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Severe periodontitis in young adults is associated with sub-clinical atherosclerosis

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Abstract

Aim: The aim of this study was to evaluate the association between severe periodontitis and sub-clinical atherosclerosis in young ($\leqslant$40 years) systemically healthy individuals.

Material and Methods: Ninety systemically healthy subjects, 45 affected by severe periodontitis (mean age 36.35 $\pm$ 3.65 years) and 45 controls without a history of periodontal disease (mean age 33.78 $\pm$ 3.28 years), were enrolled in this study. Patients and controls were paired for age, gender, body mass index and smoking habits. Carotid intima-media thickness (IMT) was bilaterally assessed by ultrasonography at the level of common carotid artery. Traditional cardiovascular risk factors for atherosclerosis were also evaluated.

Results: The overall mean carotid IMT was 0.82 $\pm$ 0.13 mm in the test group and 0.72 $\pm$ 0.07 mm in the control group ($p<0.0001$). Stepwise regression analysis showed that periodontitis ($p<0.0001$) and regular physical activity ($p=0.0009$) were predictor variables of overall mean carotid IMT. When considering an IMT $\geqslant$ 0.82 mm as the critical index of increased cardiovascular risk, periodontal patients overcame this threshold compared with healthy patients by an odds ratio = 8.55 [confidence interval 95%: 2.38; 39.81]. No investigated haemostatic variable was associated with increased carotid IMT.

Conclusion: Severe periodontitis is associated with sub-clinical atherosclerosis in young systemically healthy patients.

Key words: cardiovascular disease; carotid intima-media thickness; periodontal disease; periodontal medicine; sub-clinical atherosclerosis

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Periodontal disease is a chronic inflammatory destruction of tissue surrounding the teeth caused by specific anaerobic pathogens contained in dental plaque organized on the tooth surface (Sanz & Quirynen 2005). Over 30% of adults in United States and Northern Europe have periodontal disease (Hugoson et al. 1998, Albandar et al. 1999); in 13% of these individuals, the condition is severe (Albandar et al. 1999). Two major types of periodontal disease are recognized: *chronic periodontitis*, which has a major prevalence in adults with a low pattern of progression, and *aggressive periodontitis*, a less common type, generally affecting young patients with rapid periodontal destruction that may often result in tooth loss with aesthetic, functional and psychological problems (American Academy of Periodontology 1999).

In the past decade, significant associations between periodontal disease and the extent and severity of atherosclerosis were found, even after adjustment for a variety of potential confounders (DeStefano et al. 1993, Mattila et al. 1993, 1995, Beck et al. 1996, Desvarieux et al. 2003, 2005, Engebretson et al. 2005, D'Aiuto et al. 2007, Persson & Persson in press). Possible biological mechanisms of interaction between periodontitis and cardiovascular disease include the burden of bacterial pathogens, antigens, bacterial toxins (Chiu 1999, Haraszthy et al. 2000, Cairo et al. 2004) and the production of inflammatory mediators (Offenbacher et al. 1999). Several experimental studies support this association, demonstrating the ability of periodontal pathogens to induce platelet aggregation

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(Herzberg & Weyer 1998), foam-cell formation (Qi et al. 2003) and the development of atheroma (Lalla et al. 2003). Other studies have demonstrated the effectiveness of periodontal therapy in reducing acute-phase response markers (D'Aiuto et al. 2004, 2007) and improving endothelial function (Tonetti et al. 2007).

Cardiovascular disease and related events (e.g. stroke and myocardial infarction) may occur after decades of silent arterial wall alterations. B-mode ultrasonography is a non-invasive and highly reliable tool for assessing the early stages of atherosclerosis (Touboul et al. 2004) by measuring the thickness of the inner layers of the vessel walls (intima-media thickness – IMT). This procedure has been extensively applied in monitoring carotid artery (carotid IMT) (Touboul et al. 2004, 2007) to identify the sub-clinical atherosclerosis condition. Increased carotid IMT is associated with cardio- and cerebrovascular events (Howard et al. 1993, Burke et al. 1995, O'Leary et al. 1999). IMT values ranging from 0.75 to 0.82 mm were defined as borderline values and associated with a greater cardiovascular risk than lower values (Aminbakhsh & Mancini 1999).

Some studies evaluated carotid IMT in relation to periodontal disease in the middle-aged to elderly population (Beck et al. 2001, Leivadaros et al. 2005, Soder et al. 2005). A large epidemiological study (Atherosclerosis Risk in Communities) analysed persons of mean age 62 in the United States and reported that severe periodontitis was associated with increases in carotid IMT (Beck et al. 2001). Up to now, no data are available concerning the association between severe periodontal disease and sub-clinical atherosclerosis in young patients.

The aim of this study was to assess the association between severe periodontitis and increased carotid IMT in systemically healthy young ($\leqslant$40 years) patients evaluated for traditional risk factors for cardiovascular disease.

Material and Methods

Patients

The population consisted of patients referred to the Department of Periodontology of Florence University for periodontal evaluation and therapy from September 2002 to December 2006.

General entry criteria: Caucasian race, age ranging between 18 and 40 years, body mass index (BMI) $\leqslant$ 27 kg/m^2 and subjects with blood pressure $\leqslant$ 130/80 mmHg.

Exclusion criteria: the presence of systemic disease (e.g. diabetes mellitus or cardiovascular, kidney, liver or lung disease), a recent history or the presence of any other acute or chronic infection, as assessed on clinical examination and routine laboratory testing, systemic antibiotic treatment within the previous 3 months or any other regular medication, pregnancy and intense sporting activity.

Periodontal entry criteria: severe generalized periodontitis with at least 30% of sites with clinical loss of attachment and alveolar bone loss exceeding 1/3 of the root in at least 30% of the entire dentition.

A single periodontist (F. C.) performed a complete periodontal examination in each patient, collecting data on tooth loss, full-mouth plaque score (FMPS) and full-mouth bleeding score (FMBS). Probing depth and clinical attachment level (CAL) were recorded in six points/tooth. Periodontal disease was categorized as aggressive or chronic periodontitis according to the American Academy of Periodontology (1999). These periodontal patients comprised the *test group*. Healthy individuals without a history of periodontal disease (CAL $\leqslant$ 3 mm in each site) referred for oral hygiene procedures or for treatment of Miller Class I and II gingival recessions were assigned to the *control group*.

All subjects underwent a physical examination. An extensive medical history was determined from each patient by the interview method. The information included

- Presence of systemic disease.
- BMI.
- Smoking status, categorized as former, current or never smoker. When the patient was a current smoker, the age of starting and the number of cigarettes/day were recorded; in addition, the index *Packyear* was calculated (number of cigarette packs in a day $\times$ number of years of tobacco use).
- Family history of cardiovascular disease.
- History of oral contraceptive use.
- Physical activity: subjects were asked to indicate their usual pattern of physical activity, under the headings of regular walking or cycling, recreational activity or sporting activity. Recreational activity included gardening, pleasure walking, dancing and do-it-yourself jobs. Sporting activity included gymnastics, running, playing soccer, golf, tennis, swimming, etc. Subjects were grouped into three broad categories based on their total score: *absent or light* (i.e. inactive or walking for 30 min. less than three times per week), *regular* (i.e. walking for 30 min. three to five times per week or sporting exercise once a week) and *intense sporting activity* (i.e. sporting activity at least twice a week).
- Education level (below high school, high school and above high school).

To obtain two homogeneous groups, on the basis of medical history, test and control patients were paired according to

- Gender.
- Age (using a 5-year threshold of tolerance).
- BMI (using 1-value as the threshold of tolerance).
- Smoking habits: test and control patients were paired for number of cigarettes/day and exposure to smoking in years. To be considered smokers, patients had to be using tobacco for at least 5 years. Patients who reported to be smokers for a lesser time were excluded from the study.

Patients who fulfilled the entry criteria but could not be paired were excluded from the study. Each participant signed an informed consent in accordance with the Helsinki Declaration of 1975.

Blood collection and laboratory analysis

Venous blood samples were taken from each subject at least 2 weeks after periodontal examination by a single venipuncture in the antecubital fossa. Tubes containing blood without an anticoagulant were centrifuged at room temperature ($2000 \times g$) for 15 min. within 1 h of collection and aliquots of serum samples were stored at -80°C until samples from all participants were collected. High-sensitivity C-reactive protein (hs-CRP) levels were assessed by a high-sensitivity assay on a BNII nephelometer (Dade Behring, Marburg,

Germany) that detects values as low as 0.17 mg/l.

Glucose, triglycerides, high-density lipoprotein cholesterol (HDL-cholesterol), low-density lipoprotein cholesterol (LDL-cholesterol) and total serum cholesterol and haemoglobin A1C% were quantified by commercial enzymatic tests performed by standardized biochemical testing.

Measurements of carotid IMT

Carotid IMT was bilaterally assessed at the common carotid artery in each test and control patient in the supine position. A single experienced physician (S. C.) blinded with respect to periodontal conditions used an ATL HDI 3500 instrument applying a standardized protocol (Touboul et al. 2000). The carotid arteries were visualized with longitudinal and transverse scans to detect the possible presence of significant focal atherosclerotic lesions, defined as a localized protrusion exceeding 1.5 mm within the vascular lumen (Touboul et al. 2004). Carotid IMT was measured with an orthogonal incidence of the ultrasonic beam to the axial course of the artery, on a 10 mm segment of the far wall of the common carotid artery (longitudinal projection) avoiding non-linear segments, using a dedicated software (M'Ath, Metris, France). The real-time measurement of carotid IMT represented the mean of 10 measures on each side. The average of both right and left IMT was considered for all subsequent computations.

Data management and statistical analysis

All the calculations were performed using JMP version 5.1 and SAS-STAT 9.1. All statistical analyses were planned before enrolment of the patients.

Sample size calculation was performed assuming a clinically significant difference for carotid IMT of 0.09 mm. Using this mean difference with $\alpha = 0.05$, $\sigma = 0.14$ mm (McQuillan et al. 1999) and a power of 80%, the sample size resulted in 39 individuals for each group.

Descriptive statistics included calculation of mean and standard deviations for quantitative variables. Qualitative variables were expressed as frequency and percentage.

For bivariate analysis of test *versus* control group, the *p*-value was calculated using Student's *t*-test for quantitative variables while the Fisher exact test was used for binominal variables. The pairing variables used (gender, age, BMI, smoking status) were analysed to ascertain possible differences between the test and control groups.

A bivariate analysis was also performed considering the binary IMT as the outcome variable. Because carotid IMT values ranging from 0.75 to 0.82 mm were defined as borderline values and associated with a greater cardiovascular risk than lower values (Aminbakhsh & Mancini 1999), 0.82 mm was considered to be the threshold value. Odds ratios (OR) and confidence intervals were calculated with exact conditional logistic regression (Mehta et al. 1992).

A multivariate analysis (backward stepwise linear regression with $p = 0.10$ to enter and $p = 0.05$ to leave) was performed considering the mean IMT as the outcome variable. Predictor variables were periodontitis, gender, age, education level, pack-year, a family history of cardiovascular disease, BMI, systolic blood pressure, diastolic blood pressure, physical activity, glucose, leucocytes, triglycerides, HDL-cholesterol, LDL-cholesterol, total serum cholesterol, hs-CRP and haemoglobin A1C% levels. A sensitivity analysis was also performed considering mean IMT as the outcome variable and other variables as predictors (full model).

A backward stepwise logistic regression (with $p = 0.10$ to enter and $p = 0.05$ to leave) was performed considering the binary IMT as the outcome variable (threshold value = 0.82 mm). In the model, OR and confidence intervals were calculated with exact conditional logistic regression (Mehta et al. 1992). An approximate confidence interval 95% was used for probability of IMT $\geqslant 0.82$ (π) in the final model of the backward logistic regression (Ryan 1997).

Results

A total of 67 systemically healthy patients with severe periodontitis were consecutively selected. After pairing with comparable controls, 45 periodontal patients were included in the test group. The remaining 22 patients were excluded due to the lack of comparable controls (20 patients) or due to the fact that they were smokers for <5 years (two patients). Finally, the *test group* consisted of 45 systemically healthy individuals aged $\leqslant 40$ years showing a mean age of 36.35 ± 3.65 years [range 25.9–40.0] affected by severe periodontitis; 21 were female (47%) and 19 patients were current smokers (42%). No former smoker was enrolled. The mean tooth loss due to periodontal disease was 2.3 ± 2.3. Twenty-four (53%) patients showed aggressive periodontitis, while 21 (47%) showed chronic periodontitis. The mean loss of attachment was 4.70 ± 0.72 mm. Patients showed a mean of 72.6 ± 23.8 pockets of $\geqslant 4$ mm and a mean of 21.6 ± 9.0 pockets of $\geqslant 6$ mm.

A total of 85 potential controls without a history of periodontal disease were screened: 45 of them were used as comparable controls for the test group. Then, the *control group* consisted of 45 systemically healthy individuals aged $\leqslant 40$ years showing a mean age of 33.78 ± 3.28 years [26.8–39.8]; 21 were female (47%) and 19 were current smokers (42%). The mean tooth loss due to caries or endodontic problems was 0.3 ± 0.5, while the mean loss of attachment was 2.24 ± 0.12 mm (Table 1).

A statistically significant difference between the two groups was found for tooth loss (<0.0001), FMPS (<0.0001) and FMBS (<0.0001). Periodontal patients had lower educational levels than the control group (<0.0001) (Table 1).

The mean carotid IMT was 0.82 ± 0.13 mm in the test group [0.61–1.16] and 0.72 ± 0.07 mm in the control group [0.59–0.94]. This difference was statistically significant ($p<0.0001$). The left carotid IMT was higher in the test group (0.81 ± 0.13 mm) than in the control group (0.72 ± 0.08 mm) ($p<0.0001$); similarly, the right carotid IMT was higher in the test group (0.82 ± 0.15 mm) than in the control group (0.73 ± 0.09 mm) ($p = 0.0006$). In the test group, 20 patients (44%) showed carotid IMT $\geqslant 0.82$ mm while in the control group four patients (9%) showed carotid IMT (Table 1). When comparing aggressive (0.81 ± 0.14 mm) *versus* chronic (0.82 ± 0.11 mm) periodontitis, no significant difference was found in terms of mean IMT values ($p = 0.7693$).

Periodontal patients showed higher levels of hs-CRP ($p = 0.0002$), total serum cholesterol ($p = 0.0465$) and LDL-cholesterol ($p = 0.0203$). No statistically significant differences between the test and control groups

Table 1. Descriptive statistics and details of matching criteria for test and control group

Variables	Test group, $N = 45$	Control group, $N = 45$	p-value
Gender (females)	21 (47%)	21 (47%)	–
Age (years)	36.35 ± 3.65	33.78 ± 3.28	0.0007
Smoking habits (yes)	19 (42%)	19 (42%)	–
Number cigarettes/day	5.3 ± 6.9	5.3 ± 6.9	–
Smoking habits >5 years	18 (40%)	18 (40%)	–
Packyear	4.1 ± 5.6	3.7 ± 4.9	0.6682
Aggressive periodontitis	24 (53%)	0 (0%)	–
Mean CAL (mm)	4.70 ± 0.72	2.24 ± 0.12	–
Mean PD (mm)	4.32 ± 0.68	2.15 ± 0.12	–
Tooth loss	2.3 ± 2.3	0.3 ± 0.5	<0.0001
FMPS (%)	50.0 ± 20.4	23.0 ± 9.6	<0.0001
FMBS (%)	47.0 ± 16.4	18.0 ± 8.6	<0.0001
Number of sites with CAL ⩾4 mm	96.9 ± 27.5	0.4 ± 0.6	–
Number of sites with PD ⩾4 mm	72.6 ± 23.8	0.0 ± 0.0	–
RT IMT (mm)	0.82 ± 0.15	0.73 ± 0.09	0.0006
LT IMT (mm)	0.81 ± 0.13	0.72 ± 0.08	<0.0001
IMT mean (mm)	0.82 ± 0.13	0.72 ± 0.07	<0.0001
IMT (⩾0.82)	20 (44%)	4 (9%)	0.0002
Family history of cardiovascular disease	19 (42%)	4 (9%)	0.0005
Regular physical activity	15 (33%)	18 (40%)	0.6621
History of oral contraceptive (females)	11 (52%)	9 (43%)	0.7579
Systolic blood pressure (mmHg)	120.3 ± 7.4	119.8 ± 4.4	0.6665
Diastolic blood pressure (mmHg)	70.1 ± 5.7	70.1 ± 4.1	1.000
BMI (kg/m^2)	22.4 ± 2.0	22.5 ± 2.1	0.9551
Educational level (higher than high school)	8 (18%)	28 (62%)	<0.0001

Mean CAL, mean clinical attachment level; Mean PD, mean probing depth; FMPS, full-mouth plaque score; FMBS, full-mouth bleeding score; RT IMT, right carotid intima-media thickness; LT IMT, left carotid intima-media thickness.

Table 2. Laboratory variables in test and control groups

Variable	Test group, $N = 45$	Control group, $N = 45$	p-value
Hs-CRP (mg/l)	2.54 ± 2.59	0.95 ± 0.93	0.0002
Glucose (g/l)	0.83 ± 0.09	0.82 ± 0.11	0.7729
Leucocytes (n)	6.52 ± 1.98	6.14 ± 1.30	0.2847
Triglycerides (mg/dl)	132.20 ± 146.29	88.22 ± 46.75	0.0580
Total serum cholesterol (mg/dl)	201.22 ± 36.04	186.36 ± 33.75	0.0465
HDL-cholesterol (mg/dl)	53.07 ± 11.10	55.07 ± 16.06	0.6316
LDL-cholesterol (mg/dl)	134.64 ± 57.64	112.47 ± 25.23	0.0203
Haemoglobin A1C%	5.49 ± 0.46	5.35 ± 0.29	0.0892

Hs-CRP, high-sensitivity C-reactive protein; HDL-cholesterol, high-density lipoprotein-cholesterol; LDL-cholesterol, low-density lipoprotein cholesterol.

were detected for other haemostatic variables (Table 2).

Bivariate analysis showed that periodontal disease (OR 8.01), age, education level, regular physical activity and diastolic blood pressure were associated with binary IMT (threshold value = 0.82 mm) (Table 3).

The variables retained in the model after the backward stepwise linear regression were periodontal disease and physical activity. Severe periodontal disease ($p < 0.0001$) was a predictor variable for increased IMT. Moreover, regular physical activity was associated with a lower carotid IMT (Table 4). Sensitivity analysis (full model) performed considering mean IMT as the outcome variable and other variables as predictors confirmed that only periodontal disease directly and regular physical activity inversely were associated with carotid IMT (Table 5).

When considering an IMT of 0.82 mm as the critical index of increased cardiovascular risk, periodontal patients overcame this threshold compared with healthy patients by an OR = 8.55 (stepwise logistic regression). In addition, diastolic blood pressure was found to be associated (OR = 2.14) with the risk of exceeding the critical value (Table 6). Figure 1 graphically represents the results of Table 6, showing the probability to have carotid IMT ⩾ 0.82 mm considering diastolic blood pressure values of the test and control patients.

Discussion

A growing body of literature shows that local and/or systemic infection may contribute to the development of atherosclerosis in association with lipid unbalance, haemodynamic stress and immune reactions at the vascular wall (Ross 1999). Periodontal disease was found to be associated with the severity and progression of atherosclerosis (DeStefano et al. 1993, Mattila et al. 1993, 1995, Beck et al. 1996, Desvarieux et al. 2003, 2005, Engebretson et al. 2005, D'Aiuto et al. 2007) due to the presence of a microbial burden (Chiu 1999, Haraszthy et al. 2000, Cairo et al. 2004) and the production of inflammatory mediators (D'Aiuto et al. 2004, 2007). The association between periodontal disease and increased carotid IMT has been described only in the middle-aged to elderly population with chronic/adult periodontitis (Beck et al. 2001, Leivadaros et al. 2005, Soder et al. 2005). Although IMT increase in the elderly might have been caused by periodontitis, this finding cannot rule out the well-known influence of ageing on both IMT thickening (Howard et al. 1993) and the prevalence of periodontal disease (Hugoson et al. 1998, Albandar et al. 1999). On the other hand, young patients might have been affected by a severe form of periodontitis that results in extensive destruction of periodontal attachment and bone with precocious tooth loss. This condition may be due to genetics, impaired host response and microbiological patterns (American Academy of Periodontology 1999).

The results of this study showed that an association between severe periodontal disease and sub-clinical atherosclerosis does exist in young systemically healthy patients; stepwise regression analysis showed that severe periodontal disease is a predictor variable of increased mean IMT ($p < 0.0001$). Although the 45 patients enrolled in the test group may be regarded as a limited sample size when compared with other surveys dealing with middle-aged to elderly periodontal patients (Beck et al. 2001), one should take into account that severe forms of periodontitis affect only

Table 3. Bivariate analysis considering the binomial IMT as the outcome variable (threshold value = 0.82 mm)

Variables	Odds ratio	Lower CI 95%	Upper CI 95%	*p*-value
Periodontal disease*	8.01	2.32	35.96	0.0002
Gender (females)*	1.05	0.37	2.99	1.0000
Age†	1.25	1.07	1.48	0.0035
Packyear†	1.04	0.95	1.13	0.3702
Educational level* (higher than high school)	0.30	0.08	0.96	0.0420
Familiarity for CHD*	2.21	0.70	6.83	0.1994
Regular physical activity*	0.26	0.06	0.89	0.0287
Systolic blood pressure (mmHg)‡	1.42	0.90	2.36	0.1448
Diastolic blood pressure (mmHg)‡	2.11	1.16	4.12	0.0114
BMI (kg/m^2)†	1.12	0.89	1.42	0.3172
Hs-CRP (mg/l)†	1.19	0.96	1.48	0.1051
Glucose (g/l)†	0.18	0.01	21.77	0.4834
Leucocytes (n)†	0.83	0.60	1.13	0.2611
Triglycerides (mg/dl)†	1.00	1.00	1.01	0.2338
Total serum cholesterol (mg/dl)†	1.00	0.99	1.02	0.5270
HDL-cholesterol (mg/dl)†	0.99	0.96	1.03	0.8281
LDL-cholesterol (mg/dl)†	1.00	0.99	1.01	0.5104
Haemoglobin A1C%†	0.81	0.24	2.75	0.7564

*Binary variable (yes/no).
†Odds ratio was calculated for each 1 U of increase.
‡Odds ratio was calculated for each 5 mmHg of increase.
BMI, body mass index; hs-CRP, high-sensitivity C-reactive protein; HDL-cholesterol, high-density lipoprotein-cholesterol; LDL-cholesterol, low-density lipoprotein cholesterol; IMT, intima-media thickness.

Table 4. Final model of the stepwise linear regression using the overall mean IMT as outcome variable ($R^2 = 0.28$)

Term	Estimate	Standard error	*p*-value	95% confidence intervals	
Intercept	0.75	0.02	<0.0001	0.72	0.78
Periodontal disease	0.09	0.02	<0.0001	0.05	0.13
Regular physical activity	-0.07	0.02	0.0009	-0.12	-0.03

IMT, intima-media thickness.

Table 5. Full model for linear regression with all 18 variables as sensitivity analysis ($R^2 = 0.39$)

Term	Estimate	Standard error	Prob > |*t*|
Intercept	0.38	0.30	0.2131
Periodontal disease	0.09	0.03	0.0063
Gender	-0.01	0.04	0.7123
Age	0.004	0.004	0.3138
Packyear	0.002	0.002	0.4844
Educational level (higher than high school)	0.01	0.03	0.7616
Familiarity for CHD	0.01	0.03	0.5985
Regular physical activity	-0.05	0.03	0.0360
Systolic blood pressure (mmHg)	0.001	0.003	0.7414
Diastolic blood pressure (mmHg)	0.003	0.003	0.3357
BMI (kg/m^2)	0.01	0.01	0.5138
Hs-CRP (mg/l)	-0.01	0.01	0.3489
Glucose (g/l)	-0.13	0.12	0.2540
Leucocytes (*n*)	-0.01	0.01	0.4632
Triglycerides (mg/dl)	0.00003	0.00017	0.8734
Total serum cholesterol (mg/dl)	0.0001	0.0006	0.8364
HDL-cholesterol (mg/dl)	0.0002	0.0011	0.8313
LDL-cholesterol (mg/dl)	0.0002	0.0006	0.7245
Haemoglobin A1C%	-0.03	0.03	0.4062

BMI, body mass index; hs-CRP, high-sensitivity C-reactive protein; HDL-cholesterol, high-density lipoprotein-cholesterol; LDL-cholesterol, low-density lipoprotein cholesterol.

1–3% of Europeans in an age range similar to the one considered in this study (Hugoson et al. 1998). In addition, periodontal patients showed severe tooth loss, generalized loss of attachment and periodontal pockets and extensive gingival inflammation, with aesthetic, functional and psychological problems. When compared with the healthy individuals without signs of periodontitis, the periodontal patients overcame the carotid IMT $\geqslant 0.82$, assumed as the critical index of increased cardiovascular risk (Aminbakhsh & Mancini 1999), by an OR = 8.55.

An association between LDL, HDL, total cholesterol levels and atherosclerosis risk has been generally recognized (Sharrett et al. 1995, Crouse et al. 1996). In this study, higher levels of LDL and total serum cholesterol were detected in periodontal patients than in control subjects: these findings are in accordance with those obtained in clinical studies showing higher lipid levels associated with *Porphyromonas gingivalis* infection in periodontal patients (Cutler et al. 1999a, b). However, no association was found between these variables and carotid IMT. This might have been due to the exclusion of patients with a high BMI.

CRP, a strong predictor of the risk of myocardial infarction and stroke (Ridker et al. 1997), was higher in the test group ($p = 0.0002$), confirming that periodontal disease is associated with acute-phase response and changes of CRP serum levels (D'Aiuto et al. 2004). In the present study, no association was found between CRP and carotid IMT. This finding is in accordance with a recent longitudinal study (Lorenz et al. 2007) showing that CRP is not an independent causal factor for the initiation and progression of early atherosclerotic changes of the carotid arteries, and possible associations may be explained by confounding factors such as age, gender and cardiovascular risk factors.

This study shows that periodontal patients frequently have a lower education level, a surrogate marker of socioeconomic position, than healthy individuals ($p < 0.0001$). This finding confirms the possible influence of education level on the prevalence of periodontal disease (Klinge & Norlund 2005). Furthermore, lower socioeconomic groups have a greater cardiovascular risk than higher socioeconomic groups (Cox et al. 2006) even if no association between education level and sub-clinical atherosclerosis was observed in this study.

Table 6. Final model of the stepwise logistic regression using IMT ⩾0.82 cut-off value as outcome variable

Term	Estimate	Odds ratio	95% CI	Odds ratios	*p*-value
Periodontal disease	2.15	8.55	2.38	39.81	0.0002
Diastolic blood pressure	0.15	2.14*	1.16*	4.34*	0.0114

*Odds ratio and confidence intervals (CI) for odds ratios were considered every 5 mmHg.
IMT, intima-media thickness.

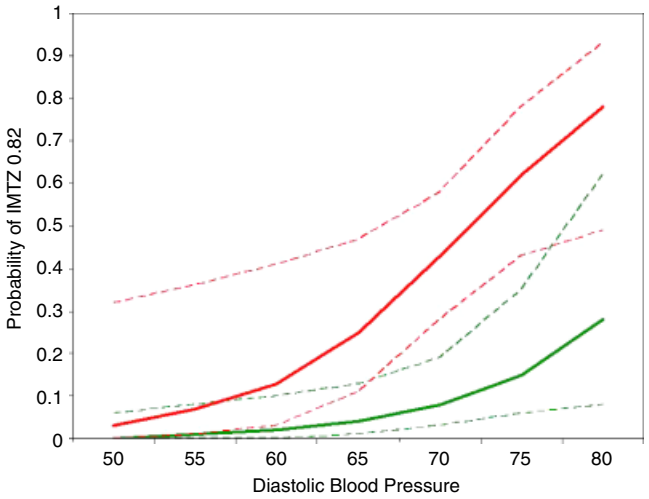

Fig. 1. Graph representing the probability to show carotid intima-media thickness (IMT) ⩾0.82 mm considering diastolic blood pressure values of the test and control groups. The green continuous line represents the probability for the control group while the red continuous line represents the likelihood for the test group. Punctuated lines show the corresponding 95% confidence intervals.

Regular physical activity was found to be related to lower carotid IMT values: patients engaged in physical activity on a regular basis (at least once a week) had IMT readings lower than sedentary patients. This observation supports previous findings demonstrating that regular physical activity may be associated with the attenuation of age-related endothelial dysfunction by restoring nitric oxide (Galetta et al. 2006) and preventing oxidative stress (DeSouza et al. 2000).

It has been recognized that smoking habit is a risk factor for both periodontal disease and atherosclerosis (Hujoel et al. 2002). In this research, no association was detected between smoking habits and sub-clinical atherosclerosis, probably due to the fact that the study design considered a similar number of cigarettes per day and a minimum of at least 5 years of smoking-exposure when pairing test and control subjects.

The limitations of this study may be related to differences in age between the test and control groups: test patients resulted older than control patients by approximately 2.5 years. Even if a minimal age-related increase (~ 0.02 mm/year) in carotid IMT was reported (Howard et al. 1993), the difference between test and control groups is only partially explained in this study by age difference. Moreover, age was included in the stepwise regression model and in the sensitivity analysis as a possible predictor variable but it was not selected in the final models.

In conclusion, this study shows that severe periodontitis is associated with sub-clinical atherosclerosis not only in the elderly population (Beck et al. 2001) but also in young systemically healthy individuals, thus supporting the hypothesis that periodontal infection may be related to atheroma development. Periodontal disease may predict a systemic atherosclerosis condition decades before the occurrence of clinical cardiovascular events.

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Clinical Relevance

Scientific rationale for the study: Information on the association between periodontitis and cardiovascular disease in systemically healthy young periodontal patients is not available.

Principal findings: Severe periodontitis is associated with increased carotid IMT in young systemically healthy patients.

Practical implications: Periodontal disease may be a strong predictor of cardiovascular disease decades before the occurrence of clinical cardiovascular events.