J Clin Periodontol 2004; 31: 402–411 doi: 10.1111/j.1600-051X.2004.00580.x
Printed in Denmark. All rights reserved

Journal of Clinical Periodontology

Periodontitis and atherogenesis: causal association or simple coincidence?

A pilot intervention study

Francesco D'Aiuto[1], Mohammed Parkar[1], Georgios Andreou[1], Peter M. Brett[1], Derren Ready[2], and Maurizio S. Tonetti[1]

[1]Department of Periodontology, Eastman Dental Institute and Hospital, University College London; [2]Microbiology Unit, Eastman Dental Hospital, University College London Hospitals, NHS Trust

D'Aiuto F, Parkar M, Andreaou G, Brett PM, Ready D, Tonetti MS: Periodontitis and atherogenesis: causal association or simple coincidence? J Clin Periodontol 2004; 31: 402–411. doi: 10.1111/j.1600-051X.2004.00580.x. © Blackwell Munksgaard, 2004.

Abstract

Objectives: The aim of this study was to assess the systemic effects of treating severe widespread periodontitis in a population of otherwise healthy individuals by examining treatment associated changes in markers of inflammation that are also implicated in cardiovascular atherosclerotic diseases. The potential impact of specific polymorphisms in cytokine genes known to influence both periodontitis and cardiovascular diseases was also examined.

Materials and Methods: A convenience sample of patients affected with severe generalised periodontitis was enrolled into a prospective single blind longitudinal intervention trial with a 6 months follow-up. Serum C-reactive protein (CRP) and interleukin-6 (IL-6) levels were assessed by high-sensitivity assays. Serological and clinical periodontal parameters were evaluated at baseline, 2 and 6 months after completion of non-surgical periodontal therapy.

Results: In the 94 subjects that completed this pilot trial improvements in all clinical periodontal parameters were achieved. These were accompanied with significant reductions in serum IL-6 and CRP concentrations. In a multivariate model, serum CRP levels were significantly associated with the outcome of periodontal treatment after correcting for potential covariates (age, body mass index, gender, smoking) and polymorphisms in the IL-6 (–174 C/G) and IL-1A (–889) genes. A median decrease in serum CRP of 0.5 mg/l (95% CI 0.4–0.7 mg/l) was observed 6 months after completion of periodontal therapy in this population. Subjects with above average response to periodontal therapy (< 30 residual pockets and < 30% of sites bleeding on probing) accounted for the observed improvement in serum CRP.

Conclusions: Control of periodontitis, achieved with non-surgical periodontal therapy, significantly decreased serum mediators and markers of acute phase response. The significance of the serum response was associated with the half of the population that responded better to non-surgical periodontal therapy. The results of this pilot study indicate that severe generalised periodontitis causes systemic inflammation. This is consistent with a causative role of periodontitis in atherogenesis.

Key words: clinical trial; CRP; IL-6; inflammation; periodontal medicine; periodontitis

Accepted for publication 24 June 2003

Atherosclerosis and its consequent cardiovascular diseases represent one of the leading causes of death in the industrialised world. Its etiological pathway is one of a chronic inflammatory disease. However the stimuli of the invoked inflammatory reaction remain incompletely understood (Ross 1999). In the past 15 years, many investigators, unable to attribute a large part of cardiovascular events to the well recognised risk factors (cholesterol, lipids, smoking, etc.), raised again the old theory that chronic infections may cause atherosclerosis (Leinonen & Saikku 2002).

Several reports and a recent meta-analysis have shown increased odds ratios for cardiovascular events in large populations of subjects harbouring hypothetical triggering chronic infective

agents (*H. pylori*, *C. pneumoniae*). The role of chronic infections on atherosclerotic cardiovascular diseases is now supported by a bulk of validating evidence that has also generated a series of antimicrobial intervention trials to establish causality in the association (Gupta & Camm 1998, Anderson et al. 1999, Danesh 1999, Fong 2000, Stone et al. 2002).

In recent years an increasing number of epidemiological studies have indicated that subjects with periodontitis may have increased risk of cardiovascular events. The association between periodontitis and cardiovascular diseases, however, remains a matter of debate. Recently, discussions have focused on the divergent results that different groups have obtained after analysis of material from the same study (Beck & Offenbacher 2001, Howell et al. 2001, Genco et al. 2002, Hujoel et al. 2002). Critics have underlined the fact that both periodontitis and atherosclerosis share commonalities in terms of established risk factors, and that the association, even if established, could be spurious. If the association was firmly established, however, the critical question of its nature in terms of possible causality will remain central to understanding the medical significance of periodontal infections.

The magnitude of the odds ratios reported in a large meta-analysis for non-oral chronic infections and the so-called dental diseases (caries and periodontal disease) was similar and, as a whole, the available evidence would seem to support the hypothesis that chronic infections, including periodontitis, may have a causative role in atherosclerosis (Danesh 1999).

While the debate on the strength and nature of the association between periodontitis and cardiovascular diseases will continue for some time, recent evidence has changed the definition of periodontitis. Subjects affected by this disease share common polymorphisms in specific genes considered important in the regulation of the inflammatory response (Kornman et al. 1999, Kornman & Duff 2001). Furthermore, patients with severe periodontitis have increased levels of serum C-reactive protein (CRP), hyperfibrinogenaemia, moderate leukocytosis, as well as increased serum levels of IL-1 and IL-6 when compared with unaffected control populations (Kweider et al. 1993, Ebersole & Cappelli 2000, Loos et al. 2000, Noack et al. 2001, Fredriksson et al. 2002). Recognised risk factors include also the important effect of cigarette smoking, and systemic diseases such as diabetes present also in the panel of the etiological agents for cardiovascular diseases (Genco 1996, Page 1998). These data have clearly indicated that a systemic inflammatory response is associated with severe periodontitis in otherwise healthy individuals.

Two mechanistic hypotheses have been proposed regarding the etiological pathways of the association between periodontitis, systemic inflammation and cardiovascular diseases: one focuses on the chronic infectious burden that periodontitis may represent for the organism via access of either microorganisms or endotoxins; the others sees the diseased periodontium as a source of systemic inflammatory mediators.

Independently of the underlying mechanism(s), systemic inflammation seems to be central for explaining the nature of the link between chronic infections and atherosclerosis (Danesh 1999, Koenig 2001, Libby et al. 2002).

In this respect, CRP is generally considered as the most sensitive marker of the acute phase response to infectious burdens and/or inflammation (Gabay & Kushner 1999). As a consequence of its kinetics it best describes the systemic individual ''inflammatory'' status. CRP hepatic production is usually elicited by an inflammatory stimulus (infectious or not) and mediated through a complex network of cytokines (mainly interleukin-6, IL-6) (de Maat & Kluft 2001). CRP serum concentrations in the upper quartiles of normality, moreover, have assumed a significant role as predictors for future coronary events in healthy populations (Ridker et al. 1997). Systemic low-grade infections with their moderate acute phase responses may accelerate the formation of atheromatous plaques with consequent increased risk of future cardiovascular events (Danesh et al. 2000).

This body of emerging evidence, however, has left unanswered a series of questions on the debated link between chronic infections, for example, periodontitis and atherosclerotic events. Is this reported modest increase of CRP serum concentration and the inflammatory condition that it represents a novel etiologic mechanism for atherosclerosis or just a coincidence?

The aim of this pilot intervention trial was primarily to assess whether the control (elimination) of active periodontal infection might have an impact on serological markers of systemic inflammation (CRP, IL-6) in otherwise healthy individuals. Clinical periodontal and microbiological outcomes were also analysed to validate the level of efficacy of periodontal treatment. Secondly, the study investigated the potential significance of specific cytokine polymorphisms known to influence both cardiovascular and periodontal diseases in terms of patterns of serum response.

Materials and Methods

Study design and experimental population

The study was a prospective, longitudinal, single blind pilot intervention trial with 6 months follow-up. Participants were recruited from subjects referred to the Department of Periodontology of the Eastman Dental Hospital for treatment of periodontitis. Inclusion criteria were the presence of severe generalised periodontitis without any other systemic infection or disease. Subjects were required to have a probing pocket depth (PPD) greater than 6 mm and marginal alveolar bone loss greater than 30% at least in 50% of the entire dentition. Exclusion criteria included (i) known systemic diseases (cardiovascular, respiratory, renal, malignancy, etc.), (ii) history and/or presence of any other infections, (iii) systemic antibiotic treatment in the preceding 3 months, (iv) treatment with any medication known to affect the serum level of inflammatory markers (e.g. non-steroidal anti-inflammatory drugs), and (v) pregnant or lactating females. All patients gave informed written consent; the study had been reviewed and approved by the Eastman/UCLH joint ethics committee.

Study outline

A baseline visit was conducted by a blind calibrated examiner who collected a complete medical history, standard clinical periodontal parameters, blood and microbial samples. A periodontal treatment phase followed and it was carried out by a periodontist. The patients were re-examined 2 and 6 months after the completion of treatment when the same clinical and serological parameters were collected.

Periodontal parameters and examiner calibration

At the three study visits (baseline, 2 and 6 months) full mouth PPDs, recessions (REC) from the free gingival margin to the cemento-enamel junction and bleeding on probing (BoP) were recorded at six sites per tooth. Furthermore full mouth plaque scores (FMPSs) were recorded as the percentages of total surfaces which revealed the presence of plaque (O'Leary et al. 1972). 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 in two different occasions full mouth PPD and REC at six sites per tooth (excluding third molars) using a manual, UNC-15 periodontal probe. Clinical attachment level (CAL) was calculated from PPD and REC. Upon completion of all measurements, intra-examiner repeatability for CAL measurement was assessed. Examiner was judged to be reproducible after meeting a percentage of agreement within ± 2 mm between repeated measurements of at least 98%.

Periodontal treatment

Patients were treated for periodontitis by means of non-surgical periodontal therapy by a periodontist. Oral hygiene instructions and subgingival scaling and root planing under local anaesthesia were performed using uniquely a piezoelectric instrument equipped with appropriate subgingival tips (EMS, Switzerland). The therapist, without limitations in terms of time or number of visits, completed this phase within 1–3 months of the baseline visit. All other dental treatments (extractions of hopeless teeth, restorative and endodontic treatments) were carried out before completion of the periodontal treatment phase.

Blood collection and analysis

Serum samples were collected from a single, clean venipuncture with minimal stasis from the antecubital fossa at baseline and 2 and 6 months after completion of treatment. Serum was obtained by centrifugation for 15 min at 2000 rpm within 1 h of collection. Samples were stored at –70°C until analysis that was performed in a standardised fashion to limit intra-individual variations. CRP serum levels were assessed by an automated immunoturbidimetric high-sensitivity assay (Cobas Integra, Roche, lower detection limit of 0.25 mg/l); IL-6 was measured by means of a commercial high-sensitivity ELISA kit (Quantikine HS, R&D System, Minneapolis, MN, USA, lower detection limit of 0.04 ng/l) according to manufacturer's instructions. Analyses were performed in duplicate and all samples for a given patient were analysed on the same ELISA plate to minimise variability.

Genetic polymorphisms analyses

Patients' DNA was extracted from peripheral leukocytes collected at the baseline and stored in an EDTA vacutainer in a –70°C freezer. A commercial kit was used according to manufacturer's instructions (Nucleon® BACC2 kit, Nucleon Bioscience, Coatbridge, UK). In brief, blood samples were thawed at room temperature and 30 ml of reagent A (red cell lysis buffer; 10 mM Tris–HCl pH 8; 0.32 M sucrose; 5 mM $MgCl_2$; 1% Triton X-100) added to 10 ml of blood in a 50 ml polypropylene centrifuge tube. The contents were mixed by inverting several times and then centrifuged at $1300 \times g$ for 5 min to sediment the leukocytes. The supernatant was discarded and 2.0 ml of reagent B (white cell lysis buffer) and 300 μg of proteinase K (Invitrogen, Paisley, UK) was added to the cell pellet. After vortexing, the tubes were incubated at 37°C for 30 min and 0.5 ml sodium perchlorate (0.5 M) was added, the contents mixed by inversion, then 2.0 ml chloroform was added and mixed again. Three hundred microliters of Nucleon® resin was added and the tubes centrifuged at $1300 \times g$ for 3 min to separate the phases, followed by removal of the upper aqueous phase containing the DNA, which was transferred to a fresh tube. Two volumes of cold ethanol were added to precipitate the DNA and the DNA was resuspended in 250 μl of sterile water. The DNA concentration was estimated by measuring absorbance at a wavelength 260 nm using a spectrophotometer. Ten nanograms of DNA were subsequently used for polymerase chain reaction (PCR). Each PCR reaction was performed in 25 μl volume containing 1 μl of DNA in buffer containing 10 mM Tris–HCl, pH 8.3, 50 mM KCl, 500 μM of each dNTPs (dATP, dCTP, dTTP, dGTP), 50 pmol of each of the primers and 1 U of taq polymerase (Abgene, UK). The $MgCl_2$ concentration varied for different primers. Sequences of the oligonucleotide primers used for PCR amplification, the size of the predicted PCR products and the PCR amplification program used were described previously (Kornman et al. 1997, Fishman et al. 1998). Briefly the following primer sets were used: for IL-1A (−889) 5′ **AAG CTT GTT CTA CCA CCT GAA CTA GGC** 3′and 5′ **TTA CAT ATG AGC CTT CCA TG** 3′; for IL-1B (−511) 5′ **TGG CAT TGA TCT GGT TCA TC** 3′ and 5′ **GTT TAG GAA ATC TTC CCA CTT** 3′; for IL-1B (+3954) 5′ **CTC AGG TGT CCT CGA AGA AAT CAA** A 3′and 5′ **GCT TTT TTG CTG TGA GTC CCG** 3′; finally for IL-6 (−174 G/C) 5′ **TGA CTT CAG CTT TAC TCT TGT** 3′, 5′ **CTG ATT GGA ACC CTT ATT AAG** 3′. Alleles were identified following digestion with restriction endonucleases on a 3% agarose gel containing 0.5 mg/ml ethidium bromide in 1 TBE buffer at 100 V for 2 h. The bands were visualised using a UV transilluminator.

Microbial samples and analysis

Samples of subgingival periodontal plaque were collected at baseline and 2 months after completion of treatment. Using a sterile curette the examiner collected subgingival plaque samples from the four deepest pockets, one in each quadrant (hopeless teeth were not included). Supragingival plaque was gently removed with a scaler, the site isolated from saliva and the curette inserted in the pocket. Samples were placed into 1 ml of reduced transport fluid (pooled sample) (Syed & Loesche 1972). Specimens were immediately transported to the Department of Microbiology at the Eastman Dental Hospital for analysis. At the 2 months visit plaque samples were collected from the same pockets irrespective of the changes in PPD. DNA was isolated from the plaque samples by the use of a commercial DNA extraction kit (Puregene, MI, USA). The DNA was then used as a template to carry out amplification of the bacterial 16S rRNA gene to confirm the presence of bacterial DNA in each sample. This 16S rRNA gene product was then used as a template using species specific primers to amplify regions of DNA specific to one of three different periodontal pathogens by the use of multiplex-PCR (Tran & Rudney 1999). The use of these

primers produced three different sized DNA products for *Tannerella forsythensis* (*Tf*) [formerly *B. forsythus*], *Porphyromonas gingivalis* (*Pg*) and *Actinobacillus actinomycetemcomitans* (*Aa*). PCR products were then visualised on an agarose gel. The presence of a DNA band at the expected size allowed confirmation of the presence of the pathogen within the plaque sample.

Statistical analysis

As pilot intervention trial, the sample size of this study was not based on formal power calculations but on logistic considerations. No other study was found in the literature at that time to perform a sample size analysis. All data were entered in a computer file, proofed for entry errors and analysed with a statistical package (SAS version 8.1, Chicago, IL, USA). Changes in serum concentrations of CRP and IL-6 following periodontal therapy were used as the primary outcome variables. Moreover, clinical and microbiological outcomes were defined in terms of changes in PPD, REC, CAL and presence of specific periodontal pathogens at different time points. Variables not normally distributed were logarithmic transformed before being used in parametric comparative analysis. Due to previously reported intra-individual variations in terms of serum CRP concentration, we included in the analysis only values within the interval of 3SD as previously suggested (Macy et al. 1997, Roberts et al. 2001). Continuous normally distributed variables were reported as mean $\pm$ SD while median and interquartile (IQ) ranges were used to describe non-normally distributed data. Between visits differences for each inflammatory marker were assessed by Wilcoxon's paired rank-sum test. Paired *t*-test was used to compare differences in clinical periodontal parameters. Presence or absence of periodontal pathogens after 2 months was assessed by McNemar's paired test. The null hypothesis of no changes in CRP and IL-6 concentrations following periodontal therapy was assessed with repeated measure analysis of variance adjusting for the known confounding factors. A significant difference was set to be at $p < 0.05$.

Results

Experimental population

Ninety-four subjects agreed on the treatment plan and reached the 6 months follow-up. Overall subjects were 46 ± 9 years of age, 54% were females, 42% were current smokers and 26% had a family history of cardiovascular diseases (see Table 1). The sample population included 75% chronic periodontitis patients while 25% were affected by an aggressive generalised form.

The frequency of different genotypes at the studied polymorphic sites for IL-1 and IL-6 is displayed in Fig. 1 and was in accordance with previous reports (Kornman et al. 1997, Fishman et al. 1998). All subjects throughout the 6 months study did not report any change in diet, medication or smoking habits. The recorded clinical periodontal parameters emphasise the severity and extent of the periodontal infection (Table 2). At baseline, patients had an average FMPS of $58 \pm 20.7\%$ and had an average of $63.5 \pm 16.4\%$ sites bleeding upon probing. The average number of deep periodontal pockets (PPD $>$ 4 mm) was 77 ± 23, with full mouth patient averages of CAL of 4.93 ± 1.13 mm, and PPDs of 4.36 ± 0.59 mm.

Multiplex PCR detectable levels of specific anaerobic pathogens were found in the majority of the cases as follows: 76.3% of the subjects were positive for *Tf*, 72.8% for *Pg* and 43.8% for *Aa*, indicating the high prevalence of infection with recognised periodontal pathogens.

Effects of treatment on periodontal outcomes

The clinical periodontal outcomes of treatment are displayed in Table 2. Oral hygiene was significantly improved with 2 and 6 months FMPS averaging about 20%. Full mouth bleeding scores (FMBS) also reached averages of 16% and 17% at 2 and 6 months, respectively. Subjects showed a significant reduction in the number of periodontal pockets from 77 ± 23 at baseline to 28 ± 16 at 2 months and 23 ± 15 at the 6 months follow-up ($p < 0.0001$, *t*-test). Changes in full mouth patient averages in terms of PPD, REC and CAL were also significant at the different time points ($p < 0.0001$, *t*-test) (Table 2). A significant reduction was achieved in the prevalence of *Pg* positive subjects (from 72.8% at baseline, to 34.2% at 2 months $p < 0.0001$ McNemar's test, Table 3) whereas, even if reduced, the number of sites positive for *Tf* did not reach statistical significance and no change was noted for *Aa*.

Table 1. Experimental population

Characteristics	
Age (years)	46 ± 8
Gender	Female 54%
Smoking	Current smokers 42%
Body mass index (kg/m^2)	25.3 ± 3.7
Family history of CVD	Positive 26%
Periodontal diagnosis	Chronic 75% Aggressive 25%
No. of teeth at baseline	27 ± 3
No. of teeth extracted	2 ± 2

Values reported are means $\pm$ SD.

Effect of periodontal treatment on serum parameters

Baseline CRP median level was 1.9 mg/l with an IQ range of 3.6 mg/l, whereas IL-6 median level was 1.82 ng/l (1.45 ng/l IQ). No significant differences in concentrations were found among different groups according to age, gender, periodontal diagnosis (chronic versus aggressive periodontitis) and smoking status. Nevertheless, a significant difference in serum level was found among sub-groups according to the carriage of specific polymorphisms in the IL-6 genes as well in the IL-1A gene (Figs. 2 and 3).

Baseline CRP concentrations were significantly associated with age, body mass index and patient average CAL in a multivariate analysis (Table 4).

Both inflammatory markers had a positive skewed distribution curve. A significant decrease in serum IL-6 concentration was found at 2 and 6 months after completion of periodontal treatment ($p = 0.021$, 0.006, respectively, Wilcoxon's test). CRP serum concentration decreased significantly only at the 6 months follow-up ($p < 0.0001$, Wilcoxon's test, Fig. 4A, B). The median change in CRP concentration between baseline and 6 months was of 0.5 mg/l with a distribution-free 95% confidence interval of 0.4–0.7 mg/l. The difference in median concentration of IL-6 was 0.18 ng/l (0.02–0.44 95% CI) between baseline and 2 months and 0.22 ng/l (0.06–0.44 95% CI) between baseline and 6 months (Table 5).

Due to heterogeneity of response to periodontal treatment (in terms of clinical periodontal parameters) and to the

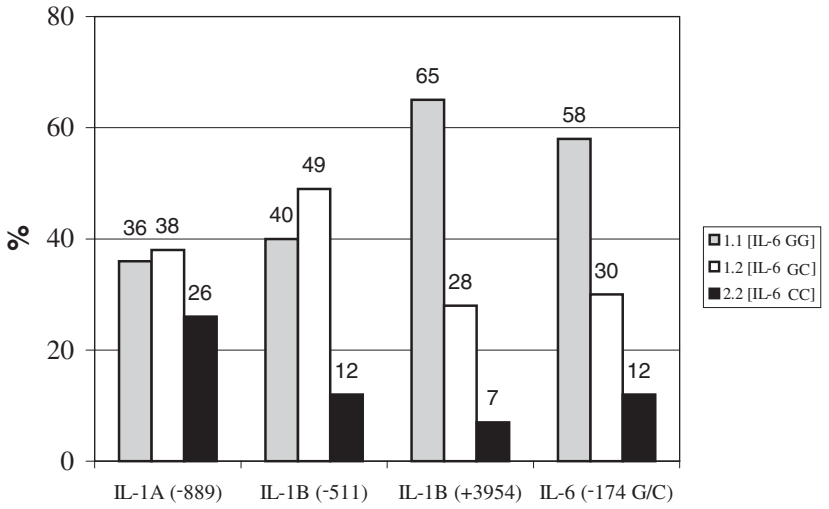

Fig. 1. Bar charts describing the prevalence of different genotypes in the population.

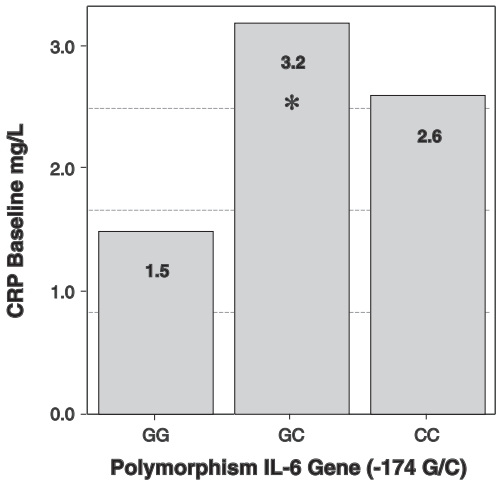

Fig. 2. Median baseline CRP levels among subgroups of patients according to specific IL-6 polymorphisms. *A significant difference was observed between heterozygous as compared with subjects homozygous for the G allele ($p = 0.027$, the Mann–Whitney test).

Table 2. Clinical periodontal parameters before and after treatment

	Baseline		2 Months		6 Months	
	Mean	SD	Mean	SD	Mean	SD
FMPS†	58.04	20.70	20.90‡	14.29	20.08‡	10.42
FMBS†	63.57	16.39	15.80‡	11.57	17.10‡	11.91
No. pockets*	77.08	23.23	27.82‡	16.36	22.91‡	15.04
PPD (mm)	4.36	0.59	3.25‡	0.47	3.19‡	0.47
REC (mm)	0.56	0.88	1.56‡	0.94	1.72‡	0.94
CAL (mm)	4.93	1.13	4.74	1.14	4.85	1.13

*Number of pockets with PPD > 4 mm. PPD, REC and CAL represent full mouth averages of each subject.
†FMPS, full mouth plaque score; FMBS, full mouth bleeding score.
‡$p < 0.001$ *t*-test compared with baseline.

presence of well-established covariates such as smoking, age, gender and body mass index, data were further analysed using a repeated measure analysis of covariance model with Log CRP as the dependent variable (Table 6). For the purposes of this analysis subjects were classified in terms of their level of response to periodontal therapy based on the median number of residual pockets and the median percentage of bleeding sites 6 months after treatment. The group with the better response to periodontal therapy was characterised by the persistence of less than 30 pockets 5 mm or deeper and less than 30% BoP. A highly significant model was constructed that explained 31% of the observed variability in Log CRP in terms of effectiveness of periodontal treatment, age, body mass index, smoking status and carriage of specific cytokine genotypes (Table 6). Data indicated that there was a significant interaction between the treatment outcome and the overall CRP levels (baseline, 2 and 6 months), and that the decrease in CRP was significant in those subjects who had the best outcomes in terms of periodontal parameters. The effect was significant after correcting for other known covariates such as age, gender, body mass index and smoking. Furthermore, the analysis indicated that serum CRP concentrations were significantly associated with carriage of specific polymorphic functional alleles in the promoter region of the IL-6 (−174G/C) and IL-1A (−889) genes. Carrying different alleles at the polymorphic sites of IL-1B gene (−511 and +3954) did not reach significance in the model.

A similar analysis using Log IL-6 as the dependent variable indicated that serum IL-6 changes were also associated with periodontal treatment effect as well as with covariates such as age of the subjects and a specific polymorphism in the IL-1B gene (−511)(Table 6).

Table 3. Prevalence of subjects positive for selected periodontal pathogens (multiplex PCR)

	Positive%	
	Baseline	2 Months
T. Forsythensis	76.3	64.4
P. Gingivalis	72.8	34.2*
A. Actinomycetemcomitans	43.8	39.5

*Significantly different from baseline $p <$ 0.0001 McNemar's test.

Discussion

This study reports the results of a pilot trial examining the effects of non-surgical periodontal therapy on systemic markers of inflammation. It demonstrated that effective control of

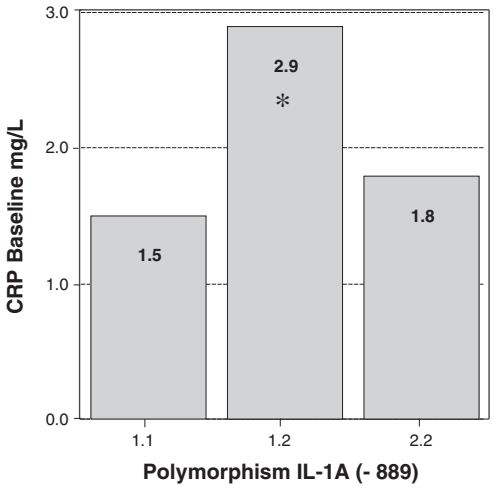

Fig. 3. Median baseline CRP levels among subgroups of patients according to specific polymorphism in the promoter region of IL-1A (−889). *A significant difference was observed between subjects carrying the 1.2 alleles (heterozygous) and those homozygous for 1.1 ($p = 0.013$, the Mann–Whitney test).

Table 4. Multiple linear regression model of Log [CRP] at baseline controlling for age, body mass index and CAL*

Model	Unstandardized coefficients		Standardized coefficients	Sig
	B	Std. error	*β*	*p*-value
(Constant)	−1.028	0.282		0.000
Age (years)	0.005	0.004	0.127	0.240
Body mass index (kg/m^2)	0.031	0.009	0.326	0.001
CAL* baseline (mm)	0.073	0.033	0.235	0.031

*CAL, clinical attachment level calculated as (PPD minus REC).
Model $R^2 = 0.22$.

periodontal infection reduced serum inflammatory markers (CRP, IL-6) in a relatively small population ($N = 94$) with severe generalised periodontitis. Within the limits of the design of this pilot study, the data clearly indicated that, in this systemically healthy population, there was a causal link between periodontitis and the systemic inflammatory status.

In this respect it seems relevant to emphasise that serum CRP and IL-6 levels detected at baseline were in the upper quartiles of the current definition of normality when determined with a high-sensitivity assay, rather than being in ranges usually associated with acute infections or systemic inflammatory diseases. This is important in providing a proper interpretation of these findings: in the context of infections, physicians have considered frankly elevated CRP values as an indication of a systemic infectious burden requiring appropriate therapeutic measures. More recent data from longitudinal studies, however, have indicated that acute phase response markers such as CRP and/or IL-6 in the upper distribution of normality seem to be predictive of atherogenesis and cardiovascular events (Ridker et al. 1997, Danesh et al. 2000). Renewed interest has been placed in understanding the role of chronic, low-grade infections and their associated inflammatory responses. Atherosclerosis is a multifactorial disease. The infection hypothesis has been on the scene since the early years of last century. Many seroepidemiological studies have supported an association between specific infections and cardiovascular events. *C. pneumoniae*, *H. pilory*, cytomegalovirus, and oral/dental infections have been implicated (Danesh 1999, Leinonen & Saikku 2002). The theory is based upon the presumable noxious effect that these pathogens may have acting directly and/or indirectly (through the inflammatory response) causing procoagulant activity, reduced fibrinolysis, increased leukocytes adhesion, increased cytokines production, and ultimately enhanced LDL and cholesterol deposition in the arterial wall (Libby et al. 2002). Among the possible mechanisms one is that the pathogens may sustain a low-grade inflammatory response in the atheroscletoric plaque (mediated by macrophages, endothelial cell, etc.). The intravascular infection may induce an endothelial injury and activate a local inflammatory response. In this vicious circle the activation of inflammatory cells and the production of inflammatory cytokines as well as of adhesion molecules may increase the risk for thrombogenesis. The increased values of systemic inflammation markers reported in the literature would therefore be an indicator of the atherosclerosis (endothelial damage) progression rather than the effect of distant infections. However a prospective study (Rotterdam Study) indicated that atherosclerosis-free individuals with chronic low-grade infections had an accelerated progression of atherosclerosis suggesting that there was chronological consistency, with infection preceding the development of atherosclerosis (van der Meer et al. 2002). Another good example of clear association between chronic infections (including periodontitis) and atherosclerosis comes from an ongoing prospective study (Kiechl et al. 2001). In atherosclerosis-free individuals the presence of a chronic infection predicted nearly 40% of new atherosclerotic lesions. This risk was also higher among subjects who showed an increased systemic inflammatory reaction. Whether this was due to particular virulence of pathogens involved or to an abnormal host reaction to them has yet been demonstrated. More support to this ''infectious'' hypothesis comes from two of the larger antibiotic secondary prevention trials. A beneficial effect of the antimicrobial therapy in terms of both serum levels of inflammatory markers (reduction at 6 months) and improvement in the endothelial function has been clearly reported (Gupta & Camm 1998, Anderson et al. 1999, Stone et al. 2002, Wiesli et al. 2002) in different population samples (mainly with pre-existing cardiovascular disease). More challenging has been demonstrating that antimicrobials are also effective in reducing the risk of new cardiovascular events. Properly sized randomised controlled clinical trials are currently under way to estab-

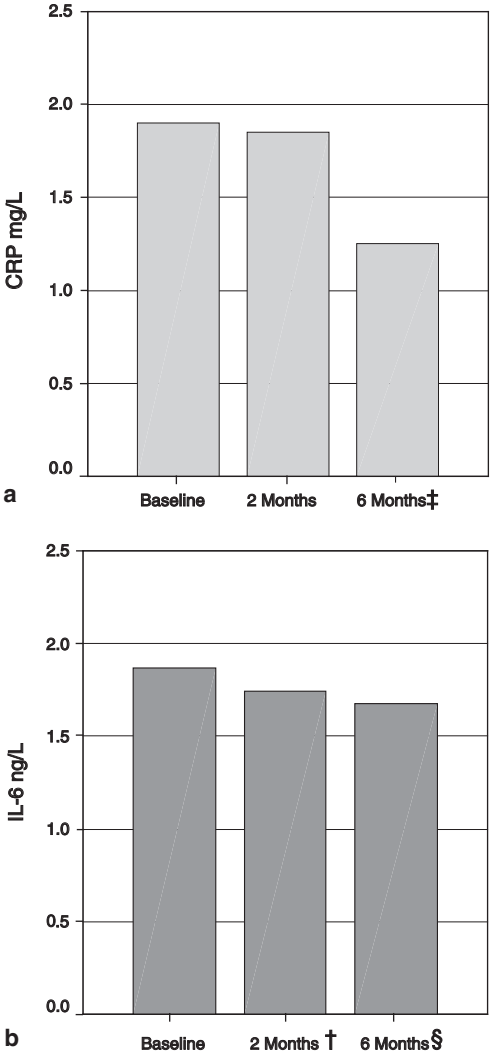

Fig. 4. (A, B) Bar charts showing median CRP and IL-6 serum levels at three different time points. ‡$p < 0.0001$ †$p = 0.021$ §$p = 0.006$ Wilcoxon's test when compared with baseline.

Table 5. Median differences of systemic inflammatory markers at 2 and 6 months follow up

	Δ Baseline – 2 months†			Δ Baseline – 6 months†		
	Median ± IQ	95%	CI*	Median ± IQ	95%	CI*
CRP (mg/l)	0.1 ± 1.0	−0.3	0	0.5 ± 1.3	0.4	0.7
IL-6 (ng/l)	0.18 ± 1.0	0.02	0.4	0.22 ± 1.0	0.06	0.4

*95% confidence interval distribution free.
†Δ = Difference of serum systemic markers between visits.

lish in a definitive way the nature of the association between chronic infections and cardiovascular events. It should be emphasised, however, that these trials will not be able to assess the role of biofilm centred infections such as periodontitis.

Evidence that periodontitis is associated with a systemic acute phase response has been relatively sparse. Periodontitis patients have higher CRP and IL-6 levels when compared with periodontally healthy matched populations (Ebersole et al. 1997, Ebersole & Cappelli 2000, Loos et al. 2000, Noack et al. 2001). This perturbation of the physiological homeostasis is also accompanied by a lower number of erythrocytes and haemoglobin concentrations (Hutter et al. 2001), higher values of haptoglobin, moderate leukocytosis (Fredriksson et al. 2002) and increased cholesterol, LDL and glucose levels (Losche et al. 2000). However it has become clear that some confounding factors (e.g. cigarette smoking) may be underlying these associations. The results of the current intervention study establish the presence of a causal link between periodontitis and systemic inflammatory response. Furthermore, a correlation between the severity of periodontitis and the serum concentrations of CRP was established in the present report, indicating that the effect of periodontitis on systemic inflammation seems to be dose dependent (Table 4).

Previous to this report, only two small trials have reported the impact of non-surgical periodontal therapy on systemic inflammation. Christgau et al. did not find any effect of periodontal treatment in a mixed small population (20 diabetics and 20 healthy) in terms of changes in CRP levels. One of the reasons may be in the assay they used for quantification of the acute phase marker (detection limit 2.8 mg/l) and secondly in the extent and severity of periodontitis in their population (Christgau et al. 1998). Non-surgical periodontal therapy had a significant effect on white blood count (specifically in terms of number of neutrophils and trombocytes) with again the confusing effect of smoking as covariate (Christan et al. 2002).

Another aspect of the results worth emphasising is the indication in the multivariate analysis (Table 6) that CRP decreases following treatment were associated with the clinical outcomes of the delivered periodontal therapy: subjects that had better outcomes than the median at 6 months (in terms of number of residual pockets and BoP) seemed to account for the observed decrease in CRP after correcting for all the other covariates. This preliminary observation seems to indicate that there may a dose-dependent effect of periodontal therapy in terms of systemic parameters. Moreover, incomplete control of periodontitis (as estimated by persistence of pockets and bleeding on probing) following non-surgical periodontal therapy alone may have precluded achieving an even bigger decrease in serum CRP levels. This observation raises the issue of new end-points in periodontal therapy in the context of prevention systemic inflammation and possibly atherosclerosis as well as the need to assess the

Table 6. Multivariate analyses explaining variability in observed serum Log [CRP] and Log [IL-6] as a function of the outcomes of periodontal treatment and controlling for potential confounding factors

	Log [CRP] (mg/l)			Log [IL-6] (ng/l)		
	β	Std. error	p	β	Std. error	p
Model[†]			<0.0001			0.001
Intercept	−0.8590	0.1814	<0.0001	0.0337	0.1455	0.8170
PERIO TX effect = 0*	−0.0218	0.0119	0.0688	−0.0065	0.0096	0.4996
PERIO TX effect = 1*	−0.0303	0.0085	0.0004	−0.0141	0.0069	0.0408
Age (years)	0.0087	0.0025	0.0005	0.0073	0.0020	0.0003
BMI (kg/m^2)	0.0236	0.0044	<0.0001	0.0033	0.0036	0.3549
Smoking [smokers versus non-smokers]	0.1124	0.0431	0.0097	−0.0061	0.0348	0.8612
IL-6 (−174 G/C) [CC versus GC]	0.1888	0.0650	0.0040	0.0098	0.0525	0.8527
IL-1A (−889) [2.2 versus 1.1]	−0.1093	0.0565	0.0540	−0.0329	0.0463	0.4781
IL-1A (−889) [2.2 versus 1.2]	0.0950	0.0544	0.0818	0.0209	0.0443	0.6372
IL-1B (−511) [2.2 versus 1.1]	−0.0390	0.0653	0.5514	−0.1648	0.0536	0.0023
IL-1B (−511) [2.2 versus 1.2]	0.0171	0.0620	0.7831	−0.1336	0.0508	0.0091
IL-1B (+3954) and Gender	…	…	NS	…	…	NS

*PERIO TX effect = 0 subjects presenting with 30 or more residual pockets (PPD > 4 mm) and ⩾30% bleeding upon probing 6 months after completion of periodontal therapy. PERIO TX effect = 1 subjects presenting with less than 30 residual pockets and less than 30% bleeding upon probing 6 months after completion of periodontal therapy. BMI, body mass index. See text for explanation of gene polymorphisms.

[†]Model: Log [CRP] Adj. $R^2 = 0.312$; Log [IL-6] Adj. $R^2 = 0.134$.

possible benefits of additional periodontal therapy.

The size of the observed improvement in CRP at 6 months is also noteworthy. The reported data indicated that in this population the observed median decrease in serum CRP was 0.5 mg/l. The 95% confidence interval, furthermore, was relatively narrow indicating that the ''true'' uncorrected median decrease is likely to be in the 0.4–0.7 mg/l range. Within the limitations of this design, a decrease of this magnitude in terms of CRP is comparable with those observed with some of the most promising medications such as statins and anti-inflammatory agents (Ridker et al. 1998, 1999). The medical significance of these changes is further emphasised by the fact that such changes would substantially decrease the predicted risk for future cardiovascular events based on serum CRP concentrations (Ridker et al. 1997).

Periodontal pathogens have been identified by PCR techniques in carotid atheromatous plaques of patients undergoing endarterectomy (Haraszthy et al. 2000). In cell culture studies, *P. gingivalis* has shown the ability to invade endothelial cells (Genco et al. 1999). Experimental animal models (ApoE mice) have also demonstrated that *P. gingivalis* chronic inoculations increased the lipid profiles, enhanced the atheroma formation and produced a calcification of the aortal atherosclerotic plaques (Li et al. 2002). A report from a large prospective study (ARIC) has associated periodontitis with an increased risk for carotid atherosclerosis (⩾1 mm) after correcting for potential confounding factors (Beck et al. 2001). The causal link reported in this study is in agreement with all these lines of accumulating evidence and supports the concept that there may be a true inflammatory link between periodontitis and atherogenesis.

Hypothetical pathogenetic models consider crucial the chronic gram-negative infectious burden (presumably LPS mediated) and the host response to it. The local inflammatory response with consequent release of proinflammatory cytokines (IL-1, TNF-α, IL-6) could explain not only the damage of the tooth supporting tissues (periodontal ligament, alveolar bone) but even the moderate systemic acute phase reaction that has been described in this study.

Since variations in the inflammatory response to the periodontal pathogens are also strictly connected with the individual genetic background, this study explored the significance of specific cytokine polymorphisms. Data indicated that specific cytokine polymorphisms were associated with serum CRP levels. These included functional polymorphisms in the promoter regions of the IL-6 and IL-1A genes. Recent studies have shown that IL-1 gene markers were predictors of serum levels of specific inflammatory markers as well as periodontal disease severity (Kornman et al. 1999, De Nardin 2001, Kornman & Duff, 2001, Berger et al. 2002). These genetic variations might produce an imbalance between agonist inflammatory mediators and influence the individual response to pathogens as well as to the treatment. Berger et al. in a population with significant atherosclerosis found that the 3954 hyperinflammatory type 2 allele for IL-1B markedly influenced (two- to threefold increase) baseline CRP serum levels. A similar but weaker effect was found in subjects carrying allele 2 at the polymorphic site (4845) of the IL-1A gene that is in 99% agreement with the IL-1A −889 polymorphism (Berger et al. 2002). In the present report, this specific genotype (IL-1A −889) showed a significant association with serum CRP levels whereas differences in the 3954 loci did not reach statistical significance. Furthermore, this study detected a significant association between IL-6 (−174 G/C) polymorphisms and both pre- and post-treatment serum values of CRP. CRP and IL-6 baseline levels have been already associated with this specific genotype. A recessive effect of the C allele on the mean levels of IL-6 was reported (Margaglione et al. 2001) whereas a dominant effect of the same was associated with increased baseline CRP levels (Vickers et al. 2002). We also found a significant association between the 511 IL-1B polymorphism and serum IL-6 levels. Given the small sample size of the current population caution should be made against overinterpreting the positive associations. In general, however, they seem to indicate that inflammatory responses to periodontal and other chronic infections may be higher in subjects carrying functional polymorphisms in IL-1 and IL-6 genes. These may predispose a subpopulation of period-

ontitis patients to cardiovascular diseases.

In this study a significant decrease in serum IL-6 was observed 2 and 6 months after completion of periodontal therapy. A significant decrease in CRP, however, could only be detected at the 6 months evaluation. Our data do not offer insights on the possible reasons for this delay. Interestingly, however, looking at the results published from antibiotic intervention trials in patients with cardiovascular diseases this time lag has also been reported (Anderson et al. 1999, Stone et al. 2002). We hypothesise that it could be related to the relative inefficiency of the mechanical periodontal treatment to fully eliminate the local infection.

Some limitations in these results have to be reported. Although serum samples were collected and handled in the best possible way, they were not taken at the same time of the day. This could have affected the analysis mainly of IL-6 serum levels. In fact this cytokine has shown a diurnal variation (Cava et al. 2000). Obesity is also a major source of inflammatory mediators' production (de Maat & Kluft 1996, Yudkin et al. 1999). We cannot exclude that lifestyle or diet changes in our population even if not reported could have affected our serological results. Another point could be related to CRP biological variability. Recognised determinants of CRP ''physiologic'' levels include weight, smoking, statin and hormone replace therapies, alcohol consumption and antibiotic treatment (Cushman et al. 1999, Kluft & de Maat 2001). There are controversial opinions with regards to biological variability. CRP is thought to have a low biological variation with few outliers (as reported in longitudinal studies) (Macy, et al. 1997). There is no diurnal variation and these outliers may presumably occur for inflammatory stimuli but their clearance should not take more than 3 days (Gabay & Kushner 1999). However a relatively wide variability ($\sim$30%) has been also reported (de Maat & Kluft 2001). More research is needed in this matter. In this respect our data open new doors in clinical periodontal research investigating the systemic impact of periodontal therapy.

Acknowledgments

Authors wish to acknowledge the contributions of Drs. Kalpesh Patel, Gareth Griffiths, Lars Laurell, Ulpee Darbar, Elaine Geydris Leeper in the management of the patients included in the study, Ms. Jean Suvan for her invaluable assistance in study organisation and administration and Professor Mike Wilson for his assistance with the microbiological analysis. Authors also wish to acknowledge support from an unrestricted grant from EMS, Switzerland, as well as from the periodontal research fund of the Eastman Dental Institute.

References

Anderson, J. L., Muhlestein, J. B., Carlquist, J., Allen, A., Trehan, S., Nielson, C., Hall, S., Brady, J., Egger, M., Horne, B. & Lim, T. (1999) Randomized secondary prevention trial of azithromycin in patients with coronary artery disease and serological evidence for Chlamydia pneumoniae infection. The azithromycin in coronary artery disease: elimination of Myocardial Infection with Chlamydia (ACADEMIC) study. *Circulation* **99**, 1540–1547.

Beck, J. D. & Offenbacher, S. (2001) The association between periodontal diseases and cardiovascular diseases: a state-of-the-science review. *Annals of Periodontology* **6**, 9–15.

Beck, J. D., Elter, J. R., Heiss, G., Couper, D., Mauriello, S. M. & Offenbacher, S. (2001) Relationship of periodontal disease to carotid artery intima-media wall thickness: the atherosclerosis risk in communities (ARIC) study. *Arteriosclerosis Thrombosis and Vascular Biology* **21**, 1816–1822.

Berger, P., McConnell, J. P., Nunn, M., Kornman, K. S., Sorrell, J., Stephenson, K. & Duff, G. W. (2002) C-reactive protein levels are influenced by common IL-1 gene variations. *Cytokine* **17**, 171–174.

Cava, F., Gonzalez, C., Pascual, M. J., Navajo, J. A. & Gonzalez-Buitrago, J. M. (2000) Biological variation of interleukin 6 (IL-6) and soluble interleukin 2 receptor (sIL2R) in serum of healthy individuals. *Cytokine* **12**, 1423–1425.

Christan, C., Dietrich, T., Hagewald, S., Kage, A. & Bernimoulin, J. P. (2002) White blood cell count in generalized aggressive periodontitis after non-surgical therapy. *Journal of Clinical Periodontology* **29**, 201–206.

Christgau, M., Palitzsch, K. D., Schmalz, G., Kreiner, U. & Frenzel, S. (1998) Healing response to non-surgical periodontal therapy in patients with diabetes mellitus: clinical, microbiological, and immunologic results. *Journal of Clinical Periodontology* **25**, 112–124.

Cushman, M., Legault, C., Barrett-Connor, E., Stefanick, M. L., Kessler, C., Judd, H. L., Sakkinen, P. A. & Tracy, R. P. (1999) Effect of postmenopausal hormones on inflammation-sensitive proteins: the postmenopausal estrogen/progestin interventions (PEPI) study. *Circulation* **100**, 717–722.

Danesh, J. (1999) Coronary heart disease, Helicobacter pylori, dental disease, Chlamydia pneumoniae, and cytomegalovirus: meta-analyses of prospective studies. *American Heart Journal* **138**, S434–S437.

Danesh, J., Whincup, P., Walker, M., Lennon, L., Thomson, A., Appleby, P., Gallimore, J. R. & Pepys, M. B. (2000) Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses. *BMJ* **321**, 199–204.

de Maat, M. P. & Kluft, C. (2001) Determinants of C-reactive protein concentration in blood. *Italian Heart Journal* **2**, 189–195.

de Maat, M. P., de Bart, A. C., Hennis, B. C., Meijer, P., Havelaar, A. C., Mulder, P. G. & Kluft, C. (1996) Interindividual and intraindividual variability in plasma fibrinogen, TPA antigen, PAI activity, and CRP in healthy, young volunteers and patients with angina pectoris. *Arteriosclerosis Thrombosis and Vascular Biology* **16**, 1156–1162.

De Nardin, E. (2001) The role of inflammatory and immunological mediators in periodontitis and cardiovascular disease. *Annales of Periodontology* **6**, 30–40.

Ebersole, J. L. & Cappelli, D. (2000) Acute-phase reactants in infections and inflammatory diseases. *Periodontology 2000* **23**, 19–49.

Ebersole, J. L., Machen, R. L., Steffen, M. J. & Willmann, D. E. (1997) Systemic acute-phase reactants, C-reactive protein and haptoglobin, in adult periodontitis. *Clinical and Experimental Immunology* **107**, 347–352.

Fishman, D., Faulds, G., Jeffery, R., Mohamed-Ali, V., Yudkin, J. S., Humphries, S. & Woo, P. (1998) The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. *Journal of Clinical Investigation* **102**, 1369–1376.

Fong, I. W. (2000) Emerging relations between infectious diseases and coronary artery disease and atherosclerosis. *Canadian Medical Association Journal* **163**, 49–56.

Fredriksson, M., Bergstrom, K. & Asman, B. (2002) IL-8 and TNF-alpha from peripheral neutrophils and acute-phase proteins in periodontitis. *Journal of Clinical Periodontology* **29**, 123–128.

Gabay, C. & Kushner, I. (1999) Acute-phase proteins and other systemic responses to inflammation. *New England Journal of Medicine* **340**, 448–454.

Genco, R. J. (1996) Current view of risk factors for periodontal diseases. *Journal of Periodontology* **67**, 1041–1049.

Genco, C. A., Potempa, J., Mikolajczyk-Pawlinska, J. & Travis, J. (1999) Role of gingipains R in the pathogenesis of Porphyromonas gingivalis-mediated periodontal disease. *Clinical Infectious Diseases* **28**, 456–465.

Genco, R., Offenbacher, S. & Beck, J. (2002) Periodontal disease and cardiovascular disease: epidemiology and possible mechanisms. *Journal of the American Dental Association* **133** (Suppl.), 14S–22S.

Gupta, S. & Camm, A. J. (1998) Chlamydia pneumoniae, antimicrobial therapy and coronary heart disease: a critical overview. *Coronary Artery Disease* **9**, 339–343.

Haraszthy, V. I., Zambon, J. J., Trevisan, M., Zeid, M. & Genco, R. J. (2000) Identification of periodontal pathogens in atheromatous plaques. *Journal of Periodontology* **71**, 1554–1560.

Howell, T. H., Ridker, P. M., Ajani, U. A., Hennekens, C. H. & Christen, W. G. (2001) Periodontal disease and risk of subsequent cardiovascular disease in US male physicians. *Journal of the American College of Cardiology* **37**, 445–450.

Hujoel, P. P., Drangsholt, M., Spiekerman, C. & DeRouen, T. A. (2002) Pre-existing cardiovascular disease and periodontitis: a follow-up study. *Journal of Dental Research* **81**, 186–191.

Hutter, J. W., van der Velden, U., Varoufaki, A., Huffels, R. A., Hoek, F. J. & Loos, B. G. (2001) Lower numbers of erythrocytes and lower levels of hemoglobin in periodontitis patients compared to control subjects. *Journal of Clinical Periodontology* **28**, 930–936.

Kiechl, S., Egger, G., Mayr, M., Wiedermann, C. J., Bonora, E., Oberhollenzer, F., Muggeo, M., Xu, Q., Wick, G., Poewe, W. & Willeit, J. (2001) Chronic infections and the risk of carotid atherosclerosis: prospective results from a large population study. *Circulation* **103**, 1064–1070.

Kluft, C. & de Maat, M. P. (2001) Determination of the habitual low blood level of C-reactive protein in individuals. *Italian Heart Journal* **2**, 172–180.

Koenig, W. (2001) Inflammation and coronary heart disease: an overview. *Cardiology Review* **9**, 31–35.

Kornman, K. S. & Duff, G. W. (2001) Candidate genes as potential links between periodontal and cardiovascular diseases. *Annales of Periodontology* **6**, 48–57.

Kornman, K. S., Crane, A., Wang, H. Y., di Giovine, F. S., Newman, M. G., Pirk, F. W., Wilson, T. G. Jr., Higginbottom, F. L. & Duff, G. W. (1997) The interleukin-1 genotype as a severity factor in adult periodontal disease. *Journal of Clinical Periodontology* **24**, 72–77.

Kornman, K. S., Pankow, J., Offenbacher, S., Beck, J., di Giovine, F. & Duff, G. W. (1999) Interleukin-1 genotypes and the association between periodontitis and cardiovascular disease. *Journal of Periodontal Research* **34**, 353–357.

Kweider, M., Lowe, G. D., Murray, G. D., Kinane, D. F. & McGowan, D. A. (1993) Dental disease, fibrinogen and white cell count; links with myocardial infarction? *Scottish Medical Journal* **38**, 73–74.

Leinonen, M. & Saikku, P. (2002) Evidence for infectious agents in cardiovascular disease and atherosclerosis. *Lancet Infectious Diseases* **2**, 11–17.

Li, L., Messas, E., Batista, E. L. Jr., Levine, R. A. & Amar, S. (2002) Porphyromonas gingivalis infection accelerates the progression of atherosclerosis in a heterozygous apolipoprotein E-deficient murine model. *Circulation* **105**, 861–867.

Libby, P., Ridker, P. M. & Maseri, A. (2002) Inflammation and atherosclerosis. *Circulation* **105**, 1135–1143.

Loos, B. G., Craandijk, J., Hoek, F. J., Wertheim-van Dillen, P. M. & van d, V (2000) Elevation of systemic markers related to cardiovascular diseases in the peripheral blood of periodontitis patients. *Journal of Periodontology* **71**, 1528–1534.

Losche, W., Karapetow, F., Pohl, A., Pohl, C. & Kocher, T. (2000) Plasma lipid and blood glucose levels in patients with destructive periodontal disease. *Journal of Clinical Periodontology* **27**, 537–541.

Macy, E. M., Hayes, T. E. & Tracy, R. P. (1997) Variability in the measurement of C-reactive protein in healthy subjects: implications for reference intervals and epidemiological applications. *Clinical Chemistry* **43**, 52–58.

Margaglione, M., Bossone, A., Cappucci, G., Colaizzo, D., Grandone, E. & Di Minno, G. (2001) The effect of the interleukin-6 c/g-174 polymorphism and circulating interleukin-6 on fibrinogen plasma levels. *Haematologica* **86**, 199–204.

Noack, B., Genco, R. J., Trevisan, M., Grossi, S., Zambon, J. J. & De Nardin, E. (2001) Periodontal infections contribute to elevated systemic C-reactive protein level. *Journal of Periodontology* **72**, 1221–1227.

O'Leary, T. J., Drake, R. B. & Naylor, J. E. (1972) The plaque control record. *Journal of Periodontology* **43**, 38.

Page, R. C. (1998) The pathobiology of periodontal diseases may affect systemic diseases: inversion of a paradigm. *Annales of Periodontology* **3**, 108–120.

Ridker, P. M., Cushman, M., Stampfer, M. J., Tracy, R. P. & Hennekens, C. H. (1997) Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *New England Journal of Medicine* **336**, 973–979.

Ridker, P. M., Rifai, N., Pfeffer, M. A., Sacks, F. M., Moye, L. A., Goldman, S., Flaker, G. C. & Braunwald, E. (1998) Inflammation, pravastatin, and the risk of coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events (CARE) Investigators. *Circulation* **98**, 839–844.

Ridker, P. M., Rifai, N., Pfeffer, M. A., Sacks, F. & Braunwald, E. (1999) Long-term effects of pravastatin on plasma concentration of C-reactive protein. The Cholesterol and Recurrent Events (CARE) Investigators. *Circulation* **100**, 230–235.

Roberts, W. L., Moulton, L., Law, T. C., Farrow, G., Cooper-Anderson, M., Savory, J. & Rifai, N. (2001) Evaluation of nine automated high-sensitivity C-reactive protein methods: implications for clinical and epidemiological applications Part 2. *Clinical Chemistry* **47**, 418–425.

Ross, R. (1999) Atherosclerosis – an inflammatory disease. *New England Journal of Medicine* **340**, 115–126.

Stone, A. F., Mendall, M. A., Kaski, J. C., Edger, T. M., Risley, P., Poloniecki, J., Camm, A. J. & Northfield, T. C. (2002) Effect of treatment for Chlamydia pneumoniae and Helicobacter pylori on markers of inflammation and cardiac events in patients with acute coronary syndromes: south Thames trial of antibiotics in myocardial infarction and unstable angina (STAMINA). *Circulation* **106**, 1219–1223.

Syed, S. A. & Loesche, W. J. (1972) Survival of human dental plaque flora in various transport media. *Applied Microbiology* **24**, 638–644.

Tran, S. D. & Rudney, J. D. (1999) Improved multiplex PCR using conserved and species-specific 16S rRNA gene primers for simultaneous detection of Actinobacillus actinomycetemcomitans, Bacteroides forsythus, and Porphyromonas gingivalis. *Journal of Clinical Microbiology* **37**, 3504–3508.

van der Meer, I., de Maat M, . P., Bots, M. L., Breteler, M. M., Meijer, J., Kiliaan, A. J., Hofman, A. & Witteman, J. C. (2002) Inflammatory mediators and cell adhesion molecules as indicators of severity of atherosclerosis: the Rotterdam Study. *Arteriosclerosis Thrombosis and Vascular Biology* **22**, 838–842.

Vickers, M. A., Green, F. R., Terry, C., Mayosi, B. M., Julier, C., Lathrop, M., Ratcliffe, P. J., Watkins, H. C. & Keavney, B. (2002) Genotype at a promoter polymorphism of the interleukin-6 gene is associated with baseline levels of plasma C-reactive protein. *Cardiovascular Research* **53**, 1029–1034.

Wiesli, P., Czerwenka, W., Meniconi, A., Maly, F. E., Hoffmann, U., Vetter, W. & Schulthess, G. (2002) Roxithromycin treatment prevents progression of peripheral arterial occlusive disease in Chlamydia pneumoniae seropositive men: a randomized, double-blind, placebo-controlled trial. *Circulation* **105**, 2646–2652.

Yudkin, J. S., Stehouwer, C. D., Emeis, J. J. & Coppack, S. W. (1999) C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? *Arteriosclerosis Thrombosis and Vascular Biology* **19**, 972–978.

Address:
Maurizio Tonetti
Department of Periodontology
Eastman Dental Institute and Hospital
256 Gray's Inn Road
London WC1X 8LD
UK
Fax: +44 20 79151137
E-mail: M.Tonetti@eastman.ucl.ac.uk