

Soluble CD14 in periodontitis

15(2) (2009) 121–128
 © SAGE Publications 2009
 ISSN 1753-4259 (print)
 10.1177/1753425908101577

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Lipopolysaccharide (LPS) binds to soluble (s)CD14. We investigated which factors contribute to variations in sCD14 levels in periodontitis, a chronic infectious disease of tooth-supporting tissues associated with endotoxemia and leading to inflammation and subsequently loss of teeth. The sCD14 levels were determined by ELISA in healthy controls ($n=57$) and untreated patients (59 moderate and 46 severe) and their relation with markers of systemic inflammation (C-reactive protein levels, and leukocyte, neutrophil and lymphocyte counts) was assessed. Anti-*Aggregatibacter actinomycetemcomitans* and anti-*Porphyromonas gingivalis* IgG levels were established by ELISA and *CD14*⁻²⁶⁰ genotype was determined in a TaqMan allelic discrimination assay. Increased levels of sCD14 were more frequent among periodontitis patients ($P=0.026$) and showed a severity-dependence with increasing levels of periodontal breakdown ($P=0.008$). In patients, levels of sCD14 correlated positively with CRP ($P=0.043$), leukocyte numbers ($P=0.011$) and negatively with anti-*A. actinomycetemcomitans* IgG ($P=0.007$). In a multivariate analysis, sCD14 levels were predicted by ethnicity, age, educational level, and in Caucasian subjects also by the severity of periodontal destruction, but not by anti-*P. gingivalis* IgG or the *CD14*⁻²⁶⁰ genotype. Periodontitis is associated with elevated levels of sCD14.

Keywords: CRP, *Aggregatibacter actinomycetemcomitans*, *CD14*⁻²⁶⁰ genotype, inflammation, periodontitis, sCD14

INTRODUCTION

Lipopolysaccharide (LPS), the common component of Gram-negative bacteria, is recognized by CD14-expressing immune cells.¹ Membrane-bound (m)CD14 is mainly expressed on mature monocytes, macrophages and activated neutrophils.² Soluble (s)CD14 can be the result of protease-mediated shedding/cleavage of mCD14. CD14 binds to LPS-binding-protein (LBP),

which is another LPS receptor and also an acute-phase protein; the CD14–LBP complex transfers LPS with high affinity to Toll-like receptor-4, leading to NF- κ B activation, and thus production of pro-inflammatory cytokines such as tumor necrosis factor (TNF)- α , interleukin (IL)-1, IL-6, and IL-8.³ Moreover, it has been suggested that sCD14 could be an acute-phase protein, as it can be produced by human hepatocytes and its levels correlate with C-reactive protein (CRP)

Received 28 August 2008; Revised 20 November 2008, 4 December 2008; Accepted 5 December 2008

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and IL-6.⁴ In addition to CD14 and LBP, circulating lipoproteins are thought to play an important role in the clearance and inactivation of LPS by binding the bioactive lipid A portion of LPS.⁵

Periodontitis is a persistent bacterial infection of the tooth-supporting tissues, leading to chronic inflammation. Periodontal infections are initiated by bacterial accumulation at the gingival margin, followed by imbalance of dental biofilm towards one where anaerobic, Gram-negative bacteria dominate; this leads to strong activation of innate host-defensive mechanisms and destruction of the tooth-supporting tissues. The disease process may ultimately lead to loss of teeth. In periodontitis, elevated serum levels of LPS have been reported,⁶ as well as elevated levels of sCD14.⁷ Moreover, in the latter Japanese study, treatment of periodontitis was shown to decrease the sCD14 levels.⁷ Gram-negative periodontal pathogens, such as *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*, contribute to the overall endotoxemic burden in periodontitis patients as a result of recurrent bacteremic episodes from the periodontal pockets.⁸ It is this kind of repeated, transient, bacterial disseminations that are currently thought to be one of the causes of chronic systemic inflammation that could underlie initiation and progression of atherosclerotic lesions, ultimately resulting in cardiovascular diseases.^{9,10} Periodontitis is also associated with elevated levels of CRP and, in epidemiological research, periodontitis is identified as one of several risk factors for cardiovascular disease.^{11,12}

The promoter region of the *CD14* gene contains a single nucleotide polymorphism (C>T) at position -260. The *CD14*⁻²⁶⁰T allele has been associated not only with increased levels of mCD14 and sCD14,¹³ myocardial infarction,¹⁴ and increased susceptibility to chronic *Chlamydia pneumoniae* infection in coronary artery disease patients,¹⁵ but also with severe periodontitis.¹⁶

We have investigated sCD14 plasma levels in relation to systemic markers of inflammation, especially CRP, lipid profile, immunoglobulin G (IgG) serum levels against the periodontal pathogens *A. actinomycetemcomitans* and *P. gingivalis*, and the *CD14*⁻²⁶⁰ genotype.

SUBJECTS AND METHODS

Study population

The study population was derived from two previous studies.^{17,18} We included all subjects for whom the plasma levels of sCD14, serology, and *CD14*⁻²⁶⁰ genotype could be determined. Fifty-seven healthy controls and untreated periodontitis patients (59 moderate and 46 severe) were included. For details

of dental inclusion criteria for patients and controls, see Bizzarro *et al.*¹⁷ Systemic health was assessed via health questionnaires and by interview. Exclusion criteria for controls and patients were: (i) the presence of any systemic disease (*e.g.* cardiovascular disorders, diabetes mellitus, allergy, gastrointestinal problems); (ii) a recent history or the presence of any acute or chronic infection; (iii) systemic antibiotic treatment within the last 3 months; (iv) the use of any medication (including sporadic NSAIDs); and (v) pregnancy. For all participants, smoking habits were recorded; non-smokers were subjects who never smoked or quit smoking >10 years previously. Educational level was used as a surrogate marker for social class and the given scores were 0 or 1 for the subjects with educational level less than high school or high school and above, respectively. From height and weight measurements, the body mass index (BMI) was calculated. Subjects with European ancestry were entered as Caucasian and individuals from other or mixed ancestry were scored as non-Caucasian.

All subjects were informed both verbally and in writing 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.

Lipid profile and systemic markers of inflammation

Plasma levels of total cholesterol, HDL cholesterol and triglycerides were determined by standard (enzymatic) methods in a hospital-based diagnostic clinical laboratory; low-density lipoprotein (LDL) cholesterol was calculated.¹⁹ Plasma levels of CRP were determined using a high sensitivity (latex enhanced) nephelometric method on the BN ProSpec analyzer (Dade Behring, Marburg, Germany), and EDTA blood was used for automated leukocyte counting and leukocyte differentiation.

Immunoglobulin G levels against *A. actinomycetemcomitans* and *P. gingivalis*

The determination of serum IgG levels against two relevant periodontal pathogens, *A. actinomycetemcomitans* and *P. gingivalis*, was a modification of the enzyme-linked immunosorbent assay (ELISA) described by Pussinen *et al.*²⁰ As antigens, we used a mixture of five strains of *A. actinomycetemcomitans* and eight strains of *P. gingivalis*. The strains were ATCC 29523, Y4, NCTC 9710, 3381 and OM2 534 for *A. actinomycetemcomitans*, representing the serotypes a, b, c, d and e, and W83, HG 184, A7A1-28, ATCC 49417, HG 1690, HG 1691 and 34-4 for *P. gingivalis*, representing the capsule serotypes K1–K7, as well as the uncapsulated strain 381.

Aggregatibacter actinomycetemcomitans strains were grown for 18 h in brain heart infusion (BHI) broth (Sigma Chemical Co, St Louis, MO, USA) aerobically at 37°C in humidified 5% CO₂. *Porphyromonas gingivalis* strains were grown anaerobically (80% N₂, 10% H₂, 10% CO₂) at 37°C for 18 h in BHI broth supplemented with haemin (5 mg/l) and menadione (1 mg/l) (Sigma). The bacteria were washed once with phosphate-buffered saline (PBS; 10 mM phosphate, 150 mM NaCl, pH 7.4) and then fixed overnight at 4°C in 0.5% paraformaldehyde-PBS. The bacterial suspensions were washed three times in PBS and brought to an optical density corresponding to an absorbance of 0.15 at 580 nm in ELISA-buffer (PBS, 0.5% bovine serum albumin, 0.05% Tween-20).

For ELISA, equal volumes of the five *A. actinomycetemcomitans* or of the eight *P. gingivalis* strains were mixed and 150 µl of the mixture was used to coat Microton ELISA plates (Greiner Bio-One B.V., Alphen a/d Rijn, The Netherlands). The unspecific binding was blocked by 5% bovine serum albumin in PBS at room temperature for 30 min. Diluted (1:1500) serum samples were tested in duplicate. The plates were incubated for 2 h at room temperature and washed three times in ELISA buffer. Horse-radish peroxidase-conjugated, goat anti-human IgG (Vector Laboratories Inc., Burlingame, CA, USA) diluted (1:2000; 150 µl) was added and the plates were incubated for 2 h at room temperature. Substrate was then added, and absorbance values were measured at 450 nm with a multilabel counter (Wallac Victor 1420, Perkin-Elmer Life Sciences, Boston, MA, USA).

CD14⁻²⁶⁰ genotype

Genomic DNA was isolated from blood samples by means of the Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. We assessed the C>T substitution in the proximal *CD14* promoter GC box at position -260 from the translation start site (NCBI SNP CLUSTER ID: rs2569190). A real-time TaqMan PCR was performed using standard conditions with the following primers and probes: forward primer GACACTGCCAGGAGACACAGAA and reverse primer GCCAGCCCCCTTCCTTT, and probes: TGTTACGGCCCCCTT-VIC-MGB (for the common allele, the C allele), TTACGGTCCCCCTCC-6-FAM-MGB (for the rare allele, the T allele).

Soluble CD14

The concentration of sCD14 was measured by a sandwich ELISA using two monoclonal antibodies (MAbs) against

different epitopes of sCD14 (R&D systems, Abingdon, UK) in accordance with the manufacturer's instructions. In brief, EDTA plasma specimens were diluted 1:200 and incubated in duplicate for 3 h in a 96-well plate precoated with the anti-CD14 MAb. The plate was incubated for 1 h with the other anti-CD14 MAb conjugated with peroxidase. Substrate was then added, and absorbance values were measured at 450 nm with a multilabel counter (Wallac Victor 1420). The concentration of each sample was determined by extrapolation from a standard curve estimated from a panel of sCD14 standards of known concentrations. Serum levels of sCD14 were expressed as milligrams per litre. The intra- and inter-assay coefficients of variation were 2.4% and 6.3%, respectively.

Statistical analysis

Data analyses were performed with the SPSS v.15.0 package (SPSS Inc., Chicago, IL, USA). Mean, SD and frequency distribution were calculated; the background characteristics were compared with one-way ANOVA or χ^2 -test (or Fisher exact test), where appropriate. Normal distribution of data was assessed by Kolmogorov-Smirnov goodness-of-fit test and, except for sCD14 values, all parameters included showed normal distributions. For parametric testing using sCD14 data, the log-transformed values were employed. Differences in sCD14 between controls, moderate and severe patients were compared with the Kruskal-Wallis test. For sCD14, the 75th percentile of values of the control subjects was used as cut-off point for a frequency distribution analysis followed by χ^2 -test. Correlations between sCD14 levels, IgG levels against *A. actinomycetemcomitans* or *P. gingivalis*, CRP, and numbers of leukocytes, neutrophils and lymphocytes, and for patients also between sCD14 and the number of teeth with $\geq 50\%$ bone loss, were investigated by Spearman correlation coefficients, ρ . A multivariate analysis (backward stepwise linear regression with $P=0.10$ to enter and $P=0.05$ to leave) was performed considering the sCD14 levels as the outcome variable. Predictor variables were age, gender, ethnicity, smoking, educational level, BMI, *CD14*⁻²⁶⁰ genotype, anti-*A. actinomycetemcomitans* IgG levels, anti-*P. gingivalis* IgG levels, total cholesterol, triglycerides, and numbers of teeth with $\geq 50\%$ bone loss.

RESULTS

Study population

Two-thirds of the study population was European Caucasian (Table 1) and the remainder had a mixed

Table 1. Study population

	Control (n = 57)	Moderate periodontitis (n = 59)	Severe periodontitis (n = 46)	P-value
Background characteristics				
Age (years)	41.5 ± 10.8	42.9 ± 7.8	45.0 ± 9.1	0.184
Gender (males)	19 (33.3%)	21 (35.6%)	20 (43.5%)	0.547
Ethnicity (Caucasian)	35 (61.4%)	38 (64.4%)	34 (73.9%)	0.389
Smoking (smokers)	9 (15.8%)	14 (23.7%)	16 (34.8%)	0.081
Education (<high school)	13 (22.8%)	32 (54.2%)	22 (47.8%)	0.002
BMI (kg/m ²)	24.8 ± 4.0	25.5 ± 5.0	24.7 ± 3.9	0.618
Teeth present (n)	28.3 ± 2.0	26.2 ± 3.4	26.1 ± 3.0	<0.001
No. of teeth with bone loss				
≥30%	0	13.6 ± 5.7	20.7 ± 3.8	
≥50%	0	2.9 ± 2.0	10.5 ± 4.0	
Total cholesterol (mmol/l)	5.4 ± 1.2	5.3 ± 1.2	5.6 ± 1.0	0.442
HDL (mmol/l)	1.4 ± 0.3	1.3 ± 0.3	1.4 ± 0.4	0.073
LDL (mmol/l)	3.2 ± 0.8	3.4 ± 0.9	3.5 ± 0.9	0.104
Triglycerides (mmol/l)	1.3 ± 0.7	1.6 ± 1.5	1.3 ± 0.7	0.175
Systemic inflammatory markers				
Leukocytes (× 10 ⁹ /l)				
Total	5.9 ± 1.5	7.2 ± 2.1	7.2 ± 2.0	<0.001
Neutrophils	3.3 ± 1.1	4.2 ± 1.4	4.2 ± 1.6	<0.001
Lymphocytes	2.0 ± 0.6	2.3 ± 0.8	2.3 ± 0.6	0.071
C-reactive protein (mg/l)	2.2 ± 2.7	3.8 ± 3.8	3.4 ± 4.8	0.072
Serology of periodontal bacteria				
Anti- <i>A. actinomycetemcomitans</i> IgG (OD ₄₅₀)	0.6 ± 0.5	1.0 ± 0.6	1.1 ± 0.7	0.002
Anti- <i>P. gingivalis</i> IgG (OD ₄₅₀)	0.7 ± 0.2	0.9 ± 0.6	1.0 ± 0.7	0.006
CD14 (-260C > T) rs2569190				
Genotype frequency (%)*				
C/C	10 (18%)	17 (29%)	11 (24%)	
C/T	32 (56%)	29 (49%)	28 (61%)	0.448
T/T	15 (26%)	13 (22%)	7 (15%)	
T allele frequency (%)	54%	47%	46%	0.324

Values are mean ± SD or numbers (%) of subjects. BMI, body mass index. P-values derived from one-way ANOVA or χ^2 -test (or Fisher exact test), where appropriate. *In Caucasians, the genotype frequencies are: for controls, 20% C/C, 54% C/T and 26% T/T; for moderate periodontitis patients, 21% C/C, 61% C/T and 18% TT; for severe periodontitis patients, 26% C/C, 59% C/T and 15% T/T ($P = 0.807$). T-allele frequencies in Caucasians were 53%, 49% and 44% in controls, moderate and severe patients, respectively ($P = 0.307$) and did not significantly deviate from the Hardy–Weinberg equilibrium ($P > 0.05$).

racial background. Periodontitis patients had a lower educational level than the healthy controls ($P = 0.002$).

Lipid profile and systemic markers of inflammation

Levels of total cholesterol, HDL, LDL, and triglycerides were roughly comparable in controls, moderate or severe periodontitis patients. The total number of leukocytes was increased in moderate and severe periodontitis compared to controls ($P < 0.001$; Table 1). The increase was largely explained by the increase in neutrophil counts in periodontitis compared to health ($P < 0.001$). CRP levels showed a tendency to be increased in moderate and severe periodontitis groups compared to the control group ($P = 0.072$).

Immunoglobulin G levels against *A. actinomycetemcomitans* and *P. gingivalis*

The anti-*A. actinomycetemcomitans* and anti-*P. gingivalis* IgG levels were increased in periodontitis compared to health ($P = 0.002$ and $P = 0.006$, respectively; Table 1).

CD14⁻²⁶⁰ genotype

Allele frequencies of CD14⁻²⁶⁰ in periodontitis patients and controls did not show a significant deviation from the Hardy–Weinberg equilibrium ($P > 0.05$). We did not observe differences in the distribution of the CD14⁻²⁶⁰ genotypes or of the allele frequencies between patients and controls, neither when the entire study population

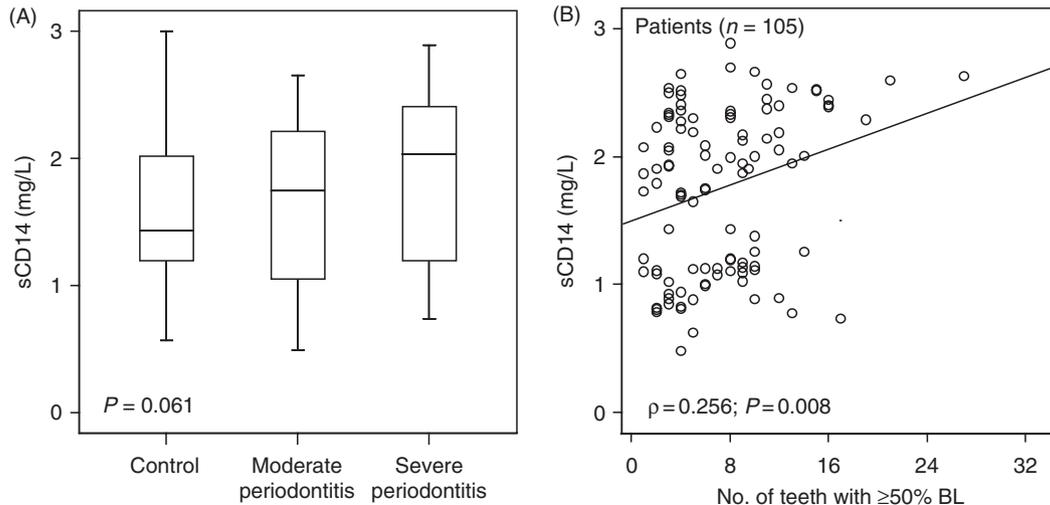


Fig. 1. (A) Box (25–75th percentiles) and whisker (10–90 percentiles) plots for sCD14 in controls, patients with moderate and severe periodontitis; the horizontal line inside the box indicates the median (the 50th percentile). *P*-value calculated by Kruskal-Wallis test. (B) Correlation between sCD14 levels and number of teeth with $\geq 50\%$ bone loss in periodontitis patients (moderate and severe). The correlation coefficient, ρ and the *P*-Value are indicated. Each symbol represents one subject. BL, bone loss.

was analyzed, nor when considering only Caucasian subjects (Table 1).

Soluble CD14 plasma levels

Periodontitis patients showed a tendency towards increased sCD14 levels compared to controls ($P = 0.061$; Fig. 1A). In a frequency distribution analysis using as threshold the 75th percentile of the sCD14 levels of the control subjects (2.03 mg/l), we observed that a higher number of moderate and severe periodontitis patients had sCD14 values above this threshold compared to healthy controls (34% and 50.0%, respectively, versus 25%; $P = 0.026$). Furthermore, in patients (moderate and severe), the sCD14 levels were positively correlated with the severity of periodontal destruction (number of teeth with $\geq 50\%$ bone loss, $\rho = 0.256$, $P = 0.008$; Fig. 1B).

Correlation between soluble CD14 and systemic parameters

For the total study population, the sCD14 levels were positively correlated with CRP plasma levels, leukocytes, neutrophils and lymphocyte counts and total cholesterol levels, although the correlation coefficients were rather low (Table 2). These correlations were present in periodontitis patients, but were not found within the control subjects ($P > 0.05$). In particular, CRP ($\rho = 0.198$, $P = 0.043$) and the total number of leukocytes ($\rho = 0.249$, $P = 0.011$) correlated positively with the sCD14 in periodontitis patients.

Interestingly, in periodontitis patients, the levels of sCD14 were negatively correlated with the anti-*A. actinomycetemcomitans* IgG levels ($\rho = -0.262$, $P = 0.007$).

Linear regression analysis of the soluble CD14 levels

In a final step, we designed a linear regression analysis to identify significant associations of sCD14 with risk factors for periodontitis. The variables retained in the model after the backward linear regression were ethnicity, age, educational level, the severity of periodontal destruction (number of teeth with $\geq 50\%$ bone loss) and the anti-*A. actinomycetemcomitans* IgG levels (Table 3). Ethnicity and age had the highest predictive value ($P < 0.001$ and $P = 0.004$, respectively), whereas educational level, severity of periodontal destruction and anti-*A. actinomycetemcomitans* IgG levels showed trends towards linear association ($P = 0.042$, $P = 0.083$ and $P = 0.098$, respectively); as ethnicity was the most significant predictor for the higher sCD14 levels in this model, we applied the regression again for the sub-group of subjects of Caucasian origin. Among these subjects, in addition to age ($P = 0.004$), severity of periodontal destruction ($P = 0.016$), anti-*A. actinomycetemcomitans* IgG levels ($P = 0.053$), and smoking status ($P = 0.074$) were retained in this model as important predictors for the sCD14 levels. The proportion of variance (R-squared) explained by the predictors retained in the final models was 25.9% for the regression model of the total study group and 22.1% for the model on Caucasian subjects.

Table 2. Correlation coefficients of sCD14 with anti-*A. actinomycetemcomitans* or anti-*P. gingivalis* IgG levels, CRP, numbers of leukocytes and neutrophils, total cholesterol and triglycerides

Correlation coefficient of sCD14 with:	All subjects (<i>n</i> = 162)	Control subjects (<i>n</i> = 57)	Periodontitis subjects (<i>n</i> = 105)	Periodontitis	
				Moderate (<i>n</i> = 59)	Severe (<i>n</i> = 46)
Anti- <i>A. actinomycetemcomitans</i> IgG	-0.149	0.005	-0.262 [‡]	-0.276 [†]	-0.279
Anti- <i>P. gingivalis</i> IgG	0.030	-0.063	-0.032	0.009	-0.110
CRP	0.173 [†]	0.053	0.198 [†]	0.311 [†]	0.164
Leukocytes	0.237 [‡]	0.107	0.249 [†]	0.245	0.228
Neutrophils	0.156 [†]	0.020	0.172	0.162	0.186
Lymphocytes	0.220 [‡]	0.203	0.186	0.182	0.198
Total cholesterol	0.173 [†]	0.229	0.159	0.284 [†]	-0.079
Triglycerides	0.062	0.156	-0.001	-0.026	0.052

Correlation coefficients calculated by Spearman rank correlation coefficient. [†]*P* < 0.05; [‡]*P* < 0.01.

Table 3. Multivariate analysis of sCD14

	All subjects (<i>n</i> = 162)		Caucasians (<i>n</i> = 107)	
	β (95% CI)	<i>P</i> -Value*	β (95% CI)	<i>P</i> -Value [‡]
Ethnicity	0.251 (0.165–0.337)	<0.001	–	–
Age	0.007 (0.002–0.011)	0.004	0.005 (0.001–0.009)	0.023
Educational level	-0.087 (-0.171 to -0.003)	0.042	n.r. [‡]	–
Teeth with ≥50% bone loss (<i>n</i>)	0.007 (-0.001 to 0.016)	0.083	0.010 (0.002–0.019)	0.016
Anti- <i>A. actinomycetemcomitans</i> IgG levels	-0.055 (-0.121 to 0.010)	0.098	-0.068 (-0.136 to 0.001)	0.053
Smoking	n.r. [‡]	–	0.098 (-0.010 to 0.206)	0.074

**P*-Values from a multivariate analysis (backward step-wise linear regression with *P* = 0.10 to enter and *P* = 0.05 to leave) using the sCD14 levels as dependent variable and age, gender, ethnicity, smoking status, educational level, body mass index (BMI), total cholesterol levels, triglycerides levels, anti-*A. actinomycetemcomitans* IgG levels, anti-*P. gingivalis* IgG levels and severity of periodontal destruction (number of teeth with ≥50% bone loss) as predictors; the regression coefficients, β, the 95% confidence intervals (CI) and the *P*-values of the predictors that remained in the final model are presented.[†]As above (*), but for subjects of Caucasian background.[‡]n.r., not retained in the final model (*P*-Value associated with this predictor was >0.10).

DISCUSSION

Several clinical studies have reported elevated levels of sCD14 in inflammatory conditions, such as Kawasaki disease,²¹ atopic dermatitis,²² liver disease,²³ rheumatoid arthritis²⁴ or systemic lupus erythematosus.²⁵ Correlations between levels of sCD14 and disease activity in systemic lupus erythematosus or the severity of trauma in polytraumatised patients have been documented.^{25,26} In our study, sCD14 plasma levels were elevated in periodontitis. Our results confirm a previous report by Hayashi *et al.*⁷ and further establish sCD14 as an acute-phase protein in periodontitis, whose level increases with disease severity. Many functions have been attributed to acute-phase proteins, including tissue repair, modulation of coagulation, neuroendocrine secretion, bacterial opsonisation and clearing, hemopoiesis, metal binding, and, in the case of CD14, fighting infection.²⁷

About 80–97% of LPS is bound to lipoproteins in circulation, and all main lipoprotein classes are involved.²⁸ sCD14 can transfer cell-bound LPS to plasma lipoproteins.²⁹ Consequently, in endotoxemic states, such as periodontitis, the effects of lipopolysaccharide will depend on the levels of plasma lipoproteins, and also on the levels of LBP and CD14. Indeed, as shown in our study, sCD14 levels positively correlated with the total cholesterol levels, especially in patients with moderate periodontitis, confirming that the lipid profile is reactive to periodontal inflammation.²⁸ Lipopolysaccharide complexed with low-density lipoprotein and very low-density lipoprotein can be taken-up by macrophages and further promote foam-cell formation,³⁰ a process involved in progression of atherosclerosis. Thus, the elevated levels of sCD14 represent a manifestation of endotoxemia in periodontitis which can be indirectly involved in atherogenesis and could partially explain the higher risk for

cardiovascular events reported for periodontitis patients.¹¹ Interestingly, automatic 'text mining' of the literature has identified the CD14 molecule as an important link between atherosclerosis and periodontitis.³¹

We also looked for alternative modifying factors that could induce variation in the sCD14 levels measured. A role for the positive infectious serology with *Helicobacter pylori*, *C. pneumoniae* and cytomegalovirus in the chronic inflammatory reaction in patients with cardiovascular disease has been proposed.¹⁰ In our periodontitis patients, we investigated the relationship between serology of two established, periodontal, Gram-negative pathogens, *A. actinomycetemcomitans* and *P. gingivalis* and the sCD14 levels. The anti-*P. gingivalis* IgG levels were not correlated with the sCD14 levels, while our results showed that the sCD14 levels were negatively associated with the levels of anti-*A. actinomycetemcomitans* IgG in periodontitis patients (Table 2). We suggest that the negative correlation demonstrates that, with a poor IgG-response to *A. actinomycetemcomitans*, sCD14 becomes increasingly important in the clearance of *A. actinomycetemcomitans*-derived LPS.

In Asian and Caucasian populations, *CD14*⁻²⁶⁰ polymorphism has been described as a modifying factor for the measured levels of CD14; the T-allele carriers have elevated levels of soluble and membrane-bound CD14.^{14,32} Albeit not conclusive, there are indications for the association of the CD14 T/T genotype with severe periodontal disease.¹⁶ In our study, the *CD14*⁻²⁶⁰ genotypes were present with similar frequency in patients and controls. Within Caucasians, the allele frequencies are similar to those reported in the literature.^{16,33} The sCD14 levels were not correlated with the *CD14*⁻²⁶⁰ genotype, neither in the entire group nor in the Caucasian sub-group. The lack of association between the *CD14*⁻²⁶⁰ genotype and sCD14 levels is not completely surprising. Contradictory reports on the correlation between *CD14*⁻²⁶⁰ genotype and sCD14 levels have been documented on even larger groups than the present relatively-small study population; Koenig *et al.*³³ found a significant effect of the *CD14*⁻²⁶⁰ genotype on sCD14 in 312 German patients suffering from coronary artery disease, but not in their age- and gender-matched 476 healthy controls. It could be speculated that it is not the *CD14*⁻²⁶⁰ polymorphism *per se* that is responsible for modified CD14 levels, but the linkage disequilibrium with other genetic variations, leading to conflicting results in different ethnic groups.³²

CONCLUSIONS

In the current study, we found elevated sCD14 levels in periodontitis and which increased with the severity of

periodontal destruction and paralleled by markers of a systemic inflammatory reaction. Our findings support the hypothesis of a pro-inflammatory state in untreated periodontitis patients resulting from recurrent bacteremic episodes. Our findings are a further step in the understanding of systemic inflammation in periodontitis, but also could help to understand further epidemiological links with cardiovascular disease. The elevated sCD14 levels confirm an endotoxemic state, LPS can be transferred to foam cells and could exacerbate atherosclerotic lesions.

ACKNOWLEDGEMENTS

This study was supported by The Netherlands Institute for Dental Sciences, Philips Oral Healthcare EMEA and the INFOBIOMED Network of Excellence (IST 2002507585). The authors thank J. Pleijsters for CD14 genotyping and J. Brunner, C.J. Bosch-Tijhof and S. Asikainen for providing the bacterial strains used in the study.

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