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Lipopolysaccharide, a possible molecular mediator between periodontitis and coronary artery disease

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Abstract

Aim: We aimed to study how lipopolysaccharide (LPS) in saliva and serum associates with each other, periodontal microbial burden, periodontitis and coronary artery disease (CAD).

Materials and methods: The used Parogene cohort comprised $N = 505$ Finnish adults. Coronary diagnosis was acquired by coronary angiography, and the main outcomes were as follows: no significant CAD ($n = 123$), stable CAD ($n = 184$) and acute coronary syndrome ($n = 169$). Periodontitis was defined according to clinical and radiographic examinations. Levels for 75 strains of subgingival bacteria were determined by checkerboard DNA–DNA hybridization. Saliva and serum LPS activity was analysed by Limulus amebocyte lysate assay.

Results: The level of 11 bacterial strains, which were mainly oral and respiratory Gram-negative species, associated with salivary LPS levels in an age- and gender-adjusted linear regression. A total of 4.9% of the serum LPS, that is endotoxemia, variation was explainable by saliva LPS among patients with periodontitis ($n = 247$, $R^2 = .049$, Pearson's $r = .222$, $p < .001$). Endotoxemia associated with stable CAD in a confounder adjusted multinomial logistic regression model (OR 1.99, 95% CI 1.04–3.81, $p = .039$, 3rd tertile).

Conclusions: In particular in periodontitis patients, subgingival microbial burden contributes to endotoxemia. LPS is a possible molecular mediator between periodontitis and CAD.

KEYWORDS

acute coronary syndrome, cardiovascular diseases, dentistry, endotoxins, pathogen-associated molecular pattern molecules, saliva

1 | INTRODUCTION

Lipopolysaccharide (LPS), often referred to as endotoxin, is located in the outer membrane of most Gram-negative bacteria. LPS is a classical trigger for septic shock, although its potential role in the progression of several non-communicable diseases has lately attained awareness (Munford, 2016). Circulating LPS, or endotoxemia, leads to systemic low-grade inflammation and has been associated with increased risk for atherosclerosis and cardiovascular diseases (Kallio et al., 2015; Miller et al., 2009; Pussinen et al., 2007;

Wiedermann et al., 1999). The term metabolic endotoxemia describes a chronic twofold to threefold increase in circulating LPS and was first proposed as a risk factor for insulin resistance, obesity and diabetes (Cani et al., 2007).

Lipopolysaccharide is constituted by the hydrophobic lipid A, which is covalently bonded to a polysaccharide moiety. While lipid A is the most conserved region of LPS, the polysaccharide consists of more heterogeneous inner and outer cores and a repetitive glycan polymer, the O-antigen domain (Raetz & Whitfield, 2002). LPS is transported throughout the body with lipoproteins or LPS binding proteins (Hailman, Albers, Wolfbauer, Tu, & Wright, 1996; Piya, Harte, & McTernan, 2013). The deleterious systemic effects of LPS depend largely on host recognition of the lipid A moiety with TLR4 membrane receptors, which in turn triggers innate immune responses for transcription of inflammatory mediators (Patel, Shah, Ferguson, & Reilly, 2015). Subsequently, increased levels of reactive oxygen species, chemotactic and pro-inflammatory cytokines and adhesion molecules affect the leucocyte invasion in the atherosclerotic vascular wall (Stoll, Denning, & Weintraub, 2004).

Circulating LPS may originate from local sites of infection or directly via bloodborne bacteria (Munford, 2016). However, a majority of the systemic LPS is arguably translocated from the small intestine, especially in subjects with increased intestinal permeability. For example, obesity, a high-fat diet or an altered gut microbiome has been shown to alter the intestinal barrier (Neves, Coelho, Couto, Leite-Moreira, & Roncon-Albuquerque, 2013; Piya et al., 2013).

It is reasonable to claim that a significant part of endotoxemia is originated from the oral cavity, bearing in mind that the oral microbiome is diverse with over 600 species, saliva is more concentrated in bacteria than the small intestine (~10^9 CFU/ml vs. 10^{0-8} CFU/ml), approximately 1 litre of saliva is swallowed daily, and that LPS possibly retains its biologic activity in acidic conditions (Dewhirst et al., 2010; Erridge, 2011). Mice with orally inoculated *Porphyromonas gingivalis* infection display higher systemic LPS levels than the controls (Kuula et al., 2009). Several periodontal pathogens have proved invasive and been found from extra-oral sites, such as endarterectomy specimens, amniotic fluid, synovial fluid and organ abscesses (Han & Wang, 2013). From this perspective, it seems likely that periodontitis could contribute to endotoxemia, while one of the hallmark features of periodontitis is oral microbial shift towards Gram-negative dominance (Darveau, 2010). Endotoxemia has also been detected after simple mastication in subjects with periodontitis, reflecting direct oral dissemination of LPS via inflamed periodontium (Geerts et al., 2002).

Our hypothesis is that the subgingival bacterial burden has an impact on salivary LPS levels, which contributes to endotoxemia. Therefore, we aimed to study the association between periodontal pathogen burden, saliva and serum LPS activity and how periodontitis and coronary artery disease (CAD) interrelates with them.

Clinical relevance

Scientific rationale for study: Periodontitis exacerbates atherosclerotic vascular disease, presumably by an increased systemic low-grade inflammation. Lipopolysaccharide is a well-known pro-inflammatory structure in the outer membrane of Gram-negative bacteria.

Principal findings: We showed that the subgingival amount of several periodontal pathogens correlated with levels of salivary lipopolysaccharide. In particular among subjects with periodontitis, this implicated higher serum LPS concentrations, which associated with increased risk of stable coronary artery disease.

Practical implications: Understanding the mechanisms between oral and systemic inflammatory diseases is vital for efficient treatment planning and prevention of these widespread diseases.

2 | MATERIALS AND METHODS

2.1 | Population

The COROGENE study is composed of 5,297 Finnish adult subjects who underwent coronary angiography for any reason at the Helsinki University Central Hospital between June 2006 and March 2008 (Vaara et al., 2012). The current study utilized the Parogene cohort, a subsample of 505 individuals who were randomly selected for comprehensive clinical and radiographic oral examinations. The population was grouped according to coronary angiography results; no significant CAD (<50% stenosis, n = 123), stable CAD (≥50% stenosis, n = 184), acute coronary syndrome (ACS; n = 169), and ACS-like but no significant CAD (n = 29). The number of significantly obstructed coronary arteries (0–3) was recorded. Data collection has been described more extensively earlier (Buhlin et al., 2011). All study individuals signed an informed consent, and the study design was approved by the Helsinki University Central Hospital ethics committee (approval reference number 106/2007). The investigation conforms to the principles of the Declaration of Helsinki, and this study complied with the STROBE guidelines for observational studies.

2.2 | Examinations

A dental assistant guided the participants to fill out a questionnaire, from which smoking habits (never/ever) were obtained. The study individuals were considered having hypertension, dyslipidemia, or diabetes if they had been prescribed medications for the respective diagnosed conditions. To verify its reliability, the acquired information was cross-checked with information from the questionnaires (Vaara et al., 2012).

Two calibrated periodontal specialists (KB and PM), unaware of the coronary diagnosis, performed oral examinations

TABLE 1 Characteristics of subjects according to coronary status

	All subjects, *n* = 505	No CAD, *n* = 123	Stable CAD, *n* = 184	ACS, *n* = 169	ACS-like, no CAD, *n* = 29	
	Mean (SD)					*p*-value*
Age (years)	63.4 (9.1)	61.7 (9.0)	65.5 (8.2)	62.9 (9.6)	58.8 (9.8)	**<.001**
	n (%)					*p*-value**
Gender (male)	328 (65.0)	61 (49.6)	136 (73.9)	122 (72.2)	9 (31.0)	**<.001**
Diabetes mellitus	118 (23.4)	24 (19.5)	55 (29.9)	37 (21.9)	2 (6.9)	**.015**
Smoking (ever)	267 (52.9)	57 (46.3)	99 (53.8)	97 (57.4)	14 (48.3)	.278
Hypertension	322 (63.8)	71 (57.7)	129 (70.1)	107 (63.3)	15 (51.7)	.061
Dyslipidaemia	404 (80.0)	90 (73.2)	172 (93.5)	125 (74.0)	17 (58.6)	**<.001**
Periodontal diagnosis						
Healthy	46 (9.1)	14 (11.4)	14 (7.6)	14 (8.3)	4 (13.8)	.222
Gingivitis	65 (12.9)	22 (17.9)	16 (8.7)	21 (12.4)	6 (20.7)	
History of periodontitis	92 (18.2)	23 (18.7)	42 (22.8)	23 (13.6)	4 (13.8)	
Active periodontitis	269 (53.3)	57 (46.3)	100 (54.3)	99 (58.6)	13 (44.8)	
Edentulous	33 (6.5)	7 (5.7)	12 (6.5)	12 (7.1)	2 (6.9)	

*One-way ANOVA.

**χ^2 test.

ACS, acute coronary syndrome; CAD, coronary artery disease; periodontal diagnosis: healthy (no ABL and BOP <25%), gingivitis (no ABL and BOP ≥25%), history of periodontitis (mild–severe ABL and BOP <25%), active periodontitis (mild–severe ABL, BOP ≥25%), periodontal diagnosis not applicable for edentulous subjects. Bold indicates $p < 0.05$.

approximately 6–20 weeks after the coronary angiography (mean ± SD: 113 ± 30 days; range, 37–224 days) (Buhlin et al., 2011). The recorded parameters included, probing pocket depth (PPD, registered from six sites of each tooth), bleeding on probing (BOP) and number of teeth present. All study individuals underwent panoramic tomography, from which alveolar bone loss (ABL) was determined. Saliva samples were collected as described earlier (Hyvärinen et al., 2012). Subgingival bacterial samples and periodontal parameters were available from dentate subjects (*n* = 473) (Mäntylä et al., 2013). Levels of subgingival bacteria (75 strains) were acquired by checkerboard DNA-DNA hybridization (Socransky et al., 2004). The bacterial samples were processed and analysed at the Oral Microbiology Laboratory, University of Bern, Switzerland (Mäntylä et al., 2013; Persson et al., 2008).

During the coronary angiographies, blood samples were drawn and stored at −80°C. Saliva and serum LPS activity was analysed by Limulus amebocyte lysate assay coupled with a chromogenic substrate. The inter-assay coefficient of variation was 6.8% and 5.5% for saliva and serum samples, respectively (Hyvärinen et al., 2012; Liljestrand et al., 2016). Both sample types (1:600, 1:5 vol/vol) were diluted with endotoxin-free water prior to the measurement. After exclusion of outliers, information on saliva/serum LPS was available for *n* = 482 and *n* = 492, respectively.

2.3 | Statistical analyses

We designed a periodontal diagnosis for each dentate subject as follows: "healthy" (no ABL and BOP <25%, *n* = 46), "gingivitis" (no ABL and BOP ≥25%, *n* = 65), "history of periodontitis" (mild–severe ABL and BOP <25%, *n* = 92) and "active periodontitis" (mild–severe ABL, BOP ≥25%, *n* = 269). A threshold of 25% for BOP was selected based on evidence that BOP percentages exceeding 20% and 30% indicate a higher risk for disease progression (Badersten, Nilvéus, & Egelberg, 1990; Joss, Adler, & Lang, 1994). The periodontal inflammatory burden index (PIBI) was calculated for each subject as "number of periodontal sites with PPD 4–5 mm" + 2x "number periodontal sites with PPD ≥6 mm" as reported earlier (Lindy, Suomalainen, Mäkelä, & Lindy, 2008).

The limit for statistical significance was defined at 0.05. Differences between groups were analysed with one-way ANOVA, Kruskal–Wallis, Mann–Whitney *U* test and chi-square test as appropriate. The effect of subgingival bacterial burden (75 strains) on serum and saliva LPS levels was analysed with age- and gender-adjusted multiple linear regression models. Subgingival bacterial levels were standardized with *Z*-score prior to the analyses. Values with $Z > 6$ were considered outliers and truncated to $Z = 6$. After standardization and truncation, subgingival bacterial levels were summed according to their usual location in the body and Gram staining, as defined in the supplementary table. Standardized beta values are reported to determine the relative influence of bacterial species on salivary LPS. R^2 values were reported for the corresponding unadjusted model. The association between saliva and serum LPS levels was analysed with a scatterplot, linear regression model and Pearson's correlation. Both saliva- and serum LPS were ln-transformed prior to analyses for increased normality.

TABLE 2 LPS levels across groups according to oral and coronary status

Oral parameter	Classes	Salivary LPS (EU/ml)	Serum LPS (EU/ml)
		Median (IQR)	
Number of missing teeth	0–1 (*n* = 9)	7,100 (24,700)	0.533 (0.772)
	2–4 (*n* = 88)	6,150 (45,000)	0.557 (0.648)
	5–8 (*n* = 141)	6,400 (11,900)	0.526 (0.587)
	9–31 (*n* = 213)	5,550 (12,000)	0.494 (0.499)
	32 (*n* = 31)	2,490 (12,300)	0.448 (0.620)
p-value*		.269	.642
Bleeding on probing (BOP, % of sites)	0–24 (*n* = 136)	5,970 (12,700)	0.503 (0.673)
	25–49 (*n* = 197)	6,250 (11,800)	0.506 (0.498)
	50–100 (*n* = 118)	5,880 (13,200)	0.565 (0.552)
p-value*		.272	.863
ABL	None (*n* = 107)	7,860 (13,700)	0.537 (0.491)
	Mild (*n* = 207)	5,670 (9,754)	0.535 (0.626)
	Moderate (*n* = 110)	4,825 (13,900)	0.451 (0.508)
	Severe or total (*n* = 27)	8,960 (16,700)	0.444 (0.359)
p-value*		**.027**	.409
PIBI	0–5 (*n* = 139)	5,800 (9,760)	0.527 (0.488)
	6–19 (*n* = 162)	5,940 (12,900)	0.519 (0.604)
	20–172 (*n* = 150)	7,100 (13,500)	0.508 (0.638)
p-value*		.157	.948
Periodontal diagnosis	Healthy	8,430 (13,000)	0.492 (0.457)
	Gingivitis	7,670 (13,300)	0.603 (0.590)
	History of periodontitis	4,140 (9,030)	0.519 (0.837)
	Active periodontitis	5,940 (12,000)	0.508 (0.499)
p-value*		.121	.468
Coronary parameter	Classes		
Coronary diagnosis	No CAD (*n* = 116)	5,160 (12,400)	0.529 (0.561)
	Stable CAD (*n* = 176)	6,150 (12,100)	0.630 (0.671)
p-value**		.426	.121
	ACS (*n* = 163)	6,300 (13,900)	0.440 (0.528)
p-value**		.272	.065
Coronary artery stenosis[a]	0 (*n* = 143)	4,610 (11,400)	0.507 (0.466)
	1 (*n* = 124)	6,650 (11,500)	0.521 (0.554)
	2 (*n* = 95)	6,190 (13,200)	0.553 (0.655)
	3 (*n* = 120)	5,760 (12,600)	0.499 (0.685)
p-value*		.392	.758

Total *N* = 482 with information on both saliva and serum LPS, oral parameters were only available for dentate subjects. Bold indicates $p < 0.05$.
*Kruskal–Wallis test.
**Mann–Whitney *U* test for two independent samples.
[a]Classes divided by number of narrowed coronary arteries; periodontal diagnosis: healthy (no ABL and BOP <25%), gingivitis (no ABL and BOP ≥25%), history of periodontitis (mild–severe ABL and BOP <25%), active periodontitis (mild–severe ABL, BOP ≥25%); ABL, alveolar bone loss; ACS, acute coronary syndrome; CAD, coronary artery disease.

The association between serum LPS levels (as tertiles) and stable CAD and ACS was studied using a multinomial logistic regression model adjusted for clinically relevant confounders: age, gender, dyslipidemia (yes/no), hypertension (yes/no), smoking (never/ever) and periodontal diagnosis (4 groups).

All statistical analyses were conducted with the SPSS statistics software (version 24; IBM Corp, Armonk, NY, USA).

3 | RESULTS

3.1 | Population characteristics

The studied population (*N* = 505) is characterized by common demographic parameters, risk factors for cardiovascular disease (CVD) and periodontal diagnosis according to CAD status (Table 1). Edentulous

TABLE 3 Associations between subgingival bacteria and salivary LPS

	Saliva LPS (Ln, EU/ml)		
Single subgingival species	**Standardized beta**	***p*-value**	**R^{2a}**
P. micra	**0.153**	**.001**	.025
P. intermedia	**0.151**	**.001**	.026
C. rectus	**0.139**	**.003**	.022
F. nucleatum sp. nucleatum	**0.130**	**.005**	.020
F. nucleatum sp. naviforme (vincentii)	**0.127**	**.007**	.017
F. periodonticum	**0.121**	**.010**	.016
P. aeruginosa	**0.113**	**.015**	.015
A. actinomycetemcomitans (ATCC 29523)	**0.112**	**.016**	.014
F. nucleatum sp. polymorphum	**0.108**	**.022**	.013
P. gingivalis	**0.106**	**.024**	.012
C. gingivalis	**0.096**	**.040**	.010
Cluster of all positively correlated bacteria (11 species)	Standardized beta	*p*-value	R^{2a}
	0.170	**<.001**	.032
Subgingival bacterial cluster according to their usual location in the body	Standardized beta	*p*-value	
Oral/oro-pharyngeal/naso-pharyngeal			
All	0.078	.099	–
Gram + (15 strains)	−0.001	.976	–
Gram − (24 strains)	**0.123**	**.009**	.017
Respiratory			
All	0.066	.162	–
Gram + (1 strains)	−0.015	.742	–
Gram − (2 strains)	**0.118**	**.012**	.016
Gastrointestinal			
All	0.060	.206	–
Gram + (7 strains)	0.080	.089	–
Gram − (4 strains)	0.012	.799	–
Urogenital/obstetrics/gynaecology			
All	0.031	.505	–
Gram + (10 strains)	0.017	.719	–
Gram − (5 strains)	0.056	.231	–
Skin			
All (Gram +, 7 strains)	−0.001	.982	–

Multiple linear regression adjusted for age and gender; all subgingival bacterial levels were transformed into *Z*-score prior to analysis and clustering. *Z*-values >6 were considered outliers in single species analyses and truncated to $Z = 6$. Only significantly associating strains are reported; see supplementary material for list of all studied bacteria (70 species, 75 strains) and cluster definitions. Bold indicates $p < 0.05$.

[a]R^2-values are reported from corresponding unadjusted simple linear regressions with fitting models; LPS, lipopolysaccharide.

subjects ($n = 33$) lack periodontal diagnosis, but are characterized along the rest of the study population. The mean age was 63 years (SD 9.1), and 65% of the participants were males. Approximately half of the population were current or former smokers. Active periodontitis, hypertension and dyslipidemia were commonly observed, with a prevalence of 53%, 64% and 80%, respectively. Due to their low number ($n = 29$), the outcome "ACS like, no significant CAD" was excluded from all further analyses.

3.2 | Saliva and serum LPS across oral and coronary status

We calculated levels of saliva and serum LPS across several groupings of oral and coronary status (Table 2). The population was grouped according to number of missing teeth, BOP, ABL, periodontal inflammatory burden (PIBI), periodontal diagnosis, coronary diagnosis and number of blocked coronary arteries. In general, the

levels of saliva LPS (magnitude of 10,000 EU/ml) were approximately 10^5-fold compared to the serum LPS levels (magnitude of 1 EU/ml). Saliva LPS levels correlated positively with ABL (p = .027). There was no evident difference in serum LPS levels across other studied groups.

3.3 | Subgingival bacteria and LPS

Based on subgingival bacterial burden calculations, multiple linear regression models (adjusted for age and gender) were studied to predict serum and saliva LPS levels. The standardized levels of each available bacteria (75 strains, Table S1) were analysed separately (Table 3). Significant regression equations and positive associations with saliva LPS level were found for 11 bacterial strains. Of the total saliva LPS variation, 2.6% could be explained by the amount of subgingival *Prevotella intermedia*, while 3.2% could be explained by the sum of all 11 strains. In further analyses, we used clusters of subgingival bacteria according to their usual location in the body and Gram staining as an independent variable. We confirmed in these analyses that saliva LPS levels associated significantly only with Gram-negative strains, with the exception *Parvimonas micra* (Table 3). In addition, significant associations were found only with oral/oro-pharyngeal/naso-pharyngeal (24 probes) and respiratory (2 probes) strains. The statistical power for all significant associations was within the range 0.89–0.99 (post hoc). No positive associations with the serum LPS level were found.

3.4 | The association between saliva and serum LPS

In a simple linear regression of the whole population (n = 469), we found a weak significant correlation between saliva/serum LPS levels, and 0.8% of the total variation of serum LPS could be explained by saliva LPS (F[1,467] = 3.991, R^2 = .01, Pearson's r = .092, p = .05). This positive correlation was enhanced in a subgroup of subjects with active periodontitis, for whom 4.9% of the serum LPS variation was explainable by saliva LPS (n = 247, F[1,2] = 12.7, R^2=.05, Pearson's r = .22, p < .001). Analyses for this subgroup yielded a good model fit in a multiple linear regression model (adjusted for age and gender); female gender (β = 0.13, p = .05) and saliva LPS (β = 0.22, p < .001) associated significantly with serum LPS levels. Age was not a significant predictor (β = −0.010, p = .88). The main results are illustrated as scatterplots (Figure 1).

3.5 | LPS and CAD diagnosis

We used a multinomial logistic regression model to analyse how serum LPS level associates with stable CAD or ACS as outcome variables (Figure 2). Subjects with high levels of serum LPS were more likely to suffer from stable CAD; the highest tertile yielding an OR-value of 2.0 (95% CI 1.04–3.81, p = .04) in the fully adjusted model. No statistically significant results were obtained regarding ACS as an outcome. Saliva LPS was not a predictor for stable CAD or ACS in similar analyses.

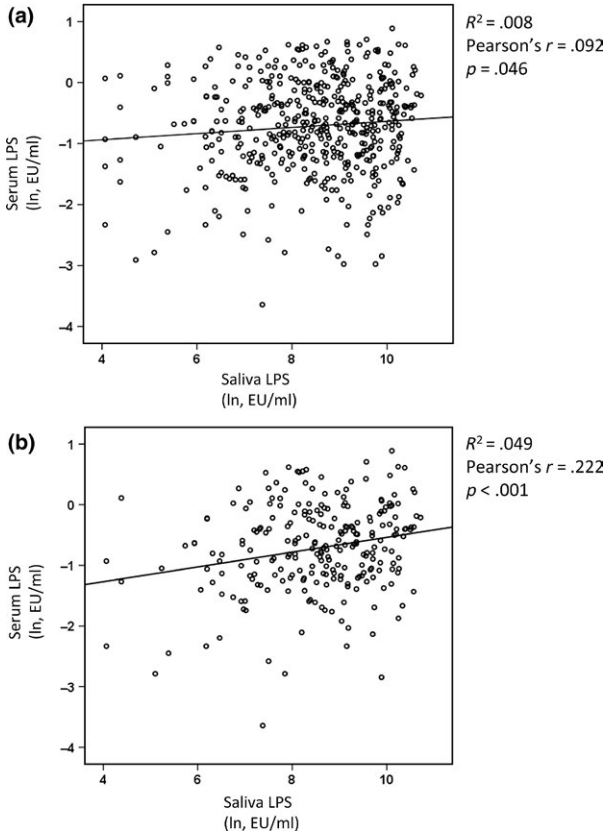

FIGURE 1 Association between salivary and serum LPS levels. (a) Salivary/serum LPS levels (ln) in the whole population (n = 469). (b) Salivary/serum LPS levels (ln) in the subjects with active periodontitis (n = 247). LPS, Lipopolysaccharide; R^2 is obtained from a simple linear regression model

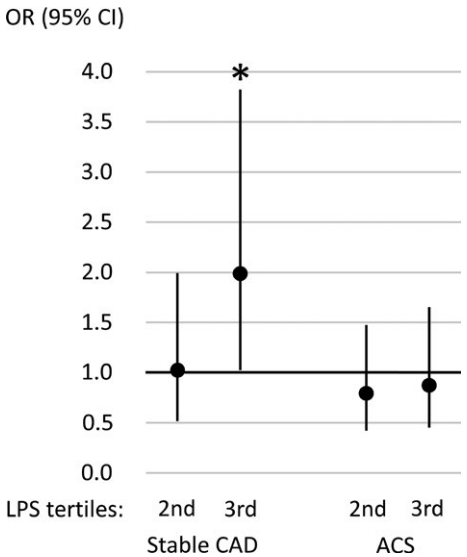

FIGURE 2 The association between serum LPS tertiles and CAD/ACS. Multinomial logistic regression adjusted for age, gender, dyslipidemia (yes/no), hypertension (yes/no), smoking (never/ever) and periodontitis (4 groups). First serum LPS tertile was used as the reference. CAD, stable coronary artery disease; ACS, acute coronary syndrome. *p < 0.05.

4 | DISCUSSION

In this cross-sectional observational cohort study, we showed that the association between periodontitis and increased risk for CAD might be partly due to LPS activity. First, the amount of several subgingival Gram-negative bacteria correlated directly with saliva LPS levels. Even though up to 75 strains were analysed, saliva LPS activity mainly correlated with the amounts of periodontal pathogens or clusters of Gram-negative oral- or respiratory species. Secondly, saliva LPS associated with serum LPS levels, especially in a subgroup of subjects with active periodontitis. Lastly, a high level of systemic endotoxin was associated with stable CAD in a confounder adjusted model, suggesting that LPS may be one of the molecular factors linking oral microbiota to atherosclerosis.

Destruction of periodontal attachment tissue is a combined result of disruption of the microbial ecology, bacterial virulence factors and aggressive immunologic host response in susceptible individuals (Darveau, 2010). Several efforts have been made to identify true pathogens for clinical measures of periodontitis, the most famous ones unarguably being the red/orange complex species introduced by Socransky et al. (Socransky, Haffajee, Cugini, Smith, & Kent, 1998). The task at hand is complicated due to factors such as the episodic nature of periodontitis, varying progressions in the subgingival microbiota and the synergy between species causing the dysbiotic biofilm (Hajishengallis, 2015; Socransky & Haffajee, 2005). Also, genetic susceptibility may contribute to bacterial colonization. However, to date, no conclusive genetic variants of the host have been found to affect the pathogenic subgingival microbiota (Divaris et al., 2012; Nibali, Di Iorio, Onabolu, & Lin, 2016). When studying associations between microbes and disease, it is encouraged to allocate more focus on shared virulence factors, such as LPS and other pathogen-associated molecular patterns, PAMPs (Ezzo & Cutler, 2003).

Periodontitis subjects have been reported to have increased salivary concentrations of TLR-4 stimulants, such as LPS (Lappin, Sherrabeh, & Erridge, 2011). In a study by Hyvärinen et al., saliva LPS correlated with number of teeth present, reflecting the importance of the surface available for the bacteria to attach. However, saliva LPS were highest among the subjects with a milder form of periodontitis (Hyvärinen et al., 2012). In a recent cross-sectional study, saliva LPS failed to differentiate various forms of periodontitis from systemically healthy subjects (Liukkonen, Gursoy, Pussinen, Suominen, & Könönen, 2016). Among the clinical periodontal parameters in the present study, only ABL associated directly with the levels of saliva LPS. Even though Gram-negative bacteria play an essential role in periodontitis, saliva LPS levels alone may not be a good biomarker for clinical disease (Hyvärinen et al., 2012; Liukkonen et al., 2016), but our results suggest that it moderately reflects the periodontal microbial burden. Factors affecting saliva LPS levels have not been studied in detail, but presumably time of sample collection, oral hygiene routines or diet might have an effect. To our knowledge, this is the first attempt to analyse the contribution of subgingival bacterial burden with saliva—and further with serum—LPS. While there are only few studies on saliva LPS in general, further research is needed.

All of the 75 studied strains were fairly prevalent (31%-93%) in the periodontal pockets, regardless of their usual location in the body. The subgingival abundance of the species and their association with periodontitis has been reported earlier (Pradhan-Palikhe et al., 2013); thus, we did not do it in this study. We concentrated on analysing their relative contribution to LPS levels after standardizations. Interestingly, although preceding assumptions were not made, most of the single species that had a statistically significant association with saliva LPS were established periodontal pathogens. All other, except *Parvimonas micra*, were Gram-negative species. This statistical association is probably explained by co-aggregation with other significant species in the biofilm ecosystem, while *P. micra* is one of the "orange complex" species (Socransky & Haffajee, 2005). Interestingly, *P. micra* has shown to increase its pro-inflammatory potential by binding LPS directly onto its cell surface (Yoshioka, Grenier, & Mayrand, 2005).

There is limited information available on the impact of subgingival bacteria on saliva/serum LPS, and further research is warranted. Traditional colour-coded bacterial clusters should be further explored while they are based on a priori data and consistently show association within clusters and severity of periodontal disease (Socransky & Haffajee, 2005). New high-throughput methods will enable more detailed profiling of the subgingival taxa, and community ordination techniques might provide insight into highly synergistic species. Clinical signs of periodontitis seem to depend on a relative abundance of traditional pathogens, opportunistic commensals and novel disease-associated taxa causing a dysbiotic microbiota (Camelo-Castillo et al., 2015; Hajishengallis, 2015) . However, considering that LPS is a membrane structure of Gram-negative bacteria, it is justified to use absolute bacterial abundance as a marker for LPS levels.

The subgingival level of *Pseudomonas aeruginosa*, a respiratory Gram-negative proteobacteria, was also significantly associated with saliva LPS. This supports earlier studies stating that *P. aeruginosa* is a common oral resident (Persson et al., 2008; Vieira Colombo, Magalhaes, Hartenbach, Martins do Souto, & Maciel da Silva-Boghossian, 2016). Hypothetically, the presence of this opportunistic species in the periodontal pocket may threaten general health, especially in immunologically compromised subjects (Peräneva et al., 2013).

The link between endotoxemia and incident CVD events has been reported in a few studies (Pussinen et al., 2007; Wiedermann et al., 1999). There is compelling evidence that the progression of atherosclerosis is exacerbated by activation of the immune system by TLR-mediated signalling (Frantz, Ertl, & Bauersachs, 2007). This is in line with our present finding that high levels of serum LPS associates with stable CAD. However, the exact origin of metabolic endotoxemia is still under debate, although translocation from the intestine has received much attention (Piya et al., 2013) and the periodontal status has usually been overlooked in earlier studies. To our knowledge, we are the first to show that saliva LPS associates with serum LPS. This was obvious among subjects suffering from active periodontitis, where saliva LPS levels explained 4.9% of the serum LPS levels. This finding supports the hypothesis of direct translocation of Gram-negative bacteria or their virulence factors from the periodontal pocket into the bloodstream, considering that subjects with periodontitis have higher levels

of oral Gram-negative bacteria and an increased surface of inflamed ulcerative attachment tissue (Forner, Larsen, Kilian, & Holmstrup, 2006; Pizzo, Guiglia, Lo Russo, & Campisi, 2010).

The main strength in our study is versatile data based on questionnaires, clinical oral parameters, salivary/serum analyses and comprehensive information on subgingival bacterial burden. Also, our study stands out with high-quality angiographically verified outcomes. While all Finnish adults have the same prerequisites for adequate treatment, the role of socio-economic status as confounder is diminished, and hence not controlled for.

The study is limited by a cross-sectional set-up; hence, no conclusions of causality or direction of events can be drawn. Population bias is evident, while our cohort consisted of Finnish adults with an initial indication for coronary angiography. While many of the studied associations might have shared risk factors, residual confounding cannot be ruled out. For example, adjustments with nutrient intake might be useful in future studies (Kallio et al., 2015). The LPS of various Gram-negative bacterial species have a rather heterogeneous structure, pro-inflammatory potential and dose–response for the non-specific Limulus amebocyte lysate assay, which might bias our results (Munford, 2016). The chain of analyses used in this study results in a possible multiple comparison error, and no formal tests of mediation are reported. Therefore, *p*-values should be interpreted with caution. The direct associations between periodontal parameters, for aetiologic periodontal species and stable CAD/ACS for this cohort, have been published earlier (Buhlin et al., 2011; Mäntylä et al., 2013) and are not reported in this study.

5 | CONCLUSIONS

Within the limitations of our study, we conclude that the salivary LPS activity correlated with subgingival bacterial burden. The bacteria with the highest contribution were oral and respiratory Gram-negative species, most of which were periodontal pathogens. Active periodontitis strengthened the positive correlation observed between saliva and serum LPS, and high levels of serum LPS had a significant association with the risk of stable CAD. As a pro-inflammatory molecule, LPS is a possible molecular mediator between periodontitis and CVDs.

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CONFLICT OF INTEREST

The authors declare that there are no conflict of interests in this study.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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