

# Acute effects of periodontal therapy on bio-markers of vascular health

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## Abstract

**Aim:** We aimed to study the impact of periodontal therapy on several bio-markers related to vascular health.

**Material and Methods:** Fifty-five consecutive subjects (age range 30–64 years) having severe periodontitis received an intensive session of periodontal therapy under local anaesthesia and provided blood samples before and 1 and 7 and 30 days following therapy. High-sensitivity assays were utilized to quantify serum concentrations of inflammatory markers [interleukin(IL)-1 $\beta$ , tumour necrosis factor- $\alpha$ ], plasma haemostatic (D-dimer) and endothelial soluble markers [soluble E-selectin(s-Es) and von Willebrand factor antigen (r-WF:Ag)].

**Results:** Periodontal therapy elicited a significant activation of the haemostatic system (38% and 68% mean increases of plasma D-dimer 1 and 7 days after therapy, respectively,  $p < 0.001$ ), together with moderate endothelial dysfunction (10% and 30% mean increases at 24 h in plasma soluble E-selectin,  $p < 0.05$  and von-Willebrand factor,  $p < 0.01$ , respectively). D-Dimer and s-Es acute changes were significantly correlated with periodontal treatment time ( $p < 0.05$ ). Cigarette smoking status and body mass index strongly influenced the acute release of IL-1 $\beta$  ( $p < 0.05$ ), D-dimer ( $p < 0.01$ ) and s-Es ( $p < 0.01$ ).

**Conclusions:** Periodontal therapy represents a novel and reliable non-drug-induced model to investigate in vivo the interplay between inflammation, coagulation and endothelial cell activation.

Key words: coagulation; endothelial cell activation; experimental model; inflammation; periodontitis

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Over the years, several experimental models have been used to identify the complex mechanisms behind the host response to infection. Haemodynamic, endothelial and inflammatory changes have been partially replicated in human experimental models including the “stre-

nuous exercise” model, the vaccination model and the endotoxin model (Martich et al. 1993, Shek & Shephard 1998, Shephard & Shek 1998, Hingorani et al. 2000, Fiuza & Suffredini 2001).

Accumulating evidence suggests that inflammation is closely linked to the onset and progression of serious chronic conditions such as arterial athero-thrombosis (Koenig 2001, Libby et al. 2002). In vitro, animal and human experimental models have provided some answers on the inflammation-induced perturbations of the haemostatic and endothelial activation systems. Extrapolation and interpretation of data obtained from these models, however, are still debatable. Indeed, the bulk of data generated may provide limited insight into the

effects of inflammation following naturally occurring acute infections or surgery. The vaccination model is associated with a mild inflammatory response (Hingorani et al. 2000, Libby et al. 2002), whereas the infusion of endotoxin in healthy subjects can be associated with substantial systemic symptoms and subjects require close monitoring (Mittermayer et al. 2005). Mechanisms linking inflammation, haemostasis and the endothelial function have also been investigated in the context of flares of autoimmune disease (DeMaria 2002, Lima et al. 2002) but the unpredictable nature of such events makes them a logistical challenge.

This prompted us to evaluate the role of periodontal therapy as a novel

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and valid experimental model to study systemic inflammation. We recently showed that a single intensive session of periodontal therapy was associated with a consistent and long-lived (1 week) acute-phase response (APR) as assessed by changes in serum levels of C-reactive protein, fibrinogen and interleukin-6 (IL-6) (D'Aiuto et al. 2004a, 2005b).

Periodontitis is a natural occurring prototype of chronic low-grade infection and inflammation (D'Aiuto et al. 2004b), and its treatment relies upon the use of mechanical instrumentation of the subgingival portion of the diseased dentition under local anaesthesia (Williams 1990). This procedure often results in an intense transient bacteraemia as well as significant local gingival soft tissue damage (Waki et al. 1990, Lofthus et al. 1991, Hartzell et al. 2005, Kinane et al. 2005, Forner et al. 2006a). Acute release of cytokines in serum such as tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) has been reported to occur as early as 60 min following periodontal therapy (Ide et al. 2004, Forner et al. 2006b).

We decided to investigate whether the APR changes following periodontal therapy were also associated with alterations of the haemostatic and endothelial cell activation systems, and whether there are any inter-individual differences in the acute release of several bio-markers of vascular health.

## Material and Methods

### Study subjects and design

This study protocol was approved by the joint ethics committees of the Eastman Dental Institute and Hospitals (University College London Hospital) and all participants included gave written informed consent before entering the study. We obtained data and biological samples from 55 consecutive systemically healthy individuals (age range 30–64 years, 26 males, body mass index (BMI) 25.8 kg/m<sup>2</sup>) referred to the Periodontology Unit of the Eastman Dental Hospital who participated in various clinical trials in which they received an intensive course of periodontal therapy (D'Aiuto et al. 2004a, D'Aiuto & Tonetti 2005, D'Aiuto et al. 2005a). Only subjects presenting with severe and generalized periodontitis were invited to participate in the study as previously described (D'Aiuto et al. 2005a). Exclusion criteria included known systemic diseases (hypertension, diabetes, dysli-

pidemia and a history of myocardial infarction or stroke) as assessed by the examining clinician, a history and/or presence of other acute or chronic infections, systemic antibiotic treatment in the preceding 3 months, treatment with any medication and pregnancy or lactation. Familial history of any cardiovascular diseases (CVD) was also recorded.

### Periodontal therapy

After a baseline visit and collection of a complete medical history, clinical periodontal parameters were recorded by a single clinician. All participants received an intensive session of subgingival periodontal therapy consisting of mechanical instrumentation of the whole diseased dentition under local anaesthesia (within 6 h) as previously described (D'Aiuto et al. 2004a). Actual treatment time was recorded by the clinician in minutes.

### Sample collection and analysis

Blood samples were obtained by a clean venipuncture from the antecubital fossa and with minimal stasis, before and 1, 7 and 30 days after intensive periodontal therapy. Blood was collected in plain vacutainer tubes (Becton Dickinson, Plymouth, UK) for serum analysis and in tubes containing 0.105 M sodium citrate in a 1:9 anticoagulant-blood ratio for plasma bio-marker analysis. Samples were immediately processed, and several aliquots stored at  $-70^{\circ}\text{C}$  for later analysis. Concentrations of inflammatory, haemostatic and endothelial cell activation markers were quantified by high-sensitivity enzyme-linked immunosorbent assays according to the manufacturer's protocol in a blind fashion: IL-1 $\beta$ , TNF- $\alpha$  and soluble E-selectin (s-Es; Quantikine HS, R&D System, Minneapolis, MI, USA), D-dimers (Zymutest, Diapharma, West Chester, OH, USA) and von-Willebrand factor antigen (v-WF:Ag; CBA ELISA, Technoclone, Vienna, Austria). The inter-assay variability for all analyses ranged between 3% and 9%.

### Statistical analysis

All values are given as means  $\pm$  SEM. Acute releases of each soluble marker were analysed by repeated measures analysis of variance. Individuals' age, gender, smoking status (recorded as

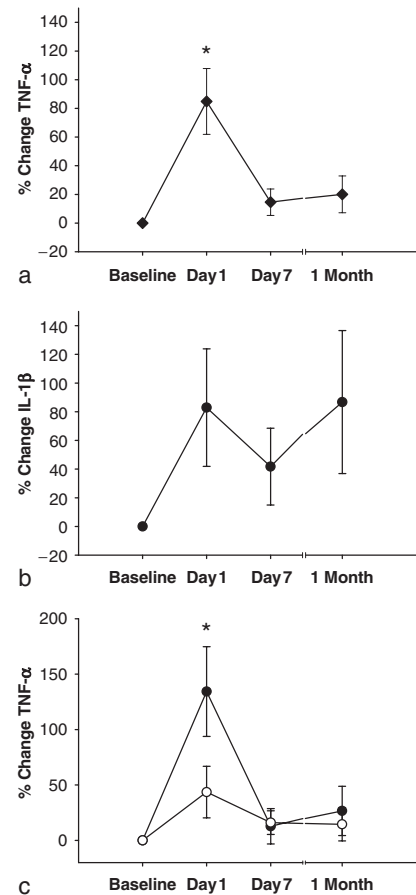


Fig. 1. Serum concentration changes in TNF- $\alpha$  (a) and IL-1 $\beta$  (b) following periodontal therapy are shown. Subjects ( $N = 55$ ) received an intensive session of periodontal therapy at baseline. A modest increase in TNF- $\alpha$  was observed 24 h following therapy. IL-1 $\beta$  levels, although increased, were not statistically different. Current smokers ( $\circ$ ,  $N = 14$ ) exhibited reduced TNF- $\alpha$  changes ( $p = 0.025$ ,  $F = 3.770$ , corrected model  $p = 0.001$ ,  $F = 3.175$ ,  $R^2 = 0.15$ ) when compared with non-smokers ( $\bullet$ )(c). Data represent mean SEM. \* $p$  value less than 0.05 versus baseline or between groups (c).

current versus no smokers), BMI (calculated as weight/(height)<sup>2</sup>), familial history of any CVD and treatment time (recorded in minutes) were included in each model as covariates. Post hoc analyses were performed by Bonferroni corrections. Correlation among different bio-markers were performed by non-parametric methods (Spearman's rank-correlation analysis). Acute release percentage of each biological marker was calculated as the difference between day 1 and baseline concentrations divided by baseline concentration values. The  $\alpha$  value was set at 0.05. Data were analysed with the statistical

software package SPSS (SPSS version 11, Chicago, IL, USA).

## Results

Periodontal therapy induced a significant increase in serum concentrations of TNF- $\alpha$  at 24 h compared with baseline [85% mean increase, 95% confidence intervals (CI) 39–131%,  $p < 0.001$ ] whereas IL-1 $\beta$  serum changes, although increased positively, did not reach statistical significance (Fig. 1a and b). One week and 1 month following therapy, both cytokines' concentration returned to values similar to baseline. TNF- $\alpha$  acute release in serum was associated with the severity of periodontal disease ( $r = 0.30$ ,  $p < 0.05$ ) as defined by the number of periodontal lesions patients presented at the treatment visit and that were subsequently instrumented. Treatment time recorded in minutes, however, was not associated with either TNF- $\alpha$  or IL-1 $\beta$  acute changes, which were particularly influenced by the smoking status of all subjects ( $p < 0.01$ ). Current smokers (56% of subjects) presented with a significantly reduced host inflammatory response for both bio-markers (Fig. 1c). In addition, individuals' BMI significantly influenced IL-1 $\beta$  serum changes after periodontal therapy ( $F = 4.604$ ,  $p < 0.05$ ; Corrected model  $p < 0.01$ ,  $F = 2.666$ ,  $R^2 = 0.21$ ). A positive CVD familial history was also associated with a reduced inflammatory reaction with respect to IL-1 $\beta$  ( $p < 0.05$ ) and TNF- $\alpha$  ( $p < 0.01$ ) serum levels (data not shown).

Periodontal therapy elicited a significant increase in plasma D-dimers (38% mean increase at day 1, 95% CI, 20–56%,  $p < 0.0001$ ), with a maximum peak at 1 week after therapy (63%, 95% CI, 44–81%,  $p < 0.0001$ ) and a decrease to baseline values only after 30 days (Fig. 2). The acute concentration (day 1) of D-dimers was proportionate to the time spent by the clinician cleaning the dentition ( $p = 0.001$ ,  $F = 13.783$ ) and moderately influenced by individuals' BMI ( $p = 0.036$ ,  $F = 4$ ; Corrected model  $p = 0.002$ ,  $F = 3.491$ ,  $R^2 = 0.45$ ).

We also found a 10% increase in s-Es plasma concentrations at day 1 (95% CI, 4–19,  $p < 0.01$ ) (Fig. 2) that was weakly correlated with treatment time ( $p = 0.038$ ,  $F = 4.600$ ) and highly influenced by subjects' BMI ( $p = 0.004$ ,  $F = 9.507$ ; Corrected model  $p < 0.001$ ,  $F = 4.836$ ,  $R^2 = 0.55$ ). s-Es baseline

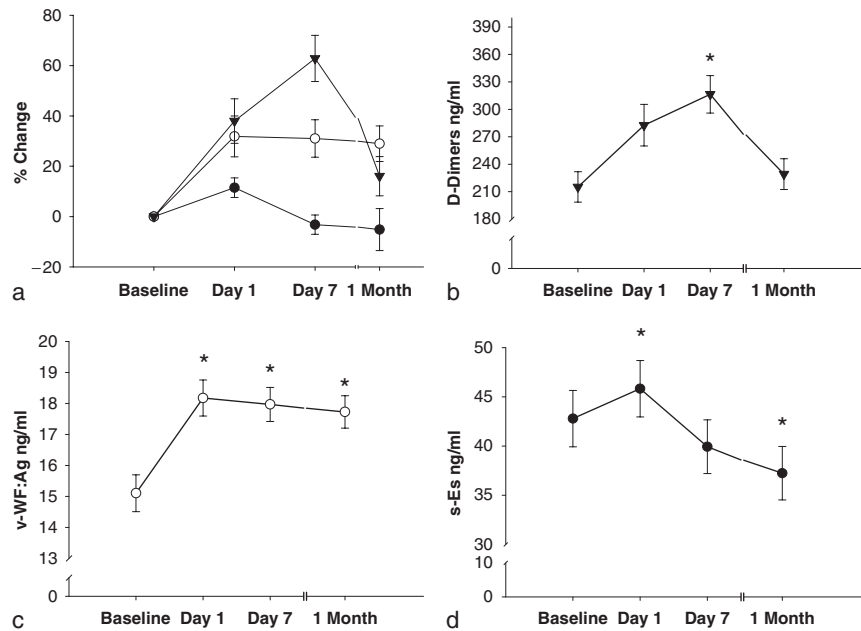


Fig. 2. Individuals with periodontitis received an intensive session of mechanical dental instrumentation at baseline ( $N = 55$ ). Plasma changes of all bio-markers (a) and actual concentrations of D-dimers (b), v-WF:Ag (c) and s-Es (d) are shown. A significant perturbation of the haemostatic system of 1-week duration was observed as reflected by enhanced cross-fibrin generation (38% increase in D-dimer,  $\blacktriangle$ ) and significant endothelial activation [v-WF:Ag 30% sustained increase ( $\circ$ ) and 10% increase of s-Es ( $\bullet$ )] (b–d). s-Es plasma concentrations were significantly reduced after 1 month of therapy when compared with baseline values (d). Data represent mean  $\pm$  SEM. \*  $p$  value less than 0.05 versus baseline.

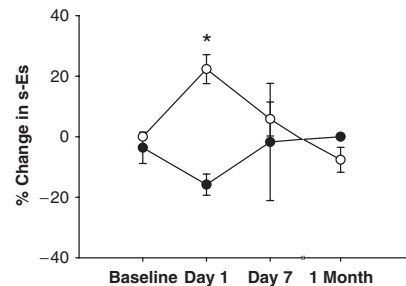


Fig. 3. Acute release in s-Es following periodontal therapy according to individuals with ( $\circ$ ,  $N = 32$ ) or without ( $\bullet$ ) a positive familial history of cardiovascular disease. Data represent mean  $\pm$  SEM. \*  $p$  value less than 0.05 between group difference.

concentrations were also correlated with BMI ( $R = 0.35$ ,  $p = 0.01$ ) as well as with TNF- $\alpha$  serum levels at all time points ( $r = 0.4$ ,  $p < 0.01$  at day 1 and  $r = 0.3$  both at 7 and 30 days,  $p < 0.05$ ). Individuals who had a positive CVD familial history ( $N = 32$ ) presented with a mean 26% (95% CI 12–40%,  $p < 0.001$ ) higher acute release of s-Es following periodontal therapy ( $p < 0.001$ ,  $F = 23.862$ ) (Fig. 3). At day 1, we also observed a significant

correlation between s-Es and D-dimer plasma levels ( $r = 0.3$ , at day 1,  $p < 0.05$ ), whereas 30 days after periodontal therapy, s-Es plasma concentrations were significantly reduced compared with baseline ( $p < 0.05$ ).

Periodontal therapy elicited a rapid and sustained 30% ( $p < 0.05$  at all visits after therapy) increase in v-WF:Ag plasma levels for the entire duration of the study (Fig. 2). These changes were not correlated with any other markers studied. Only cigarette smoking significantly influenced the acute release of this bio-marker ( $p = 0.018$ ,  $F = 4.435$ ) (corrected model  $p = 0.03$ ,  $F = 2.140$ ,  $R^2 = 0.32$ ).

## Discussion

Intensive periodontal therapy induces a moderate acute systemic inflammatory response associated with marked endothelial cell activation as demonstrated by an increase in plasma concentrations of s-Es and v-WF:Ag and alteration of the haemostatic system as reflected by a significant increase in D-dimer levels.

We previously characterized the inflammatory response associated with periodontal therapy and concluded that the stimulus was sufficient to mount a sustained (1 week) inflammatory response. This also allowed us to explore the influence of individual susceptibility in acute release of inflammatory markers and their influence on well-recognized cardiovascular risk factors (D'Aiuto & Tonetti 2005, D'Aiuto et al. 2005a).

In this report, we observed a mild increase in early inflammatory markers (IL-1, TNF) that were particularly influenced by the smoking status of participants. Several reports confirm these data by suggesting that cigarette smoking is associated with reduced serum levels of inflammatory mediators during natural and experimental models of human inflammation (Sopori et al. 1998, van der Vaart et al. 2004, D'Aiuto et al. 2005a).

Periodontal therapy strongly affected the haemostatic system in our model. Knowledge of the mechanisms behind this finding, however, is limited and our interpretation remains purely speculative. D-dimer is a composite marker of fibrinolysis and coagulation and it has been strongly associated with the presence of cardiovascular diseases in different populations and indeed has been reported as a predictable marker for future risk of coronary events independently from other conventional risk factors (Koenig 1998, 2001). Fibrin degradation products are commonly found to be elevated during systemic infections, and lipopolysaccharide is a common trigger of the activation of coagulation (Levi et al. 2002, Keller et al. 2003) as well as TNF- $\alpha$  (indirectly) or IL-6 (directly; ten Cate et al. 1997, van der Poll et al. 1997, Levi et al. 2002). Moreover, higher thrombin generation could be mediated by the extrinsic coagulation pathway involving increased tissue factor expression (ten Cate et al. 1997) or a concomitant inhibition of the fibrinolytic system dependent upon a sustained increase of plasminogen activator inhibitor-1 (Levi et al. 1993). Acute plasma D-dimer formation was mildly influenced by individuals' body weight and this finding is consistent with other reports that revealed an association between body weight measures, including BMI, and deep vein thrombosis risk as assessed by the level of several coagulation biomarkers (Abdollahi et al. 2003). We

believe, however, that this is the first time that the acute release of D-dimer following a moderate inflammatory stimulus is reported to be BMI dependent. The mechanisms behind these associations are poorly understood and will require further research.

We also observed a bimodal change in plasma s-Es levels: first, a mild acute increase after therapy, followed by a long-term significant reduction. Increased levels of s-Es are presumed to arise from excessive endothelial activation and/or damage that is perhaps cytokines driven (Newman et al. 1993, Smith 1997) and have been reported in individuals with cardiovascular diseases (Hwang et al. 1997). We presume that the acute inflammatory response following periodontal therapy was responsible for the observed endothelial cell activation. The principal regulator of s-Es concentrations in our model is perhaps TNF- $\alpha$  as its concentration was correlated at all time points with that of s-Es. A positive association between s-Es and TNF- $\alpha$  has already been reported in otherwise healthy men (Skoog et al. 2002) as well as in several cellular and animal models (Vestweber & Blanks 1999). Furthermore, the long-term reduction of s-Es that we observed supports the hypothesis that a chronic infectious disease, such as periodontitis, might be associated with a chronic systemic status of endothelial cell activation that perhaps might represent one of the mechanisms responsible of the moderate increase of cardiovascular risk in patients having periodontitis (Scannapieco et al. 2003). Our study, however, was not designed to answer these questions and therefore this hypothesis should be tested in a proper designed randomized-controlled clinical trial.

BMI significantly influenced the plasma concentration of s-Es and this finding has already been reported (Ferri et al. 1999). Our study confirms and extends these results by showing that obesity is not only related to baseline increased s-Es levels but also to its acute release following an inflammatory stimulus. A plausible explanation of such an association might be that overweight individuals produce a higher concentration of pro-inflammatory cytokines. Indeed, we observed a positive association between serum IL-1 $\beta$  and BMI that will induce higher secondary E-selectin expression (Yudkin et al. 1999, Ziccardi et al. 2002). A correlation between s-Es and D-dimer plasma concentrations

found at day 1 in our experiment might be interpreted based on some experimental evidence suggesting a possible role of s-Es in thrombus formation but this warrants further investigation (Quarumby et al. 1999).

Familial history of CVD was highly associated with the acute release of s-Es following periodontal therapy. There are no reports that address a similar question and this is why we can only speculate that this finding might be explained by a different but still unknown individual's genetic background. Nevertheless, this hypothesis should be confuted appropriately in a properly powered case-control study.

Finally, periodontal therapy produced an increase in v-WF:Ag plasma levels, considered to be the gold standard marker for endothelial damage. Raised levels of this bio-marker are found in individuals with documented damaged or dysfunctional endothelial cells as atherosclerosis (Lip & Blann 1995, 1997) or reported in association with other cardiovascular risk factors (such as smoking, hypertension or hypercholesterolaemia) (Blann & McCollum 1993, Blann et al. 1998). The acute changes in v-WF:Ag that we observed were not correlated with any other biomarkers studied at any visit including s-Es. Cigarette smoking only exhibits a significant influence and this has been previously described (Blann & McCollum 1993, Blann et al. 1998). Whether or not these findings reflect actual damage to the endothelium or merely a physiological endothelial activation warrants further research. v-WF:Ag indeed is also considered an acute-phase marker (Ruggeri 2003).

There are a series of limitations to our investigation that we ought to report. Firstly, the number of individuals included in this analysis is relatively small and these findings should be interpreted with caution. We wish to repeat the same experiment in a larger patient data set, also including individuals with less severe periodontal disease to ascertain whether there is a dose-relationship between dental instrumentation and systemic host response.

Secondly, early acute inflammatory and endothelial soluble mediators (such as IL-1 $\beta$ , TNF- $\alpha$  and s-Es) were only moderately altered 24 h following therapy in contrast with the robust changes observed in the endotoxin inflammatory model. This suggests that we have missed the peak of acute release of

most bio-markers that is probably taking place 6–8 h immediately after dental instrumentation. Further research and characterization of the early kinetics of these responses are needed. A third limitation of this study is the lack of any microbial (bacterial) serum/plasma quantification in order to assess whether the alterations seen were also correlated with the level of circulating bacterial endotoxins. This needs to be addressed in future studies as a more reliable microbiological technique will be available.

Nevertheless, this is the first study to demonstrate that intensive periodontal therapy is associated not only with a systemic inflammatory response but also with a significant perturbation of the endothelial and haemostatic system. This might be of particular interest as it has recently been proposed that acute inflammation, including that following infection, might trigger acute vascular events perhaps through a destabilization of the endothelium and coagulation system (Smeeth et al. 2004).

Periodontal therapy could represent the best model to date to study the inflammatory endothelial dysfunction association in vivo and it is the first therapeutic rather than experimental model reported. Its applicability to a large number of subjects makes it particularly useful when researchers also wish to study genetic influences or the effect of pharmacological interventions on acute inflammation.

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### Clinical Relevance

*Scientific rationale for the study:* Accumulating evidence suggests that periodontal therapy is associated with a systemic inflammatory response. Moreover, bacteraemia and local tissue damage following periodontal instrumentation might also trigger alterations of vascular health as assessed by soluble bio-markers.

*Principal findings:* We observed a significant acute increase in several bio-markers of vascular health following an intensive (whole mouth) session of periodontal non-surgical therapy. Cigarette smoking, BMI and previous history of cardiovascular diseases seem to influence the magnitude of systemic inflammation and vascular impairment. These alterations were parallel to a 1-week

systemic inflammatory response and resolved 1 month after periodontal instrumentation.

*Practical implications:* Periodontal therapy might be a useful model to study systemic inflammation and its consequences. However, a more intensive (whole mouth) approach of treating periodontitis might increase vascular risk in high-risk populations.