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Systemic inflammation following non-surgical and surgical periodontal therapy

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Abstract

Aim: To describe the kinetics of serum inflammatory markers after a course of treatment comprising surgical and non-surgical treatment of chronic periodontitis (CP).

Material and Methods: Fourteen CP cases received full-mouth non-surgical treatment and, after 6 months, at least two surgical sessions. Blood samples were collected at various time-points after treatment. Blood markers of systemic inflammation/coagulation including leucocyte counts, C-reactive protein (CRP), serum amyloid-A (SAA) and D-dimers and renal function (cystatin C) were determined using high-sensitivity assays.

Results: Periodontal treatment resulted in substantial reductions of the number of pockets, gingival bleeding and plaque at 3 and 6 months after non-surgical therapy ($p<0.001$). Surgical therapy led to an additional reduction of periodontal pockets ($p<0.01$). Marked increases in the serum levels of CRP and SAA were noted 24 h after non-surgical therapy ($p<0.01$) and periodontal surgeries ($p<0.05$). D-dimer levels increased drastically 24 h after non-surgical therapy ($p<0.05$). The drastic increase of CRP after non-surgical therapy was greater than both the surgical therapy sessions ($p<0.05$).

Conclusions: Patients undergoing periodontal treatment experience perturbations of systemic inflammation of a greater magnitude after non-surgical than surgical periodontal therapy.

Key words: C reactive protein; cystatin C; periodontal disease; periodontal surgery; systemic inflammation

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It is well established that patients suffering from periodontitis present with a low-grade systemic inflammatory state when compared with healthy subjects (Paraskevas et al. 2008). Increased concentrations of inflammatory biomarkers in both gingival tissues and serum, such as C-reactive protein (CRP) and interleukin (IL)-6, have already been reported (Loos et al. 2000). This has led to the hypothesis that subjects affected by periodontal disease may be at an increased risk of developing systemic cardio-metabolic diseases that have systemic low-grade inflammation as their common pathogenetic mechanism (Pihlstrom et al. 2005, D'Aiuto et al. 2006a).

In the contrast, inconsistent evidence shows that periodontal therapy is also associated with changes in systemic inflammation. Indeed, intensive non-surgical periodontal therapy produced an acute inflammatory response that resolved within 1 month and a marked reduction of blood markers of inflammation during the subsequent 6 months (D'Aiuto et al. 2004, 2007). Nevertheless, the exact implications of these inflammatory changes are still not completely understood. Recent evidence clearly demonstrated how the acute and long-term changes in systemic inflammation following periodontal therapy were also associated with changes in vascular function (Tonetti et al. 2007) and metabolic alterations (D'Aiuto et al. 2008).

Moreover, periodontal treatment often comprises surgical corrective sessions following the causative phase of treatment (Lindhe et al. 1982, Ramfjord et al. 1987). There is evidence on the host response after a comprehensive course of periodontal therapy, i.e.

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including both non-surgical and surgical treatment (Behle et al. 2009); however, little information is available on the contribution of each phase of treatment to the inflammatory response. It is plausible to believe that a localized periodontal surgery including mucoperiosteal flap elevation and possibly bone surgery might also represent a sharp inflammatory stimulus and may therefore produce a sustained host response.

We identified a cluster of blood markers of inflammation such as CRP, serum amyloid-A (SAA) and leucocyte counts, coagulation (D-dimers) and renal function (cystatin C) that could well describe the complexity of the host response to periodontal therapy.

CRP and SAA are recognized and stable markers of systemic inflammation (Gabay & Kushner 1999), and both are proteins of the acute-phase response. This is a non-specific rapid response to a variety of stimuli and it is part of the innate immunity (Gabay & Kushner 1999). Further leucocyte counts are a crude marker of systemic inflammation and have been used for centuries to define the host-adaptive responses to bacterial/traumatic challenges (Lowe 2005). D-dimers are composite markers of fibrinolysis and coagulation, whereas cystatin C is a reliable and sensitive serum marker of kidney function and have been used to describe the possible impact of systemic inflammation on both the coagulation (Levi et al. 2002, Keller et al. 2003) and the renal (Tanaka et al. 2007) system.

Thus, the primary aim of this study was to describe the kinetics of a commonly used and stable inflammatory marker (CRP) following a complete course of periodontal treatment (non-surgical and surgical). Secondary outcomes included changes in the leucocyte counts, SAA and other blood markers of inflammation/coagulation and renal function.

Material and Methods

Experimental design and patient selection

This study was a prospective cohort trial with a 12-month follow-up. Eligible patients were identified among those referred to the Periodontology section of the Dental and Oral Surgery clinic of the University of Pisa, Italy, and fulfilling the inclusion/exclusion criteria. Ethical approval was obtained from the local Ethics Committee and the study was conducted according to the principles outlined in the Declaration of Helsinki on experimentation involving human subjects.

A complete periodontal examination was performed including patient medical history (assessed by a questionnaire), an intra-oral examination and a full-mouth periodontal probing chart on six sites per tooth by a single calibrated examiner.

The trial included consecutive individuals with (i) generalized advanced chronic periodontitis (Armitage 1999) presenting with probing pocket depths (PPDs) $\geqslant 5$ mm on at least 30% of their dentition, (ii) at least 20 teeth present; and (iii) good general health as assessed by the examining clinician. Subjects were excluded if (i) younger than 35 years and older than 70 years; (ii) pregnant or lactating females; (iii) females using contraceptive methods; (iv) patients suffering from any systemic illnesses; (v) patients undergoing any pharmacological treatment within the 3 months before the beginning of the study; and (vi) patients already treated for periodontal disease in the previous 6 months.

Periodontal parameters

Clinical parameters were assessed using a manual UNC-15 mm periodontal probe by a single calibrated examiner (S. C.) at six sites per tooth excluding third molars. The full-mouth plaque score (FMPS) was recorded by assigning a binary score to each site and calculating the percentage of total tooth surfaces that revealed the presence of plaque detected using a periodontal probe (O'Leary et al. 1972). Similarly, a full-mouth bleeding score (FMBS) was calculated after assessing dichotomously the presence of bleeding on probing from the bottom of the pocket on gently probing (Ainamo & Bay 1975). Full-mouth PPD and recession of the gingival margin (REC) were recorded at the same time, with measurements rounded to the nearest millimetre. Clinical attachment level (CAL) was calculated as PPD plus REC (D'Aiuto et al. 2005). A total of 10 non-study subjects were recruited and used for calibration of the examiner. These subjects had periodontal disease and the examiner recorded full-mouth PPD and recessions at six sites per tooth (excluding third molars) on two different occasions using a manual, UNC-15 periodontal probe. Upon completion of all measurements, the intra-examiner repeatability for CAL measurement was assessed. The examiner was judged to be reproducible after meeting a percentage of agreement within ± 2 mm between repeated measurements of at least 98%. Complete dental and periodontal examinations were performed at baseline, 90 and 180 days after non-surgical treatment (pre-surgical examination) and 90 days after the last surgical intervention.

Periodontal therapy

A standard cycle of periodontal therapy consisting of a non-surgical and a subsequent surgical phase was performed by a single certified periodontist (F. G.). Non-surgical treatment consisted of oral hygiene instructions and supra-subgingival mechanical instrumentation of the root surface [scaling and root planing (SRP)] using a piezoelectric instrument with fine tips (EMS, Nyon, Switzerland) and hand instruments as appropriate of sites with PPD > 3 mm in two appointments within 24 h. Local anaesthesia was used as necessary.

Surgical sessions started 180 days after the non-surgical phase if patients presented with sites with PPD > 5 mm irrespective of BoP present or absent. Patients underwent at least two sessions of surgical intervention. All surgeries were performed using a modified Widman flap procedure (Ramfjord & Nissle 1974) with non-supporting osseous surgeries (osteoplasty) if needed.

After the treatment patients phases, relied on standard oral hygiene methods as instructed at the commencement of the study and no oral rinses were prescribed. Supragingival polishing was provided 90 and 180 days after non-surgical treatment and 90 days after the last surgical intervention.

Blood collection and serum analysis

Serum samples were collected from a venupuncture in the antecubital fossa at 07:30 hours and after an overnight fast for all subjects. Changes in the medical history were recorded in relation to their possible impact on the quantification of all serum markers at baseline and 1, 7, 30, 90 and 180 days after non-surgical treatment, at days 1 and 7 after the first (D180) and the second (D200) surgical interventions (D181, D187 for the first surgery and D201, D207 for the second

surgery). Finally blood samples were collected 90 days (D270) after the last surgical intervention. Blood samples were immediately processed and then stored at -70°C.

We assessed the following markers of systemic inflammation: leucocyte counts, serum CRP, plasma D-dimers, SAA and cystatin C. All biomarkers were quantified using high-sensitivity assays according to the manufacturer's instructions. The inter- and intra-assay coefficients of variation were $<7\%$ for all markers.

Vital signs such as systolic and diastolic blood pressure, cardiac frequency and temperature were also measured. All measurements were taken three times and the average was considered as the reference value.

Data management and statistical analysis

A sample size calculation suggested that a minimum of 13 subjects were needed to demonstrate a 1 mg/l increase in the serum levels of CRP after periodontal therapy (90% power, α 0.05, standard deviation of 1 (D'Aiuto et al. 2004). All data were entered in an Excel database, proofed for entry errors and analysed using a statistical package (version 16.0, SPSS Inc., Chicago, IL, USA). Variables not normally distributed were logarithmic (natural) transformed before being used in parametric comparative analysis. Data are reported as mean and standard errors unless specified. Analysis of variance for repeated measures was used to compare differences over time of each inflammatory marker. Covariates included in each model were age, gender, smoking (smokers: >5 cigarettes/day) and body mass index. Post hoc comparisons were performed using Bonferroni corrections. Drastic relative changes in biomarker serum levels were calculated according to the following formula (example on CRP levels): [(CRP day $1-$CRP at baseline) $\times$ 100]/CRP baseline. The relative differences of CRP, SAA and D-dimers at day 1 compared with baseline, day 181 compared with day 180 and day 201 compared with day 200 were analysed using ANOVA. Significance was attributed when probability p was <0.05.

Results

A total of 14 individuals were enrolled in the study. The baseline characteristics are summarized in Table 1. Over the following 12 months, no major changes in the medical history were reported at the time of all study visits.

A complete course of periodontal therapy resulted in a marked improvement in the clinical parameters over the 6 months after therapy (Table 2). Statistically significant reductions in the number of periodontal pockets >4 mm, FMBS and FMPS were recorded 3 and 6 months after non-surgical therapy ($p<0.001$ for both) (Table 2). Periodontal surgical therapy resulted in an additional statistically significant reduction in the number of periodontal pockets ($p<0.01$) as residual pockets were diminished further 50% (Table 2).

All biomarker serum levels changed over time ($p<0.001$ for all). Leucocyte counts were slightly reduced (not statistically significant) only at D1 (Fig. 1a). A marked increase in the serum levels of CRP was observed 24 h after non-surgical therapy (D1, $p<0.01$ compared with BL) and following the first (D181, $p<0.05$ compared with D180) periodontal surgery (Fig. 1b) but of a lower magnitude for the latter. Serum changes in SAA resembled those of CRP, with drastic increases at the same time points (D1, $p<0.001$ compared with BL and D181, $p<0.05$ compared with D180) (Fig. 1c). D-dimer plasma levels were drastically increased 24 h after non-surgical therapy (D1, $p<0.05$ compared with BL) but tended to decrease after both surgical therapies (Fig. 1d, not statistically significant).

Individuals presented with an average acute relative increase of $985 \pm 392\%$ of CRP (95% CI 139–1831) after non-surgical therapy, which was substantially greater than those increases observed after the first ($237 \pm 67\%$, 95% CI 88–387, $p=0.033$) and the second ($134 \pm 42\%$, 95% CI 41–228, $p=0.005$) surgical therapy sessions (Fig. 2a). Similarly, SAA acute relative increases after SRP ($587 \pm 150\%$, 95% CI 269–832) were greater than those observed after the first ($56 \pm 29\%$, 95% CI 7–119, $p=0.003$) and the second surgeries ($110 \pm 36\%$, 95% CI 29–191, $p=0.024$) (Fig. 2b). This finding was not repeated when D-dimer changes were compared between periodontal therapy effects (Fig. 2c).

Non-specific changes in arterial blood pressure were observed after both non-surgical and surgical periodontal therapy (Fig. 3a and b). The pattern of changes in the skin temperature revealed mild increases on the first day after both non-surgical and both sessions of surgical periodontal therapy, but of minimal clinical relevance (Fig. 3c).

The change in cystatin C serum levels resembled those of an acute-phase marker as after an initial moderate increase values lower than baseline were noted (Fig. 3d). Indeed, after 12 months of therapy, cystatin C levels were substantially reduced compared with the baseline levels (mean difference of 0.18 ± 0.03 mg/ml, 95% CI 0.12–0.24, $p<0.001$).

Table 1. Baseline data

Variables	Mean $\pm$ SE
Age (years) (median, IQR)	49 (15)
Gender (male) (%)	8 (57)
Smoking (current) (%)	7 (50)
BMI (kg/m^2)	26 ± 1
CRP (mg/l)	4.0 ± 2.3
SAA (mg/)	28.4 ± 21.0
D-dimers (pg/ml)	0.1 ± 0.1
WBC (10^6 cells)	6.6 ± 0.4
Systolic BP (mmHg)	116 ± 4
Dyastolic BP (mmHg)	77 ± 2
Cystatin C (mg/ml)	0.8 ± 0.02

BMI, body mass index; CRP, C-reactive protein; SAA, serum amyloid-A; WBC, leucocytes counts; BP, blood pressure; IQR, inter-quartile range.

Discussion

Non-surgical and surgical periodontal therapies induced acute systemic inflammatory responses of different magnitudes including changes of coagulation

Table 2. Periodontal parameters

	Baseline	3 months	6 months	Final
N Pockets >4 mm	64.43 ± 8.37	$16.43 \pm 5.31^{*}$	$15.29 \pm 5.05^{*}$	$7.54 \pm 2.89^{*\S}$
% Pockets >4 mm	42.23 ± 4.91	$10.77 \pm 3.33^{*}$	$10.44 \pm 3.37^{*}$	$5.05 \pm 1.96^{*\S}$
FMBS (%)	63.04 ± 5.13	$7.73 \pm .87^{*}$	$7.00 \pm 1.31^{*}$	$4.75 \pm 1.31^{*}$
FMPS (%)	68.32 ± 3.76	$9.89 \pm 1.34^{*}$	$10.91 \pm 1.35^{*}$	$14.37 \pm 7.10^{*}$

$^{*}p<0.01$ compared with baseline.
$^{\S}p<0.01$ compared with 6 months.
FMBS, full-mouth gingival bleeding scores; FMPS, full-mouth supragingival dental plaque scores.

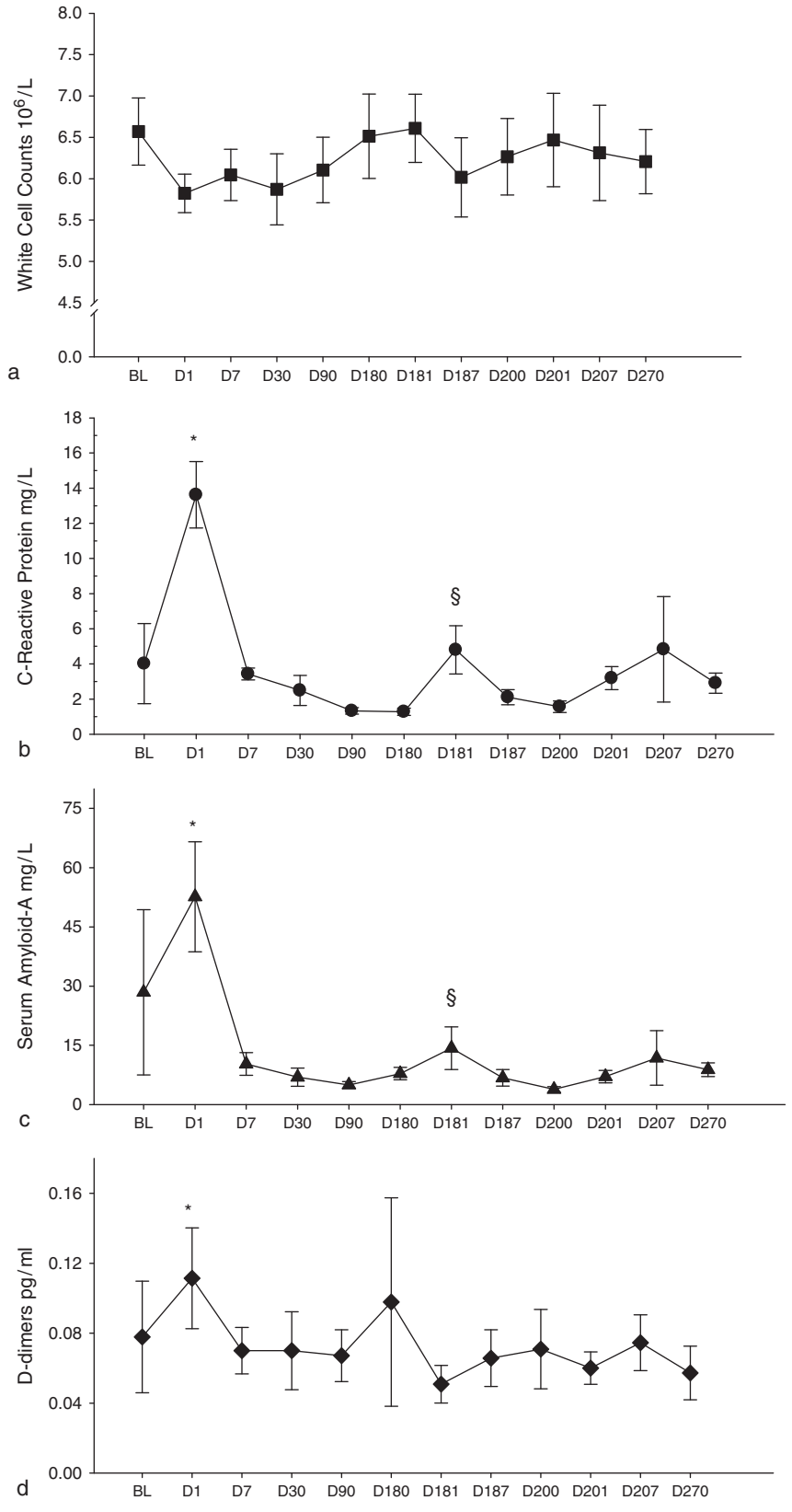

Fig. 1. Mean ± SE serum-level changes after periodontal therapy of leucocyte counts (a), C-reactive protein (b), serum amyloid-A (c) and D-dimers (d). Bonferroni adjustment: *compared with BL ($p < 0.05$) and §compared with D180 ($p < 0.05$).

and long-term reduction of renal function measures.

This is the first study to compare the magnitudes of an acute body response following different types of periodontal therapy (non-surgical and surgical). These data are of utmost importance as periodontal treatment frequently comprises both causative and corrective surgical phases. Indeed, a recent report confirmed the heterogeneous inflammatory responses observed 6 weeks following periodontal therapy (both non-surgical and surgical) (Behle et al. 2009). A cluster of biomarkers, including some of those examined in this report, was analysed and resulted in non-statistically significant changes after therapy, with an overall trend towards a reduction. The extreme biological variability of some of these biomarkers warrants further investigation in larger populations in order to define subgroups of hyper- or hyporesponder individuals both acutely or chronically following comprehensive periodontal therapy.

D'Aiuto et al. (2004) well characterized the inflammatory response associated with non-surgical periodontal therapy and concluded that the stimulus was sufficient to mount a sustained (1 week) inflammatory response. Our data confirm these findings as periodontal therapy mounted an acute response of CRP, SAA and D-dimers within 24 h. The mechanisms of this could be that both the bacteraemia and the tissue damage following subgingival instrumentation (Lofthus et al. 1991) determine an increase of pro-inflammatory mediators (Birkedal-Hansen 1993) and acute-phase proteins (Gabay & Kushner 1999). As has already been observed (D'Aiuto et al. 2007), these results are in line with those reported in other models of human inflammation (Suffredini et al. 1995, 1999).

IL-6 serum levels have also been shown to increase in a similar fashion (D'Aiuto et al. 2004), and therefore the hypotheses of both local (gingival) production and systemic dissemination are followed by the liver release of acute-phase proteins in order to protect the host against local pathological stimuli (Irwin & Myrillas 1998). Indeed, CRP and SAA acute releases in the blood stream are often used by physicians to characterize the defensive host response to local tissue trauma. It is not clear with respect to periodontal therapy whether this is due to a systemic dissemination

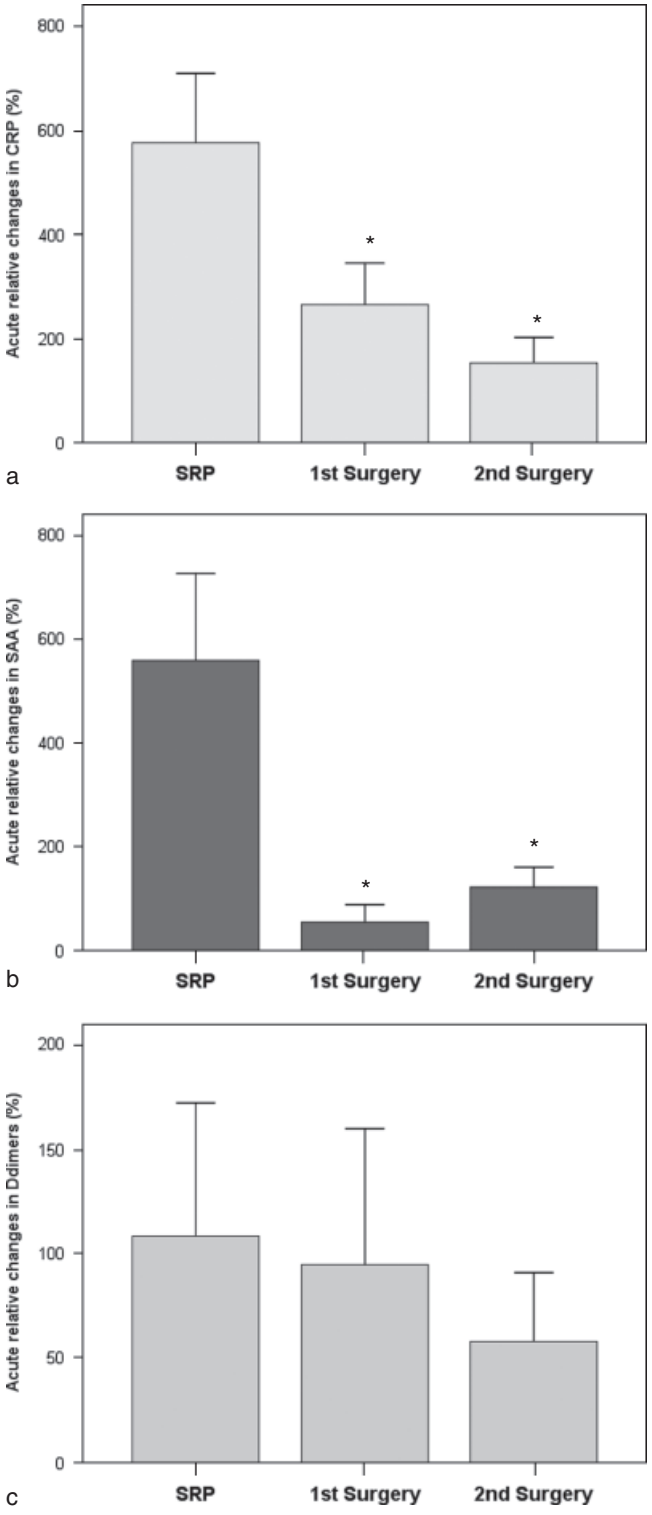

Fig. 2. Acute relative change of C-reactive protein (CRP) (a), Serum amyloid-A (SAA) (b) and D-dimers and (c) levels after non-surgical periodontal therapy [scaling and root planning (SRP), at day 1 compared with baseline], first surgery (day 181 compared with day 180) and second surgery (day 201 compared with day 200). *Bonferroni adjustment compared with SRP, $p < 0.05$.

of local cytokines or due to periodontal pathogens, bacteraemia or both. (Gabay & Kushner 1999, Pepys & Hirschfield 2003).

Perturbation of the coagulation system observed in our study is in line with those changes reported after non-surgical periodontal therapy (D'Aiuto et al. 2007). Changes in arterial blood pressure have already been reported after periodontal treatment both acutely and following 6 months after therapy (D'Aiuto et al. 2006b). Little evidence is available to comment on the possible mechanisms causing these changes. It is reasonable to believe that a reduction of patient anxiety during treatment follow-ups and possibly a direct vascular effect of periodontal therapy might be involved (Seinost et al. 2005, Tonetti et al. 2007).

Although an increase in body temperature has been reported previously when a whole-mouth non-surgical periodontal therapy approach was introduced (Quirynen et al. 1999), in our study as well in the previous reports (D'Aiuto et al. 2004, D'Aiuto et al. 2007), no statistically or clinically relevant changes in skin temperature were observed. In our study, this was also true after both non-surgical full-mouth therapy and quadrant surgical periodontal therapy.

It is noteworthy to mention that increases in CRP serum levels following both surgical periodontal sessions were lower in magnitude than those detected after non-surgical therapy. This is somehow in contrast with the clinical perception of periodontal surgical procedure being a ''trauma'' of a greater magnitude when compared with non-surgical therapy. We can only speculate on the possible facts behind these findings. Firstly, in a periodontal flap procedure, the traumatized surface area is smaller than whole-mouth non-surgical periodontal treatment. Indeed, our results indicated that periodontal surgery was performed only on 25% of all the residual diseased areas. Thus, a smaller postoperative wound could be responsible for a systemic inflammatory response of a lesser degree. Secondly, we could hypothesize that subgingival instrumentation of the entire dentition would be associated with a greater bacteraemia as opposed to periodontal surgery because of a reduction in the quantity and quality of periodontal pathogens found in the periodontal pockets due to the host habitat modifications that have already taken place after non-surgical therapy (Haffajee et al. 2006).

We also reported the short- and long-term effects of complete periodontal treatment on cystatin C serum levels. Cystatin C is an aminoacid, a member of the family of cysteine protease inhibi-

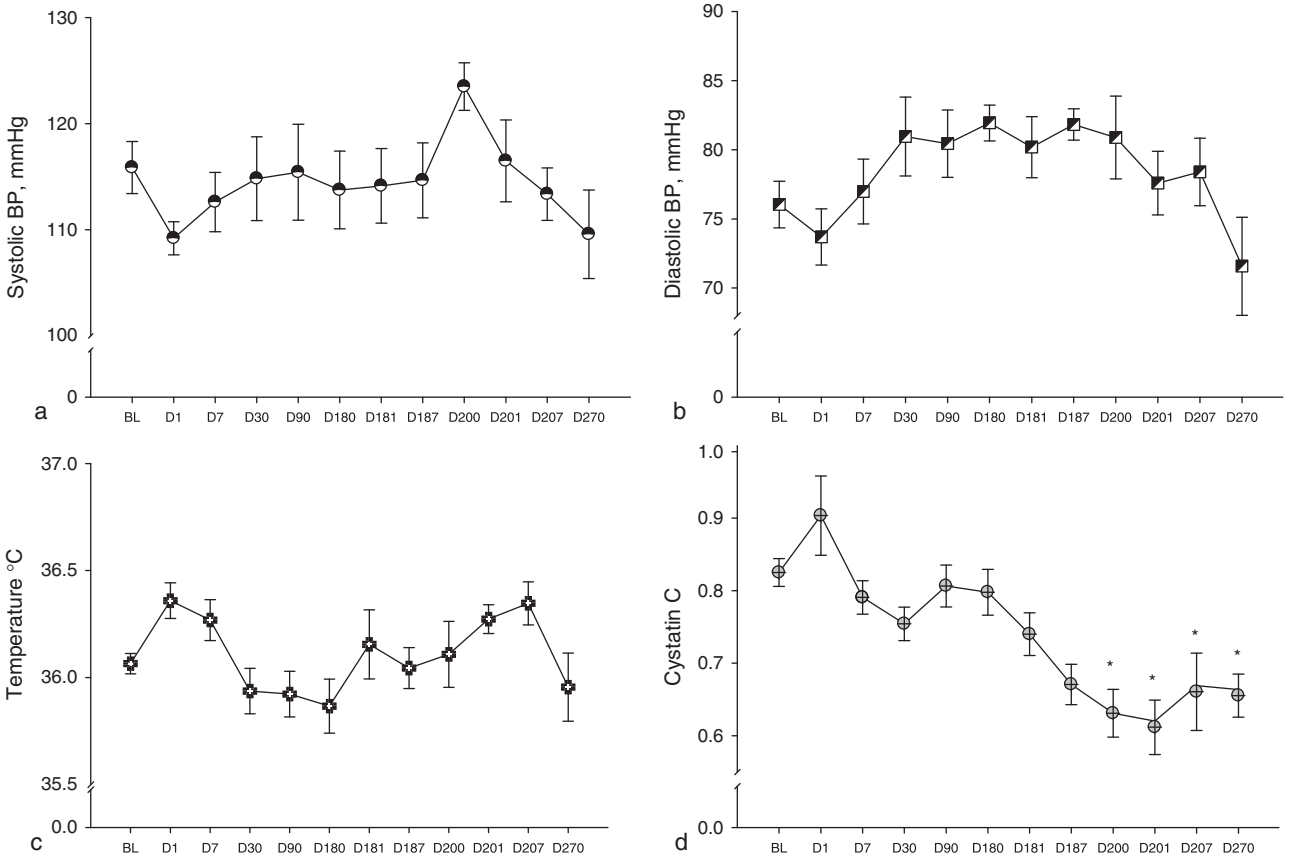

Fig. 3. Average changes of arterial blood pressure [(a) systolic, (b) diastolic] skin temperature (c) and cystatin C (d) over time. *Bonferroni adjustment compared with baseline, $p < 0.05$.

tors, and it is considered a marker of renal function in both healthy and diseased patients (Tanaka et al. 2007). Indeed, it has been reported that creatinine-based equations (glomerular filtration rate) are not as accurate and sensitive as cystatin C in measuring the glomerular filtration rate (Shlipak et al. 2003). This preliminary finding of a long-term reduction in the cystatin C serum levels would raise the hypothesis of a more efficient glomerular filtration rate (Fliser & Ritz 2001).

Lastly, the authors are aware of the intrinsic limitations of the study presented. Firstly, although formal sample size estimation was performed, the patient sample was small. Descriptive analyses should therefore be replicated in order to further confirm these findings. Secondly, no control group was enrolled to compare the long-term changes of biomarkers such as CRP and cystatin C. Nevertheless, this is the first study that characterizes and compares within the same individuals the host responses to a complete course of periodontal treatment including non-surgical and surgical periodontal therapy.

In conclusion, periodontal therapy, whether non-surgical or surgical, is associated with systemic inflammation. This might be of particular interest as inflammation could lead to an acute state of vascular dysfunction (Seinost et al. 2005, Tonetti et al. 2007) and a possibly increased risk of vascular events. Indeed, acute inflammation for instance associated with acute respiratory or urinary tract infections has been associated with a short-term increase in the risk of individuals having a first vascular event (Smeeth et al. 2004). In healthy individuals, however, the host response to inflammatory stimuli often resolves without complications. It is plausible to believe, however, that in other individuals perhaps already suffering from a vascular dysfunctional state, invasive therapy associated with substantial systemic inflammation could alter the integrity of the vasculature. The evidence on the possible detrimental vascular effects following dental and periodontal procedures is inexistent and therefore might warrant further research.

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Clinical Relevance

Scientific rationale for the study: Periodontal non-surgical treatment produces systemic inflammation. Nevertheless, no information is available on the systemic response after a complete course of periodontal therapy, i.e. non-surgical and subsequently surgical periodontal therapy.

Principal findings: CRP tends to increase significantly after every therapeutic event. Interestingly, the highest peak is obtained after periodontal non-surgical therapy.

Practical implications: Clinicians should be aware that a complete course of periodontal therapy determines frequent increases of systemic inflammatory markers. The impact of these changes, especially in patients suffering from a vascular dysfunctional condition, is yet to be determined.