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Chapter 4

Soluble CD14 as a possible molecular link between atherosclerosis and chronic infectious diseases: periodontitis as model

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Abstract

Lipopolysaccharide (LPS) binds to soluble (s) CD14. The LPS-sCD14 complexes activate endothelial and smooth muscle cells, exacerbating inflammation in atherosclerotic lesions. We hypothesized that sCD14 is an important link between atherosclerosis and low-grade infectious processes, such as chronic periodontitis. 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 (CRP levels, leukocyte, neutrophil and lymphocyte counts) was assessed. Anti-*A. actinomycetemcomitans* and anti-*P. 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. We suggest that increased sCD14 levels in periodontitis might lead to increased activation of non-CD14 expressing cells, possibly activating atherogenesis.

Introduction

Cardiovascular diseases (CVD) are a leading cause of global mortality, accounting for almost 17 million deaths annually or 30% of all global mortality (1). The underlying mechanism associated with CVD is atherosclerosis. In the recent years, a growing interest is emerging in aspects of infection and inflammation, as they may also be important risk factors in the initiation and progression of atherosclerosis (2). Longstanding, systemic chronic inflammation has been attributed to repeated, transient dissemination of certain micro-organisms in the peripheral circulation. These include *Helicobacter pylori*, *Chlamydia pneumoniae*, and cytomegalovirus (3). The systemic chronic inflammation is evidenced by elevated C-reactive protein (CRP); this acute-phase protein is regarded as a risk factor for CVD (4). Periodontitis may be a source of daily, low-grade bacteremias. The periodontal pockets form ports of entry which lead to transient bacteremias and leakage of bacterial products into blood (5). Periodontitis is also clearly associated with elevated levels of CRP and increased risk for CVD (6,7).

Lipopolysaccharide (LPS), the common component of Gram-negative bacteria, might be the trigger for the events linking some chronic infectious processes and atherosclerosis (8). LPS is recognized by CD14-expressing immune cells (9). Membrane-bound CD14 (mCD14) is mainly expressed on mature monocytes, macrophages and activated neutrophils (10). Soluble (s)CD14 can be the result of shedding / cleavage of mCD14 or be produced in the liver. Automatic text mining of the literature has identified the CD14 molecule as an important link between atherosclerosis and periodontitis (11). In periodontitis, elevated serum levels of LPS have been reported, (8) as well as elevated levels of sCD14 (12). Moreover, in the latter, Japanese study, treatment of periodontitis was shown to decrease the sCD14 levels (12). Gram-negative periodontal pathogens, such as *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*, might contribute to the overall pro-atherogenic burden in periodontitis patients as a result of recurrent bacteremic episodes (5).

CD14 binds LPS linked with LPS-binding-protein (LBP); the CD14-LPS-LBP complex activates Toll-like receptors 2 and 4 and will, through activation of downstream molecules, result in production of cytokines such as tumor necrosis factor (TNF)-alpha, interleukin (IL)-1, IL-6 and growth factors (13). The resulting pro-inflammatory milieu can accelerate atherogenesis. Endothelial and smooth muscle cells, lacking their own mCD14, are directly activated by a LPS-sCD14 complex (14,15), leading to the

expression of cell adhesion molecules, thereby increasing procoagulant activity, and exacerbating inflammation in atherosclerotic vessel walls (16).

The promotor region of the *CD14* gene contains a single nucleotide polymorphism (C>T) at position -260. The *CD14*⁻²⁶⁰ T allele has been associated not only with increased levels of sCD14 (17), myocardial infarction (18), and increased susceptibility to chronic *C. pneumoniae* infection in coronary artery disease patients (19), but also with severe periodontitis (20).

Given above, we hypothesized that CD14 is an important molecular link between atherosclerosis and low-grade infectious processes. Using periodontitis as a model, we investigated sCD14 plasma levels in relation to systemic markers of inflammation, especially CRP, infection with the periodontal pathogens *A. actinomycetemcomitans* and *P. gingivalis*, and the *CD14*⁻²⁶⁰ genotype.

Materials and methods

Study population

Our study population was derived from two previous studies.(21,22) We included all subjects for which 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 (22). Exclusion criteria for controls and patients were: (I) the presence of systemic disease (especially cardiovascular disorders, diabetes mellitus, allergy), (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 NSAID's), and (V) pregnancy. For all participants smoking habits were recorded; non-smokers were subjects who never smoked or quit smoking >10 years ago. Educational level was used as surrogate marker for social class and the given scores were 0 or 1 for the subjects with educational level < highschool or ≥ highschool, respectively. From height and weight measurements the body mass index (BMI) was calculated. Subjects from European ancestry were entered as Caucasian and individuals from other or mixed ancestry were scored as non-Caucasian.

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.

Lipids 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; LDL cholesterol was calculated (23). Plasma levels of CRP were determined using a high sensitivity (latex enhanced) nephelometric method on the BN ProSpec analyzer (Dade Behring, Marburg, Germany). EDTA blood was used for automated leukocyte counting and leukocyte differentiation.

Infection with *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.(24) 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. *A. actinomycetemcomitans* were grown for 18h in brain heart infusion (BHI) broth (Sigma Chemical Co., St.Louis, MO) aerobically at 37°C in humidified 5% CO₂. *P. gingivalis* were grown anaerobically (80% N₂, 10% H₂, 10% CO₂) at 37°C for 18h 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 Microlon 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 2h at room temperature, washed 3 times in ELISA-buffer. The horse-radish peroxidase-conjugated goat anti-human IgG (Vector Laboratories Inc., Burlingame, CA) diluted (1:2000; 150 µl)

was added and the plates were incubated for 2h 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).

We selected a cut-off point for defining seropositivity, the 75th percentile of the control population (OD=0.744 for *A. actinomycetemcomitans* and OD=0.915 for *P. gingivalis*, respectively). The subjects were considered seropositive for *A. actinomycetemcomitans* and *P. gingivalis* when the corresponding IgG value was higher than the threshold.

***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 manufacture'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 realtime TaqMan PCR was performed using standard conditions with the following primers and probes: forward primer GACACTGCCAGGAGACACAGAA and reverse primer GCCAGCCCCCTTCCTTT, and probes: TGTTACGGCCCCCCT-VIC-MGB (for the common allele, the C allele), TTACGGTCCCCCTCC-6-FAM-MGB (for the rare allele, the T allele).

Soluble CD14 (sCD14)

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 to the manufacturer's instructions. In brief, EDTA plasma specimens were diluted 1:200 and incubated in duplicate for 3h in a 96-well plate precoated with the anti-CD14 MAb. The plate was incubated for 1h 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 mg/L. The intra- and inter-assay coefficients of variation were 2.4% and 6.3%, respectively.

Statistical analysis

Data analyses were performed with the SPSS 15.0 package (SPSS Inc., Chicago, IL, USA). Means, standard deviations and frequency distributions 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 if needed, 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, infection with *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 no. of teeth with $\geq 50\%$ bone loss.

Results

Study population. Two third of the study population was European Caucasian (Table 4.1) and the remainder had a mixed racial background. Periodontitis patients had a lower educational level than the healthy controls ($P=0.002$).

Lipids 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 4.1). 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 ($P=0.072$).

Infection with *A. actinomycetemcomitans* and *P. gingivalis*. The anti-*A. actinomycetemcomitans* and the anti-*P. gingivalis* IgG levels were increased in

periodontitis compared to health ($P=0.002$ and $P=0.006$, respectively). As expected, more *A. actinomycetemcomitans*-seropositive and *P. gingivalis*-seropositive subjects were present among moderate and severe periodontitis patients than among controls ($P=0.004$ and $P=0.049$, respectively; Table 4.1).

***CD14*⁻²⁶⁰ genotype.** *CD14*⁻²⁶⁰ allele frequencies 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 was analyzed, nor when considering only Caucasian subjects (Table 4.1).

	Control (n=57)	Moderate periodontitis (n=59)	Severe periodontitis (n=46)	P-value
Background characteristics				
Age	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
No. of teeth present	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 (x10 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
Infection with periodontal bacteria				
Anti- <i>Aa</i> IgG (OD ₄₅₀)	0.6 ± 0.5	1.0 ± 0.6	1.1 ± 0.7	0.002
Anti- <i>Pg</i> IgG (OD ₄₅₀)	0.7 ± 0.2	0.9 ± 0.6	1.0 ± 0.7	0.006
<i>Aa</i> -seropositive	14 (25%)	30 (51%)	24 (52%)	0.004
<i>Pg</i> -seropositive	14 (25%)	21 (36%)	22 (48%)	0.049
<i>CD14</i> (-260C>T) rs2569190				
Genotypes 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

Table 4.1 Study population. Values are means ± standard deviation or numbers (%) of subjects. BMI, body mass index; *Aa*, *A. actinomycetemcomitans*; *Pg*, *P. gingivalis*; OD, optical density at 450nm.

†In Caucasians, the genotype frequencies: for controls 20% C/C, 54% C/T and 26% T/T; for moderate periodontitis patients 21% C/C, 61% C/T and 18%; 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 ($P>0.05$).

sCD14 plasma levels. Periodontitis patients showed a tendency towards increased sCD14 levels compared to controls ($P=0.061$; Fig. 4.1A). 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 (no. of teeth with $\geq 50\%$ bone loss, $\rho=0.256$, $P=0.008$; Fig. 4.1B).

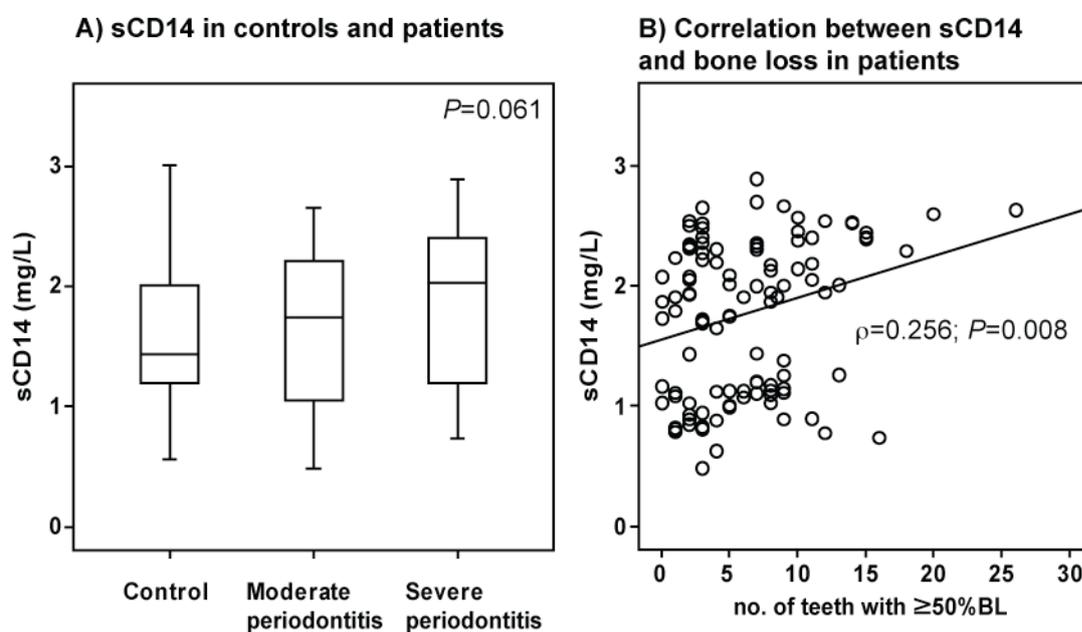


Fig. 4.1 A) Box (25 to 75 percentiles) and whisker (10 to 90 percentiles) plots for sCD14 in controls, patients with moderate periodontitis and patients with severe periodontitis; the horizontal line inside the box indicates the median (the 50th percentile). P -value calculated by Kruskal-Wallis test. B) Correlation between sCD14 levels and severity of bone loss (no. of teeth with $\geq 50\%$ BL) tested by Spearman rank correlation in periodontitis patients (moderate and severe). The correlation coefficient, ρ and the P -value are indicated. Each symbol represents one subject. BL=bone loss

Correlation between sCD14 and systemic parameters. For the total study population, the sCD14 levels were positively correlated with CRP plasma levels, leukocyte, neutrophil and lymphocyte counts (Table 4.2), although the correlation coefficients were rather low. 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 numbers 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 ($\rho=-0.262$, $P=0.007$).

Correlation coefficient of sCD14 with*	All subjects	Control subjects	Periodontitis patients	Periodontitis	
	n=162	n=57	n=105	Moderate n=59	Severe n=46
Anti- <i>Aa</i> IgG	-0.149	0.005	-0.262‡	-0.276†	-0.279
Anti- <i>Pg</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

Table 4.2 Correlation coefficients of sCD14 with infection with *A. actinomycetemcomitans*, *P. gingivalis*, CRP, numbers of leukocytes, neutrophils and lymphocytes. *Calculated by Spearman rank correlation coefficient. † $P<0.05$; ‡ $P<0.01$; *Aa*, *A. actinomycetemcomitans*; *Pg*, *P. gingivalis*

Linear regression analysis of the sCD14 levels. In a final step we designed a linear regression analysis, trying to identify significant associations of sCD14 with risk factors for periodontitis. The sCD14 levels were not normally distributed (Kolmogorov-Smirnov goodness-of-fit test $P<0.05$); hence in the linear regression analysis the log-transformed values were employed. The variables retained in the model after the backward linear regression were ethnicity, age, educational level, the severity of periodontal destruction (no. of teeth with $\geq 50\%$ bone loss) and the anti-*A. actinomycetemcomitans* IgG levels (Table 4.3). 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.

	All subjects n=162		Caucasians n=107	
	β (95% CI)	<i>P</i> *	β (95% CI)	<i>P</i> †
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, -0.003)	0.042	n.r. ‡	-
No. teeth with \geq 50%BL	0.007 (-0.001, 0.016)	0.083	0.010 (0.002, 0.019)	0.016
anti- <i>Aa</i> IgG levels	-0.055 (-0.121, 0.010)	0.098	-0.068 (-0.136, 0.001)	0.053
Smoking	n.r. ‡	-	0.098 (-0.010, 0.206)	0.074

Table 4.3 Multivariate analysis of sCD14. **P*-values from a multivariate analysis (backward stepwise 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-*Aa* IgG levels, anti-*Pg* IgG levels and severity of periodontal destruction (no. teeth with \geq 50%BL) as predictors; the regression coefficients, β , the 95% CI and the *P*-values of the predictors that remained in the final model are presented. BL=bone loss; *Aa*, *A. actinomycetemcomitans*; †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

Periodontitis is a disease where bacteremias occur regularly due to a breach in the mucosal barrier, while patients are not obviously ill. The aim of the current study was to investigate sCD14 as a possible molecular link between atherosclerosis and chronic infectious processes. Periodontitis served as a model of such chronic, underdiagnosed infectious processes. We observed that higher values of sCD14 are more often present among untreated periodontitis patients than among healthy controls. Our study is in agreement with a previous report demonstrating increased serum sCD14 values in untreated periodontitis Japanese patients (12). Furthermore, we demonstrated a positive correlation of the sCD14 levels with the severity of periodontal destruction in periodontitis patients. It is important to note that the sCD14 levels were positively correlated with established systemic inflammatory markers, such as CRP and numbers of leukocytes. It has been proposed that these markers could be elevated by undiagnosed, chronic infectious processes; the consequences of the created pro-inflammatory milieu within the vasculature could be the exacerbation of disease activity within atherosclerotic plaques and the precipitation of acute cardiovascular events (25). sCD14 is involved in LPS-clearance (26) and given the frequent low-grade bacteremias in periodontitis (5), leading to elevated

blood levels of LPS (8), the positive correlation with disease severity points into the direction that the sCD14 increases with the need to remove LPS from circulation. Therefore we propose that periodontitis is one of the undiagnosed infections inducing a systemic, sub-clinical inflammatory reaction that contributes to an increased atherothrombotic burden.

In this study we looked for alternative modifying factors that could induce variation in the sCD14 levels measured. A role for the positive infectious serology with *H. pylori*, *C. pneumoniae* and cytomegalovirus in the chronic inflammatory reaction in patients with cardiovascular disease has been proposed (3). In our periodontitis model, we investigated the relationship between history of infection with two Gram-negative established periodontal pathogens, *A. actinomycetemcomitans* and *P. gingivalis* and the sCD14 levels. The anti-*P. gingivalis* IgG were not correlated with the sCD14 levels. Our results showed that the sCD14 levels were negatively associated with the levels of anti-*A. actinomycetemcomitans* IgG in periodontitis patients (Table 4.2). 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.

The $CD14^{-260}$ polymorphism has been described as a modifying factor for myocardial infarction and atherosclerosis, in Asian and Caucasian populations; the T-allele carriers have elevated levels of soluble and membrane-bound CD14 (18,27). Albeit not conclusive, there are indications for the association of the $CD14$ T/T genotype with severe periodontal disease (20). In our study, the $CD14^{-260}$ genotypes were present with similar frequency in patients and controls. Within the Caucasians, the allele frequencies are similar to those reported in the literature (28,20). The sCD14 levels were not correlated with the $CD14^{-260}$ genotypes, neither in the entire group nor in the Caucasian sub-group. The lack of association between the $CD14^{-260}$ genotypes and sCD14 levels is not completely surprising. Contradictory reports on the correlation between $CD14^{-260}$ genotypes and sCD14 levels have been documented on even larger groups than the present relatively-small study population; Koenig et al. (28) found a significant effect of the $CD14^{-260}$ genotypes on sCD14 in 312 Germans 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 (27).

In the current study, we found elevated sCD14 levels that were increasing with the severity of periodontal destruction and were paralleled by markers of a systemic inflammatory reaction. Our findings support the hypothesis of a pro-atherogenic milieu in untreated periodontitis patients resulting from recurrent bacteremic episodes. Since periodontal therapy results in a reduction of sCD14 plasma values (12), future strategies for reducing the risk for cardiovascular events might consider periodontal therapy among the prevention methods to reduce the overall pro-atherogenic burden. However, such studies are still in demand.

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