

# Association of Endodontic Lesions with Coronary Artery Disease

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## Abstract

An endodontic lesion (EL) is a common manifestation of endodontic infection where *Porphyromonas endodontalis* is frequently encountered. EL may associate with increased risk for coronary artery disease (CAD) via similar pathways as marginal periodontitis. The aim of this cross-sectional study was to delineate the associations between EL and CAD. Subgingival *P. endodontalis*, its immune response, and serum lipopolysaccharide were examined as potential mediators between these 2 diseases. The Finnish Parogene study consists of 508 patients (mean age, 62 y) who underwent coronary angiography and extensive clinical and radiographic oral examination. The cardiovascular outcomes included no significant CAD ( $n = 123$ ), stable CAD ( $n = 184$ ), and acute coronary syndrome (ACS;  $n = 169$ ). EL was determined from a panoramic tomography. We combined data of widened periapical spaces (WPSs) and apical rarefactions to a score of EL: 1, no EL ( $n = 210$ ); 2,  $\geq 1$  WPS per 1 apical rarefaction ( $n = 222$ ); 3,  $\geq 2$  apical rarefactions ( $n = 76$ ). Subgingival *P. endodontalis* was defined by checkerboard DNA-DNA hybridization analysis, and corresponding serum antibodies were determined by ELISA. In our population, 50.4% had WPSs, and 22.8% apical rarefactions. A total of 51.2% of all teeth with apical rarefactions had received endodontic procedures. Subgingival *P. endodontalis* levels and serum immunoglobulin G were associated with a higher EL score. In the multiaadjusted model (age, sex, smoking, diabetes, body mass index, alveolar bone loss, and number of teeth), having WPSs associated with stable CAD (odds ratio [OR] = 1.94, 95% confidence interval [95% CI] = 1.13 to 3.32,  $P = 0.016$ ) and highest EL score were associated with ACS (OR = 2.46, 95% CI = 1.09 to 5.54,  $P = 0.030$ ). This association was especially notable in subjects with untreated teeth with apical rarefactions ( $n = 59$ , OR = 2.72, 95% CI = 1.16 to 6.40,  $P = 0.022$ ). Our findings support the hypothesis that ELs are independently associated with CAD and in particular with ACS. This is of high interest from a public health perspective, considering the high prevalence of ELs and CAD.

**Keywords:** dentistry, periapical periodontitis, acute coronary syndrome, immunity, *Porphyromonas endodontalis*, lipopolysaccharides

## Introduction

An endodontic lesion (EL), frequently referred to as *apical periodontitis*, is a polymicrobial endodontic infection resulting in destruction of periradicular tissue. Similar to the progression of periodontitis, the microbiota shifts toward obligate aerobes and gram-negative anaerobes (Siqueira and Rôças 2009a). The bacterial invasion and the periapical defense reactions commonly reach an equilibrium resulting in an established EL. The treatment of ELs is endodontic chemomechanical elimination of infection (root canal treatment).

The prevalence of ELs is high: approximately 10% of all teeth are endodontically treated, and 5% of all teeth have periapical radiolucencies (Pak et al. 2012). The prevalence of  $\geq 1$  periapical lesions has been reported to be up to 61% (Jiménez-Pinzón et al. 2004). The biofilm of necrotic root canals commonly consists of the genera *Fusobacterium*, *Prevotella*, *Eubacterium*, *Peptostreptococcus*, and *Porphyromonas* (Siqueira and Rôças 2009b). *Porphyromonas endodontalis* is a black-pigmented gram-negative rod and a key pathogen in apical periodontitis, in light of its high prevalence and virulence factors

(Rôças et al. 2011; Montagner et al. 2012). It is frequently encountered in ELs, with a prevalence of approximately 65% (Rôças et al. 2011). It might occupy critical niches in maintaining the microbial community's stability and virulence (Hong

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et al. 2013). Furthermore, among typical endodontic pathogens, only *P. endodontalis* is able to invade vascular endothelial and smooth muscle cells, potentially linking endodontic infection with systemic cardiovascular complications (Dorn et al. 2002). Increased levels of subgingival *P. endodontalis* also associate with marginal periodontitis but are rarely detectable in health (Paster et al. 2001).

Infection triggers the production of an array of cytokines, which are involved in the immune response of ELs (Grauanaite et al. 2012). Systemic inflammatory markers have consistently shown to be increased in patients with ELs (Gomes et al. 2013). One of the main triggers of this process may be lipopolysaccharide (LPS), which is produced by gram-negative bacteria. High-serum LPS activity is independently associated with an increased risk of future cardiometabolic disorders (Pussinen et al. 2007; Kallio et al. 2015).

The association between marginal periodontitis and cardiovascular diseases (CVDs) is well established (Lockhart et al. 2012). Periodontitis is associated with an increased risk for CVDs, most likely via bacteremia, endotoxemia, and a systemic inflammatory burden (Pussinen et al. 2007; Schenkein et al. 2013). It has been suggested that ELs could increase the risk for CVDs via comparable pathways, due to similarities in the inflammatory and microbial profiles between marginal periodontitis and ELs (Frisk et al. 2003; Siqueira and Rôças 2009a). Few studies have addressed the associations between ELs and coronary artery disease (CAD), but the results are somewhat inconsistent (Frisk et al. 2003; Caplan et al. 2006; Joshipura et al. 2006; Caplan et al. 2009; Pasqualini et al. 2012; Costa et al. 2014; Petersen et al. 2014; Gomes et al. 2016).

The aim of the present study was to delineate the associations between radiographically evident ELs and angiographically verified CAD outcomes. The presence of subgingival *P. endodontalis*, its humoral immune response, and serum LPS activities were examined as potential mediators between these 2 diseases.

## Materials and Methods

### Population

The COROGENE study consists of 5,297 Finnish adult patients who underwent coronary angiography at the Helsinki University Central Hospital between June 2006 and March 2008 and who fulfilled inclusion criteria (Vaara et al. 2012). A random sex-stratified subsample comprising approximately 10% of the COROGENE population was enrolled for comprehensive clinical and radiographic oral examinations. Altogether 508 subjects were examined in the Parogene study. The population was grouped according to coronary angiography results; no significant CAD (<50% stenosis,  $n = 123$ ), stable CAD ( $\geq 50\%$  stenosis,  $n = 184$ ), acute coronary syndrome (ACS;  $n = 169$ ), and ACS-like but no significant CAD ( $n = 32$ ). Data collection has been described more extensively (Buhlin et al. 2011). All subjects signed an informed consent, and the study design was approved by the local ethics committee. This study complied with STROBE guidelines for observational studies.

### Examinations

Patients were considered having hypertension, dyslipidemia, or diabetes if they had the respective medications. Prior to the oral examinations, a dental assistant guided the patients to fill in a questionnaire.

All examiners were blinded regarding coronary diagnosis. Two calibrated periodontal specialists performed oral examinations approximately 6 to 20 wk (mean  $\pm$  SD:  $113 \pm 30$  d; range, 37 to 224 d) after the coronary angiographies (Buhlin et al. 2011). A full dental chart was performed, which recorded carious teeth, probing pocket depth, bleeding on probing, poor filling margins, root remnants, and number of teeth. Amputation was defined as total coronal pulpectomy. Subgingival bacterial samples were collected from dentate subjects ( $n = 478$ ; Mäntylä et al. 2013). Quantitative data of subgingival *P. endodontalis* were acquired by checkerboard DNA-DNA hybridization as described earlier (Socransky et al. 2004).

All dentate ( $n = 476$ ) and edentulous ( $n = 32$ ) subjects underwent digital panoramic tomography analyzed at the Institute of Dentistry, University of Helsinki. The number of teeth with periapically widened spaces and apical rarefactions were recorded by a radiologist specialized in oral and maxillofacial radiology. The definitions are in concordance with the commonly used periapical index (PAI); widened periapical spaces were scored as PAI = 3 and apical rarefaction as PAI = 4 to 5 (Ørstavik et al. 1986). Mild to severe alveolar bone loss (ABL) was considered a proxy for mild to severe marginal periodontitis (Hyvärinen et al. 2012).

Blood samples were drawn from each patient at the time of the coronary angiography and stored at  $-80\text{ }^{\circ}\text{C}$ . The Helsinki University Central Hospital Laboratory analyzed the high-sensitivity C-reactive protein. Serum LPS activity was determined by Limulus amoebocyte lysate assay coupled with a chromogenic substrate, and the interassay coefficient of variation was 5.5% ( $n = 6$ ). Serum immunoglobulin A and G (IgA and IgG) levels against whole cell antigen of *P. endodontalis* were determined with enzyme-linked immunosorbent assay (ELISA; Pussinen et al. 2002; Pussinen et al. 2011). All samples were analyzed in 1:100 and 1:200 dilutions as duplicates along with a set of blanks and control sera, which were used to normalize the absorbances after the whole population was analyzed. The interassay coefficient of variation was 7.9%.

### Statistical Analyses

For this cross-sectional study, we designed a score for each patient according to ELs as follows: score 1, no EL ( $n = 210$ , including 32 edentulous subjects); score 2,  $\geq 1$  widened periapical space and/or 1 apical rarefaction ( $n = 222$ ); score 3,  $\geq 2$  apical rarefactions ( $n = 76$ ). The groups were selected to reflect a continuum of the patients' infectious endodontic status. The limit for statistical significance was 0.05. The statistical difference among groups were analyzed with  $\chi^2$  test for categorical variables and Kruskal-Wallis test for continuous variables. The association between endodontic status and cardiologic outcome was analyzed with multinomial logistic regression with the reference

**Table 1.** Oral Parameters and CAD Outcomes.

Parameter	Outcome, n (%)			P Value <sup>a</sup>
	No Significant CAD (n = 123)	Stable CAD (n = 184)	ACS (n = 169)	
Cariou teeth	58 (47.2)	80 (43.5)	80 (47.3)	0.722
Teeth with root canal fillings	79 (64.2)	127 (69.0)	110 (65.1)	0.620
Teeth with inadequate root fillings	63 (51.2)	95 (51.6)	90 (53.3)	0.930
Amputations	15 (12.2)	13 (7.1)	12 (7.1)	0.212
Poor filling margins	59 (48.0)	87 (47.3)	77 (45.6)	0.910
Root remnants	6 (4.9)	11 (6.0)	14 (8.3)	0.473
Alveolar bone loss (mild to severe)	80 (69.0)	142 (82.6)	122 (77.2)	<b>0.027</b>
Teeth with widened periapical space	52 (43.7)	101 (55.2)	81 (48.8)	0.138
Teeth with apical rarefactions	24 (19.5)	37 (20.1)	47 (27.8)	0.140

ACS, acute coronary syndrome; CAD, coronary artery disease.

<sup>a</sup> $\chi^2$  test for univariate analyses. Bold indicates  $P < 0.05$ .

group being patients with no significant CAD ( $n = 123$ ). The association between presence of *P. endodontalis* and bivariate periapical outcomes was similarly analyzed. All models were statistically significant in the final model fitting test, and numeric problems were not present ( $SE < 2$ ). No significant factor-by-factor interactions were observed between dependent variables in the full factorial interaction analyses, excluding collinearity issues. Subjects with ACS-like but no significant CAD ( $n = 32$ ) were excluded from the association analyses due to their low number. Three models were applied to adjust for clinically relevant confounders: model 1, adjusted with age and sex; model 2, additionally adjusted with smoking (never vs. ever), diabetes mellitus, and BMI; model 3, additionally adjusted with ABL (no vs. mild to severe) and number of teeth present.

In the characteristics of the population, skewed continuous variables are presented as medians and interquartile range. Serum LPS levels and *P. endodontalis* IgA/IgG levels across groups for ELs and cardiologic outcome were analyzed with Kruskal-Wallis tests and Jonckheere-Terpstra tests. The potential interactive effects of ELs (scores 1, 2, and 3) and cardiologic outcome (no CAD, stable CAD, ACS) on serum LPS were analyzed with 2-way analysis of variance (type 3 sum of squares). The similar interaction analyses were conducted for ELs (scores 1, 2, and 3) and presence of subgingival *P. endodontalis* (yes/no) on IgA/IgG levels. All statistical analyses were conducted with the SPSS Statistics software (version 22; IBM).

## Results

### Oral Status

Compared with those without significant CAD, subjects with stable CAD or ACS were more often male (51%, 75%, and 73%, respectively;  $P < 0.001$ ). Those with stable CAD were older ( $65 \pm 8$  vs.  $61 \pm 9$  y;  $P = 0.001$ ). Patients with ACS were more frequently smokers (ever, 58% vs. 44%,  $P = 0.031$ ). There was no significant difference regarding caries, root canal fillings, inadequate root fillings, amputations, poor filling margins, root remnants, or teeth with widened periapical space or apical rarefactions in relation to cardiologic diagnosis in univariate chi-square analyses (Table 1).

Approximately half of the population had carious lesions, and more than half had inadequate root fillings. Altogether, 50.4% of our population had at least 1 widened periapical space, and 22.8% had at least 1 apical rarefaction. Altogether, the subjects had 10,163 teeth. Of these 1,026 (10.1%) were root canal treated. There were 172 teeth with apical rarefactions; 81 (47.1%) were root canal treated; and 7 (4.1%) had the coronal pulp amputated. Thus, 51.2% of all teeth with apical rarefactions had past endodontic procedures.

### Endodontic Lesions and CAD

The subjects with stable CAD had more likely ABL (83% vs. 69%,  $P = 0.007$ ), but they also more frequently had at least 1 widened periapical space (55% vs. 44%,  $P = 0.050$ ). The adjusted associations between ELs and CAD outcomes are presented with the nonsignificant CAD as the reference group (Table 2). Having  $\geq 1$  widened periapical space (vs. 0) was significantly associated with stable CAD, with odds ratios (ORs) ranging between 1.63 and 1.94 depending on the level of adjustments. Similar analyses regarding apical rarefactions yielded nonsignificant associations. We repeated the analyses separately for subjects with  $\geq 1$  apical rarefaction (vs. 0) in teeth with/without root canal treatment. Having root canal-treated teeth with apical rarefactions ( $n = 51$ ) did not associate significantly with CAD outcome, but having untreated teeth with ELs ( $n = 57$ ) provided OR values of 2.72 to 2.91 for ACS depending on the level of adjustments. This association was especially evident in patients  $>60$  y (model 3: OR = 3.78, 95% confidence interval [95% CI] = 1.01 to 14.2,  $P = 0.049$ ).

When ELs were studied as a score combining information of widened periapical space and rarefactions, there was a significant association with score 3 and ACS (OR = 2.31 to 2.49). Scores 2 and 3 were also associated with an increased risk of stable CAD, but the results were of borderline significance or affected by the adjustments for ABL and number of teeth.

### Mediators of the Association

After the association of ELs with increased CAD risk was recovered, the groups of ELs (score, 1 to 3) were characterized

**Table 2.** Association between Endodontic Lesions and CAD Outcomes.

Lesion	Stable CAD (n = 184)		ACS (n = 169)	
	OR (95% CI)	P Value	OR (95% CI)	P Value
≥1 widened periapical space (n = 234) <sup>a</sup>				
Model 1 <sup>b</sup>	<b>1.63 (1.00 to 2.64)</b>	<b>0.047</b>	1.23 (0.76 to 2.00)	0.395
Model 2 <sup>c</sup>	<b>1.78 (1.07 to 2.95)</b>	<b>0.027</b>	1.20 (0.73 to 1.99)	0.473
Model 3 <sup>d</sup>	<b>1.94 (1.13 to 3.32)</b>	<b>0.016</b>	1.36 (0.80 to 2.32)	0.253
≥1 apical rarefaction (n = 108) <sup>e</sup>				
Model 1	0.98 (0.54 to 1.78)	0.946	1.54 (0.87 to 2.73)	0.136
Model 2	0.90 (0.48 to 1.69)	0.900	1.47 (0.81 to 2.67)	0.202
Model 3	0.89 (0.47 to 1.69)	0.723	1.55 (0.85 to 2.84)	0.156
≥1 apical rarefactions in teeth without RCF (n = 57) <sup>f</sup>	1.33 (0.60 to 3.18)	0.597	<b>2.91 (1.31 to 6.47)</b>	<b>0.009</b>
Model 1				
Model 2	1.17 (0.45 to 3.01)	0.748	<b>2.77 (1.18 to 6.50)</b>	<b>0.019</b>
Model 3	1.14 (0.44 to 2.93)	0.794	<b>2.72 (1.16 to 6.40)</b>	<b>0.022</b>
Endodontic lesion				
Model 1				
Score 1 <sup>g</sup>	1.00		1.00	
Score 2	1.41 (0.85 to 2.33)	0.187	0.97 (0.58 to 1.63)	0.919
Score 3	1.83 (0.82 to 4.11)	0.143	<b>2.49 (1.15 to 5.40)</b>	<b>0.021</b>
Model 2				
Score 1	1.00		1.00	
Score 2	1.68 (0.96 to 2.93)	0.070	1.00 (0.58 to 1.76)	0.985
Score 3	1.70 (0.72 to 3.98)	0.226	<b>2.31 (1.04 to 5.14)</b>	<b>0.040</b>
Model 3				
Score 1	1.00		1.00	
Score 2	1.75 (0.99 to 3.10)	0.053	1.06 (0.60 to 1.88)	0.835
Score 3	1.75 (0.74 to 4.15)	0.204	<b>2.46 (1.09 to 5.54)</b>	<b>0.030</b>

“No CAD” (n = 123) used as reference group in the multinomial logistic regression. Bold indicates P < 0.05.

95% CI, 95% confidence interval; ACS, acute coronary syndrome; CAD, coronary artery disease; OR, odds ratio; RCF, root canal filling.

<sup>a</sup>“No widened periapical spaces” (n = 242) used as reference group.

<sup>b</sup>Adjusted with age and sex.

<sup>c</sup>Additionally adjusted with smoking (never vs. ever), diabetes mellitus, and body mass index.

<sup>d</sup>Additionally adjusted with alveolar bone loss (none vs. mild to severe) and number of teeth present.

<sup>e</sup>“No apical rarefactions” (n = 368) used as reference group.

<sup>f</sup>“No apical rarefactions in teeth without RCF” (n = 419) used as reference group.

<sup>g</sup>Score 1 (reference score): no radiographically verified endodontic lesions (n = 200). Score 2: ≥1 widened periapical space and/or 1 apical rarefaction (n = 204). Score 3: ≥2 apical rarefactions (n = 72).

(Table 3). There was an evident increasing linear trend regarding smoking, dyslipidemia, ACS, ABL, bleeding on probing, number of teeth, and high-sensitivity C-reactive protein across groups but no age or sex differences.

The presence and amount of subgingival *P. endodontalis* and serum IgA/IgG levels were studied across scored groups of ELs (Table 4). First, a total of 65.8% of the whole population had subgingival *P. endodontalis* present. The presence and levels of subgingival *P. endodontalis* differed significantly across 3 groups with an increasing trend. The presence of *P. endodontalis* associated significantly with ≥1 widened periapical space and ≥1 apical rarefaction in a multiaadjusted binomial logistic regression (model 3: OR = 1.53, 95% CI = 1.00 to 2.33, P = 0.045; OR = 2.29, 95% CI = 1.34 to 3.90, P = 0.002, respectively). Second, the total levels and highest tertiles of bacteria-specific serum IgA/IgG were compared across the groups. Being in the highest tertile (vs. lower 2 tertiles) of *P. endodontalis* IgG was associated with a higher score of EL, and levels of IgG differed among the groups with a borderline significance (P = 0.072; Table 4). There was a statistically significant trend of higher median *P. endodontalis* IgG with higher scores of ELs (Jonckheere-Terpstra test

statistics = 41,287, z = 2.279, P = 0.023). Third, we analyzed whether EL score, presence of *P. endodontalis*, and the antibody response against it were associated. Median serum *P. endodontalis* IgA/IgG levels across groups of ELs and present subgingival *P. endodontalis* are illustrated (Fig. A). The antibody levels differed between subjects with and without subgingival *P. endodontalis* (P = 0.034 for IgA and P = 0.036 for IgG). The interaction of effects between the EL score and the presence of subgingival *P. endodontalis* on serum antibodies was, however, not significant. Finally, in a binomial logistic regression model, the presence of subgingival *P. endodontalis* or high levels of corresponding antibodies did not significantly associate with the cardiologic diagnosis (data not shown).

The levels of serum LPS across the groups with different scores of ELs and CAD outcomes are illustrated in Figure B. Serum LPS levels differed among the 3 CAD outcome groups (P = 0.002) but not with scores of ELs. Two-way analysis of variance failed to show differences by serum LPS between cardiologic outcomes and ELs. Serum LPS did not associate with subgingival *P. endodontalis* or corresponding serum antibodies (data not shown).

**Table 3.** Characteristics of the Population According to Score of Endodontic Lesions.

Variables	All Subjects (N = 508)	Score 1 <sup>a</sup> (n = 210)	Score 2 (n = 222)	Score 3 (n = 76)	P Value <sup>b</sup>
Categorical, n (%)					
Male	330 (65.0)	130 (61.9)	147 (66.2)	53 (69.7)	0.411
Diabetes mellitus	118 (23.6)	44 (21.2)	57 (25.9)	17 (23.3)	0.510
Smoking, ever	233 (53.0)	74 (44.3)	121 (59.3)	38 (55.1)	<b>0.015</b>
Hypertension	322 (63.9)	136 (64.8)	140 (63.6)	46 (62.2)	0.918
Dyslipidemia	404 (80.3)	164 (78.1)	187 (85.4)	53 (71.6)	<b>0.021</b>
Cardiologic diagnosis <sup>c</sup>					
No CAD	123 (25.8)	59 (28.1)	53 (24.1)	11 (14.7)	
Stable CAD	184 (38.7)	70 (33.3)	88 (40.0)	26 (34.7)	0.155
ACS	169 (35.5)	71 (33.8)	63 (28.6)	35 (46.7)	<b>0.024</b>
ACS-like, no CAD	29 (33.5)	10 (4.8)	16 (17.3)	3 (4.0)	0.418
Alveolar bone loss (mild to severe)	363 (76.1)	124 (69.3)	175 (78.8)	64 (84.2)	<b>0.016</b>
Continuous, mean ± SD					
Age, y	62.1 ± 10.4	60.7 ± 10.8	63.0 ± 10.7	62.9 ± 8.6	0.603
Body mass index, kg/m <sup>2</sup>	28.2 ± 5.1	27.5 ± 4.8	29.0 ± 5.4	28.0 ± 5.1	0.882
Bleeding on probing, % <sup>d</sup>	40.4 ± 19.4	35.9 ± 17.1	41.8 ± 20.7	46.9 ± 19.3	<b>0.000</b>
Continuous, median [IQR]					
Number of teeth	24 [27]	24 [13]	24 [8]	23 [12]	<b>0.014</b>
Hs-CRP (mg/L)	5.7 [24.0]	6.3 [18.9]	15.3 [30.0]	1.7 [5.3]	<b>0.001</b>

Bold indicates  $P < 0.05$ .

ACS, acute coronary syndrome; CAD, coronary artery disease; EU, ELISA unit; Hs-CRP, high-sensitivity C-reactive protein; Ig, immunoglobulin; IQR, interquartile range.

<sup>a</sup>Score 1: no radiographically verified endodontic lesions; score 2:  $\geq 1$  widened periapical space and/or 1 apical rarefaction; score 3:  $\geq 2$  apical rarefactions.

<sup>b</sup> $\chi^2$  test for categorical variables. Kruskal-Wallis test for continuous variables.

<sup>c</sup>Bivariate  $\chi^2$  test with "No CAD" group as reference.

<sup>d</sup>Measured from 4 surfaces of each tooth. No values for edentulous ( $n = 32$ ) subjects.

**Table 4.** Subgingival Presence and Serology *Porphyromonas endodontalis* According to Score of Endodontic Lesions.

Variables	All Subjects (n = 474)	Score 1 <sup>a</sup> (n = 210)	Score 2 (n = 222)	Score 3 (n = 76)	P Value <sup>b</sup>
Categorical, n (%)					
Presence of subgingival <i>P.e.</i>	312 (65.8)	100 (56.5)	151 (68.3)	61 (80.3)	<b>0.001<sup>c</sup></b>
High levels of serum <i>P.e.</i> <sup>d</sup>					
IgA	164 (33.3)	60 (29.3)	77 (35.8)	27 (37.0)	0.278
IgG	164 (33.3)	54 (26.3)	77 (35.8)	33 (45.2)	<b>0.008</b>
Continuous, median [IQR]					
Level of subgingival <i>P.e.</i> <sup>e</sup>	0.30 [1.87]	0.08 [1.19]	0.38 [2.44]	0.61 [2.85]	<b>0.006, 0.023</b>
Level of serum <i>P.e.</i> , EU					
IgA	1.63 [2.5]	1.41 [2.64]	1.78 [2.56]	1.89 [2.45]	0.294, 0.148
IgG	4.29 [5.76]	3.86 [5.37]	4.23 [6.07]	4.59 [6.71]	0.072, <b>0.023</b>

Bold indicates  $P < 0.05$ .

ACS, acute coronary syndrome; CAD, coronary artery disease; EU, ELISA unit; Ig, immunoglobulin; IQR, interquartile range; *P.e.*, *Porphyromonas endodontalis*.

<sup>a</sup>Score 1: no radiographically verified endodontic lesions (includes edentulous subjects); score 2:  $\geq 1$  widened periapical space and/or 1 apical rarefaction; score 3:  $\geq 2$  apical rarefactions.

<sup>b</sup> $\chi^2$  test for categorical variables. Kruskal-Wallis test and Jonckheere-Terpstra test for continuous variables.

<sup>c</sup>Compared with the absence of subgingival *P. endodontalis*.

<sup>d</sup>Highest vs. lower 2 tertiles.

<sup>e</sup>Count  $\times 105$ . No values for edentulous ( $n = 32$ ) subjects.

## Discussion

We demonstrated an association between ELs and cardiologic outcomes, especially ACS, which was independent of periodontitis and its risk factors. The association between apical rarefactions and ACS was more evident in patients with untreated ELs. ELs were associated with subgingival *P. endodontalis* and corresponding serum IgG levels, which

illustrates the imbalance in the oral biofilm and subsequent systemic immunologic response that may link these 2 diseases.

Widened periapical spaces reflect either symptomatic teeth with irreversible pulpitis or precursors for established ELs in necrotic teeth (Carrotte 2004). In our study, the widened periapical spaces were more likely to reflect early apical lesions while patients were not actively seeking dental treatment. We combined the data of widened periapical spaces and apical

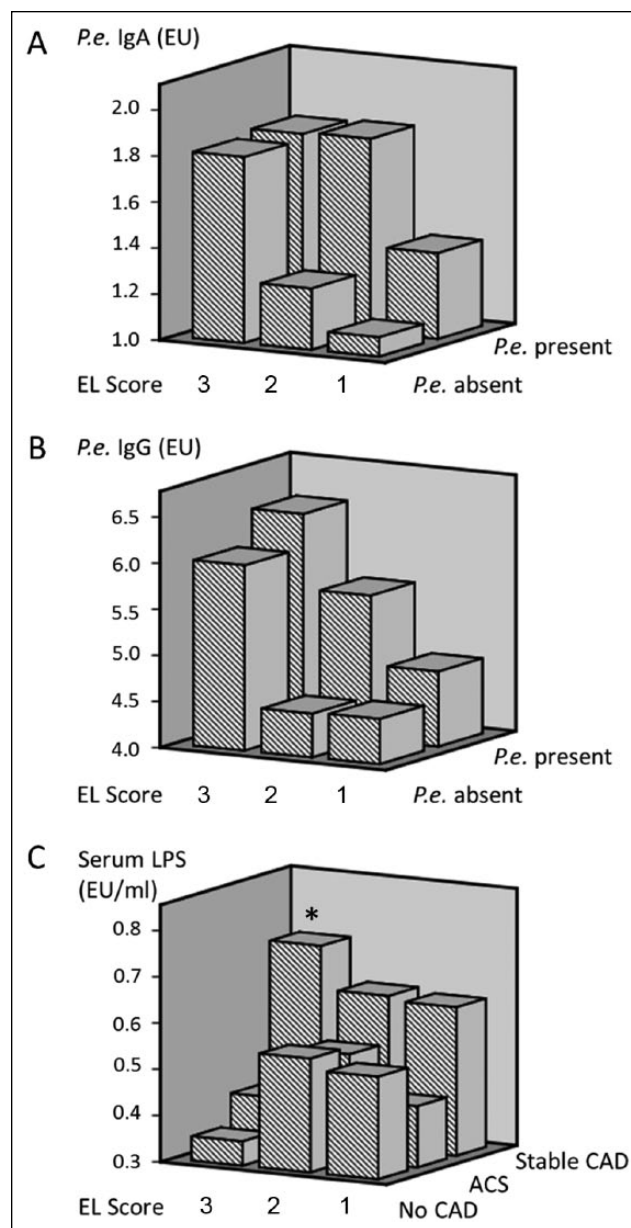
rarefactions in the EL score to simultaneously study 2 stages of the same disease entity. The group with  $\geq 2$  apical rarefactions (score 3) had a significant 131% to 149% increased OR for ACS even after extensive adjustment for confounders.

Although some studies have resulted in unconvincing results (Frisk et al. 2003), most have shown a significant association between ELs and CVD (Caplan et al. 2006; Joshipura et al. 2006; Caplan et al. 2009; Pasqualini et al. 2012; Petersen et al. 2014; Costa et al. 2014; Gomes et al. 2016). Thus, it seems plausible that ELs associate with an increased risk for CAD. Petersen et al. (2014) showed that ELs, in particular without root canal treatment, associate with the atherosclerotic burden of the abdominal aorta. In our study, having  $\geq 1$  untreated apical rarefactions was associated with ACS, with ORs ranging from 2.72 to 2.91 depending on the level of adjustments, while a respective finding with endodontically treated teeth did not. Our findings support the hypothesis that endodontic treatment might attenuate the association between ELs and CAD. To our knowledge, these are the first studies to separate treated and untreated teeth when analyzing the association between ELs and CAD.

Three models were applied in the multinomial logistic regression analyses, and the adjustments were selected due to established risk factors. Missing teeth have frequently been associated with CVDs and were included in the multiaadjusted model (Hung et al. 2004; Watt et al. 2012; Liljestrand et al. 2015). We chose to include ABL in the model as a proxy for marginal periodontitis, as it is a potential confounder and has been frequently ignored in previous studies investigating ELs and CVD. ABL and missing teeth were both associated with ELs in this study. Since ELs consistently associate with CAD after adjustment for marginal periodontitis, it might be an independent risk factor for CAD and should be accounted for in future research (Gomes et al. 2013).

In our study, the level and presence of subgingival *P. endodontalis* were associated with our score for ELs, also after adjustments for marginal periodontitis. Our findings support the hypothesis that *P. endodontalis* is a significant factor in the progression of ELs and has biomarker potential for a pathologic endodontic and periodontal biofilm community (Martinho et al. 2010; Rôças et al. 2011). We showed a statistically significant association between the presence of subgingival *P. endodontalis* and serum IgG/IgA against it, demonstrating bacteremia and systemic immunologic response. Furthermore, high levels of serum IgG, but not IgA, against *P. endodontalis* correlated with our scores for ELs. IgG is more stable than IgA, thus making it a more appealing antibody for clarifying a history of infection. In patients with ELs, the major immunoglobulin against *P. endodontalis* antigens produced at the site is IgG (Ogawa et al. 1992). Therefore, ELs might lack the nature of repeated or recent antigen exposure, which is required for elevated *P. endodontalis* IgA levels. To our knowledge, this study is the first to assess associations between *P. endodontalis*-specific antibodies and ELs. Clearly, the serology associated with ELs requires further attention.

Bacterial-derived LPS is one of the main inducers of systemic inflammation, and endotoxemia is associated with future



**Figure.** Levels of serum *P. endodontalis* antibodies and lipopolysaccharides. **(A, B)** Median levels of serum immunoglobulin A and G (IgA and IgG) against *Porphyromonas endodontalis* across groups of endodontic lesions (ELs) and present subgingival *P. endodontalis*. **(C)** The median levels of serum lipopolysaccharide across groups of ELs and cardiologic outcome. The differences across groups were analyzed with a Kruskal-Wallis test across all subgroups separately. Statistical significance among the coronary artery disease groups in EL score 3 is depicted with an asterisk (\* $P < 0.05$ ). ACS, acute coronary syndrome; EU, ELISA unit; LPS, lipopolysaccharide; *P.e.*, *Porphyromonas endodontalis*.

CVD events (Pussinen et al. 2007). Potential mechanisms linking oral infections to CVDs include bacteremia and endotoxemia (Pussinen and Könönen 2016). We analyzed these components considering subgingival *P. endodontalis* as a putative antigen, with serum antibodies and LPS reflecting systemic exposure. Median serum LPS activities were associated with CAD outcome but not with *P. endodontalis* or ELs. Taken

together, we failed to show that bacteremia or endotoxemia deriving from this single species would mediate the association between ELs and CAD. Therefore, further studies are needed, including a proper characterization of root canal biofilm and systemic response to it.

There is a high variability among determinations of CAD outcomes (Cotti and Mercurio 2015). Our data stand out, with an extensive set of oral parameters and an accurate angiographically verified cardiologic diagnosis as the outcome. In addition, panoramic tomographies have been dependable for diagnosing ELs in comparable studies (Gomes et al. 2016).

The main limitation of our study is the cross-sectional setup; hence, conclusions of causality cannot be established. The patients were limited to those with an initial indication for coronary angiography (e.g., stable and atypical chest pain, valvular heart disease, or cardiomyopathy), which may lead to selection bias without healthy subjects. Patients with cardiac complications may exhibit unhealthier behavior reflecting on oral health in general. However, our population (aged 33 to 82 y,  $62.1 \pm 10.1$ ) was not limited to any specific age group. Although our analyses were statistically adjusted, residual confounding cannot be ruled out. For instance, we did not have the information on socioeconomic status. While all Finnish adults have the same prerequisites for adequate treatment, the role of socioeconomic status as confounder is diminished. Also, we had no information available on the time when the teeth with root canal fillings had been treated, since they may have been in a healing phase or represented persistent or secondary endodontic infections with a slightly different microbial flora (Siqueira and Rôças 2009a). Furthermore, no information was available on the endodontic microflora, as discussed above.

There seems to be a paradigm shift in the field of endodontics—from a field of pain management, tooth preservation, and control of infections toward a perspective where all oral infections are risks for systemic complications (Han and Wang 2013). Further studies are needed to evaluate if ELs are a true risk factor for CVD, if other pathogens and their humoral responses mediate systemic effects, and if treatment for ELs reduces the risk for future CVDs.

## Conclusion

Our findings support the hypothesis that ELs associate independently with CAD and in particular ACS, consolidating the role of oral conditions in evaluating total cardiovascular risk. Subgingival *P. endodontalis* and high levels of corresponding serum IgG associate with EL. The potential association between ELs and CVDs is of high interest given the high prevalence of both diseases.

## Author Contributions

J.M. Liljestrand, contributed to conception, design, data acquisition, analysis, and interpretation, drafted the manuscript; P. Mäntylä and S. Paju, contributed to conception, design, data acquisition, analysis, and interpretation, critically revised the

manuscript; K. Buhlin, contributed to design and data acquisition, critically revised the manuscript; K.A.E. Kopra, contributed to data acquisition and interpretation, critically revised the manuscript; G.R. Persson, contributed to data analysis and interpretation, critically revised the manuscript; M. Hernandez, contributed to data interpretation, critically revised the manuscript; M.S. Nieminen and J. Sinisalo, contributed to conception and design, critically revised the manuscript; L. Tjäderhane, contributed to data acquisition and interpretation, critically revised the manuscript; P.J. Pussinen, contributed to conception, design, data acquisition, analysis, and interpretation, critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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