

Endotoxin Levels Are Associated With High-Density Lipoprotein, Triglycerides, and Troponin in Patients With Acute Coronary Syndrome and Angina: Possible Contributions From Periodontal Sources

David Goteiner,*† Ronald G. Craig,† Robert Ashmen,* Malvin N. Janal,§ Barnet Eskin,|| and Neal Lehrman*

Background: Recent studies have reported an association between poor dental health and acute coronary syndrome (ACS). The purpose of this study was to correlate the presence of periodontitis with serum endotoxin/lipopolysaccharides (LPS), lipid profiles, troponin, and immunoglobulin G (IgG) antibody to *Porphyromonas gingivalis* in control patients or patients with ACS or angina at the time of hospital admission.

Methods: Blood samples from 194 subjects presenting with ACS, angina, or non-cardiac chest pain were analyzed for endotoxin/LPS (*Limulus* amoebocyte lysate assay), lipid profile, troponin, and IgG antibody to *P. gingivalis*. Data were collected from hospital charts and dental records, and health questionnaire responses.

Results: Subjects with ACS or angina were more likely to have poor oral care, fewer remaining teeth, and increased alveolar radiographic bone loss compared to subjects with chest pain. In all subjects, endotoxin/LPS and IgG antibody to *P. gingivalis* tended to increase in association with increased radiographic bone loss. Endotoxin/LPS increased directly with triglyceride and troponin levels ($P = 0.04$ and $P = 0.006$, respectively) and inversely with high-density lipoprotein (HDL) levels ($P = 0.002$). IgG antibody to *P. gingivalis* levels was directly correlated with very low-density lipoprotein ($P = 0.03$) and triglycerides ($P = 0.06$) and inversely with low-density lipoprotein ($P = 0.01$).

Conclusions: Results showed more alveolar bone loss in patients with cardiac disease than in patients without cardiac disease, but there was no difference between the groups in the serum levels of endotoxin/LPS or IgG antibody to *P. gingivalis*. However, there were associations between endotoxin/LPS and levels of serum triglycerides, troponin, and HDL. *J Periodontol* 2008;79:2331-2339.

KEY WORDS

Acute coronary syndrome; endotoxin; high-density lipoprotein; periodontitis; triglycerides; troponin.

An array of risk factors has been associated with atherosclerotic complications, such as myocardial infarction and stroke. In addition to the more traditional cardiovascular risk factors, which include elevated total cholesterol, triglycerides, and low-density lipoprotein (LDL), decreased high-density lipoprotein (HDL), and diabetes, increased systemic inflammation has been proposed as a potential cardiovascular risk factor.¹ However, it is not clear whether increased systemic inflammation associated with atherosclerotic complications is merely a reflection of inflammation arising from atherosclerosis itself or is attributable to peripheral sources, such as infection.² In support of the latter possibility, inflammatory diseases, such as chronic periodontitis, have been associated with an increased incidence of atherosclerotic complications.³⁻⁶ In addition, bacterial and viral infections have been associated with atherosclerotic complications in susceptible

* Department of Periodontology, New Jersey Dental School, University of Medicine and Dentistry of New Jersey, Newark, NJ.

† Department of Dentistry, Morristown Memorial Hospital, Morristown, NJ.

‡ Department of Periodontology and Implant Dentistry, New York University College of Dentistry, New York, NY.

§ Department of Psychiatry, New Jersey Medical School, University of Medicine and Dentistry of New Jersey.

|| Emergency Department, Morristown Memorial Hospital.

populations, and subclinical infections have been associated with acute coronary syndrome (ACS).^{4,7-9}

Periodontitis is an inflammatory disease resulting from the infection and interaction of specific subgingival bacterial species with components of the host-immune response in disease-susceptible individuals.¹⁰ In particular, the Gram-negative facultative anaerobic or anaerobic bacterial species *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythia* (previously *T. forsythensis*), and *Aggregatibacter actinomycetemcomitans* (previously *Actinobacillus actinomycetemcomitans*) have been strongly associated with periodontitis disease status and progression. In addition, elevated levels of these bacteria after treatment have been strongly related to disease recurrence.¹⁰ These bacterial species can invade periodontal epithelium and connective tissues,^{11,12} can invade and persist within endothelial cells in culture, and were detected and shown to be viable in coronary endothelium and atherosclerotic plaques.¹³⁻¹⁹ In vitro, *P. gingivalis* in the presence of LDL was shown to induce macrophages to become foam cells;²⁰ in vivo, the extent of periodontitis-affected tissue has been directly associated with LDL activation of macrophages.²¹ Perhaps most supportive of the potential for these bacteria to play a role in systemic disease pathogenesis is the finding that *P. gingivalis* and *A. actinomycetemcomitans* have been recovered in human atherosclerotic plaques.¹⁹

Gram-negative bacterial components, such as endotoxin/lipopolysaccharides (LPS), are potent activators of the innate and adaptive immune response. Endotoxin/LPS is a characteristic glycolipid component of the Gram-negative bacterial outer membrane wall that can activate inflammatory cells, increase oxidative stress, and modify lipoprotein metabolism. Once released, endotoxin/LPS is rapidly cleared from the circulation by serum lipoproteins.²² Lopes-Virella²³ proposed a direct link between Gram-negative infections and atherosclerosis, mediated by LDL and LPS. He reported that endotoxin/LPS can induce endothelial cell damage with the resulting oxidized LDL (ox-LDL) becoming immunogenic. The formation of an immune complex with ox-LDL can promote the release of proinflammatory cytokines, such as interleukin-1 and tumor necrosis factor- α , which can lead to further endothelial damage, increased vascular permeability, and procoagulant activity.²³ In support of this hypothesis, rats fed a diet enriched with butter or stearic acid showed a marked predisposition to endotoxin-initiated thrombosis.²⁴

In view of the above studies linking peripheral infection, periodontitis, and altered serum lipid profiles with ACS, we asked whether endotoxin/LPS levels, perhaps derived from periodontopathic bacteria, were also elevated in ACS and angina. The purpose of this

study was to determine whether changes in the serum levels of endotoxin/LPS, lipid profiles, and immunoglobulin G (IgG) antibody to *P. gingivalis* are related to the patient's cardiovascular and oral health status.

MATERIALS AND METHODS

Subject Inclusion Criteria and Evaluation

This cross-sectional study protocol was reviewed and approved by the Institutional Review Boards of the University of Medicine and Dentistry of New Jersey and Morristown Memorial Hospital. The study began on May 21, 2001 and was completed on May 20, 2004.

The subjects were randomly recruited when they entered the Emergency Department of Morristown Memorial Hospital seeking care for chest pain. To minimize potential confounding variables, patients were excluded if they had insulin-dependent diabetes mellitus, multiple diagnoses, pregnancy, obesity (as defined by the discharge diagnosis of the attending physician), a history of chronic antibiotic therapy, antibiotic regimens, or overt infection at the time of admission.

All medical diagnoses were recorded from the discharge notes made by the attending physician. The ACS/angina group consisted of 137 subjects, each with at least six natural teeth, who were admitted and discharged from the Cardiac Care Unit at Morristown Memorial Hospital with acute myocardial infarction (AMI), unstable angina, or angina (*International Classification of Diseases* [ICD], Ninth Revision, Clinical Modification Codes 410 to 413.9). Because AMI and unstable angina make up a clinical spectrum, essentially differing only in the sequelae of cell death, they are often referred to as ACS. Fifteen edentulous subjects with a diagnosis of AMI were recruited. Thirty-three subjects whom the physician believed were not having ACS or angina and who were dismissed with a diagnosis of chest pain (ICD 786.5) were also included.

After obtaining written informed consent, the subject's medical and dental records were abstracted, and residual blood taken upon admission to the Emergency Department was collected. Serum lipid profiles measured by the clinical laboratory of the hospital at the time of admission were also recorded. If these data were unavailable, information in their cardiologist's medical records (from commercial laboratories) was used if measured ≤ 3 months before the date of admission.

Demographic and health history data were obtained from the subject's hospital records and the dentist of record. In addition, a questionnaire was sent to the subject regarding oral symptoms, home care regimens, and whether their dentist had ever given them a diagnosis of periodontitis, "gum disease," or "pyorrhea." Periapical radiographs sent by their dentist and taken <1 year prior to admission or those taken at the Dental Clinic after discharge were examined

by three periodontists for alveolar bone height as a measure of past periodontitis. If disagreements occurred in the scoring of a subject, the examiners discussed the scoring to reach a consensus. The severity of periodontitis on the radiographs was given the following scores: 0 (no disease) if there was no loss of alveolar bone; 1 (mild disease) if there was $\leq 20\%$ loss; 2 (moderate disease) if there was $>20\%$ but $\leq 50\%$ loss; and 3 (severe disease) if there was $>50\%$ loss.^{25,26}

Measurement of Serum Endotoxin/LPS

The residual blood taken at the time of admission to the Emergency Department was centrifuged, serum separated, and stored until assayed. After deplementing the samples at 56°C for 15 minutes, serum endotoxin/LPS levels were measured by the *Limulus* amebocyte lysate assay[¶] using the method described by Novitsky.²⁷ In brief, 50 μ l of each serum sample in duplicate was added to a sterile microtiter plate containing serial dilutions of an endotoxin standard and sterile water as a negative control. Fifty microliters of reconstituted chromogenic endotoxin test reagent solution was added to each well, and the microtiter plate was incubated at 37°C for 35 minutes. Following incubation, 20 μ l stop solution (50% acetic acid) was added to each well, and the optical density of the reaction product was measured using a microtiter plate reader.[#]

Measurement of Serum IgG Antibody to *P. gingivalis*

Serum was analyzed for IgG antibody against cell-surface antigens of *P. gingivalis* using a previously described enzyme-linked immunosorbent assay (ELISA).²⁸ Microtiter plates were coated with formalized, whole-cell preparations of *P. gingivalis* (American Type Culture Collection #33277). A series of three dilutions of subject sera and a series of five dilutions of standard sera were added to the microtiter plates in duplicate and incubated in succession with affinity-purified rabbit anti-human IgG and goat anti-rabbit IgG heavy and light chain specific alkaline phosphatase conjugate. End point conversion of enzyme substrate was measured by optical absorbance using a microtiter plate reader.^{**} Sample antibody activities were related to a standard reference serum curve to *P. gingivalis*. The highest standard was assigned a value of 100 ELISA units (EU), and a reference curve relating the \log_{10} EU was constructed for each plate using a curve-fitting software program.^{††} Results were reported as the average EU value \pm SEM for the up to six serum dilutions that were within the range of the reference curve.

Data Analysis

Preliminary analysis showed that several variables were not normally distributed. In the case of *Limulus*

amebocyte lysate test (LAL), these values were \log_{10} transformed for some analyses, and quartiles were used for others; in the case of ELISA, data were transformed into a quintile scale. Mean \pm SD of the untransformed values are presented, because these provide a familiar metric. However, when groups were compared or correlations were computed, the transformed values were used. Data included discrete measures summarized as proportions and continuous measures summarized as mean \pm SD. Differences between groups for the ELISA measure were evaluated by means of the Mann-Whitney test. Differences between groups for \log_{10} LAL were evaluated by analysis of variance (ANOVA). Non-significant findings were supplemented by the computation of η^2 , a measure of effect size. Associations between continuous and discrete measures were evaluated using Spearman correlation coefficients, whereas associations between two continuous measures were evaluated with Pearson correlations. Logistic regression analyses were used to test mediational hypotheses. All analyses were accomplished using statistical software.^{‡‡} Statistical significance was set at $\alpha \leq 0.05$.

RESULTS

Clinical Parameters

A complete characterization of the study population was published previously.²⁹ In summary, no statistically significant differences were found between the groups with respect to age, gender, or smoking history. Subjects with ACS/angina reported a longer median time between dental visits compared to patients who registered at the hospital for elective procedures (6.5 versus 3.0 months, $P = 0.008$). Compared to subjects discharged with a diagnosis of chest pain, subjects with ACS/angina had fewer teeth (22.9 ± 7.3 versus 26.3 ± 6.0 ; $P < 0.001$), manifested more untreated dental pathology (50.4% versus 24.2%; $P < 0.01$), and had a higher prevalence of severe periodontitis as measured by radiographic alveolar bone loss (38.5% versus 9.1%; $P < 0.05$). For dentate subjects with ACS/angina, the number of teeth present was inversely related to radiographic bone loss ($r = -0.424$; $P < 0.001$). Patients with ACS/angina had more signs of periodontal disease than those reporting only chest pain.

Serum Endotoxin/LPS

Table 1 shows endotoxin/LPS levels as a function of periodontal attachment loss. ANOVA failed to show statistical significance ($F = 0.93$; degrees of freedom = 2,133; $P = 0.40$) and estimated that only $<1.5\%$ of

¶ Associates of Cape Cod, Woods Hole, MA.

Bio Rad Laboratories, La Jolla, CA.

** Bio Rad Laboratories.

†† Biolinx, Dynatech, Chantilly, VA.

‡‡ SPSS v.14, SPSS, Chicago, IL.

Table 1.
Endotoxin Levels as a Function of Radiographic Evidence of Bone Loss

Severity of Periodontal Attachment Loss	Subjects (n)	Endotoxin/LPS (EU/ml; mean \pm SD)
Mild	44	3.72 \pm 3.29
Moderate	62	4.29 \pm 4.30
Severe	30	5.18 \pm 6.23

Table 2.
Endotoxin/LPS Levels as a Function of Cardiac Status

Cardiac Group	Subjects (n)	Endotoxin/LPS (EU/ml; mean \pm SD)
Dentate ACS/angina group	112	4.58 \pm 4.32
Subgroups		
Dentate with AMI	57	5.20 \pm 4.70
Dentate with angina	10	3.93 \pm 4.75
Dentate with unstable angina	45	3.93 \pm 4.89
Dentate with chest pain	33	3.40 \pm 3.02
Edentulous with AMI	15	4.41 \pm 4.07

Only 112 of the 137 subjects in the ACS/angina group had LAL data.

the variance in \log_{10} LAL was attributable to the variation in attachment loss. Table 2 compares endotoxin/LPS levels in patients with ACS and angina, either dentate or edentulous, to patients discharged from the Emergency Department with a diagnosis of non-cardiac chest pain. ANOVA failed to show a statistical difference among groups ($F = 1.19$; degrees of freedom = 4,155; $P = 0.32$) and estimated that only $\sim 3\%$ of the variance in \log_{10} LAL was attributable to the grouping factor. Then serum endotoxin/LPS levels were correlated with serum lipids and troponin. While there were 183 subjects with troponin data, only 120 also had HDL data, and only 105 had LAL data as well. Significant positive correlations were found between levels of \log_{10} LAL and serum triglycerides and troponin, and a significant negative correlation was found between \log_{10} LAL and HDL (Table 3). There was no apparent statistical relationship between endotoxin/LPS and LDL or very low-density lipoprotein (VLDL). Thus, although endotoxin levels vary with levels of blood lipids and troponin, they were not related to signs of cardiac or periodontal disease.

Table 3.
Pearson Correlation Between Serum Endotoxin/LPS (log LAL), Lipids, and Troponin (quartiles) in Subjects With ACS/Angina

Serum Lipid (n)	mg/dl (mean \pm SD)	r Value With Endotoxin/LPS	P Value
Cholesterol (122)	181.7 \pm 45.5	0.03	NS
Triglycerides (114)	172.5 \pm 101.7	0.19	0.04
HDL (128)	42.0 \pm 12.6	-0.29	0.002
LDL (127)	98.1 \pm 37.6	0.02	NS
VLDL (88)	43.2 \pm 31.5	0.06	NS
Troponin (105)	27.3 \pm 80.7	0.27	0.006

NS = not statistically significant.

Table 4.
Pearson Correlation Between Serum IgG Antibody to *P. gingivalis* (quintiles) and Serum Lipids

Lipid (n)	Lipid Value (mg/dl; mean \pm SD)	IgG to <i>P. gingivalis</i> (EU)	r Value	P Value
Cholesterol (48)	179.7 \pm 48.4	189.9 \pm 408.2	-0.198	NS
Triglycerides (46)	195.8 \pm 105.1	189.9 \pm 408.2	0.275	0.06
HDL (45)	41.6 \pm 15.0	189.9 \pm 408.2	-0.114	NS
LDL (41)	91.90 \pm 40.83	189.9 \pm 408.2	-0.394	0.011
VLDL (39)	56.75 \pm 42.0	189.9 \pm 408.2	0.343	0.03

NS = not statistically significant.

Forty-eight of the 64 subjects with antibody levels were available for the lipid analyses.

Serum IgG Antibody to *P. gingivalis*

Serum from 51 subjects with ACS/angina (25 dentate with AMI, 18 unstable angina, five angina, and three edentulous ACS patients) and 13 non-cardiac subjects were available for this analysis. Although the cardiac group showed higher antibody levels, there was no statistical difference between the groups (Mann-Whitney U test = 239; $P = 0.12$). However, higher levels of serum IgG antibody to *P. gingivalis* (Table 4) were associated with higher levels of triglycerides ($r = 0.275$; $P = 0.06$) and VLDL ($r = 0.343$; $P = 0.03$) and lower levels of LDL ($r = -0.394$; $P = 0.011$).

Logistic Regression Analyses

Logistic regression analyses were used to identify any mediation of the association between ACS/angina status (any cardiac chest pain versus non-cardiac chest pain) and periodontal disease severity by measures of blood lipids, troponin (quartiles), and LPS (LAL quartiles). Table 5 summarizes those analyses.

Analysis 1 included the 120 subjects with troponin data. Model 1 showed that the odds of being in the cardiac group were 3.5 times higher for those with moderate or severe attachment loss compared to those with mild loss ($P < 0.05$). Model 2 showed that the addition of HDL levels improved the prediction of ACS status; those with lower levels of HDL were more likely to be in the ACS group ($P < 0.05$). Because the magnitude of the odds ratio (OR) relating severity of radiographic bone loss or severity of periodontal disease to ACS status did not change in Model 2, we concluded that the severity and HDL levels each make an independent contribution to ACS status. A corollary of this

conclusion is that HDL does not mediate the association between ACS status and periodontal disease severity. Other analyses, not detailed here, showed that other blood lipids did not improve the prediction of ACS status above the contribution made by HDL. Finally, Model 3 showed that troponin levels neither predicted ACS status nor reduced the OR for periodontal disease severity or HDL; therefore, it did not mediate either of those effects.

Analyses 2 and 3 provided data showing that LAL levels and *P. gingivalis* antibody levels also did not mediate the association between ACS status and periodontal disease severity and HDL levels. (Because the pattern of missing data included some different subjects, the ORs in the three analyses are similar but not the same. Also, although OR levels are similar among the analyses, the levels of significance decreased in Analysis 3 because of the smaller sample.)

To summarize, patients with ACS/angina cardiac chest pain were significantly more likely to have more severe periodontal disease and lower HDL levels than those with non-cardiac chest pain. These variables were independent predictors of ACS status, and the data did not suggest that troponin, LPS, or indicators of bacterial infection mediated those effects.

Table 5.

Logistic Regression Analyses Predicting ACS/Angina Status (versus non-cardiac chest pain) as a Function of Severity of Periodontal Disease (mild versus moderate and severe), Levels of HDL, and Troponin Levels (quartiles) or LAL Levels (quartiles)

	OR (95% confidence limits)		
	Model 1	Model 2	Model 3
Analysis 1 (n = 120)			
Severity	3.48 (1.03 to 11.79)	3.49 (0.99 to 12.31)	3.51 (0.99 to 12.44)
HDL		0.95 (0.91 to 0.99)	0.95 (0.91 to 0.99)
Troponin (quartiles)			0.97 (0.46 to 2.06)
Analysis 2 (n = 101)			
Severity	3.21 (0.94 to 11.04)	3.22 (0.89 to 11.61)	3.26 (0.90 to 11.83)
HDL		0.95 (0.91 to 0.99)	0.96 (0.91 to 1.0)
LAL (quartiles)			1.29 (0.69 to 2.41)
Analysis 3 (n = 41)			
Severity	3.0 (0.44 to 20.44)	3.58 (0.45 to 28.21)	3.49 (0.44 to 27.69)
HDL		0.95 (0.90 to 1.01)	0.96 (0.90 to 1.01)
<i>P. gingivalis</i> assay (quintiles)			1.09 (0.44 to 2.71)

Analysis 1 results are based on the sample of 120 subjects with troponin data, whereas those in Analysis 2 include the 101 subjects with LAL data, and Analysis 3 includes the 41 subjects with *P. gingivalis* assay data.

DISCUSSION

The present study was designed to test whether endotoxin/LPS levels were elevated in ACS/angina populations correlated with traditional atherosclerotic risk factors, such as altered serum lipid profiles, and correlated with the presence of periodontal pathogenic bacteria, such as *P. gingivalis*. Endotoxin/LPS levels were lowest in subjects with non-cardiac chest pain; they were not statistically different from levels in subjects with ACS/angina (Table 2). In addition, endotoxin/LPS levels were associated with several traditional atherosclerotic risk factors. Endotoxin/LPS levels positively correlated with serum triglycerides and troponin and negatively correlated with serum HDL (Table 3). However, no correlation was found between endotoxin/LPS and total cholesterol, LDL, or VLDL. Together, these results suggest that endotoxin/LPS are not elevated in patients with cardiac disease but are correlated with

several serum lipid risk factors for atherosclerotic complications. In part, these findings support the results of a recently reported 10-year prospective study²² from Finland that found elevated endotoxin/LPS increased the risk for myocardial infarction. In addition, the study²² found elevated endotoxin/LPS positively correlated with total cholesterol and elevated IgG antibody to *P. gingivalis* and *A. actinomycetemcomitans* and negatively correlated with HDL. Taken together, the results from the present study and the prospective Finnish study²² suggest that an increased Gram-negative burden leading to elevated endotoxin/LPS is a risk factor for ACS.

The association between endotoxin/LPS and triglycerides found in the present study adds support to the results of previous studies. The administration of LPS by inoculation induced a rapid increase in serum triglycerides in man,²³ and elevated fasting triglyceride levels have been strongly associated with the risk for myocardial infarction, even after controlling for HDL.³⁰ Elevated plasma triglycerides and reduced HDL are key features of the metabolic syndrome; the other key features are abdominal obesity, hypertension, and impaired fasting glucose.³¹ These changes are often accompanied by an increase in small dense LDL-cholesterol. The triad of increased triglycerides and small dense LDL-cholesterol and reduced HDL is known as atherogenic dyslipidemia of the metabolic syndrome.³²⁻³⁴ Therefore, it would be interesting to determine whether there is a relationship between the metabolic syndrome and peripheral blood levels of endotoxin/LPS.

The observations that endotoxin/LPS levels significantly correlated with triglycerides and inversely correlated with HDL support the concept that, in the absence of the protective effect of HDL, endotoxin/LPS may play a role in vascular inflammation and in the acute episode leading to myocardial cell death. Serum triglyceride levels were reported to increase during viral and Gram-negative bacterial infections. The observed changes were related to the level of infection but were independent of the infective agent.³⁵ This may be a response to endotoxin/LPS, which was shown to induce a rapid increase in serum triglycerides in man.³⁶

Additional reports suggest an association between blood endotoxin/LPS levels and serum lipids. In hamsters, endotoxin/LPS increased serum cholesterol levels by increasing hepatic cholesterol synthesis.¹⁵ Antibodies to *Chlamydia*-specific endotoxin/LPS were reported in patients with ACS.⁷ After gentle mastication, endotoxin/LPS levels were significantly higher in the patients with severe periodontal disease than in the subjects with mild or moderate periodontitis.³⁷ A study^{38,39} of 10,000 military personnel found a positive association between periodontal pockets

and higher cholesterol and LDL cholesterol blood levels in men, but not women. Another study⁴⁰ confirmed this finding in patients with periodontitis. Moreover, it was shown in animal and human models that HDL protects against the effects of LPS.⁴¹ HDL, a natural component of plasma, was shown to neutralize endotoxins in vitro.¹⁵ This neutralizing effect was shown in mice using reconstituted HDL, VLDL, LDL, and other chylomicrons.⁴²

The present study also found a correlation between endotoxin/LPS (Table 3), severity of alveolar bone loss (Table 5), and troponin levels (Table 3), a measure of cardiac muscle necrosis. In vivo studies^{43,44} suggested a mechanism for endotoxin/LPS-mediated elevation of serum troponin. Increased endotoxin/LPS levels due to infection or sepsis were shown to activate apoptosis in myocardial tissues via the induction of proinflammatory acute-phase proteins and cytokines, resulting in contractile dysfunction and sarcomeric destruction. The increase in peripheral blood troponin levels clinically reflects these cellular events.^{43,44}

Although the present study found elevated endotoxin/LPS levels in the ACS/angina group, the source of endotoxin/LPS was not apparent. A limitation of this study was not determining the species specificity of the LPS. One of the hypotheses of the present study was that there would be more periodontopathic bacteria, in particular *P. gingivalis*, in periodontitis and, therefore, it would contribute to systemic endotoxin/LPS levels. All subjects with ACS/angina enrolled in the present study had radiographic alveolar bone loss, increased numbers of missing teeth, and increased levels of serum IgG antibody to *P. gingivalis*. The levels of serum IgG antibody to *P. gingivalis* in this study were among the highest measured by our laboratory.²⁸

These data suggest that periodontitis was prevalent in this ACS population. The effect of periodontitis on atherosclerotic complications in this population may derive from inflammatory load rather than past periodontal disease activity as represented by the number of teeth lost.³² However, the lack of correlation between endotoxin/LPS and serum IgG antibody to *P. gingivalis* in the present study was most likely due to the small number of serum samples available for serum antibody analysis. We observed a significant relationship between the levels of IgG antibody to *P. gingivalis* and VLDL ($P = 0.03$) and a trend with triglycerides ($P = 0.06$). The inverse relationship between levels of IgG antibody to *P. gingivalis* and LDL is puzzling and awaits further investigation. Van Lenten et al.⁴⁵ showed that LPS binds to lipoproteins in direct proportion to their cholesterol content and that the LDL-LPS complex, once taken by macrophages, is not degraded and so may no longer be available for measurement in routine lipid profiles.

A study by Bengtsson et al.⁴⁶ demonstrated that oxidative modifications of phosphatidylcholines in vascular LDL by *P. gingivalis* could result in aggregation, degradation, protein inactivation, and cellular dysfunction. They concluded that *P. gingivalis* has the ability to change the protein expression and proliferative capacity of LDL and may represent a crucial event in periodontitis-associated atherosclerosis. This study did not look at LDL cholesterol, which does increase in atherogenic dyslipidemia. In a 10-year prospective study, Pussinen et al.²² followed 6,051 individuals, of whom 185 had cardiac events. They were matched with 320 non-event controls in a case-cohort design. Elevated endotoxin/LPS and IgG antibody to *P. gingivalis* or *A. actinomycetemcomitans* seemed to predict cardiovascular events, even after adjusting for age or gender, but not cholesterol or HDL levels. Earlier clinical studies from the same group associated elevated serum IgG antibody levels to *A. actinomycetemcomitans*⁴⁷ or *P. gingivalis*⁴⁸ with an increased incidence of coronary artery disease. Clinical trials on the use of antimicrobials to prevent coronary events had *Chlamydophila pneumoniae* (previously *Chlamydia pneumoniae*) as a target pathogen, and the results have been mixed.⁴⁹ In a long-term pilot study, Paju et al.⁴⁹ reported that clarithromycin reduced recurrent cardiovascular events in subjects without periodontitis, whereas it was ineffective in subjects with periodontitis. They concluded that periodontitis may overpower the beneficial effects of antibiotics.

One of the limitations of the present study is that, because of the medically compromised status of the ACS/angina population, periodontal disease status was assessed by examination of periapical and bite-wing radiographs (a record of past periodontal disease activity) and not by clinical examination that would have included probing depth, attachment loss, and clinical signs of inflammation, such as bleeding on probing. The periapical radiographs may not have indicated the extent of disease as accurately as when the patients were first evaluated in the hospital. This limitation should be borne in mind when evaluating the results of the present study. A trend was observed between radiographic alveolar bone loss and serum endotoxin/LPS that failed to attain statistical significance (Table 1). One may speculate about whether clinical indices of periodontal disease status, if they were available, might have correlated better with the endotoxin/LPS data. In their study, Valentaviciene et al.³² concluded that ulceration of the tissue as determined by a higher bleeding index, not clinical attachment loss, determines bacterial permeability into coronary vessels.

Using regression analysis, patients with ACS/angina were significantly more likely to have more se-

vere periodontal disease and lower HDL levels than those with non-cardiac chest pain. These variables were independent predictors of ACS status, and the data did not suggest that LPS or indicators of bacterial infection mediated those effects.

CONCLUSIONS

This cross-sectional study found associations between endotoxin/LPS and serum triglycerides, troponin, and HDL. Although moderate to severe alveolar bone loss was prevalent in the ACS population and serum IgG antibody to *P. gingivalis* was elevated, this study was not able to show a contribution from these periodontopathic sources to the systemic levels of serum endotoxin/LPS or to levels of alveolar bone loss in this population.

ACKNOWLEDGMENTS

The authors thank Dr. Marc Goldman, Department of Dentistry, Newark Beth Israel Hospital, Newark, New Jersey, and all of the personnel at the Department of Emergency Medicine and Cardiac Research and the Core Laboratory, Morristown Memorial Hospital, for their invaluable assistance. We also thank Drs. Michael Deasy and Robert Zarabi, Department of Periodontology, and Drs. David Furgang and Daniel Fine, Department of Oral Biology, University of Medicine and Dentistry of New Jersey, and Dr. John Banas, Department of Cardiovascular Medicine, Morristown Memorial Hospital, for guidance and support. The authors report no conflicts of interest related to this study.

REFERENCES

1. Ridker PM, Cusman MJ, Tracy RP, Hennekens CH. Inflammation, aspirin and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med* 1997;336:973-979.
2. Ross R. Atherosclerosis – An inflammatory disease. *N Engl J Med* 1999;340:115-126.
3. Khader YS, Albashairah ZSM, Alomari MA. Periodontal disease and the risk of coronary heart and cerebrovascular diseases: A meta-analysis. *J Periodontol* 2004;75:1046-1053.
4. Renvert S, Petterson T, Ohlsson O, Persson GR. Bacterial profile and burden of periodontal infection in subjects with a diagnosis of acute coronary syndrome. *J Periodontol* 2006;77:1110-1119.
5. Mattila KJ, Nieminen MS, Yalibnen YY, et al. The association between dental health and myocardial infarction. *BMJ* 1989;298:779-782.
6. Mattila KJ, Valtonen VV, Nieminen M, Huttunen JK. Dental infection and the risk of new coronary events: Prospective study of patients with documented coronary artery disease. *Clin Infect Dis* 1995;20:588-592.
7. Shimada K, Mokuno H, Watanabe Y, Sawano M, Daida H, Yamaguchi H. High prevalence of seropositivity for antibodies to *Chlamydia*-specific lipopolysaccharide in patients with acute coronary syndrome. *J Cardiovasc Risk* 2000;7:209-213.

8. Espinola-Klein C, Rupprecht H, Blankenberg S, et al. Impact of infectious burden on extent and long-term prognosis of atherosclerosis. *Circulation* 2002;105:15-21.
9. Doğan B, Budunelli E, Emingil G, et al. Characteristics of periodontal microflora in acute myocardial infarction. *J Periodontol* 2005;76:740-748.
10. Socransky SS, Haffajee AD. Dental biofilms: Difficult therapeutic targets. *Periodontol 2000* 2002;28:12-55.
11. Fives-Taylor P, Meyer DH, Mintz KP. Characteristics of *Actinobacillus actinomycetemcomitans* invasion of and adhesion to cultured epithelial cells. *Adv Dent Res* 1995;9:55-62.
12. Frank RM. Bacterial penetration in the apical pocket wall of advanced human periodontitis. *J Periodontol Res* 1980;15:563-573.
13. Dorn BR, Donn WA Jr., Progulsk-Fox A. *Porphyromonas gingivalis* traffics to autophagosomes in human coronary artery endothelial cells. *Infect Immun* 2001;69:5698-5708.
14. Dorn BR, Dunn WA Jr., Progulsk-Fox A. Invasion of human coronary artery cells by periodontal pathogens. *Infect Immun* 1999;67:5792-5798.
15. Freudberg MA, Galanos C. Bacterial liposaccharides: Structure, metabolism and mechanisms of action. *Int Rev Immunol* 1990;6:207-221.
16. Haraszthy VI, Zambon JJ, Trevisan M, Zeid M, Genco RJ. Identification of periodontal pathogens in atheromatous plaques. *J Periodontol* 2000;71:1554-1560.
17. Dorn BR, Leung KP, Progulsk-Fox A. Invasion of aortic and heart endothelial cells by *Prevotella intermedia*. *Infect Immun* 1998;66:6054-6057.
18. Dorn BR, Burks JN, Seifert KN, Progulsk-Fox A. Invasion of endothelial and epithelial cells by strains of *Porphyromonas gingivalis*. *FEMS Microbiol Lett* 2000;187:139-144.
19. Kozarov EV, Dorn BR, Shelburne CE, Dunn WA, Progulski-Fox A. Human atherosclerotic plaque contains viable invasive *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis*. *Arterioscler Thromb Vasc Biol* 2005;25:e17-e18.
20. Kuramitsu HK, Kang IC, Qi M. Interaction of *Porphyromonas gingivalis* with host cells: Implications for cardiovascular diseases. *J Periodontol* 2003;74:85-89.
21. Pussinen PJ, Vilkkuna-Rautianen T, Alfthean G, et al. Severe periodontitis enhances macrophage activation via increased serum lipopolysaccharides. *Arterioscler Thromb Vasc Biol* 2004;24:2174-2180.
22. Pussinen PJ, Tuomisto K, Jousilahti P, Havulinna AS, Sundvall J, Salomaa V. Endotoxemia, immune response to periodontal pathogens and systemic inflammation associate with incident cardiovascular disease events. *Arterioscler Thromb Vasc Biol* 2007;27:1433-1439.
23. Lopes-Virella MF. Interaction between bacterial lipopolysaccharides and serum lipoproteins and their possible role in coronary heart disease. *Eur Heart J* 1993;14(Suppl K):118-124.
24. Renaud S, Kuba K, Goulet C, Lemire Y, Allard C. Relationship between fatty-acid composition of platelets and platelet aggregation in rat and man. Relation to thrombosis. *Circ Res* 1970;26:553-564.
25. Pitiphat W, Crohin C, Williams P, et al. Use of preexisting radiographs for assessing periodontal disease in epidemiologic studies. *J Public Health Dent* 2004;64:223-230.
26. Valachovic RW, Douglass CW, Berkey CS, McNeil BJ, Chauncey HH. Examiner reliability in dental radiography. *J Dent Res* 1986;65:432-436.
27. Novitsky TJ. *Limulus* amebocyte lysate (LAL) detection of endotoxin in human blood. *J Endotoxin Res* 1994;1:253-263.
28. Craig RG, Boylan R, Yip J, et al. Serum IgG antibody response to periodontal pathogens in minority populations: Relationship to periodontal disease status and progression. *J Periodontol Res* 2002;37:132-46.
29. Goteiner D, Ashmen R, Lehrman N, Janal MN, Eskin B. Oral health of patients entering Morristown Memorial Hospital with acute coronary syndrome and angina. *J N J Dent Assoc* 2007;78:33-37.
30. Gaziano JM, Hennekens CH, O'Donnell CJ, Breslow JL. Fasting triglycerides, high-density lipoprotein and risk of myocardial infarction. *Circulation* 1997;96:2520-2525.
31. Friedlander AH, Weinreb J, Friedlander I, Yagiela JA. Metabolic syndrome: Pathogenesis, medical care and dental implications. *J Am Dent Assoc* 2007;138:179-187.
32. Valentaviciene G, Paipaliene P, Nedzelskiene I, Zilinskas J, Anuseviciene OV. The relationship between blood serum lipids and periodontal condition. *Stomatologija* 2006;8:96-100.
33. Zaremba M, Górska R, Suwalski P, Kowalski J. Evaluation of the incidence of periodontitis-associated bacteria in atherosclerotic plaque of coronary blood vessels. *J Periodontol* 2007;78:322-327.
34. Stoll LL, Denning GM, Weintraub NL. Potential role of endotoxin as a proinflammatory mediator in atherosclerosis. *Arterioscler Thromb Vasc Biol* 2004;24:2227-2236.
35. Gallin JI, Kay D, O'Leary WM. Serum lipids in infection. *N Engl J Med* 1969;281:1081-1086.
36. Sammalkorpi K, Valtonen V, Kettula Y, Nikkilä E, Taskinen MR. Changes in serum lipoprotein pattern induced by acute infection. *Metabolism* 1988;37:859-865.
37. Geerts SO, Nys M, DeMol P, et al. Systemic release of endotoxins induced by gentle mastication: Association with periodontitis severity. *J Periodontol* 2002;73:73-78.
38. Katz J, Chaushu G, Sharabi Y. On the association between hypercholesterolemia, cardiovascular disease and severe periodontal disease. *J Clin Periodontol* 2001;28:865-868.
39. Katz J, Flugelman MY, Goldberg A, Heft M. Association between periodontal pockets and elevated cholesterol and low density lipoprotein cholesterol levels. *J Periodontol* 2002;73:494-500.
40. Craig RG, Yip JK, So MK, Boylan RJ, Socransky SS, Haffajee AD. Relationship of destructive periodontal diseases to the acute phase response. *J Periodontol* 2003;74:1007-1016.
41. Levine DM. In vivo protection against endotoxin by plasma HDL. *Proc Natl Acad Sci U S A* 1993;90:12040-12044.
42. Harris HW, Grundfield DC, Fiengold KR, Rapp JH. Human very low density lipoproteins and chylomicrons can protect against endotoxin in direct death in mice. *J Clin Invest* 1990;86:696-702.
43. Lancel S, Joulin J, Favory R, et al. Ventricular myocyte caspases are directly responsible for endotoxin-induced cardiac dysfunction. *Circulation* 2005;111:2596-2604.
44. Song Y, Song Z, Zhang L, McLain CJ, Kang YL, Cai L. Diabetes enhances lipopolysaccharides-induced cardiac toxicity in the mouse model. *Cardiovasc Toxicol* 2003;3:363-372.

45. Van Lenten BJ, Fogelman AM, Haberland ME, Edwards PA. The role of lipoproteins and receptor-mediated endocytosis in the transport of bacterial lipopolysaccharides. *Proc Natl Acad Sci USA* 1986;83:2704-2708.
46. Bengtsson T, Karlsson H, Gunnarsson P, et al. The periodontal pathogen *Porphyromonas gingivalis* cleaves apoB-100 and increases the expression of apoM in LDL in whole blood leading to cell proliferation. *J Intern Med* 2008;263:558-571.
47. Pussinen PJ, Nyyssonen K, Alfthan G, Salonen R, Laukkanen JA, Salonen JT. Serum antibody levels to *Actinobacillus actinomycetemcomitans* predict the risk of coronary heart disease. *Arterioscler Thromb Vasc Biol* 2005;25:833-838.
48. Pussinen PJ, Alfthan G, Tuomilehto J, Asikainen S, Jousilahti P. High serum antibody levels to *Porphyromonas gingivalis* predict myocardial infarction. *Eur J Cardiovasc Prev Rehabil* 2004;11:408-411.
49. Paju S, Sinasalo J, Pussinen PJ, Altonen V, Nieminen MS. Is periodontal infection behind the failure of antibiotics to prevent coronary events? *Atherosclerosis* 2007;193:193-194.

Correspondence: Dr. David Goteiner, 2A North Rd., Chester, NJ 07930. Fax: 908/879-8367; e-mail: drgoteiner@yahoo.com.

Submitted February 1, 2008; accepted for publication June 13, 2008.