventional quadrant-wise (Q-SRP) versus one-stage full-mouth scaling (FM-SRP) in patients affected by type 2 diabetes mellitus (T2DM).

Methods: Patients affected by periodontitis and T2DM were randomly allocated to receive FM-SRP or Q-SRP and followed up at Day1 and Day90. Serum samples, vital signs and flow-mediated dilation (FMD) parameters were collected at baseline, Day1 and Day90. Periodontal variables were collected at baseline and Day90. The primary outcome was the C-reactive Protein (CRP) concentration at Day1 after periodontal treatment. The Student t-test for independent samples was used for between-group comparisons (Mann Whitney U test for non-normal data), while the ANOVA with post-hoc Tukey tests (Kruskal Wallis and Dunn tests for non-normal data) were used for intragroup comparisons.

Results: 40 subjects were included. FM-SRP produced a significant increase in CRP and a significant reduction in FMD at Day1 compared to Q-SRP ($p<0.05$). The absolute change in HbA1c (mmol/mol) from baseline to Day90 was significantly improved in the Q-SRP (ΔHbA1c=-1.59 (SD=1.20)) compared to the FM-SRP group (ΔHbA1c=-0.8 (SD=0.95)) ($p=0.04$).

Conclusions: FM-SRP triggers a robust acute-phase response at 24 hours after treatment compared to Q-SRP. Such systemic acute perturbations may offset the benefic systemic effects of periodontal treatment in terms of HbA1c reduction and improvement in endothelial function in T2DM subjects.

Clinical Relevance

Scientific rationale: One-stage full-mouth non-surgical treatment determines a systemic inflammation in the first post-operative hours in systemically healthy subjects compared to conventional quadrant treatment. No information is available regarding subjects affected by type 2 diabetes mellitus.

Principal findings: One-stage full mouth treatment determined a higher CRP increase at 24 hours. Both treatments determined a three-month reduction of Hb1AC, yet these effects may be modulated by postoperative CRP levels.

Practical Implications: Quadrant treatment is indicated in subjects affected by periodontitis and type 2 diabetes as higher benefits on glycated hemoglobin were noted, presumably because a lower acute inflammation was triggered in the immediate post-operative period.

1 Introduction

Periodontal treatment encompasses a range of different treatments combined within a step-wise framework, ranging from behavioral changes to surgical interventions (Sanz et al., 2020). In particular, Step 2 of periodontal treatment includes sub-gingival non-surgical instrumentation, which represents the foundation of the management of periodontitis allowing an important reduction of key disease oral indicators such as gingival inflammation, pocket depth and clinical attachment (Sanz et al., 2020; Suvan et al., 2020). The benefits are extending over periodontal parameters with important effects on overall health (Sanz et al., 2020; Tonetti et al., 2015) and patient's perception of quality of life (Graziani & Tsakos, 2020). In addition to such relevant positive effects on overall health, non-surgical treatment was consistently shown to determine in the immediate post-operative hours a robust systemic inflammatory reaction characterized by an increase of acute-phase proteins, alteration of endothelial function, increase of body temperature, higher tendency of developing blood clot and reduction of renal function (D'Aiuto et al., 2005, 2013; Graziani et al., 2010; Ide et al., 2004; Tonetti et al., 2007). The potential effects of such transient condition have not been fully elucidated and, cautiously, it has been suggested that in subjects with comorbidities attempts on limiting post-inflammatory reactions should be implemented (Sanz et al., 2020). A successful method to avoid such reaction relies on treatment delivery. Quadrant conventional non-surgical periodontal treatment has shown not to alter systemic inflammation when compared to the one-stage full-mouth approach (Graziani et al., 2015). It appears that the length of the clinical session may be related to the post-operative systemic inflammation. Thus, shorter sessions did not lead to acute-phase reaction, and it was speculated that longer sessions would lead to an increased bacteremia. However, the impact

of such reactions in systemically healthy subjects, are likely not to determine medium and long-term consequences for the overall health. Nonetheless, questions on the effect of treatment in subjects with comorbidities, affecting the overall acute-phase response, were raised.

Thus, aim of this study was to compare one-stage full-mouth versus conventional quadrant non-surgical treatment in terms of post-operative acute phase reaction, as measured with 24-hours postoperative levels of high-sensitive C-reactive protein as primary outcome, in subjects affected by Periodontitis and type 2 diabetes mellitus.

2 Materials and Methods

2.1 *Trial design*

This study was reported following the Consolidated Standards of Reporting Trials (CONSORT) 2010 guidelines (Moher et al., 2010). The current study was a single-center, parallel group, randomized controlled clinical trial with a 3-month follow up. The study protocol was approved by the University Hospital of Pisa Ethics committee (Pisa, Italy) (protocol number 3399) and it was registered in a clinical trials database (NCT03087266).

2.2 *Participants*

All individuals attending the Unit of Periodontology at the University Hospital of Pisa (Italy) were screened for eligibility. Subjects were eligible to participate if they met the following inclusion criteria: i) age between 18 and 70 years; ii) previous diagnosis of type 2 diabetes mellitus (T2DM; glycated hemoglobin levels ≥48 mmol/mol at diagnosis) confirmed by the diabetologist and undergoing treatment (Kazi & Blonde, 2001); iii) diagnosis of periodontitis

defined as proximal attachment loss ≥3 mm in ≥2 non-adjacent teeth (Tonetti et al., 2005); iv) at least 20% of the entire dentition with Probing Depth (PD) ≥5 mm; v) Full Mouth Bleeding Score (FMBS) ≥20%. Subjects were excluded if: i) pregnant or lactating females; ii) had a diagnosis of any systemic disease other than diabetes; iii) prescribed with any pharmacological treatment within 3 months before the inclusion in the study except for diabetes medications; iv) they underwent periodontal treatment in the last 6 months. All participants were provided with the subject information sheet and gave written informed consent, which was followed by collection of medical and dental histories and comprehensive oral examination.

2.3 *Outcomes*

2.3.1 *Clinical parameters*

At baseline examination, all participants received a full periodontal evaluation by a single masked calibrated examiner. One examiner was trained for repeatability for Clinical Attachment Level (CAL) measurement. Consistency of probing was judged adequate when reaching a percentage of agreement within ±2 mm between repeated measurements of at least 98% (Graziani et al., 2010); in particular, the agreement within 1 mm was 95.3%. Periodontal parameters such as PD and recession (REC) were assessed full-mouth and rounded to the nearest millimeter (UNC15 mm periodontal probe); CAL values were obtained by the sum of PD and REC. Plaque and Bleeding on Probing (BoP) were recorded dichotomously six sites per tooth in order to calculate the Full Mouth Plaque Score (FMPS) and the Full Mouth Bleeding Score (FMBS), respectively (Ainamo & Bay, 1975; O'Leary et al., 1972). During the *post-hoc* analysis, periodontal charts and intraoral radiographs were used

in order to further classify participants using the 2018 EFP/AAP Classification System (Tonetti et al., 2018).

2.3.2 *Blood collection and serum markers*

Blood samples were collected from a venipuncture in the antecubital fossa before 8am after an overnight fast for all participants. Blood samples were processed immediately, and serum aliquots were stored at -80°C until analyses were performed. All analyses were performed by the ISO-certified laboratory at the University Hospital of Pisa (Pisa, Italy) by an operator blinded to group allocation. High-sensitivity C-reactive protein (CRP), interleukin-6 (IL-6), total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglycerides, as well as glycated hemoglobin (HbA1C), insulin and glucose levels, were measured following standard laboratory procedures.

Serum CRP was measured by immunoturbidimetry (Cobas, Roche Diagnostic, Manheim, Germany) and a Multiplex array (Meso Scale Discovery, Rockville, Maryland, MD, USA) was employed to assay interleukin 6 (IL-6) levels (lower detection limit of 0.92 pg/mL) using the ELISA method.

Intra-and inter-assay coefficient of variations were all <6% and CRP standard deviation of the measurement was 0.83.

2.3.3 *Vital signs*

At baseline examination, vital signs were collected by an experienced operator and encompassed. Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP) were measured in triplicates using an automatic oscillometric device (OMRON-705IT, Omron, Kyoto, Japan); the mean blood pressure (BP) was then measured as the mean of the last two

measurements. Heart rate (beats per minute-bpm) was calculated as the average of two measurements. Body temperature (Celsius degree-°C) was measured twice with tympanic reading (Genius TM 2, Covidien LLC, USA) and the average measurement was registered.

2.3.4 *Endothelial function*

The Flow-Mediated Dilation (FMD), together with other measures (baseline/maximum/release diameter, baseline shear rate, maximum shear rate, best diameter, GTN-baseline/maximum diameter, FMD) of endothelial function, were recorded by assessing the endothelium-dependent vasodilation of the brachial artery by means of ultrasound imaging (Acuson XP 128/10, Siemens), as previously performed (Czesnikiewicz-Guzik et al., 2019; Tonetti et al., 2007). Measurements were performed by a single experienced examiner blinded to treatment assignment. FMD was calculated as the change (expressed as percentage) from baseline to the peak diameter 45 to 75 seconds after the release of the blood-pressure cuff of the sphygmomanometer. After 10 minutes of rest, FMD was measured also after sublingual administration of 25µg of nitroglycerin, following the same protocol.

2.3.5 *Health related quality of life*

The impact of periodontal treatment on quality of life in both groups was assessed using the the Oral Health Impact Profile-14 (OHIP-14) (Slade 1997) questionnaire at baseline, Day 1 and Day 90. The questionnaire was fulfilled independently by the patient in a quiet, separate room.

2.4 *Randomization and allocation concealment*

Participants were randomly allocated in a 1:1 ratio to receive either FM-SRP or Q-SRP therapy by using a computer-generated random sequence. Allocation to treatment was concealed in consecutively numbered opaque envelopes and revealed to the therapist and patient on the day of the treatment.

2.5 *Interventions*

2.5.1 *Periodontal treatment*

All patients received Step 1 and Step 2 of treatment according to EFP guidelines (Sanz et al., 2020). Thus Step 1 was implemented with oral Hygiene Instructions (OHI) and motivation sessions. Patients were instructed to use a rotating oscillating toothbrush (Oral B®) and interproximal brushes; OHIs were reinforced during each treatment session and follow up visits. Supra and subgingival mechanical instrumentation (Step 2) of the root surface was performed by a single periodontist, using both hand and ultrasonic instrumentation with fine tips (EMS, Nyon, Switzerland); local anesthesia was performed only when needed. During the clinical sessions, treatment time was measured with a chronometer by a research nurse. FM-SRP was performed within a single session. Q-SRP was performed one quadrant per session, with an interval on 1 week in between instrumentation sessions for a total of 3 weeks. The first session always comprised the instrumentation of the upper right quadrant; alternatively, the left maxillary quadrant was chosen if less than 6 teeth were present.

2.6 *Follow ups*

Figure 1 shows the study outline. All patients were re-examined at Day1 and Day90. Day1 in the FM-SRP group was defined as 24 hours upon completion of the instrumentation, while Day1 in the Q-SRP group was defined as 24 hours after the first session of treatment. On the

other hand, Day90 in the FM-SRP was 90 days after the completion of the treatment, and Day90 in the Q-SRP was 90 days after the completion of the last session of treatment. Recorded variables at each timepoint are shown in Figure 1.

2.7 *Sample size*

Serum CRP levels at Day1 was the primary outcome. Sample size was estimated in order to detect an intergroup 3.5mg/l difference (with a Standard Deviation - SD - of 3) in serum CRP levels at Day1 (D'Aiuto et al., 2004; Graziani et al., 2015). The magnitude of the intergroup difference of the primary outcome was chosen on the basis of previous evidence of perturbation of the systemic inflammation and considering the physiological fluctuations of CRP values (Bogaty et al., 2005; D'Aiuto et al., 2004; Koenig et al., 2003). A power of 90% and an error alpha of 0.05 were considered in order to compensate for a 10% drop-out rate, a final sample of 20 participants per group was planned (STATA BE, version 17, StataCorp LP, Texas, USA).

2.8 *Statistical methods*

Before performing statistical analysis, data were verified through double data entry and proofreading. All analyses were performed through an *ad hoc* statistical software (STATA BE, version 17, StataCorp LP, Texas, USA) setting the level of significance at 5%. Continuous variables were expressed as mean and SD, while categorical variables were expressed as proportions. Variables were adjusted for confounders known to plausibly influence the outcome (*i.e.* age, BMI, gender, smoking, and baseline body temperature) (Fernandez-Real et al., 2001; Hajishengallis & Chavakis, 2021; Kahn et al., 2006; Luetragoon et al., 2018; Sebastiani et al., 2017). After verification of data distribution, the Student t-test for

independent samples was used for between-group comparisons (Mann Whitney U test for non-normal data), while the ANOVA with post-hoc Tukey tests (Kruskal Wallis and Dunn tests for non-normal data) were used for intragroup comparisons. Correlations between inflammatory markers and treatment time, periodontal parameters, vital signs, and endothelial function were performed with Spearman rank analyses. A multiple regression model (secondary analysis) was built to evaluate the predictive ability of the relative CRP difference at Day1 (Day1 concentration minus baseline, divided by baseline and multiplied by 100) on the absolute change in HbA1c (ΔHbA1c) between Day90 and baseline. The best model was chosen according to the lowest value of Cp Mallow's coefficient. Results of the OHIP-14 were presented as severity, *i.e.* the sum of the response codes to all 14 items, and as extent, *i.e.* the number of items identified as 'Fairly often' or 'Very often' (Tsakos et al., 2011).

3 Results

3.1 *Participant characteristics*

Three-hundred and twenty-seven individuals were screened for eligibility; then, 40 individuals were eventually enrolled in the current study (Supplementary Figure 1). No participant was lost to follow up at Day 1/Day90 and all the sample was included in the final analysis. The study sample was constituted mainly by males, obese, non-smoking subjects approximately in their sixth decade of life; no differences were present between the two groups at baseline (Table 1). In total 29 patients were diabetes-controlled as 10% percent of the participants in the control group and 18% of the test group had a Hb1Ac value higher than 53 mmol/M.

3.2 *Clinical parameters*

Treatment determined significant benefits in terms of periodontal parameters in both groups. Reductions in the percentage and number of pockets, plaque accumulation and bleeding on probing were noted at Day90 (p<0.01), with no intergroup differences (p>0.05) (Table 2).

3.2.1 *Serum markers and vital signs*

Results of serum makers and vital signs are shown in Table 3. FM-SRP group showed significantly higher CRP values compared to Q-SRP group (p<0.05) (5.55 (SD=6.27) and 4.33 (SD=6.76), respectively) (Table 3); the relative CRP increase at Day1 was significantly higher in the FM-SRP group than in the Q-SRP group and it was positively associated with treatment time (R=0.53; p<0.01) (Supplementary Table 1). FM-SRP group showed a significant increase in IL-6 concentration at Day1 (p<0.01).

At Day 90, HbA1c levels were significantly reduced by both treatment protocols (p<0.05) with no intergroup differences; the HbA1c reduction between baseline and Day90 (ΔHbA1c) was almost two times higher in the Q-SRP group (ΔHbA1c=-1.59 (SD=1.20)) compared to the FM-SRP group (ΔHbA1c=-0.8 (SD=0.95)) (p=0.04) (Table 4). Lipid fractions, SBP, DBP, heart rate and body temperature did not show any significant differences between study groups and across timepoints (p>0.05) (Table 3).

3.2.2 *Endothelial function*

Results of the endothelial function are reported in Supplementary Table 2. The FM-SRP group demonstrated a significant worsening in the absolute difference in FMD between baseline and Day1 (ΔFMD=-0.86 (SD=0.92)) compared to the Q-SRP group (ΔFMD=0.54 (SD=0.39))

(*p*=0.04) (Table 4). The absolute difference in FMD between Day90 and baseline was also improved, even though not significantly (Table 4).

3.2.3 *OHIP-14*

At 24 hours, the FM-SRP group demonstrated an increase in the OHIP-14 extent compared to baseline (*p*=0.04). Overall, both Q-SRP group and FM-SRP group showed a significant reduction in the OHIP-14 extent between baseline and Day90 (*p*=0.04), which was comparable across groups (*p*>0.05). Periodontal treatment significantly and comparably reduced the OHIP-14 severity at Day90 when performed with either the Q-SRP (*p*=0.03) or the FM-SRP protocol (*p*=0.04) (Table 2).

3.3 *Linear multiple regression model*

Results of the linear multiple regression model are shown in Table 5. The higher the relative CRP increase at Day1, the lower the reduction in HbA1c levels at Day90 (ΔHbA1c Day90-baseline) (*p*=0.02). Smoking status (smokers, *p*=0.01) and treatment group (FM-SRP, *p*=0.03) were significant predictors in this observation. The model was statistically significant (F=2.12; *p*=0.04) with an adjusted R^2 of 41.04%. Linear predictions of HbA1c levels at 3 months (ΔHbA1c Day 90-baseline) on the relative CRP increase at Day 1 are graphed in Figure 2.

4. Discussion

One-stage full-mouth non-surgical periodontal treatment is associated with a higher acute inflammatory response compared to quadrant delivery in subjects affected by periodontitis and type 2 diabetes mellitus in the first 24 post-operative hours. At 3 months, periodontal treatment was capable to ameliorate glycemic control and periodontal conditions in both

groups with no differences among them. However, subjects that experienced a higher acute systemic perturbation after treatment showed a lower reduction of glycated hemoglobin. Thus, patients undergoing quadrant treatment reduced glycated hemoglobin levels to a higher extent and showed an improvement in endothelial function.

One day after treatment, patients undergoing one-stage full-mouth instrumentation showed a significant perturbation of the acute-phase response characterized by an increase of CRP and IL-6 values, as already been noted in systemically healthy subjects (D'Aiuto et al., 2013; Graziani et al., 2015). The reason for such findings may be related to both post-operative bacteremia and the local trauma derived from the sub-gingival instrumentation, triggering a higher production of local cytokines, which finally induce a liver-originated acute phase proteins release (Heinrich et al., 1990; Ide et al., 2004). One-stage full-mouth deliveries may be associated with higher trauma as longer time of instrumentation is needed. Accordingly, treatment time was associated with higher post-operative increase of CRP, as noted in the current and in our previous trial (Graziani et al., 2015).

Conversely, conventional quadrant scaling determined only modest non-significant variations of acute phase proteins, thus confirming the findings comparing the same two protocols in systemically healthy subjects (Graziani et al., 2015). Plausibly, the extent of trauma, as measured by the reduced treatment time, and the bacteremia produced after instrumentation of one quadrant may not constitute a sufficient trigger to the liver-induced inflammation.

The acute inflammation noted after one-stage full-mouth treatment may determine some acute state of vascular dysfunction as it was noted by the significant reduction of FMD noted in this group in the first 24 hours after treatment and already noted in systemically healthy patients (Seinost et al., 2005; Tonetti, Aiuto, et al., 2007). This might be of importance if considering that subjects with diabetes retain an increased risk for cardiovascular events (Sarwar et al., 2010). Cautiously, cardiovascular safety of the longer sessions of periodontal treatment in co-morbid patients has been questioned due to the higher level of systemic perturbation noted and the transient endothelial impairment (Sanz et al., 2020).

Three months after treatment significant reductions of glycated hemoglobin was noted in both groups. This was expected and in line with the current knowledge (D'Aiuto et al., 2018; Madianos & Koromantzos, 2018). At 3 months, no difference among the two groups were noted. Nevertheless, a secondary analysis, indicated that significantly higher reduction was noted in the quadrant group, when compared to the full-mouth. Indeed, the multiple regression analysis indicated that the higher increase of post-operative CRP noted in the one-stage full-mouth delivery is correlated with a lower reduction of glycated hemoglobin 3 months after treatment. This might suggest that acute inflammatory events taking place in the 24 hours after treatment may determine metabolic alterations extending over few months. Smoking also appeared to be predictors of HbA1c changes at 3 months confirming its role as a major modifiable risk factor for glycemic control (Peng et al., 2018.; Szwarcbard et al., 2020). The study design allows only speculative attempts to understand such findings. Chronic higher levels of inflammatory markers are associated with metabolic deficiencies as noted in both diabetes and obesity-affected patients (Hotamisligil, 2017). The reasons for such correlation are not entirely understood, but it is likely that insulin sensitivity may be

influenced by systemic inflammation and CRP levels by alteration of various key steps in the insulin signaling pathway (Festa et al., 2000). This is particularly evident in obese subjects bearing in mind the fact that the population of our trial appeared to be borderline obese. Thus, it may be speculated that the perturbation of systemic inflammation may exert a temporary action on such pathways and thus further analysis is advocated.

In the current trial, periodontal treatment was successfully conducted in both groups. No differences among the two groups were also noted, further confirming the available data indicating the comparable performance of both treatments (Eberhard et al., 2008; Suvan et al., 2020). Moreover, periodontal treatment determined a significant amelioration of oral health-related quality of life both in terms of severity and extent of the involved psychometric testing. The reduction noted is considered to a level that is clinically meaningful for the patient (Graziani & Tsakos, 2020). Once again, no differences were noted among the two groups except for some alteration of quality of life noted one day after treatment in the one-stage full-mouth group.

No other trial investigated the acute inflammatory effects of periodontal treatment in patients affected by type 2 diabetes mellitus. However, the reader should bear in mind some obvious limitations. The observed treatment effect - in terms of CRP levels at Day 1 - was smaller than the one hypothesized in the sample size calculation (3.5 mg/L vs 1.22 mg/L), and this may have reduced the statistical power for some estimates. From a biological standpoint, this might be due to the already high level of baseline CRP values that was found confirming that patients affected by diabetes have a higher level of systemic inflammation when compared to non-affected ones. Thus, it might be speculated that the intergroup difference

noted in this trial might be hampered by an already high baseline value of CRP. Nonetheless from a statistical standpoint, despite the high risk of Type II error, the current study was able to note a statistically significant difference CRP levels at Day 1 between groups; in addition, the non-standardized *post-hoc* power calculation - performed considering 1.22 mg/L as intergroup CRP difference at Day 1 - resulted in a power >90%. Furthermore, the comparison is made between the acute-phase response after full-mouth instrumentation and the one recorded after instrumentation of the first quadrant, assuming that after instrumentation of the other quadrants a similar inflammatory reaction might occur; however, this data was not collected and intermediate follow-ups between Day 1 and Day 90 were not performed. Another limitation regards the use of surrogate variables of systemic inflammation and endothelial function (i.e., CRP and FMD), whose inherent inter- and intra-subject variability hampers their application in the daily clinical practice within the medical field.

In addition, the relationship between the immediate postoperative acute inflammatory reaction and the HbA1c changes was evaluated on a medium-term follow up (3 months); therefore, the possibility that these detected differences between the two treatment deliveries may level out during longer follow ups (6 months/1 year) cannot be excluded.

Lastly, the reader should consider that the study was designed before the implementation of the current classification system; hence, participants were staged and graded in a *post-hoc* fashion using the available periodontal charts and intraoral radiographs.

Conclusions

In conclusion, one-stage full-mouth periodontal treatment may determine a higher transient acute systemic inflammation and an endothelial dysfunction in the first day after treatment to an extent that is approximately 7-times higher than the one noted after quadrant

treatment. Moreover, an inferior reduction of glycated hemoglobin was noted in this group and further analysis indicated that subjects with higher level of immediate post-operative inflammation are related with inferior benefits in terms of glycemic control. Hence, these findings possibly suggest that in subjects affected by periodontitis and type 2 diabetes mellitus a conventional treatment should be preferred in order to maximize the systemic benefits of periodontal treatment.

References

Bogaty, P., Brophy, J. M., Boyer, ; Luce, Simard, S., Joseph, L., Fernand Bertrand, ;, & Dagenais, G. R. (n.d.). *Fluctuating Inflammatory Markers in Patients With Stable Ischemic Heart Disease*. http://archinte.jamanetwork.com/

D'Aiuto, F., Gkranias, N., Bhowruth, D., Khan, T., Orlandi, M., Suvan, J., Masi, S., Tsakos, G., Hurel, S., Hingorani, A. D., Donos, N., Deanfield, J. E., Lomax, A., Horvath, A., Zambon, R., Tay, S., Tatarakis, N., Spratt, D., Kingston, I., … Mellor, T. (2018). Systemic effects of periodontitis treatment in patients with type 2 diabetes: a 12 month, single-centre, investigator-masked, randomised trial. *The Lancet Diabetes and Endocrinology*, *6*(12), 954–965. https://doi.org/10.1016/S2213-8587(18)30038-X

D'Aiuto, F., Nibali, L., Mohamed-Ali, V., Vallance, P., & Tonetti, M. S. (2004). Periodontal therapy: a novel non-drug-induced experimental model to study human inflammation. *Journal of Periodontal Research*, *39*(5), 294–299. https://doi.org/10.1111/j.1600-0765.2004.00741.x

D'Aiuto, F., Orlandi, M., & Gunsolley, J. C. (2013a). Evidence that periodontal treatment improves biomarkers and CVD outcomes. *Journal of Clinical Periodontology*, *40*(SUPPL. 14). https://doi.org/10.1111/jcpe.12061

D'Aiuto, F., Orlandi, M., & Gunsolley, J. C. (2013b). Evidence that periodontal treatment improves biomarkers and CVD outcomes. In *Journal of Clinical Periodontology* (Vol. 40, Issue SUPPL. 14). https://doi.org/10.1111/jcpe.12061

D'Aiuto, F., Parkar, M., & Tonetti, M. S. (2005). Periodontal therapy: A novel acute inflammatory model. *Inflammation Research*, *54*(10), 412–414. https://doi.org/10.1007/s00011-005-1375-4

Eberhard, J., Jervøe-Storm, P. M., Needleman, I., Worthington, H., & Jepsen, S. (2008). Full-mouth treatment concepts for chronic periodontitis: A systematic review. *Journal of Clinical Periodontology*, *35*(7), 591–604. https://doi.org/10.1111/j.1600-051X.2008.01239.x

Fernandez-Real, J.-M., Vayreda, M., Richart, C., Gutierrez, C., Broch, M., Vendrell, J., & Ricart, W. (2001). *Circulating Interleukin 6 Levels, Blood Pressure, and Insulin Sensitivity in Apparently Healthy Men and Women*. https://academic.oup.com/jcem/article/86/3/1154/2847633

Festa, A., D'Agostino, R., Howard, G., Mykkänen, L., Tracy, R. P., & Haffner, S. M. (2000). Chronic subclinical inflammation as part of the insulin resistance syndrome: The insulin resistance atherosclerosis study (IRAS). *Circulation*, *102*(1), 42–47. https://doi.org/10.1161/01.CIR.102.1.42

Graziani, F., Cei, S., la Ferla, F., Vano, M., Gabriele, M., & Tonetti, M. (2010). Effects of non-surgical periodontal therapy on the glomerular filtration rate of the kidney: An exploratory trial. *Journal of Clinical Periodontology*, *37*(7), 638–643. https://doi.org/10.1111/j.1600-051X.2010.01578.x

Graziani, F., Cei, S., Orlandi, M., Gennai, S., Gabriele, M., Filice, N., Nisi, M., & D'Aiuto, F. (2015). Acute-phase response following full-mouth versus quadrant non-surgical periodontal treatment: A randomized clinical trial. *Journal of Clinical Periodontology*, *42*(9), 843–852. https://doi.org/10.1111/jcpe.12451

Graziani, F., & Tsakos, G. (2020). Patient-based outcomes and quality of life. In *Periodontology 2000* (Vol. 83, Issue 1, pp. 277–294). Blackwell Munksgaard. https://doi.org/10.1111/prd.12305

Hajishengallis, G., & Chavakis, T. (2021). Local and systemic mechanisms linking periodontal disease and inflammatory comorbidities. In *Nature Reviews Immunology* (Vol. 21, Issue 7, pp. 426–440). Nature Research. https://doi.org/10.1038/s41577-020-00488-6

Heinrich, P. C., Castellt, J. v, & Andust, T. (1990). and the acute phase response. In *Printed in Great Britain) REVIEW ARTICLE Interleukin* (Vol. 265, Issue 6).

Hotamisligil, G. S. (2017). Inflammation, metaflammation and immunometabolic disorders. *Nature*, *542*(7640), 177–185. https://doi.org/10.1038/nature21363

Ide, M., Jagdev, D., Coward, P. Y., Crook, M., Barclay, G. R., & Wilson, R. F. (2004). The Short-Term Effects of Treatment of Chronic Periodontitis on Circulating Levels of Endotoxin, C-Reactive

Protein, Tumor Necrosis Factor-α, and Interleukin-6. *Journal of Periodontology*, *75*(3), 420–428. https://doi.org/10.1902/jop.2004.75.3.420

Kahn, S. E., Hull, R. L., & Utzschneider, K. M. (2006). Mechanisms linking obesity to insulin resistance and type 2 diabetes. In *Nature* (Vol. 444, Issue 7121, pp. 840–846). Nature Publishing Group. https://doi.org/10.1038/nature05482

Koenig, W., Sund, M., Fröhlich, M., Löwel, H., Hutchinson, W. L., & Pepys, M. B. (2003). Refinement of the association of serum C-reactive protein concentration and coronary heart disease risk by correction for within-subject variation over time: The MONICA Augsburg studies, 1984 and 1987. *American Journal of Epidemiology*, *158*(4), 357–364. https://doi.org/10.1093/aje/kwg135

Luetragoon, T., Rutqvist, L. E., Tangvarasittichai, O., Andersson, B. Å., Löfgren, S., Usuwanthim, K., & Lewin, N. L. (2018). Interaction among smoking status, single nucleotide polymorphisms and markers of systemic inflammation in healthy individuals. *Immunology*, *154*(1), 98–103. https://doi.org/10.1111/imm.12864

Madianos, P. N., & Koromantzos, P. A. (2018). An update of the evidence on the potential impact of periodontal therapy on diabetes outcomes. *Journal of Clinical Periodontology*, *45*(2), 188–195. https://doi.org/10.1111/jcpe.12836

Moher, D., Hopewell, S., Schulz, K. F., Montori, V., Gøtzsche, P. C., Devereaux, P. J., Elbourne, D., Egger, M., & Altman, D. G. (2010). CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. *BMJ (Clinical Research Ed.)*, *340*, c869. https://doi.org/10.1136/bmj.c869

Peng, K., Chen, G., Liu, C., Mu, Y., Ye, Z., Shi, L., Zhao, J., Chen, L., Li, Q., Yang, T., Yan, L., Wan, Q., Wu, S., Wang, G., Luo, Z., Tang, X., Huo, Y., Gao, Z., Su, Q., … Ning, G. (n.d.). *Association between Smoking and Glycemic Control in Diabetic Patients: Results from the REACTION study Runing title: Smoking and Glycemic control*. https://doi.org/10.1111/jdb.12625

Sanz, M., Herrera, D., Kebschull, M., Chapple, I., Jepsen, S., Beglundh, T., Sculean, A., Tonetti, M. S., Merete Aass, A., Aimetti, M., Kuru, B. E., Belibasakis, G., Blanco, J., Bol-van den Hil, E., Bostanci, N., Bozic, D., Bouchard, P., Buduneli, N., Cairo, F., … Wennström, J. (2020a). Treatment of stage I–III periodontitis—The EFP S3 level clinical practice guideline. *Journal of Clinical Periodontology*, *47*(S22), 4–60. https://doi.org/10.1111/jcpe.13290

Sanz, M., Herrera, D., Kebschull, M., Chapple, I., Jepsen, S., Beglundh, T., Sculean, A., Tonetti, M. S., Merete Aass, A., Aimetti, M., Kuru, B. E., Belibasakis, G., Blanco, J., Bol-van den Hil, E., Bostanci, N., Bozic, D., Bouchard, P., Buduneli, N., Cairo, F., … Wennström, J. (2020b). Treatment of stage I–III periodontitis—The EFP S3 level clinical practice guideline. *Journal of Clinical Periodontology*, *47*(S22), 4–60. https://doi.org/10.1111/jcpe.13290

Sanz, M., Marco del Castillo, A., Jepsen, S., Gonzalez-Juanatey, J. R., D'Aiuto, F., Bouchard, P., Chapple, I., Dietrich, T., Gotsman, I., Graziani, F., Herrera, D., Loos, B., Madianos, P., Michel, J. B., Perel, P., Pieske, B., Shapira, L., Shechter, M., Tonetti, M., … Wimmer, G. (2020). Periodontitis and cardiovascular diseases: Consensus report. *Journal of Clinical Periodontology*, *47*(3), 268–288. https://doi.org/10.1111/jcpe.13189

Sarwar, N., Gao, P., Kondapally Seshasai, S. R., Gobin, R., Kaptoge, S., di Angelantonio, E., Ingelsson, E., Lawlor, D. A., Selvin, E., Stampfer, M., Stehouwer, C. D. A., Lewington, S., Pennells, L., Thompson, A., Sattar, N., White, I. R., Ray, K. K., Danesh, J., Tipping, R. W., … Wormser, D. (2010). Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: A collaborative meta-analysis of 102 prospective studies. *The Lancet*, *375*(9733), 2215–2222. https://doi.org/10.1016/S0140-6736(10)60484-9

Sebastiani, P., Thyagarajan, B., Sun, F., Schupf, N., Newman, A. B., Montano, M., & Perls, T. T. (2017). Biomarker signatures of aging. *Aging Cell*, *16*(2), 329–338. https://doi.org/10.1111/acel.12557

Seinost, G., Wimmer, G., Skerget, M., Thaller, E., Brodmann, M., Gasser, R., Bratschko, R. O., & Pilger, E. (2005). Periodontal treatment improves endothelial dysfunction in patients with severe periodontitis. *American Heart Journal*, *149*(6), 1050–1054. https://doi.org/10.1016/j.ahj.2004.09.059

Suvan, J., Leira, Y., Moreno Sancho, F. M., Graziani, F., Derks, J., & Tomasi, C. (2020a). Subgingival instrumentation for treatment of periodontitis. A systematic review. *Journal of Clinical Periodontology*, *47 Suppl 2*, 155–175. https://doi.org/10.1111/jcpe.13245

Suvan, J., Leira, Y., Moreno Sancho, F. M., Graziani, F., Derks, J., & Tomasi, C. (2020b). Subgingival instrumentation for treatment of periodontitis. A systematic review. In *Journal of Clinical Periodontology* (Vol. 47, Issue S22, pp. 155–175). Blackwell Munksgaard. https://doi.org/10.1111/jcpe.13245

Szwarcbard, N., Villani, M., Earnest, A., Flack, J., Andrikopoulos, S., Wischer, N., Soldatos, G., Gasevic, D., & Zoungas, S. (2020). The association of smoking status with glycemic control, metabolic profile and diabetic complications– Results of the Australian National Diabetes Audit (ANDA). *Journal of Diabetes and Its Complications*, *34*(9). https://doi.org/10.1016/j.jdiacomp.2020.107626

Tonetti, M. S., Aiuto, F. D. ', Nibali, L., Donald, A., Storry, C., Parkar, M., Phil, M., Suvan, J., Hingorani, A. D., Vallance, P., & Deanfield, J. (2007). *Treatment of Periodontitis and Endothelial Function A BS T R AC T*. www.nejm.org

Tonetti, M. S., D'Aiuto, F., Nibali, L., Donald, A., Storry, C., Parkar, M., Suvan, J., Hingorani, A. D., Vallance, P., & Deanfield, J. (2007). Treatment of periodontitis and endothelial function. *The New England Journal of Medicine*, *356*(9), 911–920. https://doi.org/10.1056/NEJMoa063186

Tonetti, M. S., Eickholz, P., Loos, B. G., Papapanou, P., van der Velden, U., Armitage, G., Bouchard, P., Deinzer, R., Dietrich, T., Hughes, F., Kocher, T., Lang, N. P., Lopez, R., Needleman, I., Newton, T., Nibali, L., Pretzl, B., Ramseier, C., Sanz-Sanchez, I., … Suvan, J. E. (2015). Principles in prevention of periodontal diseases: Consensus report of group 1 of the 11th European Workshop on Periodontology on effective prevention of periodontal and peri-implant diseases. *Journal of Clinical Periodontology*, *42*(S16), S5–S11. https://doi.org/10.1111/jcpe.12368

Tonetti, M. S., Greenwell, H., & Kornman, K. S. (2018). Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. *Journal of Clinical Periodontology*, *45*(January), S149–S161. https://doi.org/10.1111/jcpe.12945

Figure legends

Figure 1. Flow chart of the study design.

Figure 2. linear predictions of HbA1c levels at 3 months (Day 90) (ΔHbA1c Day 90-baseline) (y axis) on the relative C-reactive protein (CRP) increase at Day 1 (x axis). Covariates were dichotomized as follows: i) Group (0=Q-SRP; 1=FM-SRP); ii) Age (0 if age<55 years old; 1 if age≥55 years old); iii) Smoking Habit (SH) (0=non-smokers; 1=smokers); iv) BMI (0 if BMI<25; 1 if BMI≥25).

Table 1: patients characteristics by study group.

Variable	Quadrant Mean (SD)/N(%)	Full-mouth Mean (SD)/N(%)	p-value
Age (years)	62.45 (9.60)	56.89 (10.37)	0.09
Gender			
Males	12 (60%)	12 (63.16%)	0.55
Females	8 (40%)	7 (36.84%)	
BMI	29.22 (6.00)	29.47 (7.45)	
Regular weight	2 (10%)	1 (5%)	
Overweight	4 (20%)	7 (35%)	0.93
Obese	14 (70%)	12 (60%)	
Smoking			
Yes	5 (25%)	8 (42.11%)	0.32
No	15 (75%)	11 (57.89%)	
Smoking duration (years)	22.33 (9.29)	26.83 (18.82)	
Number of cigarettes/day	21.25 (6.29)	12.38 (6.55)	0.51
Number of teeth	22.4 (4.42)	24.3 (4.78)	0.19
Patients undergoing statins	6 (30.0%)	4 (20.0%)	0.39
Periodontitis stage (EFP/AAP)			
Stage III	9 (45.0%)	12 (60.0%)	0.19
Stage IV	11 (55.0%)	8 (40.0%)	
Periodontitis grade (EFP/AAP)			
Grade B	9 (45.0%)	10 (50.0%)	0.32
Grade C	11 (55.0%)	10 (50.0%)	
Treatment time per session (min)	27.25 (7.39)	57.59 (13.22)	***<0.01***

Abbreviations: BMI, Body Mass Index; SD, Standard Deviation;

Table 2. periodontal parameters and Oral Health Impact Profile 14 between groups and at each timepoint. Values with different superscript letters are different at the 5% level.

Variable	Timepoint	Mean (SD)		*p*-value between groups	MD (95% CI)
		Q-SRP	FM-SRP		
N. pockets<4mm	*Baseline*	58.9 (32.96)	78.4 (36.92)	0.09	19.50 (-2.90, 41.90)
	Day90	113.67 (26.44)	108.55 (39.03)	0.06	-5.12 (-26.45, 16.22)
	p-value intragroup	***<0.01***	***<0.01***		
N. pockets≥4mm	*Baseline*	75.5 (37.85)	66.95 (21.21)	0.38	-8.55 (-28.19, 11.09)
	Day90	22.67 (19.37)	37.25 (24.62)	0.07	14.58 (0.39, 28.76)
	p-value intragroup	***<0.01***	***<0.01***		
N. pockets≥5mm	*Baseline*	56.65 (40.72)	43.5 (24.66)	0.22	-13.15 (-34.70, 8.40)
	Day90	10.11 (10.34)	20.9 (16.10)	0.08	10.79 (2.13, 19.45)
	p-value intragroup	***<0.01***	***<0.01***		
N. pockets≥6mm	*Baseline*	15.75 (14.37)	15.65 (10.89)	0.98	-0.10 (-8.26, 8.06)
	Day90	2.78 (2.28)	4.65 (3.86)	0.08	1.87 (-0.16, 3.90)
	p-value intragroup	***<0.01***	***<0.01***		
%PD<4mm	*Baseline*	44.21 (23.92)	51.64 (18.39)	0.28	7.43 (-6.23, 21.09)
	Day90	83.31 (13.31)	73.06 (18.53)	0.09	-10.25 (-10.58, 0.78)
	p-value intragroup	***<0.01***	***<0.01***		
%PD≥4mm	*Baseline*	55.78 (23.92)	47.94 (17.60)	0.24	-7.84 (-21.28, 5.60)
	Day90	16.58 (13.36)	26.94 (18.53)	0.06	10.36 (0.02, 20.70)
	p-value intragroup	***<0.01***	***<0.01***		
%PD≥5mm	*Baseline*	41.61 (27.39)	32.14 (19.96)	0.22	-9.47 (-24.81, 5.87)
	Day90	7.52 (7.46)	15.11 (12.06)	0.10	7.59 (1.17, 14.01)
	p-value intragroup	***0.01***	***<0.01***		
%PD≥6mm	*Baseline*	11.63 (9.34)	11.52 (8.33)	0.97	-0.11 (-5.78, 5.56)
	Day90	2.11 (1.77)	3.52 (3.02)	0.07	1.41 (-0.17, 2.99)
	p-value intragroup	***<0.01***	***<0.01***		
FMPS (%)	*Baseline*	83.58 (30.54)	79.51 (26.39)	0.65	-4.07 (-22.34, 14.20)
	Day90	33.36 (23.13)	37.25 (26.89)	0.28	3.89 (-12.17, 19.95)
	p-value intragroup	***<0.01***	***<0.01***		
FMBS (%)	*Baseline*	68.98 (32.59)	56.73 (21.83)	0.17	-12.25 (-30.01, 5.51)
	Day90	14.00 (12.60)	23.85 (18.11)	0.78	9.85 (-0.14, 19.84)
	p-value intragroup	***<0.01***	***<0.01***		
Mean PD (mm)	*Baseline*	3.96 (0.69)	3.74 (0.64)	0.30	-0.22 (-0.65, 0.21)
	Day90	2.78 (0.34)	3.04 (0.55)	0.06	0.26 (-0.03, 0.55)
	p-value intragroup	***<0.01***	***<0.01***		
Mean REC (mm)	*Baseline*	0.52 (0.76)	0.54 (0.62)	0.93	0.02 (-0.42, 0.46)
	Day90	0.89 (0.85)	0.99 (0.92)	0.10	0.10 (-0.47, 0.67)
	p-value intragroup	***<0.001***	***0.001***		
Mean CAL (mm)	*Baseline*	4.48 (1.08)	4.29 (1.01)	0.55	-0.19 (-0.86, 0.48)
	Day90	3.68 (0.91)	4.03 (1.26)	0.07	0.35 (-0.35, 1.05)
	p-value intragroup	***<0.01***	***<0.01***		

OHIP-14 Severity†‡	*Baseline*	8.56 (4.89)[a]	7.14 (4.48)[a]	0.19	-1.42 (-4.42, 1.58)
	Day1	5.81 (3.35)[a]	6.54 (4.28)[a,b]	0.35	0.73 (-1.73, 3.19)
	Day90	4.75 (2.14)[b]	5.59 (2.32)[b]	0.61	0.84 (-0.59, 2.27)
	p-value intragroup	***0.03***	***0.03***		
OHIP-14 Extent†‡	*Baseline*	0.55 (0.23)[a]	0.60 (0.68)[a]	0.09	0.05 (-0.27, 0.37)
	Day1	0.67 (1.03)[a]	1.35 (1.09)[b]	***0.04***	0.68 (0.001, 1.36)
	Day90	0.32 (1.44)[b]	0.43 (1.09)[c]	0.39	0.11 (-0.71, 0.93)
	p-value intragroup	***0.04***	***0.04***		
Perceived Pain†‡	*Day1*	5.37 (2.55)	5.43 (1.79)	0.99	0.06 (-1.35, 1.47)

Abbreviations: CAL, Clinical Attachment Level; FM-SRP, Full-Mouth Scaling and Root Planing; FMPS, Full Mouth Plaque Score, FMBS, Full Mouth Bleeding Score; MD, difference in means; OHIP, Oral Health Impact Profile; PD, Probing Depth; Q-SRP, Quadrant Scaling and Root Planing; REC, Recession; SD, Standard Deviation.

Notes: All *p*-values refer to intra- and inter-group differences adjusted for baseline characteristics (age, BMI, gender, smoking and baseline body temperature), except when otherwise specified. Values with different superscript letters are different at the 5% level.

† Unadjusted *p*-values.

‡ n=38 at Day 90.

Table 3: vital signs and serum makers between study groups and at each timepoint. Values with different superscript letters are different at the 5% level.

Variable	Timepoint	Mean (SD)		*p*-value	MD (95% CI)
		Q-SRP	FM-SRP	between groups	
Systolic blood pressure (mmHg)	*Baseline*	128.5 (14.15)	130.79 (14.93)	0.63	2.29 (-7.02, 11.60)
	Day1	128.61 (13.91)	132.22 (16.29)	0.26	3.61 (-6.09, 13.31)
	Day90	125.29 (27.35)	129.75 (18.88)	0.12	4.46 (-10.58, 19.50)
	p-value intragroup	1.00	0.26		
Diastolic blood pressure (mmHg)	*Baseline*	78.75 (8.72)	75.11 (11.09)	0.26	-3.64 (-10.03, 2.75)
	Day1	77.78 (9.43)	72.22 (9.11)	0.15	-5.56 (-11.50, 0.38)
	Day90	74.94 (12.50)	74.6 (11.32)	0.17	-0.34 (-7.98, 7.29)
	p-value intragroup	0.43	0.45		
Heart rate (bpm)	*Baseline*	72.6 (13.36)	73.58 (13.83)	0.82	-5.02 (-13.72, 3.68)
	Day1	71.55 (8.75)	74.11 (13.61)	0.31	2.56 (-4.76, 9.88)
	Day90	71 (12.28)	70.65 (11.66)	0.08	-0.35 (-8.02, 7.32)
	p-value intragroup	0.19	0.84		
Body temperature (°C)	*Baseline*	36.46 (0.35)	36.47 (0.38)	0.30	0.01 (-0.22, 0.24)
	Day1	36.42 (0.39)	36.59 (0.31)	0.41	0.17 (-0.06, 0.40)
	Day90	36.38 (0.37)	36.47 (0.45)	0.97	0.09 (-0.17, 0.35)
	p-value intragroup	0.96	0.64		
Glucose (mg/dL)	*Baseline*	105.37 (16.76)	115.53 (36.50)	0.28	10.16 (-8.02, 28.34)
	Day1	113.79 (46.05)	120.5 (44.95)	0.21	6.71 (-22.42, 35.84)
	Day90	108.94 (18.98)	114.1 (40.53)	0.12	5.16 (-15.10, 25.42)
	p-value intragroup	0.19	0.06		
Cholesterol (mg/dL)	*Baseline*	165 (38.78)	187.55 (39.23)	0.08	22.55 (-2.42, 47.52)
	Day1	165.05 (37.74)	184.4 (34.59)	0.28	19.35 (-3.82, 42.52)
	Day90	162.66 (40.69)	182.15 (33.95)	0.08	19.49 (-4.49, 43.48)
	p-value intragroup	1.00	0.84		
HDL (mg/dL)	*Baseline*	48.6 (10.97)	50.5 (12.90)	0.62	1.90 (-5.77, 9.57)
	Day1	47.95 (9.55)	50.85 (13.01)	0.69	2.90 (-4.41, 10.21)
	Day90	46.55 (12.24)	50.55 (9.87)	0.78	4.00 (-3.12, 11.12)
	p-value intragroup	0.80	0.50		
Triglycerides (mg/dL)	*Baseline*	136.8 (90.13)	156 (46.70)	0.62	19.20 (-26.75, 65.15)
	Day1	128.35 (67.42)	142.45 (96.41)	0.48	14.10 (-39.15, 67.35)
	Day90	146.77 (58.14)	142.35 (51.30)	0.97	-4.42 (-39.52, 30.68)
	p-value intragroup	0.43	0.59		
HbA1c, mmol/M	*Baseline*	48.2 (9.01)	51.4 (15.20)	0.42	3.20 (-4.79, 11.19)
	Day1	-	-		
	Day90	46.61 (6.52)	50.6 (11.42)	0.74	3.99 (-1.96, 9.94)
	p-value intragroup	***0.02***	***0.04***		
HbA1c, %	*Baseline*	6.1 (1.09)	6.9 (2.50)	0.39	0.80 (-0.43, 2.03)
	Day1	-	-		
	Day90	6.4 (1.7)	6.8 (3.2)	0.74	0.40 (-1.2, 2.00)

	p-value intragroup	***0.04***	0.06		
Insulin (μU/mL)	*Baseline*	13.35 (11.10)	14.12 (10.66)	0.83	0.77 (-6.19, 7.74)
	Day1	9.65 (5.35)	12.15 (9.02)	0.42	2.5 (-2.25, 7.25)
	Day90	12.44 (7.98)	12.63 (8.62)	0.15	0.19 (-5.13, 5.51)
	p-value intragroup	0.50	0.48		
CRP (mg/L)	*Baseline*	4.20 (4.48)	3.32 (6.18)[a]	0.61	-0.88 (-4.33, 2.58)
	Day1	4.33 (3.76)	5.55 (4.27)[b]	***0.04***	1.22 (0.95, 5.39)
	Day90	3.98 (6.16)	3.67 (5.73)[a,b]	0.19	0.31 (-4.12, 3.50)
	p-value intragroup	0.06	***<0.01***		
Relative CRP increase (Day 1, %)†	-	17.87 [-3.45, 80.00]	86.47 [32.01, 233.10]	***0.03***	215.39 (10.93, 419.85)
IL-6 (pg/mL)	*Baseline*	5.21 (4.74)	3.63 (3.28)[a]	0.25	-1.58 (-4.19, 1.03)
	Day1	5.45 (6.37)	4.69 (5.34)[b]	0.26	-0.76 (-4.52, 3.00)
	Day90	4.38 (4.30)	4.52 (7.75)[a,b]	0.18	0.14 (-3.87, 4.15)
	p-value intragroup	0.92	***<0.01***		
Relative IL-6 increase (Day 1, %)†	-	-18.46 [-54.84, 71.43]	-10.26 [-33.52, 38.58]	0.69	17.60 (-73.61, 108.81)

Abbreviations: bpm, beats per minute; FM-SRP, Full-Mouth Scaling and Root Planing; Q-SRP, Quadrant Scaling and Root Planing; SD, Standard Deviation.

Notes: All *p*-values refer to intra- and inter-group differences adjusted for baseline characteristics (age, BMI, gender, smoking and baseline body temperature). Values with different superscript letters are different at the 5% level.

† Variable expressed as median [interquartile range].

Table 4: absolute changes of specific parameters between groups over timepoints.

Variables	Group	Mean±SD Day1-BL	*p*-value	Mean±SD Day90-Day1	*p*-value	Mean±SD Day90-BL	*p*-value
Periodontal parameters							
%PD≥4mm	*Q-SRP*	-	-	-	-	-39.20 (27.4)	0.06†
	FM-SRP	-		-		-21.00 (25.57)	
%PD≥5mm	*Q-SRP*	-	-	-	-	-34.09 (28.38)	0.07†
	FM-SRP	-		-		-17.03 (23.34)	
%PD≥6mm	*Q-SRP*	-	-	-	-	-9.52 (8.47)	0.58†
	FM-SRP	-		-		-8.00 (8.85)	
Serum markers							
ΔHbA1c (mmol/M)	*Q-SRP*	-	-	-	-	-1.59 (1.20)	***0.04***†
	FM-SRP	-		-		-0.8 (0.95)	
ΔCRP (mg/L)	*Q-SRP*	0.13±0.82	***0.02***†	-0.35 (0.82)	0.09†	-0.22 (1.41)	0.22†
	FM-SRP	2.23±1.05		-1.88 (1.74)		0.35 (1.26)	
ΔIL-6 (pg/mL)	*Q-SRP*	0.24±1.22	0.61†	-1.07 (2.02)	0.25†	-0.83 (2.79)	0.40†
	FM-SRP	1.06±3.79		0.83 (4.37)		0.89 (3.15)	
Endothelial function							
ΔFMD (%)	*Q-SRP*	0.54±0.39	***0.04***‡	0.4 (1.22)	0.58‡	0.94 (1.49)	0.95‡
	FM-SRP	-0.86±0.92		0.86 (1.08)		0 (2.52)	
ΔGTN-FMD	*Q-SRP*	-0.58±1.86	0.42§	1.58 (2.68)	0.51§	1 (2.24)	0.97§
	FM-SRP	-1.44±2.78		2.34 (3.10)		0.9 (1.35)	

Abbreviations: SD, Standard Deviation; HbA1c, glycated hemoglobin; CRP, C-reactive protein; FMD, flow-mediated dilation; GTN-FMD, glyceryl-trinitrate Flow Mediated Dilation.

† p-values adjusted for baseline characteristics (age, BMI, smoking, gender, baseline body temperature)

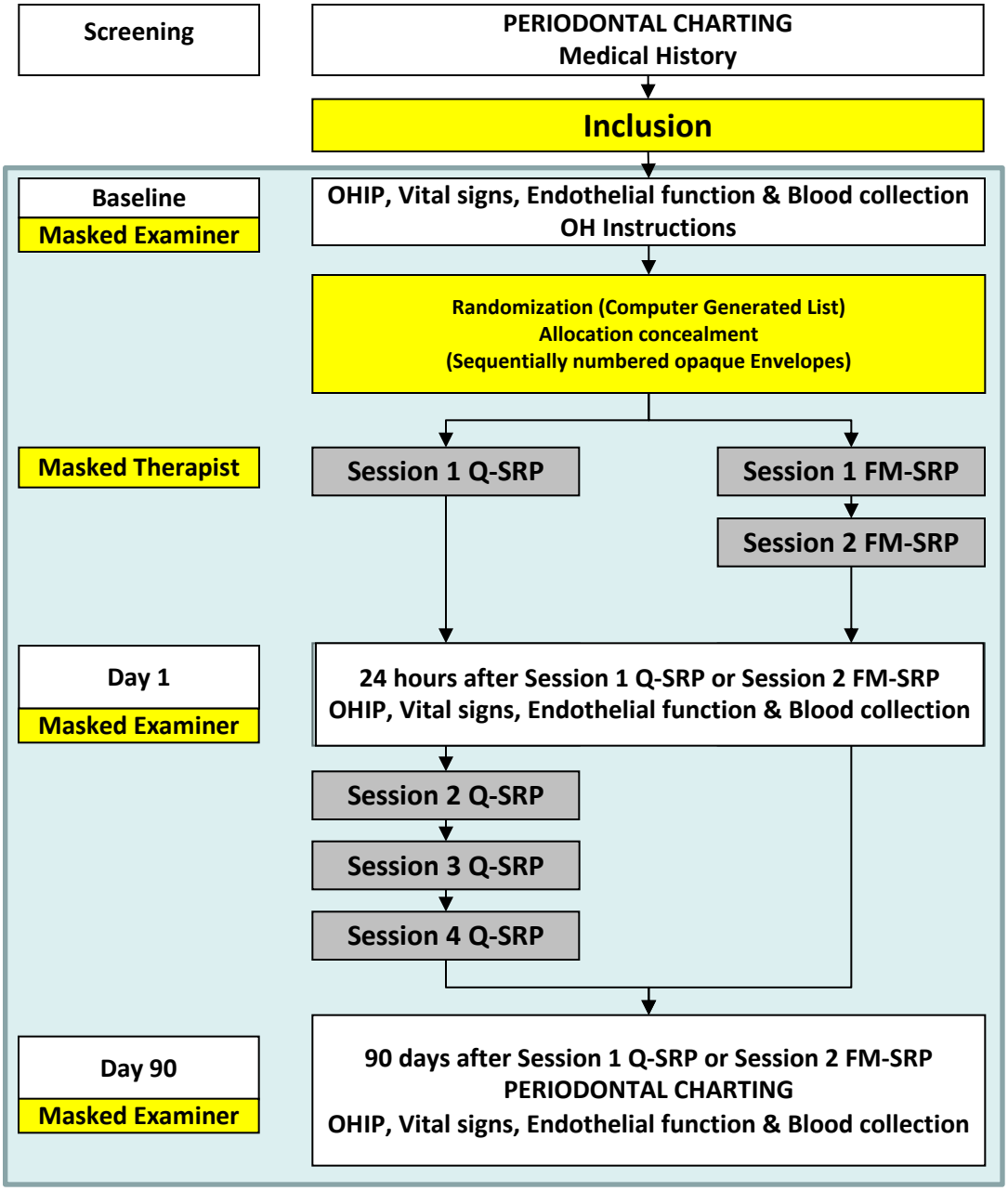
‡ p-values adjusted for baseline diameter.

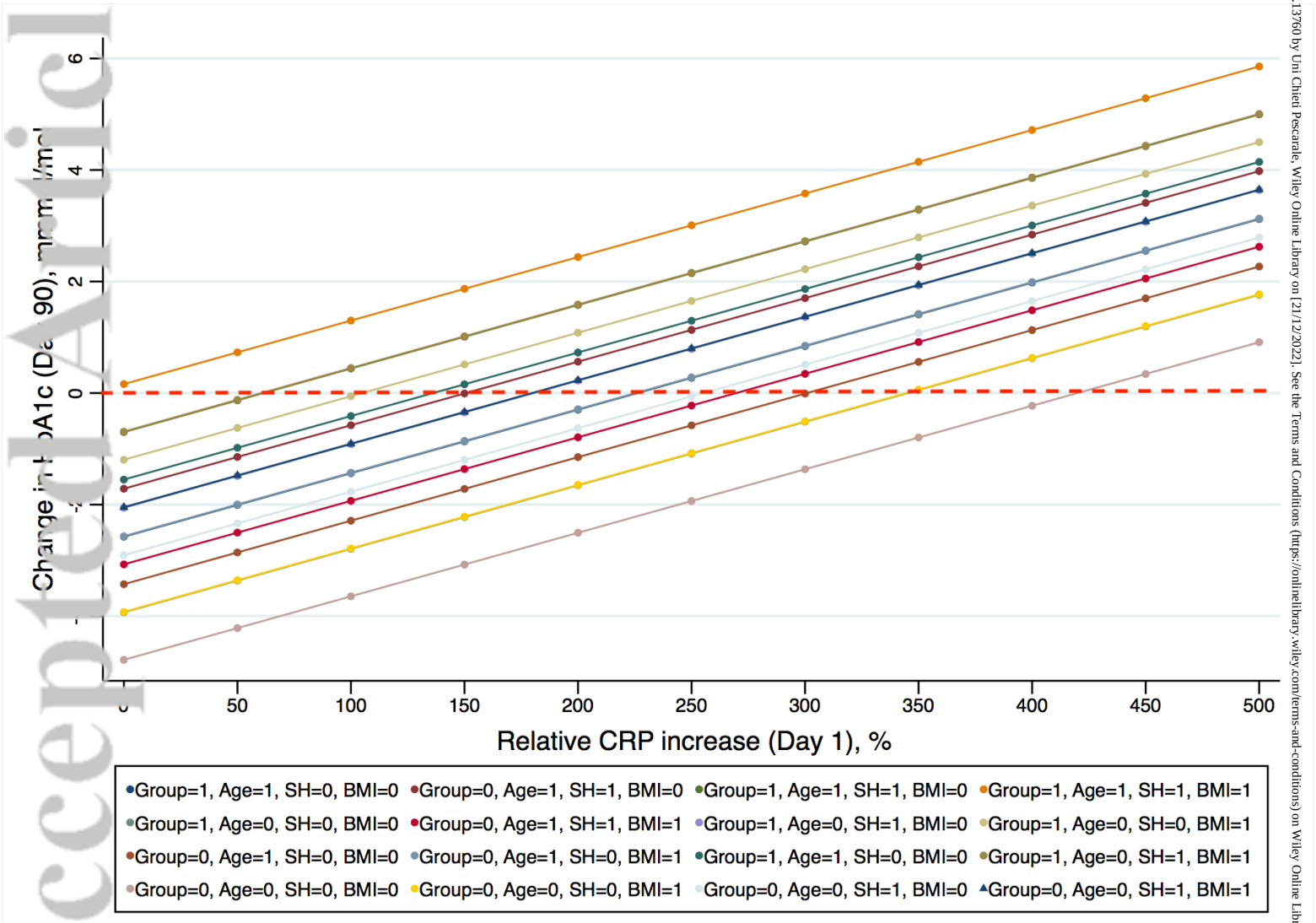
§ p-values adjusted for baseline GTN diameter.

Table 5: best model of the linear multivariate regression analysis evaluating the impact of the relative CRP increase at Day1 on the change in HbA1c levels at Day90 (ΔHbA1c baseline-Day90).

Best Model (Mallow's Cp= 4.31)						
					95% CI	
Predictors	**Coefficients**	**SE**	**t**	***p*-value**	**Lower**	**Upper**
(Constant)	-1.73	7.44	-0.23	0.82	-17.52	14.06
Relative CRP increase (% - Day1)	0.02	0.004	2.069	*0.02*	0.002	0.03
Age	0.03	0.11	0.32	0.75	0.19	0.26
Smoking	1.90	1.46	1.30	*0.01*	1.20	5.00
Group	0.95	1.31	0.49	*0.03*	0.43	2.13
BMI	1.04	1.27	0.82	0.43	-1.66	3.73
				Analysis of Variance		
			Source	**Sum of squares**	**df**	**Mean Square**
R^2	0.4984		Model	75.50	5	15.10
Adjusted R^2	0.4104		Residual	113.99	16	7.12
Root MSE	2.6692		Total	189.5	21	9.02
			*F=2.12; p=0.04**			

Abbreviations: CI, Confidence Interval; SE, Standard Error; Root MSE, Root Mean Square Error; df, degrees of freedom.





JCPE_13760_Figure 2.tif