

# Periodontitis is characterized by elevated PAI-1 activity

Bizzarro S, van der Velden U, ten Heggeler J, Leivadaros E, Hoek FJ, Gerdes VEA, Bakker SJL, Gans ROB, ten Cate H, Loos BG. Periodontitis is characterized by elevated PAI-1 activity. *J Clin Periodontol* 2007; 34: 574–580. doi: 10.1111/j.1600-051X.2007.01095.x.

## Abstract

**Objectives:** Periodontitis is a chronic infectious disease and has been associated with cardiovascular diseases (CVD). We investigated whether plasma levels of markers of a prothrombotic state were elevated in patients with periodontitis in comparison with healthy controls.

**Materials and methods:** Untreated patients with moderate ( $n = 53$ ) and severe periodontitis ( $n = 38$ ) and healthy controls ( $n = 39$ ) were recruited. Levels of von Willebrand factor (vWF), prothrombin fragment 1+2 (F1+2), plasminogen activator inhibitor-1 (PAI-1) activity and D-dimer were measured as markers of a prothrombotic state.

**Results:** The erythrocyte sedimentation rate (ESR), plasma C-reactive protein (CRP) and leucocyte counts (WBC) were significantly higher in patients with periodontitis. No statistically significant difference was found among the three groups for vWF ( $p = 0.264$ ), F1+2 ( $p = 0.295$ ) and D-dimer ( $p = 0.572$ ). However, PAI-1 was clearly elevated in the severe periodontitis group ( $p = 0.001$ ), even after adjusting for potential confounding factors ( $p_{\text{adj}} = 0.004$ ). Moreover, more patients than controls were having vWF and PAI-1 levels above the respective population medians.

**Conclusions:** In periodontitis, elevated levels of PAI-1 activity are observed compared with healthy controls. This may increase the potential for impaired fibrinolysis, a condition that results in a prothrombotic state. We suggest that this state, if left untreated, may contribute to an increased risk for CVD.

S. Bizzarro<sup>1</sup>, U. van der Velden<sup>1</sup>, J. M. A. G. ten Heggeler<sup>1</sup>, E. Leivadaros<sup>1</sup>, F. J. Hoek<sup>2</sup>, V. E. A. Gerdes<sup>3</sup>, S. J. L. Bakker<sup>4</sup>, R. O. B. Gans<sup>4</sup>, H. ten Cate<sup>5</sup> and B. G. Loos<sup>1</sup>

<sup>1</sup>Department of Periodontology, Academic Center for Dentistry Amsterdam (ACTA), University of Amsterdam and Vrije University, Amsterdam, The Netherlands; <sup>2</sup>Department of Clinical Chemistry and <sup>3</sup>Department of Vascular Medicine, Academic Medical Center (AMC), University of Amsterdam, Amsterdam, The Netherlands; <sup>4</sup>Department of Internal Medicine, Academic Hospital Groningen, Groningen, The Netherlands; <sup>5</sup>Department of Internal Medicine and Cardiovascular Research Institute Maastricht, University of Maastricht, Maastricht, The Netherlands

Key words: D-dimer; F1+2; PAI-1; periodontitis; vWF

Accepted for publication 19 March 2007

Periodontitis is a chronic infectious disease of the supporting tissues of the teeth. The aetiology of this disease is strongly related to the colonization of the periodontal tissues by a complex mix of anaerobic, Gram-negative bacteria. A few Gram-negative bacterial species have been specifically associated with periodontitis, including

*Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans* and *Tannerella forsythensis* (Pihlstrom et al. 2005).

Interestingly, epidemiological studies have reported associations between periodontitis and cardiovascular diseases (CVD), especially myocardial infarction and stroke, and the association seems stronger in patients with severe periodontitis (Janket et al. 2003). One explanation for these epidemiological observations is the notion that in periodontitis, markers for CVD are elevated. Several studies have reported that patients with chronic periodontitis show elevated blood levels of leucocytes, especially neutrophils, elevated levels of C-reactive protein (CRP) and interleukin-6 (IL-6) (Loos

et al. 2000, Noack et al. 2001, Buhlin et al. 2003). Thus, a mild acute-phase response is present in periodontitis. Furthermore, intervention studies have shown that successful non-surgical periodontal treatment can decrease levels of CRP and IL-6 (D'Aiuto et al. 2004a, Seinost et al. 2005). These same factors are associated with the process of atherosclerosis, the underlying cause of CVD (Thompson et al. 1995, Folsom et al. 1997, Smith et al. 1997, Lowe 1998, Tuomisto et al. 2006). It has been suggested that periodontitis as a chronic infectious disease could contribute to CVD via CRP and IL-6 (Ajwani et al. 2003, Buhlin et al. 2003, D'Aiuto et al. 2004b). Most likely, plasma CRP and IL-6 are elevated due to a trigger from

## Conflict of interest and source of funding statement

The authors declare that they have no conflict of interests.  
The authors thank Philips Oral Healthcare EMEA for their support of this investigation.

the periodontal lesion; in periodontitis, the ulcerated pocket epithelium forms a porte d'entrée for bacterial antigens, lipopolysaccharide and whole bacteria, resulting in episodes of bacteraemia (Herzberg & Weyer 1998, Li et al. 2000, Daly et al. 2001, Geerts et al. 2002, Kinane et al. 2005).

An acute-phase response may contribute to a prothrombotic state (Munford 2001) as a result of endothelial perturbation, increased activity of blood coagulation and attenuated fibrinolysis. A prothrombotic state is reflected by the presence of increased concentrations of proteins like von Willebrand factor (vWF) and plasminogen activator inhibitor-1 (PAI-1), or protein cleavage products as prothrombin fragment 1+2 (F1+2) and D-dimer (Sagripanti et al. 1996). Indeed, elevated plasma levels of these markers have been associated with a higher risk for recurrent ischaemic events in patients with a history of coronary heart disease or ischaemic stroke (Lindgren et al. 1996, Cote et al. 2000, Danesh et al. 2001, Lowe et al. 2001, Barber et al. 2004).

While there is good evidence for close interactions between inflammatory proteins, endothelial activation and prothrombotic alterations in diseases like sepsis, it has only been scarcely established whether a more subtle chronic infectious state, like periodontitis, leads to prothrombotic changes in the blood compartment. A recent study on the systemic effects of full-mouth tooth extraction in 67 individuals showed a significant reduction of PAI-1 antigen and fibrinogen, highly suggestive that periodontitis may indeed result in a prothrombotic state (Taylor et al. 2006). To further investigate the association between periodontitis and a prothrombotic state, we set out to analyse four thrombotic markers, in conjunction with established markers of inflammation, in patients with periodontitis and healthy controls.

## Material and Methods

### Study population

We selected a consecutive series of periodontitis patients who were referred to the Department of Periodontology of the Academic Center for Dentistry Amsterdam (ACTA) for diagnosis and treatment of periodontitis. Controls were selected among subjects registered for restorative dental procedures or who

visited the dental school for regular dental check-ups. Control subjects were included if they were not missing more than one tooth per quadrant (3rd molar excluded). These subjects showed on dental bitewing radiographs  $\leq 1$ -year-old a distance between the cemento-enamel junction (CEJ) and the alveolar bone crest of  $\leq 3$  mm on all teeth. All subjects were both verbally and written informed about the purpose of the study and gave written informed consent to participate. The Medical Ethical Committee of the Academic Medical Center of the University of Amsterdam approved the study.

On the basis of an extensive medical history by a written questionnaire and an interview, the following subjects were not included in the study: pregnant women and individuals who suffered from any given disease or chronic medical condition, apart from periodontitis, or had trauma or tooth extractions in the last 2 weeks, or received antibiotics within the last 3 months. For all participants, smoking habits were recorded as current smoker, former smoker or non-smoker. Former smokers were defined as those subjects who quit smoking  $\leq 10$  years ago, and non-smokers were defined as those subjects who never smoked or quit smoking  $> 10$  years ago. Educational level was used as a surrogate marker for social class. From height and weight measurements, the body mass index (BMI) was calculated. Ethnicity was recorded and scored as Caucasian or non-Caucasian.

We used dental radiographs to estimate the severity of periodontal destruction as described before. Patients with  $\geq 7$  teeth with  $\geq 50\%$  bone loss were classified as having severe periodontitis ( $n = 38$ ). The remainder of the periodontitis patients were classified as having moderate periodontitis ( $n = 53$ ) (Leivadaros et al. 2005).

### Clinical and laboratory examinations

All participants were asked to fast at least for 12 h before the clinical visit. Fasting venous blood samples were obtained between 8.30 and 11.30 AM by venipuncture in the antecubital fossa, without excessive venous stasis. The venous blood was collected into ethylenediaminetetraacetic acid (EDTA) (10 and 5 ml) and citrate (5 ml)-containing vacuum tubes (BD Vacutainer System, Plymouth, UK). One tube of EDTA blood was used for automated leucocyte

counting, leucocyte differentiation and the erythrocyte sedimentation rate (ESR). The other tubes of EDTA blood and citrate blood were centrifuged at 3000 r.p.m. for 10 min., and the obtained plasma was stored in aliquots at 80°C until further analysis.

The EDTA plasma was used for measurements of plasma total cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides and CRP. Plasma levels of total cholesterol, HDL cholesterol and triglycerides as well as the ESR were determined by standard (enzymatic) methods in a hospital-based diagnostic clinical laboratory as described before (Loos et al. 2000); low density lipoprotein (LDL) cholesterol was calculated (Friedewald et al. 1972). Plasma levels of CRP were determined using a high-sensitivity (latex enhanced) nephelometric method on a BN ProSpec analyzer (Dade Behring, Marburg, Germany) (Loos et al. 2000).

### Analysis of markers of a prothrombotic state

Citrate plasma samples were used for analysis of markers of a prothrombotic state. Plasma F1+2 levels were determined using commercially available enzyme linked immunosorbent assay (ELISA) kits, according to the manufacturer's instructions (Enzygnost F1+2 micro, Dade Behring, Marburg, Germany). PAI-1 activity was measured with a commercially available ELISA kit (Spectrolyse, Kordia NV, the Netherlands). VWF was measured by means of an in-house enzyme immunoassay (sandwich ELISA-based assay) developed in the laboratory for Special Hemostasis Research, Academic Hospital Maastricht, the Netherlands. In this assay, Dako polyclonal antibodies (Dako, Glostrup, Denmark) (rabbit anti-human, A082) were used as capture antibodies in a 96-well MaxiSorp plate (Nunc, Roskilde, Denmark). Horseradish peroxidase (HRP)-conjugated polyclonal antibodies (Dako) (rabbit anti-human, P0226) were used as detection antibodies. 'Normal' pool plasma (0.105 mol/l citrated plasma, pooled from 87 healthy volunteers without any medication, mean age 45) was used as a calibrator (100% vWf:ag). The range in the 'normal' population is 40–175%. Plasma D-dimer was measured by means of a commercially available enzyme immunoassay (TintElize, Biopool International, Umea, Sweden).

### Statistical analysis

Statistical analysis of data was performed with the SPSS package version 11.0 (SPSS, Chicago, IL, USA). Means, standard deviations, medians and frequency distributions were calculated. Differences between the three study groups for all the background variables were analysed by analyses of variance (ANOVA) or by  $\chi^2$  tests. Missing data for BMI ( $n = 1$ ), total cholesterol and triglycerides ( $n = 13$ ), F1+2 ( $n = 4$ ) and vWF ( $n = 2$ ) were estimated by model-based imputation. Raw data for vWF, F1+2, PAI-1 and D-dimer are presented in box and whisker plots generated in the statistical programme and were initially analysed by non-parametric testing (Kruskal–Wallis test). Differences among the three study groups in the plasma levels of vWF, F1+2, PAI-1 and D-dimer were further explored: first raw means and standard deviations are presented, analysed by ANOVA and subsequently tested by analyses of co-variance (ANCOVA), with periodontal status (three groups) as the factor and smoking and education level as the co-factors, while age, gender, BMI, total cholesterol, triglycerides and ethnicity were entered in the model as co-variables. The  $p$ -values obtained from these models are referred to as  $p_{\text{adj}}$ . Moreover, from the ANCOVA models adjusted means and confidence intervals (CI) were calculated and the distribution of the unstandardized residuals was checked for normality. In the nonparametric analyses and ANCOVA's, when overall significant differences were found, post hoc testing between any two groups was performed using the same models and the  $p$ -values obtained were multiplied by 3 to correct for multiple testing. The median values for vWF, F1+2, PAI-1 and D-dimer from the whole study population were used as cut-off points; the number of control subjects and the number of patients above and below the respective population medians were calculated, differences were analysed with Fisher's exact tests and odds ratios (OR) and 95% CI were determined.  $p < 0.05$  were considered to be statistically significant.

### Results

A total of 130 subjects, 91 untreated periodontitis patients and 39 controls, between the ages of 19 and 63 years, participated in the study. Of the 91 periodontitis patients, 38 were included

in the severe group and 53 in the moderate group. The mean ages for controls, moderate periodontitis and severe periodontitis were 39.3, 43.8 and 45.9 years, respectively (Table 1). Periodontitis patients more often had a low education level. Among periodontitis patients, >70% were either current or former smokers, while this was 44% among controls. The mean values for cholesterol and triglycerides were not different among the three groups, while BMI was slightly higher in moderate periodontitis patients (Table 1).

Periodontitis patients had lower number of teeth than controls. Further, the severe group presented on average 20.7 teeth with  $\geq 30\%$  bone loss and 10.5 teeth with  $\geq 50\%$  bone loss. For the moderate periodontitis group, the corresponding values were 13.5 and 2.4 teeth. Controls had, per definition, no bone loss (Table 2).

The markers of systemic inflammation for the three study groups are presented in Table 3; leucocytes, ESR and CRP were significantly higher in patients than controls.

### Markers of a prothrombotic state

The median values for vWF were 76.0%, 92.0% and 91.0% for controls, moderate periodontitis and severe periodontitis, respectively (Fig. 1A). There was no statistically significant difference among the three groups ( $p = 0.264$ ). The analysis for F1+2 shows median values of 0.88, 1.01 and 1.00 nmol/l for controls, periodontitis and severe periodontitis, respectively (Fig. 1B). No significant difference was found among the three groups ( $p = 0.295$ ). Figure 1C shows box plots of the plasma levels of PAI-1 activity

Table 1. Characteristics for the three study groups (control, moderate periodontitis, severe periodontitis); values are means  $\pm$  standard deviations, or number of subjects (in parentheses % of subjects)

	Control ( $n = 39$ )	Moderate periodontitis ( $n = 53$ )	Severe periodontitis ( $n = 38$ )	$p$ -value
Age (years)	39.3 $\pm$ 10.6	43.8 $\pm$ 8.8	45.9 $\pm$ 8.3	0.007
Gender				NS
Male	14 (36%)	24 (45%)	19 (50%)	
Female	25 (64%)	29 (55%)	19 (50%)	
Ethnicity				NS
Caucasian	35 (90%)	41 (77%)	34 (90%)	
Other	4 (10%)	12 (23%)	4 (10%)	
Education level				0.030
<High school	13 (33%)	34 (64%)	15 (39%)	
= High school	7 (18%)	3 (6%)	6 (16%)	
>High school	19 (49%)	16 (30%)	17 (45%)	
Smoking habits				0.006
Current smoker	12 (31%)	21 (40%)	21 (55%)	
Former smoker	5 (13%)	16 (30%)	10 (26%)	
Non-smoker	22 (56%)	16 (30%)	7 (19%)	
BMI ( $\text{kg}/\text{m}^2$ )	24.3 $\pm$ 3.4	26.1 $\pm$ 5.1	24.1 $\pm$ 2.7	0.031
Total cholesterol (mmol/l)	5.2 $\pm$ 1.0	5.4 $\pm$ 1.1	5.6 $\pm$ 1.1	NS
HDL/cholesterol (mmol/l)	1.5 $\pm$ 0.3	1.3 $\pm$ 0.4	1.4 $\pm$ 0.4	NS
LDL/cholesterol (mmol/l)	3.1 $\pm$ 0.9	3.4 $\pm$ 1.0	3.6 $\pm$ 1.0	NS
Triglycerides (mmol/l)	1.2 $\pm$ 0.5	1.5 $\pm$ 0.8	1.4 $\pm$ 0.9	NS

Table 2. Radiographic findings regarding numbers of teeth and alveolar bone loss for the three study groups (control, moderate periodontitis and severe periodontitis); values are means  $\pm$  standard deviations

	Control ( $n = 39$ )	Moderate periodontitis ( $n = 53$ )	Severe periodontitis ( $n = 38$ )
Total no. of teeth*	28.1 $\pm$ 2.1	26.2 $\pm$ 3.6	25.7 $\pm$ 3.2
No. of teeth			
$\geq 30\%$ bone loss	0 $\pm$ 0	13.5 $\pm$ 6.0	20.7 $\pm$ 3.7
$\geq 50\%$ bone loss	0 $\pm$ 0	2.4 $\pm$ 1.6	10.5 $\pm$ 4.2

\* $p = 0.002$ .

Table 3. Markers of systemic inflammation for the three study groups (control, moderate periodontitis and severe periodontitis); values are means  $\pm$  standard deviations

	Control (n = 39)	Moderate periodontitis (n = 53)	Severe periodontitis (n = 38)	p-value
Leukocytes ( $\times 10^9/l$ )				
Total	5.7 $\pm$ 1.3	7.1 $\pm$ 2.5	7.5 $\pm$ 2.0	<0.001
Neutrophils	3.1 $\pm$ 1.2	4.1 $\pm$ 1.9	4.5 $\pm$ 1.7	0.001
Lymphocytes	1.9 $\pm$ 0.4	2.3 $\pm$ 0.8	2.3 $\pm$ 0.6	0.011
Erythrocyte Sedimentation Rate (mm/h)				0.028*
Males	3.5 $\pm$ 2.1	8.2 $\pm$ 7.6	6.4 $\pm$ 5.3	
Females	9.2 $\pm$ 6.5	13.2 $\pm$ 9.3	11.8 $\pm$ 8.9	
C-reactive protein (mg/l)	1.9 $\pm$ 2.0	3.1 $\pm$ 3.3	3.1 $\pm$ 4.4	0.034

\*Overall ANOVA, adjusted for gender.

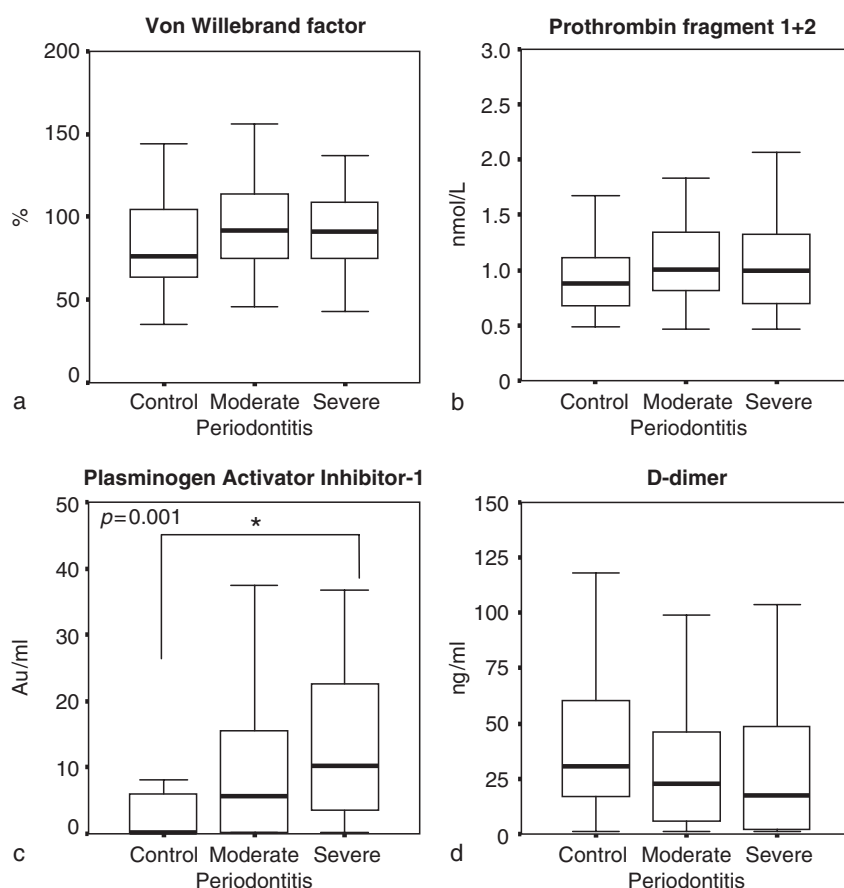


Fig. 1. Box and whisker plots for the three study groups for von Willebrand factor (vWF) (A), prothrombin fragment 1+2 (F1+2) (B), plasminogen activator inhibitor-1 (PAI-1) activity (C) and D-dimer (D). The boxes represent 25–75th percentiles. The horizontal line inside each box indicates the median (50th percentile). Overall nonparametric analyses revealed a statistically significant difference for PAI-1 activity among the three groups ( $p = 0.001$ ). \*Post hoc testing showed a significant difference between the severe periodontitis group and the control group ( $p = 0.003$ ).

for the three groups; the median plasma levels of PAI-1 activity were 0.20, 5.60, 10.20 AU/ml for controls, moderate periodontitis and severe periodontitis, respectively. Statistical analysis showed a significant difference among the three

groups ( $p = 0.001$ ). Post-hoc testing demonstrated higher values in the severe group than in controls ( $p = 0.003$ ) and a trend for higher values than in the moderate periodontitis group ( $p = 0.051$ ). Plasma levels of D-dimer

were not different among the three groups (Fig. 1D); the median values for D-dimer were 31.0, 23.0 and 18.0 ng/ml for controls, moderate periodontitis and severe periodontitis, respectively ( $p = 0.572$ ).

Table 4 presents the distribution of subjects with values above and below the total study population median for the four tested markers. OR and 95% CI are also presented. The following cut-off points were used: 88%, 0.97 nmol/l, 4.75 AU/ml and 25.5 ng/ml for vWF, F1+2, PAI-1 and D-dimer, respectively. From this analysis, it appears that more patients (58%) than controls (33%) had vWF levels above the population median ( $p = 0.013$ ) (OR 2.79). Also, for PAI-1, a clear difference in distribution was found ( $p = 0.002$ ); almost 60% of periodontitis patients *versus* 28% of controls had PAI-1 values above the population median (OR 3.71). The analyses showed no differences for F1+2 and D-dimer (Table 4).

We further analysed the differences of the markers of a prothrombotic state between the three study groups by adjusting for possible confounding factors in an ANCOVA model. We took age, gender, ethnicity, educational level, BMI, cholesterol, triglycerides and smoking into account. On the basis of this model, in addition to raw means  $\pm$  standard deviations, adjusted means and adjusted  $p$ -values are presented in Table 5. The overall analysis showed no statistical difference for plasma levels of vWF ( $p_{\text{adj}} = 0.407$ ), F1+2 ( $p_{\text{adj}} = 0.866$ ) and D-dimer ( $p_{\text{adj}} = 0.958$ ). In contrast, the ANCOVA model showed that there was a difference among the three groups for PAI-1 ( $p_{\text{adj}} = 0.004$ ); post hoc analysis showed that the severe periodontitis group had a higher PAI-1 activity than the control group (Table 5). In addition to periodontal disease status, in the statistical model, a significant association was found for PAI-1 with BMI ( $p = 0.002$ ), ethnicity ( $p = 0.039$ ) and triglycerides ( $p = 0.049$ ). The skewness of the unstandardized residuals of the mean was found to be  $<1$  and therefore PAI-1 values were assumed to be normally distributed.

## Discussion

The present study shows that in periodontitis, there is an indication for the presence of a prothrombotic state. This state seems to be primarily due to diminished potential for fibrinolytic

Table 4. Numbers (%) of control subjects and periodontitis patients with values of markers of a prothrombotic state below (<) and (≥) above the median of the whole study population. *p*-values, odds ratio's (OR) and 95% confidence intervals (CI) are reported

	Control (n = 39)	Periodontitis (n = 91)	OR (CI)	<i>p</i> value
VWF			2.79 [1.27–6.12]	0.013
< 88%	26 (66.7%)	38 (41.8%)		
≥ 88%	13 (33.3%)	53 (58.2%)		
F1+2			1.45 [0.64–3.28]	0.423
< 0.97 nmol/l	28 (71.8%)	58 (63.7%)		
≥ 0.97 nmol/l	11 (28.2%)	33 (36.3%)		
PAI-1			3.71 [1.65–8.39]	0.002
< 4.75 AU/ml	28 (71.8%)	37 (40.7%)		
≥ 4.75 AU/ml	11 (28.2%)	54 (59.3%)		
D-dimer			0.44 [0.20–0.95]	0.055
< 25.5 mg/ml	14 (35.9%)	51 (56.0%)		
≥ 25.5 mg/ml	25 (64.1%)	40 (44)		

Table 5. Means ± standard deviations and *p*-values calculated from ANOVA for plasma levels of vWF, F1+2, PAI-1 activity and D-dimer for the three study groups (control, moderate periodontitis, and severe periodontitis) are reported in the first line of each parameter

	Control (n = 39)	Moderate periodontitis (n = 53)	Severe periodontitis (n = 38)	<i>p/p<sub>adj</sub></i> -value
VWF (%)	85.0 ± 31.8	94.6 ± 31.6	96.2 ± 35.8	<i>p</i> = 0.204
	92.0 (80.6–103.5)	87.5 (77.2–97.8)	97.2 (86.2–108.3)	<i>p<sub>adj</sub></i> = 0.407*
F1+2 (nmol/l)	0.98 ± 0.42	1.21 ± 0.84	1.19 ± 0.88	<i>p</i> = 0.866
	1.08 (0.81–1.36)	1.17 (0.93–1.42)	1.10 (0.84–1.37)	<i>p<sub>adj</sub></i> = 0.866†
PAI-1 (AU/ml)	5.03 ± 8.62	8.30 ± 9.55	13.69 ± 11.75	<i>p</i> = 0.001
	7.66 (4.29–11.03)	7.00 (3.98–10.03)	13.82 (10.58–17.07) ‡	<i>p<sub>adj</sub></i> = 0.004‡
D-dimer (ng/ml)	48.0 ± 57.1	44.6 ± 65.6	34.7 ± 43.2	<i>p</i> = 0.572
	45.3 (26.0–64.5)	42.7 (25.4–59.9)	41.4 (22.9–60.0)	<i>p<sub>adj</sub></i> = 0.958§

\*Significant co-variate: BMI (*p* = 0.011).

†Significant co-variate: age (*p* = 0.010).

‡Significant co-variables: ethnicity (*p* = 0.039), BMI (*p* = 0.002) and tryglicerides (*p* = 0.049).

§Significant co-variables: gender (*p* = 0.005), BMI (*p* < 0.001) and total cholesterol (*p* = 0.006).

||Significantly different from controls (*p* = 0.003) in post-hoc testing.

In the line below adjusted mean values (95% confidence interval) calculated from the ANCOVA model and *p<sub>adj</sub>*-values are reported.

activity, caused by elevated PAI-1 levels in blood. These findings are still significantly present after correcting for several potential confounding factors. In addition, we observed a greater proportion of patients with elevated vWF levels, indicating endothelial cell activation, also known as endothelial dysfunction (*p<sub>adj</sub>* = 0.004). We did not observe a significant increase in thrombin production as expressed by the concentration of F1+2 or fibrin cleavage, although the lack of increased D-dimer concentration may in part be due to attenuated fibrinolysis. However, the observed association between the increase of plasma levels of PAI-1 activity and periodontitis may have to be interpreted with caution, as the current association study does not prove that periodontitis is actually “causing” the elevation of

plasma PAI-1 activity (according to the criteria stated by Bradford Hill) (Hofler, 2005). Nevertheless, there are biological explanations for our findings.

PAI-1 is an inhibitor of fibrinolysis through inhibition of the tissue-plasminogen activator (t-PA). It is a protein released by several cell types, including endothelial cells, hepatocytes and adipocytes and it is present in platelets, placenta and serum. Although PAI-1 is considered a marker of fibrinolysis, it is also a protein involved in the acute-phase response (De Taeye et al. 2005). In the current study, the periodontitis patients seemed to have a mild systemic inflammation as evidenced by an increased ESR and the presence of higher levels of leucocytes and CRP. Elevation of cytokines, including IL-6 and tumour necrosis factor-α, leads to the

activation of hepatic cells and their systemic release of acute-phase proteins as CRP. These cytokines also trigger the production and secretion of PAI-1 by hepatocytes. Thus, a systemic inflammatory state such as in periodontitis may also lead to elevation of PAI-1 as part of the acute-phase reaction. The possibility that the occurrence of bacteraemia in periodontitis (Daly et al. 2001, Kinane et al. 2005, Loos 2005) can stimulate the release of PAI-1 should be considered. Nevertheless, we suggest that the elevated PAI-1 is not merely a marker of systemic inflammation but also has the potential to inhibit fibrinolytic activity. This interpretation fits with clinical observations where periodontitis is associated with CVD events (Janket et al. 2003, Andriankaja et al. 2006). Moreover, PAI-1 is an established haemostatic factor associated with the development of CVD (Kannel 2005). Thus, it may be postulated that elevated plasma levels of PAI-1 predispose to a chronic prothrombotic state, and therefore to an increased risk for CVD. It has also been suggested that extravascular stimuli inducing chronic activation of inflammation, such as smoking, stress, obesity and infections including bronchitis, gastritis and periodontitis, may contribute over a long period to the formation of atherosclerotic lesions in susceptible subjects before the occurrence of a CVD event (Munford 2001).

A recent study by Bretz et al. (2005) failed to find any association between periodontitis and plasma levels of PAI-1 antigen. However, a comparison between the latter study and the current one is difficult because of several reasons. First of all, the Bretz et al. study was a population-based cross-sectional epidemiological study, while the current study is a convenient sample consisting of consecutive referred periodontitis patients and control subjects from the same dental institute, who were not affected by periodontal breakdown. Further, the Bretz et al. study included a much older population (mean age 72.7 years) with a different racial background (66% white). Finally, the population of the current study was as per selection criteria, systemically healthy. On the other hand, the currently reported elevation of PAI-1 activity in periodontitis corroborates longitudinal findings that in subjects with periodontitis, elimination of all teeth resulted in lower levels of PAI-1 antigen (Taylor et al. 2006).

Interestingly, in the current study, a higher proportion of patients showed plasma levels of vWF above the population median (Table 4). Also, in another study, vWF has been associated with periodontitis (Montebugnoli et al. 2004). Elevated levels of vWF are observed in conditions of damaged or dysfunctional endothelial cells such as in atherosclerotic vascular disease (Lip & Blann 1997). Because of the role of vWF in both adhesion and aggregation of platelets and in coagulation, the elevated levels of vWF may increase the risk of thrombus formation in patients with pre-existing disease of the vascular wall, such as in atherosclerosis (Lip & Blann 1997). It may be speculated that bacteria, endotoxin, and other inflammatory stimuli, leaking from the periodontal infection into periodontal tissues and into the circulation, may induce the release of vWF from human endothelial cells and activate platelets (Lowe 1998).

F1+2 and D-dimer are peptides released during activation of coagulation and fibrinolysis, respectively. In the current study, no significant differences were found between periodontitis patients and controls for these latter factors. The results are in accordance with another study (Montebugnoli et al. 2004), where, also, no association was found between periodontal parameters and plasma levels of F1+2 and D-dimer.

In conclusion, the current study is highly suggestive that patients with periodontitis have impaired fibrinolysis, and a mild degree of endothelial activation. These conditions may then lead to a prothrombotic state. Therefore, it seems reasonable to postulate that periodontitis, in addition to other well-known risk factors, may contribute to an increased risk of CVD in the long run. These data, in accordance with previously published findings, corroborate the need for proper oral health care, particularly in those individuals at risk for CVD.

## References

- Ajwani, S., Mattila, K. J., Narhi, T. O., Tilvis, R. S. & Ainamo, A. (2003) Oral health status, C-reactive protein and mortality – a 10 year follow-up study. *Gerodontology* **20**, 32–40.
- Andriankaja, O. M., Genco, R. J., Dorn, J., Dmochowski, J., Hovey, K., Falkner, K. L., Scannapieco, F. & Trevisan, M. (2006) The use of different measurements and definitions of periodontal disease in the study of the association between periodontal disease and risk of myocardial infarction. *Journal of Periodontology* **77**, 1067–1073.
- Barber, M., Langhorne, P., Rumley, A., Lowe, G. D. & Stott, D. J. (2004) Hemostatic function and progressing ischemic stroke: D-dimer predicts early clinical progression. *Stroke* **35**, 1421–1425.
- Bretz, W. A., Weyant, R. J., Corby, P. M., Ren, D., Weissfeld, L., Kritchevsky, S. B., Harris, T., Kurella, M., Satterfield, S., Visser, M. & Newman, A. B. (2005) Systemic inflammatory markers, periodontal diseases, and periodontal infections in an elderly population. *Journal of the American Geriatrics Society* **53**, 1532–1537.
- Buhlin, K., Gustafsson, A., Pockley, A. G., Frostegard, J. & Klinge, B. (2003) Risk factors for cardiovascular disease in patients with periodontitis. *European Heart Journal* **24**, 2099–2107.
- Cote, R., Wolfson, C., Solymoss, S., Mackey, A., Leclerc, J. R., Simard, D., Rouah, F., Bourque, F. & Leger, B. (2000) Hemostatic markers in patients at risk of cerebral ischemia. *Stroke* **31**, 1856–1862.
- D'Aiuto, F., Parkar, M., Andreou, G., Brett, P. M., Ready, D. & Tonetti, M. S. (2004a) Periodontitis and atherogenesis: causal association or simple coincidence? *Journal of Clinical Periodontology* **31**, 402–411.
- D'Aiuto, F., Ready, D. & Tonetti, M. S. (2004b) Periodontal disease and C-reactive protein-associated cardiovascular risk. *Journal of Periodontal Research* **39**, 236–241.
- Daly, C. G., Mitchell, D. H., Highfield, J. E., Grossberg, D. E. & Stewart, D. (2001) Bacteremia due to periodontal probing: a clinical and microbiological investigation. *Journal of Periodontology* **72**, 210–214.
- Danesh, J., Whincup, P., Walker, M., Lennon, L., Thomson, A., Appleby, P., Rumley, A. & Lowe, G. D. (2001) Fibrin D-dimer and coronary heart disease: prospective study and meta-analysis. *Circulation* **103**, 2323–2327.
- De Taeye, B., Smith, L. H. & Vaughan, D. E. (2005) Plasminogen activator inhibitor-1: a common denominator in obesity, diabetes and cardiovascular disease. *Current Opinion in Pharmacology* **5**, 149–154.
- Folsom, A. R., Wu, K. K., Rosamond, W. D., Sharrett, A. R. & Chambless, L. E. (1997) Prospective study of hemostatic factors and incidence of coronary heart disease: the Atherosclerosis Risk in Communities (ARIC) Study. *Circulation* **96**, 1102–1108.
- Friedewald, W. T., Levy, R. I. & Fredrickson, D. S. (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry* **18**, 499–502.
- Geerts, S. O., Nys, M., De, M. P., Charpentier, J., Albert, A., Legrand, V. & Rompen, E. H. (2002) Systemic release of endotoxins induced by gentle mastication: association with periodontitis severity. *Journal of Periodontology* **73**, 73–78.
- Herzberg, M. C. & Weyer, M. W. (1998) Dental plaque, platelets, and cardiovascular diseases. *Annals of Periodontology* **3**, 151–160.
- Hoffer, M. (2005) The Bradford Hill considerations on causality: a counterfactual perspective. *Emerging Themes in Epidemiology* **2**, 11.
- Janket, S. J., Baird, A. E., Chuang, S. K. & Jones, J. A. (2003) Meta-analysis of periodontal disease and risk of coronary heart disease and stroke. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics* **95**, 559–569.
- Kannel, W. B. (2005) Overview of hemostatic factors involved in atherosclerotic cardiovascular disease. *Lipids* **40**, 1215–1220.
- Kinane, D. F., Riggio, M. P., Walker, K. F., MacKenzie, D. & Shearer, B. (2005) Bacteremia following periodontal procedures. *Journal of Clinical Periodontology* **32**, 708–713.
- Leivadarios, E., van der Velden, U., Bizzarro, S., ten Heggeler, J. M., Gerdes, V. E., Hoek, F. J., Nagy, T. O., Scholma, J., Bakker, S. J., Gans, R. O., ten Cate, H. & Loos, B. G. (2005) A pilot study into measurements of markers of atherosclerosis in periodontitis. *Journal of Periodontology* **76**, 121–128.
- Li, X., Kolltveit, K. M., Tronstad, L. & Olsen, I. (2000) Systemic diseases caused by oral infection. *Clinical Microbiology Reviews* **13**, 547–558.
- Lindgren, A., Lindoff, C., Norrving, B., Astedt, B. & Johansson, B. B. (1996) Tissue plasminogen activator and plasminogen activator inhibitor-1 in stroke patients. *Stroke* **27**, 1066–1071.
- Lip, G. Y. & Blann, A. (1997) von Willebrand factor: a marker of endothelial dysfunction in vascular disorders? *Cardiovascular Research* **34**, 255–265.
- Loos, B. G. (2005) Systemic markers of inflammation in periodontitis. *Journal of Periodontology* **76**, 2106–2115.
- Loos, B. G., Craandijk, J., Hoek, F. J., Wertheim-van Dillen, P. M. & van der Velden, U. (2000) Elevation of systemic markers related to cardiovascular diseases in the peripheral blood of periodontitis patients. *Journal of Periodontology* **71**, 1528–1534.
- Lowe, G. D. (1998) Etiopathogenesis of cardiovascular disease: hemostasis, thrombosis, and vascular medicine. *Annals of Periodontology* **3**, 121–126.
- Lowe, G. D., Rumley, A., Sweetnam, P. M., Yarnell, J. W. & Rumley, J. (2001) Fibrin D-dimer, markers of coagulation activation and the risk of major ischaemic heart disease in the caerphilly study. *Thrombosis and Haemostasis* **86**, 822–827.
- Montebugnoli, L., Servidio, D., Miaton, R. A., Prati, C., Tricoci, P. & Melloni, C. (2004) Poor oral health is associated with coronary heart disease and elevated systemic inflammatory and haemostatic factors. *Journal of Clinical Periodontology* **31**, 25–29.
- Munford, R. S. (2001) Statins and the acute-phase response. *New England Journal of Medicine* **344**, 2016–2018.
- 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.
- Pihlstrom, B. L., Michalowicz, B. S. & Johnson, N. W. (2005) Periodontal diseases. *Lancet* **366**, 1809–1820.
- Sagripanti, A., Carpi, A., Baicchi, U., Morganti, M., Rosaia, B., Nicolini, A. & Mittermayer, C. (1996) Plasmatic parameters of coagulation activation in thrombotic microangiopathy. *Biomedicine and Pharmacotherapy* **50**, 357–362.
- Seinost, G., Wimmer, G., Skerget, M., Thaller, E., Brodmann, M., Gasser, R., Bratschko, R. O. & Pilger, E. (2005) Periodontal treatment improves endothelial dysfunction in patients with severe periodontitis. *American Heart Journal* **149**, 1050–1054.
- Smith, F. B., Lee, A. J., Fowkes, F. G., Price, J. F., Rumley, A. & Lowe, G. D. (1997) Hemostatic factors as predictors of ischemic heart disease and stroke in the Edinburgh Artery Study. *Arteriosclerosis, Thrombosis, and Vascular Biology* **17**, 3321–3325.
- Taylor, B. A., Tofler, G. H., Carey, H. M., Morel-Kopp, M. C., Philcox, S., Carter, T. R., Elliott, M. J., Kull, A. D., Ward, C. & Schenck, K. (2006) Full-mouth tooth extraction lowers systemic inflammatory and thrombotic markers of cardiovascular risk. *Journal of Dental Research* **85**, 74–78.
- Thompson, S. G., Kienast, J., Pyke, S. D., Haverkate, F. & van de Loo, J. C. (1995) Hemostatic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. European Concerted Action on Thrombosis and Disabilities Angina Pectoris Study Group. *New England Journal of Medicine* **332**, 635–641.
- Tuomisto, K., Jousilahti, P., Sundvall, J., Pajunen, P. & Salomaa, V. (2006) C-reactive protein, interleukin-6 and tumor necrosis factor alpha as predictors of incident coronary and cardiovascular events and total mortality. A population-based, prospective study. *Thrombosis and Haemostasis* **95**, 511–518.

## Address:

S. Bizzarro

Department of Periodontology

Academic Center for Dentistry Amsterdam

(ACTA)

1066EA

Amsterdam

Louwesweg 1

The Netherlands

E-mail: S.Bizzarro@acta.nl

**Clinical Relevance**

*Scientific rationale for the study:* Periodontitis has been associated with cardiovascular diseases, but the underlying biological mechanisms are not clear. A prothrombotic state is a condition that predisposes for acute-thrombotic events. Infections can enhance a prothrombotic

state. This study investigates whether periodontitis is associated with a prothrombotic state.

*Principal findings:* Inflammatory markers were elevated in peripheral blood in periodontitis patients. Among the four prothrombotic factors investigated in blood plasma, the activity of plasminogen activator

inhibitor-1 was significantly elevated.

*Practical implications:* This study confirms that periodontitis can have systemic effects, and the link with cardiovascular diseases may, among others, be through a prothrombotic state.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.