Journal of
PERIODONTAL RESEARCH

J Periodont Res 2013; 48: 350–356

JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/jre.12014

Anti-apolipoprotein A-1 autoantibodies as biomarker for atherosclerosis burden in patients with periodontitis

**P. A. Wick[1], A. Mombelli[1]
S. Pagano[2], X. Moren[2]
C. Giannopoulou[1], F. Mach[3]
P. Roux-Lombard[2,4]
N. Vuilleumier[2]**

[1]Department of Periodontology, School of Dental Medicine, University of Geneva, Geneva, Switzerland, [2]Service of Laboratory Medicine, Department of Genetics and Laboratory Medicine, Geneva University Hospitals, Geneva, Switzerland, [3]Division of Cardiology, Department of Internal Medicine, Geneva University Hospitals and University of Geneva, Geneva, Switzerland and [4]Division of Immunology and Allergy, Department of Internal Medicine, Geneva University Hospitals and University of Geneva, Geneva, Switzerland

Wick PA, Mombelli A, Pagano S, Moren X, Giannopoulou C, Mach F, Roux-Lombard P, Vuilleumier N. Anti-apolipoprotein A-1 autoantibodies as biomarker for atherosclerosis burden in patients with periodontitis. J Periodont Res 2013; 48: 350–356. © 2012 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd

Background and Objective: Anti-apolipoprotein A-1 (anti-apoA-1) IgG is a potential marker of atherosclerotic plaque vulnerability and cardiovascular complications. In patients with periodontitis the presence of anti-apoA-1 IgGs in serum and their association with atherosclerosis is unknown.

Material and Methods: One-hundred and thirty subjects with periodontal disease and 46 healthy subjects, matched for age and gender, participated in this study. Anti-apoA-1 IgG, high-sensitivity C-reactive protein (hsCRP) and matrix metalloproteinase (MMP) -2, -3, -8 and -9 were measured in serum samples. An ankle-brachial index (ABI) value below 1.11 served as a surrogate marker of atherosclerosis. Predictive accuracies of biomarkers for abnormal ABI were determined using receiver–operating characteristics curves and logistic regression analyses.

Results: Compared with healthy controls, periodontitis patients showed lower median ABI values (1.10 vs. 1.15; $p < 0.0001$), a higher prevalence of anti-apoA-1 IgG positivity (23.8% vs. 6.5%; $p = 0.009$) and higher concentrations of hsCRP (1.62 mg/L vs. 0.85 mg/L; $p = 0.02$) and MMP-9 (435 μg/mL vs. 283 μg/mL; $p < 0.0001$). In patients younger than 50 years of age ($n = 66$), anti-apoA-1 IgG was found to be the best predictor for an abnormal ABI (area under the curve = 0.63; $p = 0.03$). Anti-apoA-1 IgG positivity increased the risk of having an abnormal ABI (odds ratio = 4.20; $p = 0.04$), independently of diabetes, smoking and body mass index.

Conclusions: Anti-apoA-1 IgG positivity and atherosclerosis, as reflected by abnormal ABI, were more prevalent in periodontitis patients than in age- and gender-matched controls. In younger periodontitis patients, anti-apoA-1 IgG was found to be the best predictor of atherosclerosis burden.

Andrea Mombelli, Department of Periodontology, School of Dental Medicine, University of Geneva, Rue Barthélemy-Menn 19, CH-1205 Geneva, Switzerland
Tel: +41 22 379 4030
Fax: +41 22 379 4032
e-mail: Andrea.Mombelli@unige.ch

Key words: ankle-brachial index; anti-apolipoprotein A-1 autoantibodies; atherosclerosis; autoimmunity; matrix metalloproteinases; periodontitis

Accepted for publication August 28, 2012

Periodontitis is a chronic inflammatory disease associated with an increased risk for cardiovascular disease (CVD; 1–4) and with an increased coronary atherosclerosis burden (5). Initially controversial (6), the association has been substantiated by the results of two recent meta-analyses and one prospective longitudinal study with 1203 patients followed up to 35 years, demonstrating periodontitis to increase the risk for CVD by about twofold, independently of other established cardiovascular risk factors (7–9). Well-established and yet-to-be elucidated factors may act synergistically to enhance immune-mediated inflammatory processes and endothelial

dysfunction common to both atherogenesis and periodontitis (10–12). From a clinical perspective, the question arises if and how cardiovascular risks should be stratified in periodontitis patients (10).

Some autoantibodies may be prognostic markers for cardiovascular risk stratification (13), indicative of cardiac dysfunction (14), and at the same time may be active mediators in the pathogenesis of periodontal diseases (15,16). Anti-apolipoprotein A-1 (anti-apoA-1) IgG autoantibodies are independently associated with increased risk of CVD in high-risk populations (17–19) and with increased atherosclerotic plaque vulnerability in humans and mice (20). They may be the best humoral autoimmune candidates for cardiovascular prognosis after myocardial infarction (21). Nevertheless, in subjects with periodontitis, their existence and their relationship to CVD risk are unknown.

The ankle-brachial index (ABI) is a noninvasive method for cardiovascular risk screening. The ABI, which is the ratio of systolic blood pressure at the ankle to that in the arm, was originally used as a diagnostic test for lower-extremity peripheral arterial disease (22). Preclinical changes in peripheral arterial structures that can be measured by the ABI are associated with coronary atherosclerosis (23). An abnormal ABI is considered as a validated surrogate marker of atherosclerosis burden and a good cardiovascular risk predictor in general populations (24,25).

Endothelial dysfunction is known to precede atherosclerosis (26). The hallmark of endothelial dysfunction is impaired endothelium-dependent vasodilation, which is mediated by endothelium-derived nitric oxide (27), the most potent endogenous vasodilator known (28). Asymmetric dimethylarginine (ADMA) is an endogenous competitive inhibitor of nitric oxide synthases. Elevated levels of ADMA have been associated with endothelial dysfunction and atherosclerosis (28). ADMA is a marker of endothelial-dependent dysfunction and has a strong cardiovascular prognostic value (29,30), reflecting early atherogenesis.

Anti-apoA-1 IgG and circulating mediators of atherosclerotic plaque instability, such as matrix metalloproteinases (MMPs) 2, 3, 8 and 9 have been shown to be involved in late atherogenesis processes such as atherosclerotic plaque fragilization (17,20,31), which ultimately lead to atherosclerotic plaque rupture, clinically manifested as myocardial infarction or stroke.

The purpose of this study was to investigate whether anti-apoA-1 IgGs are present in periodontitis, whether they predict the occurrence of a low ABI and whether they are associated with higher levels of MMP-2, -3, -8 and -9, high-sensitivity C-reactive protein (hsCRP) (31) and ADMA.

Material and methods

This was a cross-sectional study. The Ethics Committee of the University Hospitals of Geneva (Geneva, Switzerland) approved the protocol. Research was conducted according to the principles outlined in the Declaration of Helsinki on human medical experimentation. Written informed consent was obtained from all participants.

Subjects

Participants were recruited among patients seeking periodontal treatment or consulting for a routine dental check-up at the School of Dental Medicine of the University of Geneva between April 2009 and August 2011. We investigated 130 periodontitis patients and 46 control subjects who were matched for age and gender. Inclusion criteria for the test group were a diagnosis of periodontitis, the presence of at least four teeth with a probing pocket depth of > 4 mm, clinical attachment loss of at least 2 mm and radiographic evidence of bone loss. Inclusion criteria for the control group were no evidence of past or present periodontal disease, defined by the absence of periodontal pockets with a probing pocket depth of > 3 mm, absence of clinical attachment loss of > 1 mm and no radiographic evidence of bone loss. Subjects with autoimmune diseases, including systemic lupus erythematosus, anti-phospholipid syndrome, rheumatoid arthritis and Sjögren syndrome, were excluded.

The sample size was computed based on an expected prevalence of anti-apoA-1 IgG in periodontitis of 20%, as determined in an unpublished pilot study, and of 1% in a healthy population. A sample size of 130 periodontitis patients and 45 healthy control subjects was needed to detect a difference in the prevalence of anti-apoA-1 IgG with a power of 90% and an alpha error of 5%.

Clinical protocol

Demographic data, cardiovascular risk factors and health history were recorded first. After a rest of 5 min in a horizontal position, the ABI was assessed and the heart rate was measured. Next, venous blood samples were drawn. After collection, serum samples were aliquoted and frozen at $-80°C$. Finally, probing pocket depth, clinical attachment level and bleeding on probing were assessed on six sites of all teeth, excluding the third molars.

The following cardiovascular risk factors were recorded: systolic hypertension, dyslipidemia, diabetes, smoking, obesity [body mass index (BMI) > 30], known CVD and a positive familial history of CVD.

The ABI was measured using Vista ABI^{TM} L450 (Summit Doppler Systems Inc., Golden, CO, USA) on both sides, as the ratio of the ankle systolic blood pressure and the brachial systolic blood pressure, with the subject in a horizontal position. Results were reported as the mean of the right and the left ABI. An ABI value of < 1.11 was chosen as the surrogate marker for atherosclerosis. The risk for CVD has been shown to be higher if the ABI falls below this cut-off value (24). ABI values of ≥ 1.30 are considered to be nondiagnostic owing to vascular incompressibility (24,25).

Biochemical analyses

Anti-apoA-1 IgG autoantibodies were measured using an ELISA, as previously

described (17–20). Briefly, MaxiSorp™ plates (Nalge Nunc International, Rochester, NY, USA) were coated with purified, human-derived delipidated ApoA-1 (20 μg/mL; 50 μL/well) for 1 h at 37°C. After three washes with phosphate-buffered saline (PBS)/2% bovine serum albumin (BSA) (100 μL/well), all wells were blocked for 1 h with 2% BSA at 37°C. Samples were diluted 1 : 50 in PBS/2% BSA and incubated for 60 min. Additional patient samples at the same dilution were also added to an uncoated well to assess individual nonspecific binding. After six further washes, 50 μL/well of signal antibody (alkaline phosphatase-conjugated anti-human IgG; Sigma-Aldrich Corp., St Louis, MO, USA), diluted 1 : 1000 in PBS/2% BSA solution, was incubated for 1 h at 37°C. After six more washes (200 μL/well) with PBS/2% BSA solution, the phosphatase substrate, p-nitrophenyl phosphate disodium (100 μL/well; Sigma-Aldrich), dissolved in diethanolamine buffer (pH 9.8), was added. Each sample was tested in duplicate, and the absorbance at 405 nm was determined after 20 min of incubation at 37°C (VersaMax™; Molecular Devices, Sunnyvale, CA, USA). The corresponding nonspecific binding value was subtracted from the mean absorbance value for each sample. The positivity cut-off was predefined as previously validated and set at a value of 0.6 and 37% of the positive control value, as described earlier (17–20). Optical density values ranged from 0 to 1.78, and corresponding index values were between 0 and 99.2%.

The levels of hsCRP, MMP-2, MMP-3, MMP-8 and MMP-9 were determined in serum using a commercially available multiplex beads immunoassay (Fluorokine® MAP Multiplex Human Cytokine Panel and Human MMP Panel; R&D Systems, Minneapolis, MN, USA), according to the supplier's instructions, using a Bio-Plex™ 200 System array reader with Luminex® xMAP™ Technology (Bio-Rad, Hercules, CA, USA).

The level of ADMA in human serum was measured using a competitive enzyme linked immunoassay kit (Immundiagnostik AG, Bensheim, Germany), according to the manufacturer's instructions.

Study end-points

Three predetermined aims were considered for this explorative study: the first end-point was to explore whether periodontitis patients had an increase in the prevalence of anti-apoA-1 IgG positivity as well as in the levels of hsCRP, ADMA and MMP-2, -3, -8 and -9 when compared with age- and gender-matched healthy subjects. The second end-point consisted of evaluating the diagnostic accuracy of anti-apoA-1 IgG and the aforementioned biomarkers to predict an ABI value of < 1.11 as a surrogate marker of atherosclerosis. The third end-point consisted of exploring potential associations between anti-apoA-1 IgG and the biomarkers of interest.

Statistics

Fisher's bilateral exact test and the Mann–Whitney *U*-test were used to determine differences between healthy and diseased subjects. A receiver–operating characteristics (ROC) curve analysis was performed for all biomarkers to assess the prediction of ABI < 1.11 and to confirm the validity of the anti-apoA-1 IgG cut-off value in periodontitis patients. Logistic regression analyses for abnormal ABI were performed using diabetes, smoking status and BMI as confounders. Associations between anti-apoA-1 IgG positivity, and study end-points are presented as odds ratio (OR) and corresponding 95% confidence interval (95% CI). Spearman correlations between anti-apoA-1 IgG, ABI and markers of inflammation and endothelial dysfunction were calculated where appropriate. Because younger age has been shown to be associated with an increased CVD risk in periodontitis patients (9), analyses were performed separately in subjects below or above the median age of the cohort.

ROC curve analyses were performed using analyse-it software for EXCEL (Microsoft, Redmond, WA, USA). The remaining analyses were performed using STATISTICA software (StatSoft, Tulsa, OK, USA). $p < 0.05$ was considered significant.

Results

The demographic and clinical characteristics of periodontitis and control subjects are summarized in Table 1. There were no significant differences between the two groups for age and gender. Periodontitis patients presented significantly more sites with probing pocket depth > 4 mm and bleeding on probing, a higher percentage of sites with bleeding on probing and had more missing teeth. Furthermore, periodontitis patients showed a higher prevalence of smoking, a greater number of pack years, higher systolic blood pressure and were more likely to be under antihypertensive and statin treatment when compared with age- and gender-matched controls. No other significant differences for cardiovascular risk factors and medical treatment were found.

As shown in Table 2, the prevalence of anti-apoA-1 IgG positivity was found to be higher in periodontitis patients than in control subjects (23.8% vs. 6.5%, $p = 0.009$), whereas no significant differences were observed in the median anti-apoA-1 IgG index between those two groups.

Periodontitis patients had lower median ABI values (1.10 vs. 1.15; $p < 0.0001$) and a higher proportion of abnormal ABI (< 1.11) (48% vs. 20%, $p = 0.0008$) when compared with healthy subjects. No significant differences were observed in ADMA levels between the two groups (Table 2).

The median levels of hsCRP, MMP-8 and MMP-9 were significantly higher in periodontitis patients than in healthy subjects, whereas the median levels of MMP-2 were lower in periodontitis patients than in controls. No significant differences were found between the groups for MMP-3 levels.

Table 3 shows the ROC curve and logistic regression analyses for an ABI < 1.11 prediction in the whole cohort of the periodontitis group as well as in the subgroups of patients of < 50 (median age of the periodontitis

Table 1. Demographic and clinical characteristics of the study participants

Parameters	Periodontitis patients (n = 130)	Control subjects (n = 46)	p-value
Age (years)	49.3 (44.6–58.5)	47.3 (42.9–58.4)	0.72
Male	56 (73)	54 (25)	0.86
Female	44 (57)	46 (21)	–
Missing teeth (n)	3 (1–7)	1 (0–2)	< 0.0001
Bleeding on probing (%)	69.4 (49–95)	12.4 (8–24)	< 0.0001
Probing pocket depth > 4 mm and bleeding on probing-positive (n)	26 (14–46)	0 (0–0)	< 0.0001
Systolic blood pressure (mmHg)	128 (119–137)	123 (113–130)	0.01
Heart rate (bpm)	72 (66–78)	69 (64–75)	0.15
Body mass index (kg/m^2)	26.2 (22.9–29.3)	26.1 (23.4–28.4)	0.72
Cardiovascular risk factors			
Systolic hypertension	24 (31)	13 (6)	0.14
Dyslipidemia	18 (23)	9 (4)	0.23
Diabetes	10 (13)	7 (3)	0.56
Smoking	44 (57)	15 (7)	0.0003
Pack years (median)	10 (0–20)	0 (0–6)	< 0.0001
Obesity	19 (25)	17 (8)	1
Known CVD	7 (9)	11 (5)	0.53
Familial history of CVD	17 (22)	7 (3)	0.14
Treatments			
Anti-aggregant	8 (10)	2 (1)	0.29
Antihypertensive agents	38 (49)	20 (9)	0.02
Antidiabetic agents	10 (13)	9 (4)	0.52
Oral anticoagulation	0.7 (1)	0 (0)	0.39
Statins	12 (16)	2 (1)	0.04

Continuous variables are expressed as median (interquartile range); all other values are expressed as % (n).
bpm, beats per minute; CVD, cardiovascular disease (including acute coronary syndromes and ischemic or hemorrhagic stroke).

Table 2. Anti-apolipoprotein A-1 (anti-apoA-1) IgG, endothelial function and inflammation in periodontitis patients and control subjects

Parameters	Periodontitis patients (n = 130)	Control subjects (n = 46)	p
Anti-apoA-1			
Anti-apoA-1 IgG, index	24.3 (16.3–35.0)	22.1 (15.2–28.8)	0.16
Anti-apoA-1 IgG positivity	23.8 (31)	6.5 (3)	0.009
Endothelial function			
ADMA (mM)	0.39 (0.36–0.42)	0.39 (0.35–0.44)	0.93
ABI	1.10 (1.05–1.16)	1.15 (1.12–1.21)	< 0.0001
ABI < 1.11	48 (63)	20 (9)	0.0008
Inflammation			
hsCRP (mg/L)	1.62 (0.78–4.56)	0.85 (0.57–2.23)	0.02
MMPs			
MMP-2 (μg/mL)	186.4 (162.0–213.5)	199.7 (181.7–225.3)	0.03
MMP-3 (μg/mL)	15.2 (10.1–19.8)	14.7 (10.0–20.9)	0.94
MMP-8 (μg/mL)	11.3 (7.0–17.1)	8.9 (4.8–13.4)	0.01
MMP-9 (μg/mL)	434.6 (323.5–605.7)	282.7 (201.0–383.5)	< 0.0001

Continuous variables are expressed as median (interquartile range); all other values are expressed as % (n).
ABI, ankle-brachial index; ADMA, asymmetric dimethylarginine; hsCRP, high-sensitivity C-reactive protein; MMP, matrix metalloproteinase.

patients = 49.3 years) and ≥ 50 years of age. For the whole periodontitis cohort, none of the studied biomarkers was predictive of an ABI of < 1.11.

In the subgroup of periodontitis patients of ≥ 50 years of age (n = 64), only ADMA levels were found to be predictive of an ABI of < 1.11. In the subgroup of patients < 50 years of age (n = 66), anti-apoA-1 IgG, MMP-8 and MMP-9 were found to be significant predictors of ABI < 1.11. At this predefined cut-off, ROC curve analyses indicated that anti-apoA-1 IgG positivity had a specificity of 90% (95% CI: 0.73–0.98), a sensitivity of 32% (95% CI: 0.18–0.50), a negative predictive value of 51% (95% CI: 37–65) and a positive predictive value of 80% (95% CI: 51–95). Combining anti-apoA-1 IgG with MMP-9 values did not increase diagnostic accuracy, as it yielded an area under the curve of 0.63 (95% CI: 0.51–0.77; p = 0.03). Logistic regression analysis demonstrated, in the subgroup of younger periodontitis patients, that being positive for anti-apoA-1 IgG increased, by fourfold, the risk of an abnormal ABI (OR = 4.20; p = 0.04), which remained unchanged after adjusting for diabetes, smoking status and BMI (OR = 4.15; p = 0.04). Corroborating the ROC curve and logistic regression analyses, periodontitis patients < 50 years of age tended to have lower ABI values if they were positive for anti-apoA-1 IgG than if they tested negative (1.08 vs. 1.11; p = 0.06; data not shown). This trend was not significant in the older periodontitis patients or in the whole periodontitis cohort.

For MMP-9 in the same subgroup, ROC curve analysis indicated that the optimal cut-off for prediction of an abnormal ABI was at 290 μg/mL, with a sensitivity of 92% (95% CI: 78–98), a specificity of 24% (95% CI: 10–44), a negative predictive value of 70% (95% CI: 35–92) and a positive predictive value of 61% (95% CI: 47–73). Nevertheless, at this cut-off, logistic regression analysis failed to demonstrate a significant increase in the risk of having an abnormal ABI (OR = 3.60; 95% CI: 0.84–15.4; p = 0.08) and this remained unchanged after the adjustment for diabetes, smoking and BMI (data not shown).

As shown in Table 4, anti-apoA-1 IgG showed a significant and positive correlation with ADMA levels on the whole periodontitis cohort and in the

Table 3. Receiver–operating characteristics (ROC) curve and logistic regression analyses for abnormal ankle-brachial index (ABI) prediction in periodontitis patients

ROC curve analyses for abnormal ABI (< 1.11) prediction			
Parameter	Whole cohort (n = 130) AUC (95% CI)	Age < 50 years(n = 66) AUC (95% CI)	Age ≥ 50 years (n = 64) AUC (95% CI)
Anti-apoA-1 IgG index	0.54 (0.44–0.64); p = 0.20	0.63 (0.51–0.76); p = 0.03	0.43 (0.27–0.59); p = 0.80
hsCRP (mg/L)	0.51 (0.41–0.62); p = 0.61	0.47 (0.33–0.62); p = 0.64	0.50 (0.35–0.65); p = 0.51
ADMA (mM)	0.54 (0.44–0.64); p = 0.23	0.59 (0.45–0.74); p = 0.10	0.66 (0.52–0.80); p = 0.01
MMP-2 (μg/mL)	0.55 (0.45–0.65); p = 0.16	0.43 (0.29–0.57); p = 0.83	0.51 (0.36–0.66); p = 0.45
MMP-3 (μg/mL)	0.57 (0.47–0.67); p = 0.09	0.59 (0.45–0.73); p = 0.10	0.49 (0.34–0.54); p = 0.54
MMP-8 (μg/mL)	0.55 (0.45–0.65); p = 0.16	0.62 (0.48–0.76); p = 0.05	0.46 (0.30–0.61); p = 0.70
MMP-9 (μg/mL)	0.55 (0.45–0.65); p = 0.16	0.62 (0.50–0.75); p = 0.04	0.46 (0.31–0.61); p = 0.69

Logistic regression analyses to predict the risk of abnormal ABI (< 1.11)						
	Whole cohort (n = 130)		Age < 50 years(n = 66)		Age ≥ 50 years (n = 64)	
	Univariate OR (95% CI)	Adjusted OR (95% CI)*	Univariate OR (95% CI)	Adjusted OR (95% CI)*	Univariate OR (95% CI)	Adjusted OR (95% CI)*
Anti-apoA-1 IgG positivity	1.63 (0.72–3.69); p = 0.24	2.47 (0.48–12.7); p = 0.27	4.20 (1.05–16.5); p = 0.04	4.15 (1.02–16.84); p = 0.04	1.02 (0.31–3.32); p = 0.97	1.00 (0.32–3.56); p = 0.92

*Adjusted for body mass index, diabetes and smoking.
95% CI, 95% confidence interval; ADMA, asymmetric dimethylarginine; anti-apoA-1, anti-apolipoprotein A-1; AUC, area under the curve; hsCRP, high-sensitivity C-reactive protein; MMP, matrix metalloproteinase; OR, odds ratio.

subgroup of younger patients (r = 0.20, p = 0.03 and r = 0.36, p = 0.01, respectively). No significant correlations were found between anti-apoA-1 IgG and mean ABI or any inflammatory markers. No correlations were found between ABI and ADMA levels, either in the whole cohort or in the age-related subgroups (data not shown).

Discussion

This study shows that low ABI and anti-ApoA-1 IgG positivity are more prevalent in periodontitis patients than in age- and gender-matched controls. The median ABI value of periodontitis patients was below 1.11, the cut-off at which the CVD risk significantly increases according to a recent meta-analysis (24), whereas the median ABI was above 1.11 in matched healthy subjects. Because low ABI values are considered as an independent predictor of atherosclerosis burden and related complications in humans (24,25), our findings are in line with current data suggesting that periodontitis could be an independent risk factor for CVD (7–9). The 24.6% prevalence of high anti-apoA-1 IgG levels in periodontitis patients was similar to or higher than prevalence figures reported from high CVD risk situations (17–21). Anti-apoA-1 IgGs have been shown to be an independent CVD risk factor in myocardial infarction and in patients with rheumatoid arthritis (17,18,21,32). By promoting sterile inflammation through the toll-like receptor 2/CD14 complex (33), and the occurrence of arrhythmia by activating L-type calcium channels (34), they are a potential mediator of atherogenesis (20) and related complications.

Endothelial dysfunction is known to precede atherosclerosis (26,27). ADMA is an established marker of CVD risk and a mediator of impaired endothelium-dependent vasorelaxation (29,30). ADMA levels were found to be predictive of an ABI < 1.11 in the older periodontitis patients only. However, anti-apoA-1 IgG showed a significant and positive correlation with ADMA levels in the periodontitis cohort overall and specifically in the subgroup of younger patients. As anti-apoA-1 IgG levels were significant predictors of an abnormal ABI in younger periodontitis patients and were, at the same time, correlated with ADMA levels, our results suggest that anti-apoA-1 IgG may be involved in the early stages of atherogenesis and in atherosclerotic plaque fragilization processes (20). However, causality between anti-apoA-1 IgG and endothelial dysfunction remains to be demonstrated.

In the present study, higher median levels of circulating MMP-8 and MMP-9, and a lower median level of circulating MMP-2, were measured in periodontitis patients than in healthy subjects. ROC curve analysis showed MMP-9 to have a predictive value for atherosclerotic burden in younger periodontitis patients. These findings are in line with previous work suggesting that circulating MMP-9 levels are raised in periodontitis patients when compared with controls (35) and constitute a marker of early atherosclerosis, as measured by carotid intima-media thickness (36). Owing to lack of power we could not define an MMP-9 cut-off for abnormal ABI prediction.

The results of the present study contrast previous work in patients with myocardial infarction and rheumatoid

Table 4. Spearman correlations between anti-apolipoprotein A-1 (anti-apoA-1) IgG and mean ankle-brachial index (ABI), markers of inflammation and endothelial dysfunction

Anti-apoA-1 IgG vs.	Whole cohort (n = 130)		Age < 50 years (n = 66)		Age ≥ 50 years (n = 64)	
	r	p	r	p	r	p
Mean ABI	−0.08	0.30	−0.16	0.19	0.05	0.69
ADMA (mM)	0.20	0.03	0.36	0.01	0.04	0.75
hsCRP (mg/L)	−0.08	0.30	−0.10	0.45	−0.07	0.80
MMP-2 (μg/mL)	0.10	0.25	0.18	0.15	0.05	0.69
MMP-3 (μg/mL)	0.09	0.30	0.18	0.15	0.03	0.78
MMP-8 (μg/mL)	0.00	0.91	0.00	1	0.04	0.77
MMP-9 (μg/mL)	0.01	0.76	0.01	1	0.01	0.85

ADMA, asymmetric dimethylarginine; hsCRP, high-sensitivity C-reactive protein; MMP, matrix metalloproteinase.

arthritis, where high levels of anti-apoA-1 IgG were significantly associated with higher levels of MMP-9 (17,33). The reason why no such association could be seen in periodontitis patients is unclear but could be related to differences in the distribution of polymorphisms related to MMPs between different populations (37,38). If our results are in line with several studies demonstrating that hsCRP levels are higher in periodontitis-affected patients than in control subjects (39), our data also indicate that this parameter is not a good discriminant biomarker for abnormal ABI prediction.

Because of the relatively good positive predictive value (80%) of anti-apoA-1 IgG in younger patients to predict an abnormal ABI, we hypothesize that anti-apoA-1 IgG positivity could help to identify periodontitis patients with a high CVD risk in whom further CVD investigations should be undertaken. Larger, prospective studies are needed to clarify this hypothesis.

Our explorative study has several limitations. Owing to the cross-sectional nature of the data, a causal relationship cannot be established between anti-apoA-1 IgG and atherosclerosis. Furthermore, as surrogate markers cannot substitute for clinical end-points, our study subjects need to be followed up longitudinally, to confirm the CVD prognostic value of anti-apoA-1 IgG in this clinical setting. Another limitation is that we chose an ABI cut-off of 1.11, which is higher than the ABI cut-off (< 0.90) commonly used for diagnosing peripheral artery disease (25). This choice was motivated by the fact that CVD risk has been shown to increase significantly below this value (24), and that the number of periodontitis patients with an ABI of < 0.90 would have been too small for proper statistical analyses. Indeed, only three patients had an ABI value of < 0.90 (data not shown).

In conclusion, anti-apoA-1 IgG positivity and atherosclerosis burden, as reflected by abnormal ABI, were more prevalent in periodontitis patients than in age- and gender-matched controls. In younger periodontitis patients, anti-apoA-1 IgG was found to be the best predictor of atherosclerosis burden. These preliminary results point to anti-apoA-1 IgG as a possible biomarker for CVD risk stratification in periodontitis patients.

Acknowledgements

This work was supported by the Swiss National Science Foundation, Grant Nos 320000-122089 (to AM) and 310030-140736 (to NV), and in part by the Lucie and Ernst Schmidheiny foundation (to PRL), the Telemaque Foundation, the Gustave and Simone Prevot Foundation and the DeReuters foundation (to NV).

References

1. Persson GR, Persson RE. Cardiovascular disease and periodontitis: an update on the associations and risk. *J Clin Periodontol* 2008;**35**(suppl 8):362–379.
2. Mattila KJ, Nieminen MS, Valtonen VV *et al*. Association between dental health and acute myocardial-infarction. *Brit Med J* 1989;**298**:779–781.
3. DeStefano F, Anda RF, Kahn HS, Williamson DF, Russell CM. Dental disease and risk of coronary heart-disease and mortality. *Brit Med J* 1993;**306**:688–691.
4. Beck J, Garcia R, Heiss G, Vokonas PS, Offenbacher S. Periodontal disease and cardiovascular disease. *J Periodontol* 1996;**67**:1123–1137.
5. Buhlin K, Mantyla P, Paju S *et al*. Periodontitis is associated with angiographically verified coronary artery disease. *J Clin Periodontol* 2011;**38**:1007–1014.
6. Beck JD, Eke P, Heiss G *et al*. Periodontal disease and coronary heart disease – a reappraisal of the exposure. *Circulation* 2005;**112**:19–24.
7. Humphrey LL, Fu RW, Buckley DI, Freeman M, Helfand M. Periodontal disease and coronary heart disease incidence: a systematic review and meta-analysis. *J Gen Intern Med* 2008; **23**: 2079–2086.
8. Bahekar AA, Singh S, Saha S, Molnar J, Arora R. The prevalence and incidence of coronary heart disease is significantly increased in periodontitis: a meta-analysis. *Am Heart J* 2007;**154**:830–837.
9. Dietrich T, Jimenez M, Kaye EAK, Vokonas PS, Garcia RI. Age-dependent associations between chronic periodontitis/edentulism and risk of coronary heart disease. *Circulation* 2008;**117**:1668–1674.
10. Friedewald VE, Kornman KS, Beck JD *et al*. The American Journal of Cardiology and Journal of Periodontology editors' consensus: periodontitis and atherosclerotic cardiovascular disease. *J Periodontol* 2009;**80**:1021–1032.
11. Higashi Y, Goto C, Jitsuiki D *et al*. Periodontal infection is associated with endothelial dysfunction in healthy subjects and hypertensive patients. *Hypertension* 2008;**51**:446–453.
12. Higashi Y, Goto C, Hidaka T *et al*. Oral infection-inflammatory pathway, periodontitis, is a risk factor for endothelial dysfunction in patients with coronary artery disease. *Atherosclerosis* 2009;**206**: 604–610.

13. Roux-Lombard P, Pagano S, Montecucco F, Satta N, Vuilleumier N. Auto-antibodies as emergent prognostic markers and possible mediators of ischemic cardiovascular diseases. *Clin Rev Allergy Immunol* 2010;doi: 10.1007/s12 016-010-8233-z. [Epub ahead of print].
14. Segovia M, Ganzinelli S, Reina S, Borda E, Sterin-Borda L. Role of anti-beta1 adrenergic antibodies from patients with periodontitis in cardiac dysfunction. *J Oral Pathol Med* 2012;**41**:242–248.
15. Hendler A, Mulli TK, Hughes FJ *et al*. Involvement of autoimmunity in the pathogenesis of aggressive periodontitis. *J Dent Res* 2010;**89**:1389–1394.
16. Buhlin K, Hultin M, Norderyd O *et al*. Risk factors for atherosclerosis in cases with severe periodontitis. *J Clin Periodontol* 2009;**36**:541–549.
17. Vuilleumier N, Bas S, Pagano S *et al*. Anti-apolipoprotein A-1 IgG predicts major cardiovascular events in patients with rheumatoid arthritis. *Arthritis Rheum* 2010;**62**:2640–2650.
18. Vuilleumier N, Rossier MF, Pagano S *et al*. Anti-apolipoprotein A-1 IgG as an independent cardiovascular prognostic marker affecting basal heart rate in myocardial infarction. *Eur Heart J* 2010;**31**: 815–823.
19. Keller PF, Pagano S, Roux-Lombard P *et al*. Autoantibodies against apolipoprotein A-1 and phosphorylcholine for diagnosis of non-ST-segment elevation myocardial infarction. *J Intern Med* 2012;**271**:451–462.
20. Montecucco F, Vuilleumier N, Pagano S *et al*. Anti-Apolipoprotein A-1 auto-antibodies are active mediators of atherosclerotic plaque vulnerability. *Eur Heart J* 2011;**32**:412–421.
21. Vuilleumier N, Pagano S, Lahlou K *et al*. Head-to-head comparison of auto-antibodies for cardiovascular outcome prediction after myocardial infarction: a prospective study. *J Clinic Experiment Cardiol* 2011;**2**:169.
22. Ouriel K, McDonnell AE, Metz CE, Zarins CK. Critical evaluation of stress testing in the diagnosis of peripheral vascular disease. *Surgery* 1982;**91**:686–693.
23. Megnien JL, Simon A, Gariepy J *et al*. Preclinical changes of extracoronary arterial structures as indicators of coronary atherosclerosis in men. *J Hypertens* 1998;**16**:157–163.
24. Fowkes FG, Murray GD, Butcher I *et al*. Ankle brachial index combined with Framingham risk score to predict cardiovascular events and mortality: a meta-analysis. *JAMA* 2008;**300**:197–208.
25. Tziomalos K, Athyros VG, Karagiannis A, Mikhailidis DP. The role of ankle brachial index and carotid intima-media thickness in vascular risk stratification. *Curr Opin Cardiol* 2010;**25**:394–398.
26. Davignon J, Ganz P. Role of endothelial dysfunction in atherosclerosis. *Circulation* 2004;**109**:III27–III32.
27. von Eckardstein A, Rohrer L. Transendothelial lipoprotein transport and regulation of endothelial permeability and integrity by lipoproteins. *Curr Opin Lipidol* 2009;**20**:197–205.
28. Cooke JP. Does ADMA cause endothelial dysfunction? *Arterioscler Thromb Vasc Biol* 2000; **20**: 2032–2037.
29. Anderssohn M, Schwedhelm E, Luneburg N, Vasan RS, Boger RH. Asymmetric dimethylarginine as a mediator of vascular dysfunction and a marker of cardiovascular disease and mortality: an intriguing interaction with diabetes mellitus. *Diab Vasc Dis Res* 2010;**7**:105–118.
30. Böger RH, Maas R, Schulze F, Schwedhelm E. Asymmetric dimethylarginine (ADMA) as a prospective marker of cardiovascular disease and mortality – an update on patient populations with a wide range of cardiovascular risk. *Pharmacol Res* 2009;**60**:481–487.
31. Ridker PM, Morrow DA. C-reactive protein, inflammation, and coronary risk. *Cardiol Clin* 2003;**21**:315–325.
32. Finckh A, Courvoisier DS, Pagano S *et al*. Evaluation of cardiovascular risk in patients with rheumatoid arthritis. Do cardiovascular biomarkers offer added predictive ability over established clinical risk scores? *Arthritis Care Res (Hoboken)* 2012;**64**:817–825.
33. Pagano S, Satta N, Werling D *et al*. Anti-apolipoprotein A-1 IgG in patients with myocardial infarction promotes inflammation through TLR2/CD14 complex. *J Intern Med* 2012;**272**:344–357.
34. Rossier MF, Pagano S, Python M *et al*. Antiapolipoprotein A-1 IgG chronotropic effects require nongenomic action of aldosterone on L-type calcium channels. *Endocrinology* 2012;**153**:1269–1278.
35. Söder PO, Meurman JH, Jogestrand T, Nowak J, Söder B. Matrix metalloproteinase-9 and tissue inhibitor of matrix metalloproteinase-1 in blood as markers for early atherosclerosis in subjects with chronic periodontitis. *J Periodontal Res* 2009;**44**:452–458.
36. Lopez Jornet P, JD B-M, Berna-Serna J, Camacho-Alonso F, Fernandez-Millan S, Reus-Pintado M. Measurement of atherosclerosis markers in patients with periodontitis: a case-control study. *J Periodontol* 2012;**83**:690–698.
37. Loo WT, Wang M, Jin LJ, Cheung MN, Li GR. Association of matrix metalloproteinase (MMP-1, MMP-3 and MMP-9) and cyclooxygenase-2 gene polymorphisms and their proteins with chronic periodontitis. *Arch Oral Biol* 2011;**56**:1081–1090.
38. Blankenberg S, Rupprecht HJ, Poirier O *et al*. Plasma concentrations and genetic variation of matrix metalloproteinase 9 and prognosis of patients with cardiovascular disease. *Circulation* 2003;**107**:1579–1585.
39. Paraskevas S, Huizinga JD, Loos BG. A systematic review and meta-analyses on C-reactive protein in relation to periodontitis. *J Clin Periodontol* 2008;**35**:277–290.