Carotid artery calcification in panoramic radiographs associates with oral infections and mortality

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Abstract

Aim To study the prevalence of carotid artery calcification (CAC) in relation to apical and marginal periodontitis, subgingival dysbiotic bacterial species and serum and saliva immune responses against them. In addition, the aim was to analyze the association of CAC with angiographically verified coronary artery disease (CAD) and mortality.

Methodology In the present random Parogene cohort, the patients had an indication for coronary angiography. Apical and marginal periodontitis were diagnosed in clinical and radiographic oral examinations, and CAC on panoramic radiographies (n=492). Presence and severity of CAD were registered from angiography. Subgingival dysbiotic bacterial species were quantitated using checkerboard DNA-DNA-hybridization, and serum and saliva antibody levels were determined by immunoassays. The cohort was followed-up for 10 years or until death (median 9.9, range 0.21-10.4) via linkage to the national death register. The statistical models were adjusted for age, gender, smoking, hypertension, diabetes, and dyslipidemia.

Results A total of 102 (20.7%) patients had detectable CAC, which was moderate in 81 (16.4%) and severe in 21 (4.3%). CAC was associated (OR, 95% CI) with severe apical periodontitis (2.25, 1.15-4.41), root canal fillings (1.15, 1.04-1.26), alveolar bone loss (2.66, 1.21-5.84), severe periodontal inflammation (2.23, 1.11-4.47), high level of gram-negative subgingival species (2.73, 1.34-5.50), saliva IgG against dysbiotic species (1.05, 1.01-1.10 / unit), and severe (2.58, 1.36-4.90) and chronic (2.13, 1.15-3.93) CAD. A total of 105 (20.7%) patients died during the follow-up and 53 (10.4%) deaths were because of cardiovascular diseases (CVD). Severe CAC predicted worse survival with HRs (95% CI) of 3.08 (1.58-6.06) for all-cause and 3.43 (1.42-8.25) for CVD death.

Conclusions CAC on panoramic tomography was associated with i) apical and marginal periodontitis and dysbiotic bacterial species giving rise to an immunological response, and with ii) severe, chronic CAD and increased mortality. The results further emphasize the role of oral infections in CAD and the importance of referring a patient with CAC for a cardiovascular evaluation.

Introduction

Carotid artery calcification (CAC) can be identifiedand even gradedon panoramic dental radiographs of the maxilla and medulla (Friedlander 1995). Just as with increased carotid artery intima-media thickness, CAC may be a sign of the broader condition of atherosclerosis. In case-control studies, patients with CAC were more likely to demonstrate extended calcifications in abdominal aorta (Friedlander *et al.* 2015), aortic arch (Friedlander *et al.* 2014), and kidneys (Yeluri et al. 2015). Furthermore, patients with CAC suffer more often from stroke and ischemic heart disease (Bengtsson *et al.* 2019). According to a meta-analysis, calcifications observed with CT-scans, plain radiography, or ultrasound predict cardiovascular events and mortality (Rennenberg *et al.* 2009). In literature reviews, panoramic radiographs had a variable sensitivity, but acceptable specificity for detecting CAC (Khosropanah *et al.* 2009, Alves *et al.* 2014, Bengtsson *et al.* 2014). Therefore, the patients with CAC on dental radiographs should be referred for cardiovascular evaluation (Friedlander & Cohen 2007, Gustafsson *et al.* 2018, Bengtsson *et al.* 2019).

The association of marginal periodontitis with several systemic conditions, such as cardiovascular diseases (CVD), is well-established (Lockhard *et al.* 2012). Apical periodontitis has also been regarded as a CVD risk factor (Liljestrand *et al.* 2016, Khalighinejad *et al.* 2016, Berlin-Broner *et al.* 2017). These two oral diseases share inflammatory, immunological, and microbiological profiles (Gomes *et al.* 2015, Pietiäinen *et al.* 2019) which link them to atherosclerosis through systemic inflammation (Hansson *et al.* 2015).

Marginal periodontitis has been associated with CAC in multiple studies (Ravon *et al.* 2003, Beckström *et al.* 2007, Kamak *et al.* 2015, Bengtsson *et al.* 2016, Gustafsson *et al.* 2020), while some studies suggest no significant association (Nakib *et al.* 2004). No studies regarding putative association between CAC and dysbiotic oral bacterial species or immune responses against them have been published. In addition, studies investigating the association between CAC and apical periodontitis or follow-up studies of patients with or without CAC in dental radiographs are scarce.

The aim of the study was to investigate the prevalence of CAC visible on panoramic radiographs in a random cohort of patients undergoing coronary angiography. The following hypotheses were investigated: i) In a cross-sectional setting, CAC is associated with oral infections/inflammations, dysbiotic oral bacteria and immune response against them. ii) CAC is associated with diagnosis and severity of coronary artery disease (CAD). iii) In a longitudinal setting, CAC and mortality are associated in a 10-year follow up.

Materials and Methods

Study design

Parogene, is a subpopulation of the prospective Corogene cohort which aims to identify coronary disease risk factors and genetics. The study is based on symptomatic Finnish patients (n=5,788), who underwent a coronary angiography for any reason in Helsinki University Hospital between June 2006 and March 2008 (Vaara *et al.* 2012). The data includes information on previous medical conditions, CVD risk factors, medications, and coronary angiogram results. A random subpopulation of approximately 10% of the cohort, forming the Parogene sample, was invited to a clinical and radiographic oral examination.

The cohort has been followed-up for 10 years or until death (median 9.9, IQR 0.45, range 0.21-10.4 years) with data on deaths being obtained through record linkage to the National Causes-of-Death Register. Death due CVD was defined based on ICD-10 codes with the underlying, direct, or contributing cause of death as I00-I99. This research has been conducted in full accordance with ethical principles including the Declaration of Helsinki. The Helsinki University Hospital ethics committee approved the study protocol (Dnro 106/2007), and all patients signed an informed consent. The study complies with 'strengthening the reporting of observational studies in Epidemiology' (STROBE) guidelines.

Study population

The Parogene study included 508 patients who were examined at the Institute of Dentistry, University of Helsinki, between October 2007 and May 2008 (Buhlin *et al.* 2011). Clinical and radiographical oral examinations were performed at least 6 weeks but no later than 5 months after the angiography. Presence or absence of carotid artery calcification (CAC) could be technically registered from 492 (96.8% of the entire population) patients. Based on the angiography, symptoms and cardiac biomarkers, patients were assigned to groups of chronic CAD (n=181, 36.8%), acute coronary syndrome (ACS) (n=164, 33.3%), and no significant CAD (n=147, 29.9%). Severity of CAD was defined as a number of arteries with ≥50% stenosis. The patients were considered to have hypertension, dyslipidemia or diabetes, if they had medications for these disorders. Information on smoking habits were collected by a questionnaire before the oral examination.

Clinical oral examinations

Clinical examination for detailed oral and periodontal status was performed in a standard dental office setting by two specialists in periodontology (P. M. and K. B.) (Buhlin *et al.* 2011). They had been inter-individually calibrated before the study. Extra oral and intra oral palpation was done to detect swelling or firmness, and oral structures were viewed for any abnormal findings, such as sinus tracts. Periodontal probing depths (PPD) were measured from six sites around each tooth with a manual periodontal probe and numbers of deepened periodontal pockets of 4-5 mm and ≥6 mm were recorded. Periodontal inflammation burden index (PIBI) was calculated as [number of 4-5 mm pockets + 2*(≥6 mm pockets)] (Lindy *et al.* 2008). Bleeding on probing (BOP) and possible suppuration were recorded from four sites of each tooth. PPD and BOP were calculated as proportions of sites examined.

Radiographic oral examination

Digital panoramic tomographs were taken after the clinical oral examination using ProOne unit (Planmeca Co., Helsinki, Finland). A single specialist of dental radiology analyzed the radiologic data including the prevalence of carotid artery calcification (CAC). CAC was differentiated from other

calcified soft tissues and cartilage as reported earlier (Carter 2000). Calcification of any size and shape as a radiopaque nodular mass adjacent to or just below the intervertebral space between C3 and C4 in the carotid artery area was registered as CAC. Calcification could be present in left, right or both sides simultaneously and appear single or scattered (Friedlander 1995, Garoff *et al.* 2016). Heterogeneous areas of radiopacities on either side were graded as 'moderate CAC' and masses exceeding 10 mm as 'severe CAC' (Ravon *et al.* 2003).

The tooth with the most severe attachment loss was chosen from each dentate sextant for calculations of horizontal alveolar bone loss (ABL). According to the mean of each sextant, ABL was graded: i) no ABL; ii) mild, ABL in cervical third of the root; iii) moderate, ABL in the middle third of the root; and iv) from severe to total loss, ABL from the apical third of the root to total ABL.

Endodontic lesions were diagnosed from the radiographs as described in detail earlier (Liljestrand *et al.* 2016). The recorded findings included root canal fillings, widened periapical space indicating irreversible pulpitis or precursors for endodontic lesions (Carrotte 2004), and apical periodontitis seen as periradicular destruction in the tip of the root. An endodontic lesion score was defined to describe the severity of apical periodontitis in dentate patients (Liljestrand *et al.* 2016): i) no endodontic lesions; ii) moderate apical periodontitis, if ≥1 widened periapical space (Huumonen *et al.* 2017) and/or 1 tooth with apical periodontitis was present; and iii) severe apical periodontitis in patients with ≥2 teeth with apical periodontitis. Treatment of apical periodontitis was considered by dividing the patients in groups: i) no endodontic lesions; ii) all endodontic lesions in teeth with root canal filling; and iii) untreated apical periodontitis (Liljestrand *et al.* 2016).

Subgingival bacterial sampling, and bacterial and antibody analyses

Pooled subgingival samples were taken from dentate subjects from the deepest pathologic periodontal pocket in each dentate quadrant as previously described (Mäntylä *et al.* 2013, Pradhan-Palikhe *et al.* 2013). In patients with no pockets exceeding 3 mm depth, the deepest possible site or site with most visible inflammation was selected for sampling. The checkerboard DNA–DNA hybridization assay for 78 oral bacterial species was performed as previously reported (Socransky

et al. 2004, Pradhan-Palikhe *et al.* 2013). The detected species are given in a supplementary table (Table S1). The species were divided into gram-positive (n=45) and gram-negative (n=33) (Pietiäinen *et al.* 2019). The bacterial levels were logarithmically transformed and summed up.

The serum and saliva IgA- and IgG-class antibody levels against periodontitis-associated dysbiotic species were determined by ELISA and as described previously (Pussinen *et al.* 2002,2011, Akhi *et al.* 2017, Liljestrand *et al.* 2018). All together, 13 strains representing five species were used as antigens. The strains and the laboratory quality information are presented in Table S2.

Statistical analysis

The significance of the trends observed between patients classified according to the presence and severity of CAC were analyzed by Chi-square, ANOVA or Jonckheere-Terpstra. The association between the presence of CAC and CAD diagnosis, parameters from clinical and radiographical oral examination, bacterial and antibody data were analyzed with multiple logistic regression model adjusted for age, gender, dyslipidemia, diabetes, hypertension, and smoking (never/ever). Cox regression models were used to calculate the prediction models for cumulative survival in groups of CAC severity. The outcomes were CVD death and all-cause death and the models were adjusted for age, gender, dyslipidemia, diabetes, hypertension, and smoking (never/ever).

Results

Characteristics of the study population (N = 492) are shown in Table 1. A total of 102 (20.7%) patients had detectable CAC in the panoramic radiographs; in 81 (16.4%) patients the CAC was moderate and in 21 (4.3%) severe. Age, hypertension, and smoking increased significantly with more severe CAC. Clinical, radiographic, and biochemical characteristics stratified according to CAC severity are presented in Table 2. While number of remaining teeth decreased, widened periapical spaces, apical rarefactions, root canal fillings, alveolar bone loss, periodontitis, and high level of gram-negative subgingival bacterial species increased with more severe CAC.

Frequencies of CAC according to CAD diagnosis and severity are presented in Figure 1A. Patients with chronic CAD (28.1%) had more often CAC compared to patients with no CAD (12.8%) or with ACS (21.3%) (p=0.022). CAC severity correlated with CAD severity increasing from 12.0% in patients without stenosis into 16.0%, 24.7%, and 32.5% in patients with 1, 2, and 3 stenosed arteries, respectively (p=0.001) (Figure 1B). Those who died during the follow-up due to CVD (35.8%) or other cause (29.2%), had more frequently CAC at baseline than the survivors (17.5%) (p=0.003) (Figure 1C).

The association of CAC with measured parameters was examined using logistic regression adjusted for age, gender, dyslipidemia, hypertension, diabetes, and smoking (Table 3). Presence of CAC was associated with mild and moderate to total alveolar bone loss, severe periodontal inflammation, severe apical periodontitis, root canal fillings, high level of gram-negative subgingival species, saliva IgG against dysbiotic species, severe CAD with stenosed arteries, and chronic CAD.

A total of 105 (20.7%) of patients died during the 10 years of follow-up and 53 (10.4%) deaths were because of CVD. Predicted cumulative survival of patients with moderate or severe CAC was analysed by using Cox regression adjusted for age, gender, dyslipidemia, hypertension, diabetes, and smoking (Figure 2). Moderate and severe CAC were associated with all-cause death with HRs (95% CI, p) of 1.52 (0.95-2.54, 0.097) and 3.08 (1.58-6.06, 0.001), respectively. Severe CAC was associated with CVD-death with an HR of 3.43 (1.42-8.25, 0.006).

Discussion

In this relatively large population, it was possible to demonstrate associations of apical periodontitis, dysbiotic oral species, and immune response against them with the presence of CAC in panoramic dental radiographs. In addition, CAC, registered from dental radiographs, is associated with chronic, severe coronary artery disease and predicts increased risk for cardiovascular and all-cause mortality. The associations were independent of established CVD risk factors, including age, sex, hypertension, dyslipidemia, diabetes, and smoking.

Overall, 21% of patients in this cohort had detectable CAC, and in most patients (16%) the finding was moderate, while in fewer patients (4.3%) it was severe. The frequency of CAC was 21-28% in patients with coronary artery disease, which is lower than reported earlier in populations with medical conditions such as a recent stroke or AMI (34-37%) (Friedlander *et al.* 1994, Gustafsson *et al.* 2020) or dilated cardiomyopathy (33%) (Sung *et al.* 2004), but higher than in patients with type 2 diabetes (20%) (Friedlander & Maeder 2000), systemic risk factors (15%) (Friedlander & Golub 2006) or renal diseases (15-17%) (Kansu *et al.* 2005). The prevalence of CAC in panoramic images in an asymptomatic middle-aged population is in the range of 2–5% (Mupparapu & Kim 2007), while in this study population 12.8% of patients without significant CAD had detectable CAC. This prevalence is higher than the CAC observed in general population, because the population consisted of elderly patients seeking medical care for heart-related symptoms. However, this prevalence is considerably lower than in a recent study, where 28% of apparently healthy, elderly controls, who did not undergo angiography, had CAC (Gustafsson *et al.* 2020). In the present cohort, CAC was significantly associated with chronic CAD affecting all coronaries, while the association with ACS was not significant. The observations are in line with a systematic review, where calcification measured with conventional modalities was usually found in more advanced lesions (Rennenberg *et al.* 2009).

Only a few studies have previously investigated associations between apical periodontitis and CAC. Friedlander *et al.* (2010) stated that CAC patients (n=36) had more radiological signs of oral infections compared to controls (n=36), although the statistical significance was diluted when periapical lesions were regarded separately in this rather small population (Friedlander *et al.* 2010). Another case-control study, including 20-40 -year-old males without advanced tooth loss, marginal periodontitis or major CVD risk-factors, found that patients with apical periodontitis (n=60) had a significantly higher ultrasonically verified carotid artery intima-media thickness than controls (n=60) (Chauhan *et al.* 2019). The present study is unique with a detailed radiological characterization of the endodontic status, and the results support an association between apical periodontitis and CAC.

Vigorous research is currently conducted regarding potential associations between apical periodontitis and CVD. Two recent systematic reviews and one meta-analysis all state similar conclusions: most studies suggest that apical periodontitis is an independent CVD risk-factor, although the level of evidence is low─moderate and biased by significant heterogeneity in design (Khalighinejad *et al.* 2016, Berlin-Broner *et al.* 2017, Aminoshariae *et al.* 2018). A positive cross-sectional association has been proposed for apical lesions and CAD, defined *inter alia* as hospital diagnoses (Caplan *et al.* 2006, Pasqualini *et al.* 2012), computed tomography (Petersen *et al.* 2014), or coronary angiography (Costa *et al.* 2014, Liljestrand *et al.* 2016). The present study shows an independent association between severe apical periodontitis and CAC further suggesting a role of endodontic infections in atherosclerosis.

The oral cavity represents a point of entry into the blood circulation for pathogenic bacteria and their virulence factors. The antimicrobial strategies against microbial antigens include innate and adaptive immune responses and oxidative stress (Hernandez-Rios *et al.* 2017, Pietiäinen *et al.* 2019). Although the exact mechanisms are unclear, oral pathogens may mediate systemic inflammation contributing to the development of atherosclerosis (Pietiäinen *et al*. 2018). Increased amounts of salivary or subgingival periodontal pathogens and high levels of local and systemic antibodies produced against them are associated with the increased risk of both subclinical atherosclerosis and CAD (Pussinen *et al.* 2005, Desvarieux *et al.* 2005, Liljestrand *et al.* 2018). Infected root canals habit polymicrobial communities (Siqueira & Rôças 2009), and similarly to the marginal periodontitis, the abundance of gram-negative species increase in endodontic lesions and surrounding tissues compared to the healthy situation (Dingsdag *et al.* 2016, Qian *et al.* 2019). The inflamed tissues produce high concentrations of local immunoglobulins, IgG and especially IgA (Greening & Schönfeld 1980, Torres *et al.* 1994), and most studies suggest at least a modest contribution of endodontic infections to the systemic inflammatory markers (Gomes *et al.* 2013, Garrido *et al.* 2019). It has been demonstrated that increasing amounts of gram-negative bacteria in subgingival plaque are associated with the severity of endodontic findings and that high levels of salivary IgG against dysbiotic bacteria are associated with apical periodontitis independently of marginal periodontitis

(Pietiäinen *et al.* 2019). High levels of serum antibodies binding to *P. endodontalis* have also been demonstrated in patients with endodontic lesions (Liljestrand *et al.* 2016). In the present study, CAC is associated with high levels of gram-negative subgingival species and saliva IgG against dysbiotic species. This supports the current view that oral pathogens may have an impact on the systemic atherosclerosis risk. However, no significant association between CAC and serum IgG against dysbiotic species was found. Thus, the possible link between immune responses to oral pathogens and CAC remains to be elucidated.

The strengths of the study include detailed characterizations of the population and the long follow-up. Another follow-up study found an association between CAC and incident stroke and/or ischemic heart diseases (Bengtsson *et al.* 2019). Despite the general nature of detecting CAC in the present study and differences in effect sizes, the hazards for all-cause and CVD mortality were of the same magnitude (3.08 and 3.94) as in a systematic review (Rennenberg *et al.* 2009). In this review the presence of calcifications detected with any modality presented odds of 3.41 (95% CI 2.71-4.30) for any cardiovascular event, 4.62 (2.24-9.53) for all-cause mortality, and 3.94 (2.39-6.50) for CVD mortality (Rennenberg *et al.* 2009). The main limitation of the present study is that important features of carotid plaque, such as morphology (soft, echogenic, hard, irregular), precise location (adventitia, media), composition (stable, unstable), or degree of stenosis were not possible to determine. Moreover, it was not known whether the patients were symptomatic or not due to the presence of CAC. The cohort is composed of symptomatic patients undergoing coronary angiography and thus, the frequencies of positive CAC findings may be higher than in the general population. Further, endodontic disease was assessed only radiographically and the teeth with widened periapical spaces were not examined further.

Conclusions

Both dental and medical professionals should pay be aware of the potential of an association between CAC and oral infections. Physicians should suggest dental examination to their cardiovascular patients since oral infections, dysbiotic bacteria, and immune response are

associated with CAC. If CAC is visible in panoramic tomography during a dental examination, the patient should be referredfor cardiovascular evaluation, since CAC may cause further diseases and severe complications predisposing to death.

Conflict of interest statement

The authors have nothing to declare.

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Figure legends

Figure 1. Frequencies of carotid artery calcification (CAC) according to A) CAD diagnosis, B) CAD severity in coronary angiography, and C) death in the follow-up. The p-values are calculated by Chi-square test.

Figure 2. Predicted cumulative survival of the patients from A) all-cause and B) CVD death in the follow-up of 10 years (median 9.9, IQR 0.45, range 0.21-10.4). The survival curves are produced by Cox regression models adjusted for age, gender, hypertension, dyslipidemia, diabetes, and smoking (never/ever) and the HR with p-value is shown for patients with moderate and severe CAC compared to those without.

Table 1. Characteristics of subjects with and without carotid artery calcification (CAC).

Variable		No CAC N=390	Moderate CAC N=81	Severe CAC N=21	
			Mean (SD)		**p-value [1]**
Age (years)		62.6 (9.4)	66.7 (7.3)	66.5 (7.5)	**<0.001**
BMI (kg/m^2)		27.8 (5.0)	28.1 (5.3)	29.0 (4.9)	0.298
			Median (IQR)		**p-value [2]**
hs-CRP (mg/l)[3]		2.09 (5.74)	2.70 (8.95)	3.00 (14.2)	0.432
			N (%)		**p-value [4]**
Gender	Males	260 (66.2)	53 (65.4)	11 (52.4)	0.318
Dyslipidemia		313 (80.7)	65 (80.2)	18 (85.7)	0.727
Hypertension		237 (60.8)	62 (77.5)	16 (76.2)	**0.005**
Diabetes		90 (23.2)	19 (24.1)	6 (28.6)	0.612
Smoking	Ever	195 (49.6)	49 (60.5)	14 (66.7)	**0.026**

[1] ANOVA, weighted linear terms; [2] Jonckheere-Terpstra test; [3] high-sensitivity C-reactive protein; [4] Chi-square test, linear-by-linear association

Table 2. Clinical, radiographic, and laboratory results of patients with and without carotid artery calcification (CAC).

Variable		No CAC N=390	Moderate CAC N=81	Severe CAC N=21	
		Mean (95% CI)			p-value[1]
Number of teeth		21.5 (20.8-22.3)	20.8 (19.2-22.4)	18.6 (15.4-21.7)	**0.005**
PPD 4-5 mm (%)		12.9 (11.6-14.3)	15.1 (11.8-18.3)	14.7 (7.7-21.7)	0.178
PPD ≥ 6 mm (%)		3.24 (2.42-4.06)	3.48 (1.61-5.35)	7.15 (0.33-14.0)	0.357
BOP (%)		37.5 (35.5-39.4)	37.3 (33.0-41.5)	41.4 (32.2-50.5)	0.602
Carious teeth		1.01 (0.84-1.18)	0.97 (0.58-1.36)	1.06 (0.27-1.85)	0.884
Widened periapical space		0.71 (0.62-0.79)	1.10 (0.83-1.37)	1.30 (0.79-1.81)	**<0.001**
Apical rarefactions		0.34 (0.24-0.43)	0.48 (0.27-0.69)	0.50 (0.03-0.97)	**0.048**
Root canal fillings (RCF)		2.02 (1.81-2.24)	2.99 (2.30-3.67)	2.35 (1.49-3.21)	**0.022**
Saliva IgA against dysbiotic species (rlu/100 ms / 10^5)		3.48 (3.23-3.72)	4.16 (3.32-5.00)	3.75 (2.42-5.08)	0.200
Saliva IgG against dysbiotic species (rlu/100 ms / 10^5)		4.12 (3.57-4.66)	5.58 (3.95-7.22)	6.09 (3.79-8.36)	0.296
Serum IgA against dysbiotic species (EU)		3.24 (3.02-3.47)	3.51 (2.99-4.03)	3.29 (2.46-4.12)	0.365
Serum IgG against dysbiotic species (EU)		4.47 (4.23-4.71)	4.66 (4.09-5.22)	4.53 (3.50-5.55)	0.601
			N (%)		p-value[2]
Alveolar bone loss	None	103 (27.5)	8 (10.8)	0 (0)	**0.001**
	Mild	160 (42.7)	41 (55.4)	10 (50.0)	
	Moderate to total	112 (29.9)	25 (33.8)	10 (50.0)	

Periodontal inflammation[3]	Low	95 (24.9)	13 (17.3)	4 (20.0)	0.127
	Mild	100 (26.2)	20 (26.7)	5 (25.0)	
	Moderate	95 (24.9)	18 (24.0)	5 (25.0)	
	Severe	91 (23.9)	24 (32.0)	6 (30.0)	
Severity of apical periodontitis	No endodontic lesions	147 (42.0)	27 (38.0)	6 (30.0)	**0.048**
	Moderate	158 (45.1)	26 (36.6)	10 (50.0)	
	Severe	45 (12.9)	18 (25.4)	4 (20.0)	
Treatment of apical periodontitis	No endodontic lesions	281 (80.3)	38 (10.9)	31 (8.9)	0.060
	All with RCF	50 (70.4)	12 (16.9)	9 (12.7)	
	Untreated apical periodontitis	15 (75.0)	1 (5.0)	4 (20.0)	
Level of gram-positive subgingival species	Below median	171 (51.4)	28 (43.1)	9 (47.4)	0.318
	Above median	162 (48.6)	37 (56.9)	10 (52.6)	
Level of gram-negative subgingival species	Below median	175 (52.7)	25 (38.5)	6 (31.6)	**0.009**
	Above median	157 (47.3)	40 (61.5)	13 (68.4)	

[1] Jonckheere-Terpstra test for linear trend; [2] Chi-square test, linear-by-linear association; [3] PIBI (periodontal inflammatory burden index) divided into quartiles

Table 3. Association between presence of carotid artery calcification (CAC) and periodontal, microbiological, and serological parameters.

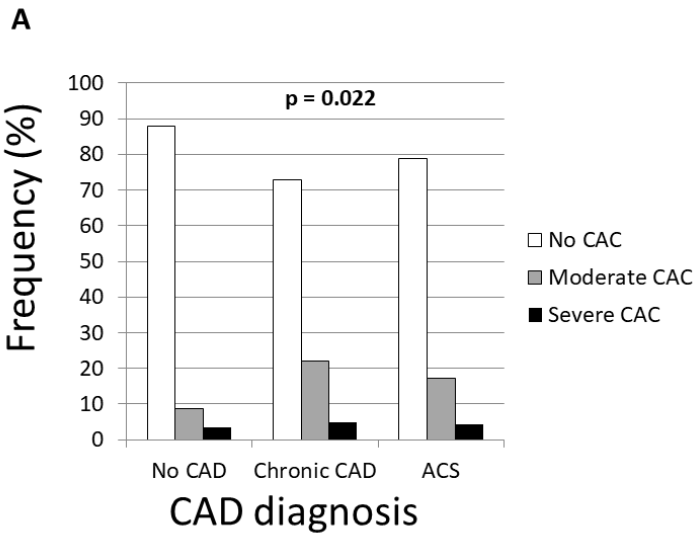
Logistic regression model	Odds ratio (95% CI)
Confounders	
Age (years)	**1.06 (1.030-1.091)**
Male gender	0.82 (0.502-1.334)
Dyslipidemia	0.76 (0.411-1.419)
DM	0.84 (0.485-1.451)
Hypertension	**2.28 (1.315-3.963)**
Smoking (ever)	**1.92 (1.179-3.121)**
Model 1	
Alveolar bone loss	
None	1.00
Mild	**2.81 (1.251-6.305)**
Moderate to total	**2.44 (1.043-5.708)**
Model 2	
Root canal fillings	**1.15 (1.042-1.262)**
Model 3	
Periodontal inflammation (PIBI)	
Low	1.00
Mild	1.69 (0.832-3.424)
Moderate	1.53 (0.745-3.155)
Severe	**2.23 (1.110-4.473)**
Model 4	
Severity of apical periodontitis	
No endodontic lesions	1.00
Moderate	1.10 (0.635-1.887)
Severe	**2.25 (1.148-4.410)**
Model 5	
Treatment of apical periodontitis	

No endodontic lesions	1.00
Endodontic lesions with RCF	1.69 (0.826-3.467)
Untreated endodontic lesions	1.73 (0.819-3.645)
Model 6	
Level of gram-positive species	
Below median	1.00
Above median	0.80 (0.407-1.584)
Level of gram-negative species	
Below median	1.00
Above median	**2.73 (1.359-5.497)**
Model 7	
Saliva IgA against dysbiotic species (rlu/100 ms / 10^5)	1.05 (0.963-1.152)
Model 8	
Saliva IgG against dysbiotic species (rlu/100 ms / 10^5)	**1.05 (1.005-1.096)**
Model 9	
Serum IgA against dysbiotic species (EU)	0.98 (0.871-1.108)
Model 10	
Serum IgG against dysbiotic species (EU)	0.99 (0.883-1.103)
Model 11	
CAD severity in angiography	
None/mild	1.00
1 stenosed artery	1.45 (0.718-2.918)
2 stenosed arteries	1.72 (0.850-3.471)
3 stenosed arteries	**2.58 (1.356-4.902)**
Model 12	
CAD diagnosis	
No significant CAD	1.00

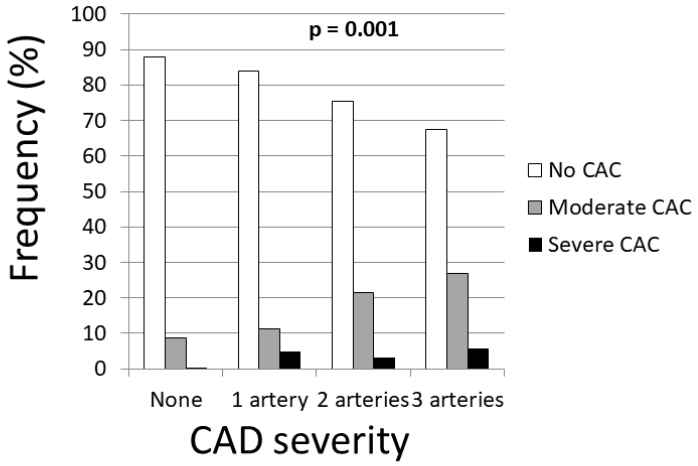
Chronic CAD	**2.13 (1.153-3.930)**
ACS	1.68 (0.893-3.177)

The twelve logistic regression models were adjusted for the covariates above (confounders). Significant effect sizes are bolded.

Figure 1 A - C


A
p = 0.022
Frequency (%)
100
90
80
70
60
50
40
30
20
10
0
No CAD
Chronic CAD
ACS
CAD diagnosis
No CAC
Moderate CAC
Severe CAC

B


p = 0.001
Frequency (%)
100
90
80
70
60
50
40
30
20
10
0
None
1 artery
2 arteries
3 arteries
CAD severity
No CAC
Moderate CAC
Severe CAC

C

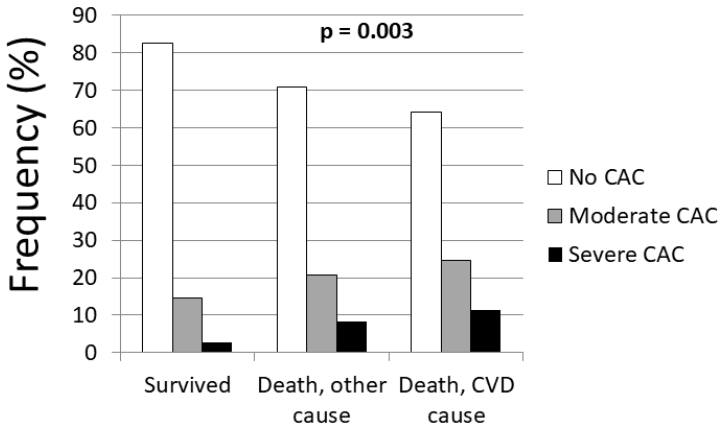
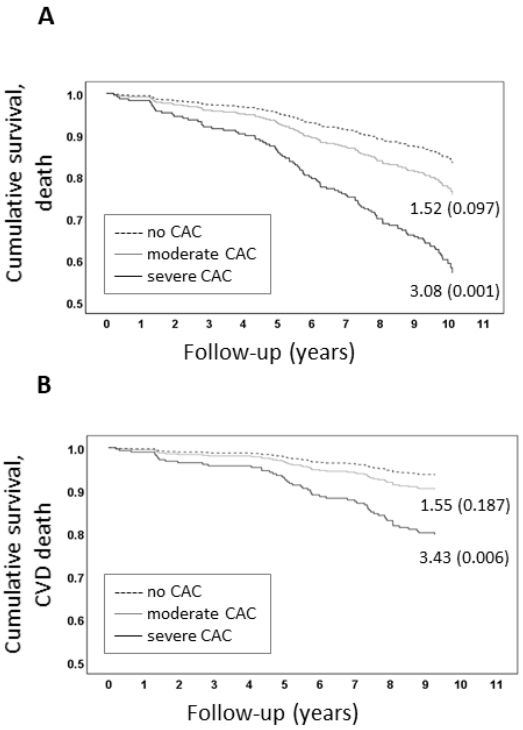

p = 0.003
Frequency (%)
90
80
70
60
50
40
30
20
10
0
Survived
Death, other cause
Death, CVD cause
No CAC
Moderate CAC
Severe CAC

Figure 2.


A
Cumulative survival, death
1.0
0.9
0.8
0.7
0.6
0.5
0 1 2 3 4 5 6 7 8 9 10 11
Follow-up (years)
----- no CAC
moderate CAC
severe CAC
1.52 (0.097)
3.08 (0.001)
B
Cumulative survival, CVD death
1.0
0.9
0.8
0.7
0.6
0.5
0 1 2 3 4 5 6 7 8 9 10 11
Follow-up (years)
----- no CAC
moderate CAC
severe CAC
1.55 (0.187)
3.43 (0.006)