Clinical research

Risk factors for cardiovascular disease in patients with periodontitis

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Aims
This study compared plasma levels of established risk markers for atherosclerosis and indices of inflammation in 50 patients with severe periodontitis to those in 46 healthy cases.

Methods and results
Full blood counts were performed and levels of high density lipoproteins (HDL), total cholesterol, haptoglobin, elastase, C-reactive protein (CRP), IL-6, TNF\textsubscript{α} receptor-1, α1-antitrypsin and antibodies against human heat shock protein (Hsp) 60, mycobacterial Hsp65 and oxLDL were determined. Total cholesterol levels were similar in both groups, whereas HDL levels were lower (P=0.007) and the lipid profile (total cholesterol/HDL) was consequentially higher (P=0.03) in patients. Monocyte counts were elevated (0.56 vs 0.44×10\textsuperscript{9}/l; P=0.002) and CRP levels were higher in patients, but TNF\textsubscript{α} receptor-1 and elastase levels were not. Anti-oxLDL antibody levels were similar, as were levels of haptoglobin, IgG anti HSP60, IgA and IgG anti-Hsp65 antibodies. Levels of IgA anti-Hsp60 antibodies were lower in patients (P=0.0001). Logistic regression analysis revealed a relationship between periodontitis and HDL (OR 2.15/0.5 mmol/l, P=0.02) and body mass index (OR 4.54 P=0.005)

Conclusion
Serological differences in subjects with periodontitis, some of which involve established risk factors for atherosclerosis, might provide insight into the reported epidemiological association between periodontitis and cardiovascular disease.

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KEYWORDS
Atherosclerosis; Risk factors; Inflammation and periodontitis

Introduction
Periodontitis is a common disease, the clinical signs of which are seen in early middle age. It is a chronic, tissue-destructive inflammation, which degrades the attachment apparatus of the teeth, causing tooth loss and, in its most severe form, edentulosity. The more severe form of the disease is present in approximately 10–15% of an adult population,\textsuperscript{1} whereas 35%\textsuperscript{2} exhibit moderate or mild signs of the disease. Atherosclerosis is also very common and starts early in life, however, since disease progression is usually slow, clinical symptoms or hospitalization are rare before 40 years of age.\textsuperscript{3}

Over the last 15 years, several studies have reported epidemiological associations between periodontitis and cardiovascular disease.\textsuperscript{4–7} However, detailed analyses suggest that this relationship is weak, and that an over-estimation of this association might result from
insufficient compensation for lifestyle differences and common risk factors in the statistical models used to evaluate the relationship. Both diseases have several risk factors in common—e.g., smoking and diabetes (see Beck et al 1998 for review), and controlling for smoking history and health awareness poses a particular challenge.

Another possible mechanism accounting for the reported association between periodontitis and cardiovascular disease could be the release of bacteria, bacterial products or pro-inflammatory cytokines from the chronic periodontal lesion into the blood stream. This might lead to a systemic inflammatory response, which resembles a risk factor profile that is consistent with cardiovascular disease. Although DNA from oral bacteria has been found in atherosclerotic plaques, a bacterial contribution to this plaque formation has yet to be demonstrated.

The aim of this study was to determine whether patients with severe periodontitis have higher plasma concentrations of established risk markers for atherosclerosis, such as lipoproteins and CRP. Levels of pro-inflammatory cytokines, antibodies against heat shock proteins and oxidized LDL, and markers of a systemic inflammatory reaction (leukocyte number, neutrophil activation (plasma elastase) and monocyte activation (plasma TNFα-receptor 1) were also determined. Blood pressure, body mass index (BMI) and working capacity (assessed with an exercise test) were also measured.

Materials and methods

Patients and healthy, non periodontal cases

The periodontitis group consisted of 50 subjects, (29 men, 21 women; group mean age 52.7 years, range 37–68 years) having a minimum of seven sites exhibiting at least 6 mm loss of clinical attachment. All had been referred to a specialist clinic in Stockholm, Sweden for treatment for this condition. The patients had periodontitis grade 3 in which there is a horizontal loss of supporting tissue by 1/3 or more of the root-length with bleeding on probing, furcation involvements of the multi-rooted teeth and/or angular bony defects with pus. Seven patients had localized abscesses to a lesser extent. Patients were excluded from the study if they had a known history of cardiovascular disease. The healthy, non-periodontal cases (comparison group) comprised 47 subjects (18 men, 29 women; group mean age 50.2 years, range 36–70 years), none of whom exhibited clinical signs of periodontal disease as measured by pocket depths over 5 mm (13.8±7.3 cigarettes/day). All of the smokers had been smoking for 10 years or more. Educational levels were evenly distributed and there was an equivalent profile of university (3 years or more) and lower educational standards in both groups. The distribution of high, intermediate and low employment grades was also equivalent, and none were unemployed.

In none of the participants was cardiovascular disease or any other ongoing general disease or infections diagnosed. Cardiovascular health was assessed on the basis of medical history, and those with a normal blood pressure and exercise test capacity were regarded as being healthy in this regard. Working capacity was calculated by dividing the actual working capacity measured in Watts with the expected working capacity value for that age and weight. The maximum cardiac output was calculated by dividing the actual heart frequency (pulse/min) with the expected heart frequency value for that age and weight.

Clinical examination for periodontal disease

Patients underwent a comprehensive periodontal examination including radiographs and the oral health status of the comparison group was verified by clinical examination. A single dentist with extensive experience undertook all clinical examinations. Teeth and gingivae were evaluated, and the pocket depth was measured using a calibrated periodontal probe. Probing depth is the distance between the gingival margin and to the bottom of the gingival pocket measured from six angles of each tooth. The presence or absence of dental plaque at the gingival margin along the mesial, buccal, distal and lingual aspects was determined and the hygiene index (HI-index, %) was calculated. The dental plaque was made visible by gently moving the tip of the probe along the gingival margin of the four sides of each tooth. Gingival pockets 4 mm or deeper were considered to be pathogenic. Gingival inflammation was noted as bleeding on probing and data are expressed as the proportion of bleeding sites among the total number of sites in the dentition.

This study was approved by the Regional Ethics Committee of the Karolinska Institutet and was conducted in accordance with the Helsinki Declaration. All participants gave their informed consent. All blood samples analyses were performed blindly.

Haematological, blood lipid and inflammatory indices analysis

Blood was collected into Vacutainer™ tubes containing EDTA and haematological indices were determined using a Coulter STKS analyzer (Coulter Electronics Inc., Hialeah, FL, USA). Plasma was obtained after centrifugation at 1500 g for 10 min and stored at −70 °C until analysis. Cholesterol and high-density lipoprotein (HDL) levels were determined using standard clinical chemistry procedures with a Hitachi 917 analyzer (Roche AG Diagnostics, Mannheim, Germany). The lipid profile was calculated as the ratio between total cholesterol and HDL. C-reactive protein (CRP) levels were determined using a high-sensitivity commercial assay kit (DADE Behring, Deerfield, IL, USA), and haptoglobin and α-1-antitrypsin (AAT) levels were measured using a BNA nephelometer (Behringwerke AG Diagnostica, Marburg, Germany).

IL-6 and TNFα-receptor 1 (R&D Systems Europe Ltd., Abingdon, UK) and elastase-A1AT complex (Merck KgaA Diagnostica, Darmstadt, Germany) levels were determined using commercial immunoassays according to the manufacturers’ recommended protocols. Plasma elastase levels reflect neutrophil activation and TNFα-receptor 1 levels reflect the anti-inflammatory response.
Levels of circulating antibodies against human Hsp60 and mycobacterial Hsp65 were determined as described previously, except that the isotype (IgA, IgG) of the anti-heat shock protein antibodies was determined, and data are presented as the absorbance values generated by samples diluted 1/100. Anti-oxidized LDL antibody levels were determined essentially as described previously. Positive reference sera were included on each plate and absorbance readings were related to this reference value (transformed to arbitrary units).

Statistical methods

Statistical analyses were performed using Statistica 6.0 and SAS 8.2 software. Parametric (normally distributed) and non-parametric data (non-normal distributed) were analysed using the Student-t test or Mann-Whitney U-test respectively.

Associations between the periodontal and serological parameters were evaluated using the Spearman Rank order correlation coefficient. No adjustment for the repeated analyses has been performed. Associations between the incidence of periodontitis and independent confounders were calculated using a logistic regression model adjusted for age, gender and smoking. These variables were chosen since they are known risk factors for cardiovascular disease. Age and smoking are also known to be important risk factors for periodontal disease. Confidence intervals were calculated using the Wald’s method.

Odds ratios were calculated using the following categorical criteria: age (change/year), gender (women vs men), smoking (yes/no), body mass index (BMI; >26 in men; >25 in women these are, the accepted values for over-weight individuals), HDL (change/unit (mmol/l)), lipid profile (change/unit) and CRP (change/unit (mg/l)). In addition to being analysed as a continuous variable, the association between periodontitis and CRP in individuals having high CRP levels (fourth quartile->2.30 mg/l) has been evaluated.

Results

The characteristics of the patient and comparison groups are given in Table 1. Apart from monocyte counts, which were higher in patients with periodontal disease (0.56 vs 0.44×10^9/l; P=0.002), haematological parameters were similar in the patient and comparison groups (data not shown). Although statistically significant, the differences in monocyte counts are unlikely to be of any clinical significance as counts in both groups are within the normal range. All other comparisons between the different groups (Tables 1–3) which are not mentioned in the text are non-significant (data not shown).

Regression analysis

The analysis of primary interest is the multiple logistic regression since this is adjusted for age, gender and smoking. Multiple logistic regression was used to calculate the odds ratios for associations between periodontal disease and serological variables. Significant associations between periodontitis and levels of HDL, and between periodontitis and body mass index were apparent (Table 4). A trend towards an association between periodontitis and 2.0 mg/l increases in CRP was also apparent (OR 1.55; P=0.07; Table 4). There was a significant association between CRP and periodontitis (OR 3.3) in those patients having CRP levels greater than 2.30 mg/l (fourth quartile).

Anthropometric measurements

There were no differences in blood pressure between the two groups. Patients exhibited a lower working capacity (lower per cent values of working capacity; P=0.004), and this difference remained when the men, women, smoker and non-smoker subgroups were analysed separately. Maximum cardiac output and systolic working capacity (the systolic blood pressure at maximum working capacity) of the groups were similar (Table 2). The patient group had a higher BMI (P=0.026), and this difference was greatest in the non-smoker subgroup (P=0.002). Although male patients exhibited higher BMI than male non-patients, and the BMI of female patients was higher than that of female non-patients, these differences were not of statistical significance (Table 2).

Lipoprotein-lipid profiles

Total cholesterol levels were similar in patients and non-patients, whereas HDL levels were lower in the patients (P=0.007, Table 2). A trend for lower HDL concentrations in patients was apparent when the subgroups were analysed separately (Table 2). HDL levels were ≤0.9 mmol/l in 13 patients (26%) with periodontitis and five individuals in the comparison group (11%; P=0.052; chi-square). This difference was also found when the groups were divided into smokers and non-smokers (15% vs 6%; 33% vs 14% respectively). Weak, but significant correlations between HDL and both bleeding on probing (R=−0.35, P=0.001) and the number of pockets (R=−0.35, P=0.001) were apparent. The lipid profile was higher in the patient group (P=0.033) and this difference was more marked in women (P=0.021).

Inflammatory mediators

Although plasma CRP levels were higher in the main and all patient subgroups, the differences were only of statistical significance in the main, smoking and male subgroups (Table 3). Weak, but significant correlations between CRP and both bleeding on probing (R=0.26, P=0.02) and the number of pockets (R=0.27, P=0.01) were also apparent. Plasma IL-6 levels were higher in patients, whereas levels of TNFα R1, haptoglobin, α-1-antitrypsin and elastase were similar in both groups (Table 3).

Anti-heat shock protein and anti-oxLDL antibody levels

Contrary to expectations, serum levels of IgA and IgG anti-Hsp65 antibodies were similar in patients and non-patients when analysed as a group, although IgA anti-Hsp65 antibody levels were lower in patient smoking subgroup. IgG anti-Hsp60 levels were also similar, whereas IgA anti-human Hsp60 antibody levels were
Table 1  Characteristics of subjects with and without periodontitis

<table>
<thead>
<tr>
<th>Gender F/M</th>
<th>Mean age (yrs.)</th>
<th>No. of teeth</th>
<th>No. of cigarettes/day</th>
<th>No. of sites with ≥4 mm of pocket depth</th>
<th>Depth of pathological pockets (mm)</th>
<th>Bleeding on probing (%)</th>
<th>Hygiene (oral plaque) index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with severe periodontitis n=50</td>
<td>21/29</td>
<td>52.7(7.1)</td>
<td>24.5(4.5)</td>
<td>40(18)</td>
<td>5.4(0.7)</td>
<td>31(20)</td>
<td>30(18)</td>
</tr>
<tr>
<td>Healthy non-patients n=46</td>
<td>28/18</td>
<td>50.3(7.0)</td>
<td>28.0(2.7)</td>
<td>5(4)</td>
<td>4.1(0.1)</td>
<td>10(6)</td>
<td>20(11)</td>
</tr>
<tr>
<td>Smokers, patients n=20</td>
<td>10/10</td>
<td>50.6(6.7)</td>
<td>24.2(5.1)</td>
<td>42(21)</td>
<td>5.4(0.8)</td>
<td>32(21)</td>
<td>32(19)</td>
</tr>
<tr>
<td>Smokers, healthy non-patients n=17</td>
<td>11/6</td>
<td>48.2(6.6)</td>
<td>13.4(5.9)</td>
<td>6(5)</td>
<td>4.0(1.1)</td>
<td>12(7)</td>
<td>26(11)</td>
</tr>
<tr>
<td>Non-smokers, patients n=30</td>
<td>11/19</td>
<td>54.1(7.2)</td>
<td>27.5(2.3)</td>
<td>35(15)</td>
<td>5.4(0.6)</td>
<td>31(19)</td>
<td>28(17)</td>
</tr>
<tr>
<td>Non-smokers, healthy non-patients n=29</td>
<td>17/12</td>
<td>51.5(7.0)</td>
<td>13.8(7.3)</td>
<td>5(4)</td>
<td>4.1(0.1)</td>
<td>9(5)</td>
<td>17(17)</td>
</tr>
<tr>
<td>Men with severe periodontitis n=29</td>
<td>29</td>
<td>53.6(7.2)</td>
<td>24.7(4.1)</td>
<td>40(19)</td>
<td>5.4(0.7)</td>
<td>32(20)</td>
<td>31(18)</td>
</tr>
<tr>
<td>Healthy men n=18</td>
<td>18</td>
<td>49.7(9.3)</td>
<td>28.2(2.4)</td>
<td>5(3)</td>
<td>4.0(0.0)</td>
<td>11(5)</td>
<td>24(13)</td>
</tr>
<tr>
<td>Women with severe periodontitis n=21</td>
<td>21</td>
<td>51.5(6.9)</td>
<td>23.9(5.2)</td>
<td>36(17)</td>
<td>5.4(0.6)</td>
<td>30(20)</td>
<td>28(17)</td>
</tr>
<tr>
<td>Healthy women n=28</td>
<td>28</td>
<td>50.6(5.2)</td>
<td>27.2(2.2)</td>
<td>5(4)</td>
<td>4.0(0.8)</td>
<td>10(6)</td>
<td>19(10)</td>
</tr>
</tbody>
</table>

aData are means (SD).
Table 2  Blood pressure, working capacity, cardiac output, plasma lipoproteins and BMI in subjects with and without periodontitis

<table>
<thead>
<tr>
<th></th>
<th>Patients with severe periodontitis n=50</th>
<th>Healthy non-patients n=46</th>
<th>Smokers, patients n=20</th>
<th>Smokers, healthy non-patients n=17</th>
<th>Non-smokers, patients n=30</th>
<th>Non-smokers, non-patients n=29</th>
<th>Men with severe periodontitis n=29</th>
<th>Healthy men n=18</th>
<th>Women with severe periodontitis n=21</th>
<th>Healthy women n=28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>135(25)</td>
<td>141(25)</td>
<td>136(24)</td>
<td>128(16)</td>
<td>134(26)</td>
<td>145(26)</td>
<td>136(24)</td>
<td>153(34)</td>
<td>133(26)</td>
<td>136(17)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>79(12)</td>
<td>83(11)</td>
<td>78(11)</td>
<td>76(5.6)</td>
<td>79(12)</td>
<td>85(11)</td>
<td>79(10)</td>
<td>88(14)</td>
<td>78(14)</td>
<td>81(9.0)</td>
</tr>
<tr>
<td>Working capacity (%)</td>
<td>97.8(21.4)</td>
<td>94.6(8.9)</td>
<td>95.8(8.2)</td>
<td>95.1(8.3)</td>
<td>101.2(21)</td>
<td>104(22)</td>
<td>119(27)</td>
<td>99.9(25)</td>
<td>117(28)</td>
<td>94.9(15.0)</td>
</tr>
<tr>
<td>Max. cardiac output (%)</td>
<td>94.6(8.9)</td>
<td>200(28)</td>
<td>114.8(26.9)</td>
<td>95.8(8.2)</td>
<td>182(27.2)</td>
<td>202(27)</td>
<td>208(27)</td>
<td>190(27)</td>
<td>222(27)</td>
<td>222(29)</td>
</tr>
<tr>
<td>Systolic working capacity (mmHg)</td>
<td>200(28)</td>
<td>197(19.8)</td>
<td>182(27.2)</td>
<td>197(19.8)</td>
<td>215(22)</td>
<td>210(22)</td>
<td>220(27)</td>
<td>229(27)</td>
<td>222(27)</td>
<td>222(29)</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.76(0.94)</td>
<td>4.83(1.00)</td>
<td>4.70(0.68)</td>
<td>5.19(1.08)</td>
<td>4.71(0.96)</td>
<td>4.61(0.89)</td>
<td>4.77(0.90)</td>
<td>5.21(1.09)</td>
<td>4.74(1.01)</td>
<td>4.58(0.86)</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>1.15(0.37)</td>
<td>1.37(0.42)</td>
<td>1.12(0.28)</td>
<td>1.44(0.45)</td>
<td>1.17(0.42)</td>
<td>1.33(0.41)</td>
<td>1.04(0.30)</td>
<td>1.17(0.38)</td>
<td>1.30(0.40)</td>
<td>1.51(0.40)</td>
</tr>
<tr>
<td>Lipid profile</td>
<td>4.49(1.48)</td>
<td>3.84(1.44)</td>
<td>4.46(1.32)</td>
<td>3.95(1.25)</td>
<td>4.51(1.64)</td>
<td>4.37(1.46)</td>
<td>4.93(1.59)</td>
<td>4.83(1.62)</td>
<td>3.87(1.06)</td>
<td>3.20(0.85)</td>
</tr>
<tr>
<td>Anti-oxLDL (units)</td>
<td>170.7(89.9)</td>
<td>186.4(126.2)</td>
<td>160.0(75.5)</td>
<td>209.4(167.5)</td>
<td>178.0(99.2)</td>
<td>181.0(118.2)</td>
<td>169.9(105.7)</td>
<td>174.7(119.4)</td>
<td>171.9(65.5)</td>
<td>191.6(131.5)</td>
</tr>
<tr>
<td>BMI</td>
<td>25.7(3.3)</td>
<td>24.1(3.0)</td>
<td>24.4(2.8)</td>
<td>24.3(4.3)</td>
<td>26.6(3.3)</td>
<td>24.0(2.3)</td>
<td>25.8(2.9)</td>
<td>24.3(3.8)</td>
<td>25.6(2.9)</td>
<td>24.0(3.5)</td>
</tr>
</tbody>
</table>

aData are means (SD).
Table 3  Levels of heat shock protein antibodies and inflammatory mediators in subjects with and without periodontitis

<table>
<thead>
<tr>
<th></th>
<th>Patients with severe periodontitis n=50</th>
<th>Healthy non-patients n=46</th>
<th>Smokers, patients n=20</th>
<th>Smokers, healthy non-patients n=17</th>
<th>Non-smokers, patients n=30</th>
<th>Non-smokers, healthy non-patients n=29</th>
<th>Men with severe periodontitis n=29</th>
<th>Healthy men n=18</th>
<th>Women with severe periodontitis n=21</th>
<th>Healthy women n=28</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA anti-HSP60</td>
<td>0.09(0.04)</td>
<td>0.17(0.12)</td>
<td>0.08(0.04)</td>
<td>0.14(0.06)</td>
<td>0.10(0.04)</td>
<td>0.17(0.13)</td>
<td>0.09(0.04)</td>
<td>0.22(0.19)</td>
<td>0.08(0.04)</td>
<td>0.14(0.06)</td>
</tr>
<tr>
<td>IgG anti-HSP60</td>
<td>0.15(0.15)</td>
<td>0.18(0.17)</td>
<td>0.13(0.09)</td>
<td>0.18(0.19)</td>
<td>0.16(0.18)</td>
<td>0.18(0.17)</td>
<td>0.15(0.16)</td>
<td>0.22(0.23)</td>
<td>0.14(0.13)</td>
<td>0.16(0.14)</td>
</tr>
<tr>
<td>IgG anti-HSP65</td>
<td>0.20(0.22)</td>
<td>0.15(0.14)</td>
<td>0.21(0.21)</td>
<td>0.06(0.04)</td>
<td>0.18(0.23)</td>
<td>0.17(0.15)</td>
<td>0.19(0.23)</td>
<td>0.17(0.13)</td>
<td>0.20(0.20)</td>
<td>0.14(0.15)</td>
</tr>
<tr>
<td>IgA anti-HSP65</td>
<td>0.13(0.07)</td>
<td>0.17(0.12)</td>
<td>0.12(0.07)</td>
<td>0.21(0.15)</td>
<td>0.13(0.07)</td>
<td>0.16(0.12)</td>
<td>0.12(0.05)</td>
<td>0.21(0.17)</td>
<td>0.14(0.09)</td>
<td>0.15(0.09)</td>
</tr>
<tr>
<td>Elastase-A1AT complex (pg/ml)</td>
<td>1.43(0.61)</td>
<td>1.40(0.73)</td>
<td>1.39(0.62)</td>
<td>1.65(1.02)</td>
<td>1.46(0.60)</td>
<td>1.24(0.41)</td>
<td>1.45(0.60)</td>
<td>1.61(0.51)</td>
<td>1.41(0.63)</td>
<td>1.27(0.82)</td>
</tr>
<tr>
<td>TNFα receptor 1 (µg/l)</td>
<td>1.86(2.29)</td>
<td>1.57(1.01)</td>
<td>1.40(1.18)</td>
<td>1.25(0.42)</td>
<td>2.16(2.78)</td>
<td>1.76(1.20)</td>
<td>1.55(1.10)</td>
<td>1.50(1.27)</td>
<td>2.27(3.29)</td>
<td>1.61(0.85)</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>3.28(4.64)</td>
<td>1.74(1.68)</td>
<td>3.27(3.64)</td>
<td>1.76(1.83)</td>
<td>3.28(5.27)</td>
<td>1.73(1.61)</td>
<td>3.45(5.57)</td>
<td>1.62(1.58)</td>
<td>3.03(3.06)</td>
<td>1.82(1.76)</td>
</tr>
<tr>
<td>IL-6 (µg/l)</td>
<td>3.66(9.74)</td>
<td>1.57(2.10)</td>
<td>6.14(14.7)</td>
<td>1.64(1.98)</td>
<td>2.01(3.36)</td>
<td>1.53(2.19)</td>
<td>4.93(12.4)</td>
<td>1.75(1.94)</td>
<td>1.91(3.51)</td>
<td>1.45(2.21)</td>
</tr>
<tr>
<td>Haptoglobin (g/l)</td>
<td>1.13(0.41)</td>
<td>1.04(0.35)</td>
<td>1.32(0.48)</td>
<td>1.25(0.35)</td>
<td>1.00(0.29)</td>
<td>0.92(0.29)</td>
<td>1.12(0.38)</td>
<td>1.10(0.41)</td>
<td>1.14(0.44)</td>
<td>1.00(0.31)</td>
</tr>
<tr>
<td>α-1-antitrypsin (g/l)</td>
<td>1.25(0.21)</td>
<td>1.22(0.15)</td>
<td>1.34(0.14)</td>
<td>1.31(0.11)</td>
<td>1.18(0.23)</td>
<td>1.17(0.15)</td>
<td>1.24(0.24)</td>
<td>1.16(0.17)</td>
<td>1.26(0.17)</td>
<td>1.26(0.12)</td>
</tr>
</tbody>
</table>

aData are means (SD)
lower in the patient group (P=0.0001; Table 3). This difference was apparent in all subgroup analyses (Table 3). Levels of anti-oxLDL antibody were similar in all groups (Table 2).

Discussion

This study has reported (for the first time to our knowledge) a significant association between periodontitis and low HDL levels (OR 2.15/0.5 mmol/l). A weak, but statistically significant relationship between HDL levels and the number of gingival pockets and gingival inflammation (bleeding on probing) has also been observed. Albeit not very strong, this correlation is important, as it suggests that periodontal disease might influence blood lipid concentrations and thereby the risk of cardiovascular disease. Mortality in individuals with HDL levels below 0.9 mmol/l is higher than that in individuals with levels over 0.9 mmol/l, regardless of total cholesterol levels.20

Our study demonstrated that 26% of the patients had an HDL concentration ≤0.9 mmol/l, as compared to 11% in the comparison group, although this difference failed to reach statistical significance. One explanation for the relationship between periodontitis and low HDL levels might be that chronic inflammation in the periodontium leads to the release of lipopolysaccharide and pro-inflammatory cytokines such as IL-1β and TNFα, which have the capacity to influence lipid metabolism.21,22 Furthermore, HDL has anti-inflammatory properties and can decrease the adhesivity of endothelial cells.21 Thus, low HDL levels may also indirectly contribute to inflammatory processes.

Unlike previous studies,23–26 we found no difference in total cholesterol levels between the periodontitis and non-periodontitis cases. One reason for this could be that our study population was younger, as dystipidemia increases with age (Kannel 200227 for review). The atherogenic lipid profile has been shown to be a better measure than total cholesterol or LDL of the risk for developing coronary heart disease, especially in individuals less than 60 years of age.28,29 Our findings that this parameter is significantly higher in patients with periodontitis suggests that this group might be at an increased risk of cardiovascular disease. Although the differences remained when the participants were divided into men/women and smoker/non-smoker subgroups, their statistical significance was lost. These data might suggest that the association between periodontal breakdown and changes in cholesterol are only partially influenced by gender and smoking status. There are differences regarding the gender composition of the groups, and it was for this reason that subgroup data are also presented. These demonstrate that similar trends are apparent when males and females are considered separately. One uncertainty about the study is the subgroup analyses and the multiplicity of testing, false-positive results might occur. However, the regression analysis, which was adjusted for smoking, age and gender demonstrates an association between periodontitis and low levels of HDL. A tendency towards an association between periodontitis and high levels of CRP is also apparent. This is important as both HDL and CRP are accepted risk factors for atherosclerosis.

Differences in levels of anti-oxLDL antibodies in the patient and comparison groups were not observed. The role of anti-oxLDL antibodies is likely to be complex and may depend on disease stage. For example, in early human cardiovascular disease these antibodies are decreased18 and immunization with oxLDL leading to raised antibody levels reduces atherosclerosis.30 In contrast, anti-oxLDL antibody levels are raised in advanced and late stage cardiovascular disease. Further studies are needed to assess the importance of oxLDL-related immune reactions in periodontitis-related cardiovascular disease.

In agreement with previous studies,31–34 CRP levels were significantly higher in the patient group. Although levels were also elevated in the non-smoking patient subgroup, the difference was not of statistical significance. According to a recent study, slightly elevated levels of CRP can be considered as an independent risk factor for developing cardiovascular disease.34 Our study showed a weak, but statistically significant correlation between CRP levels and the degree of gingival inflammation. This is in agreement with a previous study, which has reported higher CRP levels in patients with more severe periodontitis.33

Like a previous study,35 body weight was also significantly higher in patients with periodontitis. This finding most likely reflects different lifestyles of the subject groups, as patients performed less well in the exercising

### Table 4 Odds ratios (95% confidence interval (CI)) for the association between various indicators of cardiovascular disease (BMI, HDL, lipid profile, CRP) and the periodontal disease group

<table>
<thead>
<tr>
<th>Category</th>
<th>BMI Number n=96</th>
<th>HDL Number n=96</th>
<th>Lipid profile Number n=96</th>
<th>CRP Number n=96</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ratio 95% CI</td>
<td>Ratio 95% CI</td>
<td>Ratio 95% CI</td>
<td>Ratio 95% CI</td>
</tr>
<tr>
<td>Periodontitis group</td>
<td>4.54 1.588–13.00</td>
<td>0.22/unit 0.064–0.742</td>
<td>1.27/unit 0.905–1.777</td>
<td>1.25/unit 0.983–1.578</td>
</tr>
</tbody>
</table>

The associations are shown for all participants. As regards the indicators, they are all adjusted for gender, age and tobacco smoking, we assessed the following categories: BMI (+BMI 26 in men or >25 in women), HDL (change/unit (mmol/l)), lipid profile (change/unit) and CRP (change/unit (mg/l)).
test, regardless of body weight expressed as BMI. The BMI was strongly associated with smoking, as there was no difference between smoking patients and smoking non-patients, whereas a difference was present between the non-smoking subgroups.

The parameters which appeared to demonstrate the most consistent difference between the patient and comparison groups and subgroups in this study were serum IgA anti-human Hsp60 and anti-mycobacterial Hsp65 antibody levels, although the latter failed to reach statistical significance in the majority of groups. In contrast the levels of IgG anti-Hsp60/65 were similar in the patient and comparison groups. These findings contrast with previous studies which have shown elevated levels of IgA anti-Hsp65 antibodies in the serum of patients with periodontitis. However, consistent with our data is the observation that levels of bacterial antigen-specific IgA antibody are lower in patients with periodontitis.

The robust finding that serum levels of IgA anti-Hsp60/65 antibodies are lower in patients with periodontitis is intriguing. Heat shock proteins are immuno-dominant molecules and a significant element of the immune response to pathogenic microorganisms is directed towards peptides derived from heat shock proteins.

The data suggest that there is a deficiency in the ability to mount humoral responses to Hsp60/65 in these patients and this might promote the establishment of chronic inflammation. Porphyromonas gingivalis expresses heat shock proteins on its cell surface. Our findings, in part at least, are consistent with the observation that peripheral blood mononuclear cell responses to heat shock proteins is depressed in patients with periodontitis, and this might lead to qualitative differences in humoral immune responses to oral bacteria. Whether a reduced immune capacity pre-disposes individuals to periodontitis or arises as a consequence of the disease has yet to be established. Chlamydia pneumoniae infection has been implicated in cardiovascular disease and serological tests for this and other infections might provide additional insight into the possible relationship between periodontitis and cardiovascular disease.

In this study we report several serological differences between subjects with and without periodontitis, some of which involve established risk factors for atherosclerosis. These findings might offer an etiological explanation for the epidemiological association between periodontitis and cardiovascular disease, which has been reported in a number of studies.

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References


