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Low levels of antibodies for the oral bacterium *Tannerella forsythia* predict cardiovascular disease mortality in men with myocardial infarction: A prospective cohort study

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ABSTRACT

Antibody levels to periodontal pathogens in prediction of cardiovascular disease (CVD) mortality were explored using data from a health survey in Oslo in 2000 (Oslo II-study) with 12 1/2 years follow-up. IgG antibodies to four common periodontal pathogens; *Tannerella forsythia* (TF), *Porphyromonas gingivalis* (PG), and *Treponema denticola* (TD) all termed collectively the "red complex", and *Aggregatibacter actinomycetemcomitans* (AA) were analysed. The study sample consisted of 1172 men drawn from a cohort of 6,530 men who participated in the Oslo II-study, where they provided information on medical and dental history. Of the study sample, 548 men had reported prior myocardial infarction (MI) at baseline whereas the remaining 624 men were randomly drawn from the ostensibly healthy participants for comparative analyses. Dental anamnestic information included tooth extractions and oral infections. An inverse relation was found for trend by the quartile risk level of TF predicting CVD mortality, p-value for trend = 0.017. Comparison of the first to fourth quartile of TF antibodies resulted in hazard ratio (HR) = 1.82, 95% confidence interval 1.12–2.94, p = 0.015, adjusted for age, education, diabetes, daily smoking, and systolic blood pressure. Specificity comparing decile 1 to deciles 2–10 of TF predicting mortality was 92.3%. We found an increased HR by low levels of antibodies to the bacterium *T. forsythia* predicting CVD mortality in a 12 ½ years follow-up in persons who had experienced an MI but not among non-MI men. This novel finding constitutes a plausible causal link between oral infections and CVD mortality.

Introduction

Many bacteria have been identified in advanced "chronic" periodontal disease but three bacteria are commonly identified and are jointly termed the 'Red complex' [1–3]. The latter comprises the strict anaerobic bacteria *Tannerella forsythia* (TF), *Porphyromonas gingivalis* (PG), and *Treponema denticola* (TD). These bacteria act in symbiosis under the progression of the infection through their production of several virulence factors and they possess the ability to evade host reactions resulting in soft and hard tissue destruction in the oral cavity [4–8]. Their numbers increase with increasing periodontal pocket depth. The facultative anaerobic *Aggregatibacter actinomycetemcomitans* (AA) is associated with gingivitis and localized periodontitis (juvenile periodontitis) [9,10].

Multiple pathways for exploring and linking periodontal disease to cardiovascular disease (CVD) and atherosclerosis, in particular, have been published [4,6,7,11–19]. Both conditions are closely related to immunologic responses and inflammation. The exact mechanisms linking the two disorders have, however, not been firmly established, but several studies during the last decades provide evidence for an association between oral microbiota and CVD. Examples are DeStafano et al. who found an increased risk of atherosclerotic plaque formation associated with dental disease in 9760 patients over a 14 years follow-up [11], and in a case-control study Mattila et al. showed an association between periodontal disease and heart disease [12].

Periodontitis is well described and classified [20]. Periodontitis and other oral infections of dental origin in various stages occur in millions of people around the world [21]. It has been estimated by WHO that

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5–15% of the world's population suffer from chronic periodontitis and juvenile periodontitis occur in about 2% of youths. Dental caries in industrialized countries affect approximately 60–90% of schoolchildren and the vast majority of adults. However, there are differences between populations. Bacterial DNA of more than 700 bacteria has been identified in the mouth but approximately 35% have not been possible to cultivate [22]. Several dental procedures may cause bacteraemia [23,24]. Exposure to oral bacterial infections may occur at an early age in deciduous teeth with severe caries and pulpal exposure to oral bacteria. Juvenile periodontitis occur in some young people. Hence, oral infections may occur at an early age and expose individuals over many years [24,25].The close proximity of the periodontal bacterial infection to the bloodstream makes it highly plausible that bacteria themselves or bacterial products spread to distant sites in the cardiovascular system [26–29]. In fact, oral bacteria or their DNA have been identified in atheromas, heart valves, and arterial walls [16,30–39]. The first line of defence in these sites is the macrophages that are involved in the process of autophagy in bacterial infections [4]. Bacterial products such as lipopolysaccharides (LPSs) and increased low-density lipoprotein (LDL) accumulate in atheromas, initiating the transfer of macrophages into atheromas and their transformation into foam cells when absorbing LDL [7]. Andriankaja used indirect immunofluorescence microscopy with species-specific polyclonal and monoclonal serodiagnostic reagents when isolating six periodontal pathogens and assessing the odds of having a myocardial infarction (MI) in 1,060 men and women [14]. They found *Prevotella intermedia* and *T. forsythia* to be significantly associated with MI, OR = 1.40 (95% confidence interval (CI) 1.02–1.92) in adjusted analyses. In addition, three or more bacteria present in periodontal pockets gave an increased risk of MI, OR = 2.01 (95% CI 1.31–3.08).

The hypothesis

We hypothesize that anaerobe oral bacteria metastasize infection into the cardiovascular system as observed by IgG antibody levels. Using the Oslo II-cohort, we want in this sub-study to estimate the prediction for CVD mortality over 12 ½ years of follow-up by level of bacterial IgG antibodies to PG, TD, TF, and AA in men with a history of myocardial infarction (MI) versus men with no known history of myocardial infarction (non-MI). Knowledge of antibody levels to oral bacteria may provide important support to an etiologic explanation of the incidence and mortality of myocardial infarction related to oral infections and lead to the development of preventive measures such as a diagnostic test and a vaccine.

Evaluation of the hypothesis

The hypothesis was tested as follows: In 2000 the health survey named the Oslo II-study, a follow-up study of men invited to the Oslo Study of 1972/73 was undertaken [40-42]. Men of this cohort living in Oslo or in the surrounding county Akershus were invited to attend (n = 12,764). After screening 55 men withdrew and results from 6,530 participating men aged 48–77 years are eligible for analyses. At the screening, the men filled in a detailed questionnaire on medical history, oral health, medication, health service use, food- and drinking habits, physical activity, smoking, stress, and mental health. Oral health was defined according to the number, and cause of tooth extractions and current oral infections. Height, weight, waist, hip, and blood pressure were recorded (41). Blood samples were drawn for total cholesterol, HDL-C, glucose in the non-fasting state, and triglycerides analyses. EDTA-blood was stored at the HUNT biobank, Levanger, Norway, and the remaining serum after analyses were frozen and stored at −80 °C at the Norwegian Institute for Public Health, Oslo, Norway.

A study sample of 1172 men was drawn from the Oslo II cohort to assess prospectively the association between antibodies to four oral bacteria and mortality in a 12 ½ – years follow-up (42). The study sample consisted of 548 men who had reported a history of myocardial infarction at baseline in 2000 and 624 men randomly selected from the ostensibly healthy men in the cohort. All men had attended both health screenings and had hs-CRP measured. All procedures performed were in accordance with the ethical standards of the Norwegian institutional and national research committee REK (Helse Sør-øst) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study. The SPSS random generator program was used in selecting healthy subjects.

The serum samples were analysed for IgG antibodies to the oral bacteria TF, PG, TD, and AA by the ELISA method. The ELISA and IgG antibody assay used in the Oslo II-study have previously been described [42]. In short, bacteria were cultivated anaerobically at the Institute for Oral Biology, Dental faculty, University of Oslo. The ELISA was performed at the Norwegian Institute for Public Health. ELISA plates were coated with the bacterial antigen at a concentration of 5 μg protein of each of the bacteria to capture serum antibodies. Then each well was incubated overnight with 100 μl solution. Thereafter the coated wells were stored for up to 14 days at 4 °C. The human serum calibrator was diluted to 1/40, 1/80, 1/160, 1/320, 1/640, 1/1280, and 1/2560. The samples were diluted to 1/160, 1/320, 1/640, and the 170 first were also diluted to 1/80. To the washed plates were added samples or serum calibrator. These were then incubated for 2 h at room temperature. After washing, conjugated secondary antibody was added and the plates were incubated at room temperature for another 2 h. The secondary antibody was Polyclonal Rabbit Anti Human IgG/AP, D0336, 1:1000 dilution. Into each well was pipetted one hundred microliter substrate. The colour development was registered by optical density (OD) and the result was recorded as a percentage of the serum calibrator. Sample dilution curves were compared for deviation from parallelism. Mean values were used unless large deviations were observed and then median values were chosen. For more details see publication (42).

The covariates used in the analyses were selected according to known predictors for CVD and periodontal infection namely age, daily smoking, education and diabetes, which are common to oral health and CVD and systolic blood pressure, an important predictor of CVD, for prediction analyses and to adjust for confounding. In addition, health service utilization and medication use were reported. Hs-CRP was analysed in the serum samples in 2004 at the same time as the antibody analyses. Missing data were limited. The outcome during follow-up was CVD mortality. All Norwegians have a personal identification number and this was used in the matching of study participants to the Norwegian Cause of Death Registry which provides complete follow up with regard to date and cause of death according to ICD-10 codes and I21 to I99 defines all CVD diagnoses used in this study. Mortality data were provided by Statistics Norway, and the mortality information was given in one linkage after 12 ½ years follow-up with the last date of follow-up 31.12.2012.

Baseline characteristics are presented by mean and standard deviations (SD) or number and percentage. Differences between characteristics of men with or without a history of MI were examined by *t*-test for equality of means of independent groups for continuous variables or by Fisher's Exact Test for dichotomous variables, two-sided test values only. The antibody variables were analyzed both as observed values, by ln-transformation due to their skewed distribution, stratified by quartile values (quartiles), and tested for correlation by the Pearson statistic. The quartiles were used on an ordinal scale applying the lowest quartile as reference alternating with using the highest quartile as reference. Cox proportional hazards regression analyses were used to estimate survival and the results are presented by the hazard ratio (HR), 95% confidence interval (95% CI). Covariates were used to adjust for confounding and selection bias. Sensitivity analyses for combinations of the 'Red complex' bacteria with AA were performed. Interaction analyses of self-reported MI by antibodies (quartile values) were

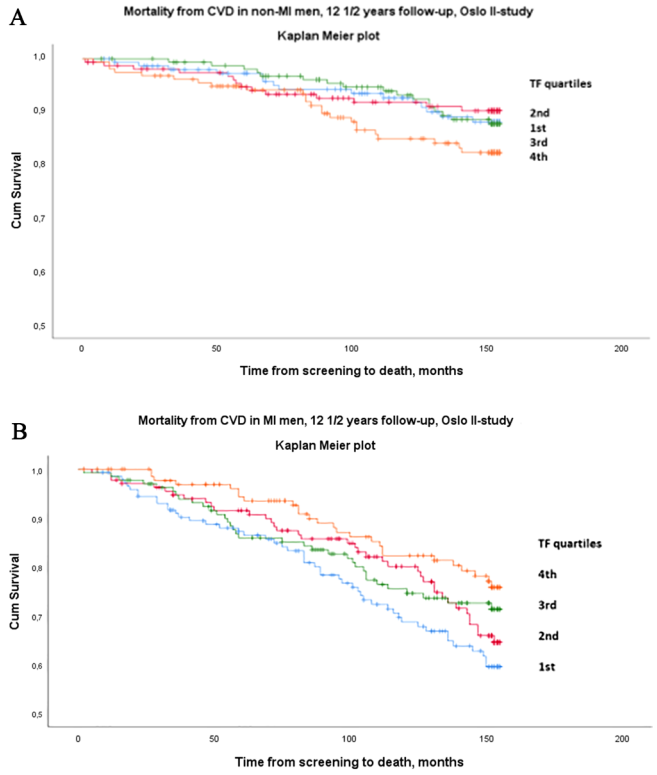

Fig. 1. Kaplan Meier plots of CVD mortality by quartile antibody levels to oral bacteria *Tannerella forsythia* (TF), in Fig. 1a men with no MI history and Fig. 1b men with a MI history. The Oslo II-study.

performed. Sensitivity and specificity for mortality were tested at decile and quartile values of the four antigen series. The receiver operating curve (ROC) was used to detect any cut off value of the antibody readings of diagnostic importance. In addition, the area under the curve (AUC) was calculated. A P-value < 0.05 was considered significant. IBM SPSS version 25 was used for the statistical analyses. Kaplan Meier plots Fig. 1 A) and 1B) illustrate cumulative survival according to quartile levels of TF for men with or without a history of MI.

Results

The mean, SD, minimum and maximum values, and quartile values of the antibodies towards the four bacteria according to the ELISA IgG results are shown in Table 1. The highest maximum IgG values were against PG (8,710) for MI and 5,299 for non-MI, and the lowest were those against TD with 2,166 for MI and 1,204 for non-MI. The mean values did not differ significantly for any of the IgG measurements of the four bacteria. All the antibody readings displayed large standard deviations (SD).

The men with self-reported MI were found to be slightly older, less educated, and more likely to suffer from diabetes compared to non-MI men (Table 2). Other significant differences were observed for systolic blood pressure, total cholesterol, HDL-C, non-fasting glucose, BMI, and triglycerides. Significantly more of the MI-men had visited the general practitioner four times or more last 12 months (40.4 versus 23.3%), but this was not the case for dental visits. The two groups did not differ regarding being on current antihypertensive drugs, 27.3 versus 28.5% whereas the percentage on cholesterol-reducing drugs (as expected) was much higher in the MI- group 63.8 versus 12.8%.

Cox proportional hazards regression analyses were performed to study which variables predicted CVD mortality over the 12 ½-years follow-up (Table 3). In age and age-adjusted univariate analyses, age

Table 2
Baseline characteristics, health service use, and medication for men of self-reported myocardial infarction (MI) versus randomly selected men with no MI reported. A limited cohort study originating from the Oslo II-study in 2000.

Covariates	Disease status in 2000		P-value
	Myocardial infarction N = 548	No myocardial infarction N = 624	
Basic characteristics			
Age, year, mean SD	71.5 (3.6)	70.2 (5.3)	< 0.001
Education, year, mean SD	11.5 (3.4)	12.1 (3.4)	0.006
Diabetes, n (%)	71 (13.0%)	35 (5.6%)	< 0.001
Systolic blood pressure, mean SD	141.3 (21.5)	144.3 (19.2)	0.013
Daily smoking, n (%)	91 (16.6%)	129 (20.7%)	0.085
Total cholesterol, mean SD	5.3 (1.1)	6.0 (1.0)	< 0.001
HDL-C, mean SD	1.3 (0.4)	1.5 (0.4)	< 0.001
Glucose, mean SD	6.3 (2.2)	5.8 (1.8)	< 0.001
Triglyceride, mean SD	2.1 (1.2)	1.9 (0.9)	< 0.001
BMI, mean SD	27.1 (3.5)	26.3 (3.2)	< 0.001
Hs-CRP, mean SD	4.3 (6.6)	3.7 (8.4)	0.204
Alcohol, drink two or more times per week, n (%)	370 (67.5%)	407 (65.2%)	0.421
Health service use, p-value for trend across response categories			
Response categories:			
General practitioner visit last 12 months, 4 visits or more versus less, n (%)	214 (40.4%)	143 (23.3%)	< 0.001
Dentist visit last 12 months, 4 visits or more versus less; n (%)	38 (7.2%)	48 (8.0%)	0.293
Medication use, p-value for trend across all response categories			
Response categories:			
Current, other, before, or never: Antihypertensiva, current n (%)	311 (27.3%)	175 (28.5%)	< 0.001
Cholesterol reduction drugs, current, n (%)	332 (63.8%)	77 (12.8%)	< 0.001
Response categories: *Daily, other, never use, or last 4 weeks:* Painkillers, daily use	32 (6.8%)	23 (2.4%)	0.564
Insomnia, daily use	28 (5.7%)	19 (3.3%)	0.018
Tranquillisers, daily use	26 (5.3%)	13 (2.2%)	0.006
Antidepressants, daily use	21 (4.4%)	11 (1.9%)	0.117

Table 1
Bacterial antibodies measured in 2000; mean, min, max, and quartile values for observed values of *Tannerella forsythia, Porphyromonas gingivalis*, *Treponema denticola*, and *Aggregatibacter actinomycetemcomitans* in persons with self-reported myocardial infarction (MI) versus non-MI reported.

Bacterial antibodies	Myocardial infarction N = 548			No myocardial infarction N = 624			p-value
	Mean (SD)*	Min–max	Quartile values	Mean (SD)*	Min–max	Quartile values	
Porphyromonas gingivalis	251.6 (603.7)	12–8710	54, 95, 201	217.6 (426.6)	9–5299	52, 86, 195	0.096
Tannerella forsythia	94.6 (146.2)	10–2166	33, 53, 102	96.8 (121.5)	9–1204	35, 58, 103	0.883
Treponema denticola	74.2 (201.9)	3–3593	26, 44, 74	69.3 (93.3)	1–1240	28, 46, 75	0.258
Aggregatibacter actinomycetem-comitans	115.1 (201.9)	5–3166	33, 62, 126	101.2 (179.7)	7–3042	32, 54, 104	0.130

* SD = Standard deviation.

Table 3
Cox-analyses; age and age-adjusted univariate analyses for prediction on total CVD mortality. A 12 ½ years follow-up of the Oslo II-study in 2000.

Risk factor	Myocardial infarction N = 548 CVD mortality n = 148		No myocardial infarction N = 624 CVD mortality n = 70	
	HR	95% CI	HR	95% CI
Age, year	1.16	1.10–1.23	1.23	1.14–1.32
Education, year	1.001	0.96–1.05	0.91	0.85–0.97
Daily smoking, yes	1.26	0.81–1.95	1.66	0.93–2.97
Diabetes, yes	1.51	0.96–2.38	2.66	1.21–5.89
Total cholesterol, mmol/l	1.01	0.86–1.17	1.21	0.97–1.52
HDL-C, mmol/l	0.48	0.29–0.80	0.90	0.50–1.64
Systolic blood pressure	0.998	0.99–1.01	1.01	1.00–1.02
Body mass index, Kg/m^2	1.03	0.99–1.08	1.04	0.97–1.12
Glucose, non-fasting, mmol/l	1.09	1.02–1.17	1.10	1.00–1.21
Triglycerides, mmol/l	1.10	0.96–1.27	1.11	0.87–1.41
Hs-C-reactive protein, mmol/l	1.01	0.99–1.03	1.02	0.997–1.03
Alcohol, drink two or more times per week	1.17	0.81–1.69	1.27	0.74–2.19
Hypertensive use, yes	0.84	0.70–1.01	0.72	0.56–0.92
Cholesterol reducing drugs, yes	0.982	0.815–1.163	0.880	0.628–1.232

was a significant factor in both groups of men as was non-fasting glucose. Further, in MI, HDL-C predicted mortality inversely (HR = 0.48, 95% CI 0.29–0.80). In non-MI, education (inversely) (HR = 0.92, 95% confidence interval (95% CI) 0.85–0.97), diabetes (HR = 2.66, 95% CI 1.21–5.89), taking antihypertensive medication (inversely) (HR = 0.72, 95% CI 0.56–0.92), and systolic blood pressure (HR = 1.01, 95% CI 1.00–1.02) predicted CVD mortality.

Using multivariate Cox analyses we studied quartile values of antibodies of each bacterium adjusted by age, education, diabetes, daily smoking, and systolic blood pressure (Table 4). We found no significant trend for quartile values on the ordinal scale of 1–4 for any of the antibodies examined separately. Then we studied quartile values using the lowest quartile as reference comparing it to the other quartiles. The results for trend were non-significant for all antibodies for both MI and non-MI except *T. forsythia* for MI where a highly significant inverse trend was found, p = 0.017. The results for Q2 versus Q1 was HR = 0.73, 95% CI 0.47–1.12, for Q3 versus Q1 was HR = 0.63, 95% CI 0.40–0.98, for Q4 versus Q1 HR = 0.47, 95% CI 0.29–0.77. We then reversed the analysis using the highest quartile as reference group for ease of interpretation which gave the following results: Q1 versus Q4 was HR = 1.82, 95% CI 1.12–2.94, for Q2 versus Q4 was HR = 1.42, 95% CI 0.86–2.35, for Q3 versus Q4 HR = 1.27, 95% CI 0.76–2.11, and trend p = 0.09. Interaction was significant for self-reported MI to antibodies of TF (quartile values), but not for any of the other three bacteria studied. Fig. 1a and b show the differences of the prediction for CVD mortality of TF antibodies by quartile values between MI and non-MI, respectively. In Fig. 1b can be observed the increased risk almost throughout the follow-up period for MI men as opposed to non-MI men shown in Fig. 1a.

It was of interest to study any potential association of the common AA to the 'Red complex' bacteria in relation to CVD mortality. AA was assessed together with the 'Red complex', each bacterium separately, in combinations with two of these bacteria, and all three bacteria. The Cox analyses were adjusted for age, education, diabetes, daily smoking, and antihypertensive drugs. The results showed associations in non-MI but not MI. Significant relations were observed for the 'Red complex' (HR = 1.71, 95% CI 1.03–2.82), 'Red complex' + AA (HR = 1.72, 95% CI 1.01–2.92), PG + AA (HR = 1.67, 95% CI 1.02–2.74), and PG + TF + AA (HR = 1.69, 95% CI 1.02–2.79).

Table 4
Cox-analyses of CVD mortality by observed quartile values of each antibody separately for self-reported myocardial infarction and control persons. Risk by quartile values relative to fourth quartile. The analyses were adjusted for age, education, diabetes, daily smoking, and systolic blood pressure. A 12 ½ years follow-up of the Oslo II-study in 2000.

Risk factor	Myocardial infarction N = 548 CVD mortality n = 148		No myocardial infarction N = 624 CVD mortality n = 70	
	HR	95% CI	HR	95% CI
TF quartiles				
1st versus 4th	1.82	1.12–2.94	0.54	0.28–1.03
2nd versus 4th	1.42	0.86–2.35	0.59	0.30–1.14
3rd versus 4th	1.27	0.76–2.11	0.63	0.34–1.18
4th quartile	1.00 (ref)		1.00 (ref)	
PG quartiles				
1st versus 4th	0.88	0.56–1.39	0.74	0.40–1.39
2nd versus 4th	1.04	0.66–1.65	0.72	0.38–1.35
3rd versus 4th	0.77	0.48–1.23	0.48	0.23–0.99
4th quartile	1.00 (ref)		1.00 (ref)	
TD quartiles				
1st versus 4th	1.11	0.70–1.76	0.93	0.47–1.84
2nd versus 4th	1.31	0.82–2.07	0.85	0.43–1.71
3rd versus 4th	0.89	0.53–1.48	0.85	0.45–1.61
4th quartile	1.00 (ref)		1.00 (ref)	
AA quartiles				
1st versus 4th	0.88	0.56–1.39	1.04	0.53–2.07
2nd versus 4th	0.90	0.57–1.41	0.85	0.44–1.62
3rd versus 4th	0.83	0.53–1.32	0.79	0.39–1.59
4th quartile	1.00 (ref)		1.00 (ref)	

The first decile of *T. forsythia* versus higher decile values among men reporting MI showed a specificity of 92.3% (369 tested for the second to the tenth decile among 400 men alive) and a sensitivity of 11.5% (17 tested for the first decile among 147 men who died during follow-up). The ROC curve did not show a distinct threshold value but the AUC was significant for *T. forsythia* (p = 0.010).

Discussion

More men reporting MI at the health screening in 2000 that later died from CVD were found to have low levels of *T. forsythia* antibodies in predictive analyses of 12 ½ years follow-up than non-MI. An 82% increased risk was found when comparing first to the fourth quartile of antibodies for *T. forsythia*. The Kaplan-Meier plot indicate that this risk was maintained throughout the follow-up period. The results showed a high specificity of the first decile to 92.3% and sensitivity to 11.5% indicating that a test for *T. forsythia* has a high probability of distinguishing men with the lowest risk for CVD mortality among men with a history of MI. The ROC curve did not display a distinct threshold but AUC did show a significant difference between the curves. The reduced ability to produce antibodies to *T. forsythia* may indicate that the 'Red complex' bacteria of "chronic" periodontal disease proceed from localized oral infection by possibly initiating or aggravating already existing coronary atherosclerosis or other cardiovascular conditions. The baseline characteristics for the study population reflect the medical diagnostic difference of men with a history of myocardial infarction versus non-MI not reporting such history (Table 1). More MI men took cholesterol reducing drugs but not more than non-MI with respect to antihypertensive drugs.

The main strength of this study is the clear temporal association with a follow-up of 12 ½ years from the Oslo II health survey in 2000, and the strength of the association with a high HR to indicate a causal relation. Another strength of the study is the complete register of cause and date of death ascertained by Statistics Norway. Further, we have shown a risk gradient by quartile values of antibodies to TF. Some

degree of multiple comparisons of the bacteria were done in order to explore their risk pattern. Microbiologic and epidemiologic studies provide plausible immunologic pathways, and it could potentially support the known notion of a hereditary risk for CVD through the antibody response pattern. One of the limitations of this study is that it included elderly men only. This reduces the generalizability of the results to women. Another limitation is that the MI diagnosis was self-reported at the health screening. However, MI is a major event in a person's life including hospitalization, and we have confidence in this information.

The association and possible causal relation between "chronic" periodontitis and CVD have been explored in a number of studies. Antibodies to periodontal pathogens were associated with different outcomes of CVD, CHD, stroke or MACE. Pussinen et al investigated antibodies to AA and PG in a random sample (n = 1163) of men aged 45–74 years in Finland. From this cross-sectional study, they reported that in persons with a high combined antibody response to PG and AA, coronary heart disease (CHD) was more common than in persons with a low response [44]. Aoyama et al examined 364 persons with diagnosed CVD for tooth extraction status [45]. In comparison of the four groups of remaining teeth, they found that the level of PG IgG in the group with 10–19 teeth was statistically higher than that in the group with ≥20 teeth. In their case-control study on stroke, Pussinen et al found that IgA-seropositive for *A.* actinomycetemcomitans was higher among the controls, non-stroke patients, and IgA-seropositive for *P. gingivalis* higher among patients with recurrent stroke during 13 years of follow-up [46]. Beck et al found that systemic antibody response to 17 periodontal bacteria rather than periodontal status was relevant to atherothrombotic coronary events (35). The bacteria involved differed between smokers and non-smokers. Differences between strains of bacteria indicate differences in virulence factors as observed by Yamazaki et al for prevalence of serum IgG positivity to 12 periodontal pathogens [47]. They observed that antibody positivity for - *P.gingivalis* FDC381 and *P. gingivalis* Su63 was higher than the other 11 bacteria and differed between the three groups of 51 CHD patients with variable degrees of periodontitis, 55 had periodontitis, and 37 were controls.

Periodontitis has been shown to be related to lower levels of HDL-cholesterol (HDL-C), a situation that may be reversed after treatment of periodontitis [17,43]. D'Aiuto et al. have shown in randomized controlled trials that in a study of 6 months duration periodontal therapy achieved a reduction in CRP, IL-6, total cholesterol, systolic blood pressure, and the Framingham cardiovascular disease risk score [48]. In a second trial on persons with type 2 diabetes, periodontal treatment over a period of 12 months significantly reduced HbA1c [49]. Ramirez et al. studied subgingival "Red complex" bacteria and biomarkers for CVD. Comparing patients with periodontitis and periodontally healthy controls they found E-selectin, MPO, and ICAM-1 to be increased among patients with periodontitis. Other CVD markers studied were flow-mediated dilatation, CRP, VCAM-1, MMP-9, Adiponectin, and tPAI-1 [50]. The Danish Nationwide Cohort Study included 17,691 patients who received a hospital diagnosis of periodontitis and compared them to 83,003 controls from the general population [51]. Hansen et al. concluded that periodontitis may be an independent risk factor for CVD measured by incidence rate ratios, all significant, for myocardial infarction, ischemic stroke, CVD death, major adverse CVD events (MACE), and all-cause mortality.

Multiple microbiology studies using several approaches have been performed in order to understand periodontal disease development - the metastatic spread of bacteria, the effect, and function of bacterial virulence factors, and the immune system response [52]. The three bacteria of the 'Red complex' show both competitive and cooperative interactions [5,53]. They also show genetic variability but according to Amano et al. changes have not been observed during persistent colonization. The bacteria of the "Red complex" possess the common feature of expressing neuraminidases. This enables them to scavenge sialic acid from host glycoconjugates. The cleaved sialic acid serves as a nutrient for bacterial growth and aid the bacteria to evade host immune attack. Another important feature of periodontal pathogens is their ability to enter into host cells. It has been shown that TD and PG display symbiosis in protein degradation, nutrient utilization, and growth promotion [54]. The reduced antibody response to TF might be due to the unusual S-layer of this bacterium where two S-layer glycoproteins are assembled into a single S-layer [54–56]. The S-layer may be used as a strategy for this bacterium to evade recognition by the innate immune system particularly by suppressing Th17 responses [56]. The surface glycosylation provides a means of manipulation of the cytokine response of macrophages and T-cells. The present findings relate myocardial infarction to the metastatic consequences of advanced periodontal/dental bacteria and/or bacterial products.

Conclusion

The novel and main finding of this study is the inverse relation for antibodies towards the periodontopathogen *T. forsythia* to increased risk for CVD mortality. *T. forsythia* is an initiator of advanced "chronic" periodontal infection, allowing for the progression of periodontitis by the joint effect of the 'Red complex' bacteria on cardiovascular structures. This throws new light on the relative importance of red complex members in the development of CVD where *P. gingivalis*, the keystone bacterium of periodontitis, has attracted most attention so far. The reduced ability to form antibodies to *T. forsythia* in relation to MI needs to be studied further to establish whether it represents a general immunological deficiency contributing to the pathophysiological mechanism resulting in myocardial infarction. The consequence is the development of a vaccine for persons identified with low levels of antibodies to *T. forsythia* by a specific ELISA-test particularly in association with chronic periodontitis. This finding supports the reports of a suggested causal link between "chronic" periodontitis to mortality in persons with myocardial infarction.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] Holt SC, Ebersole JL. *Porphymonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia*: the 'red complex', a prototype polybacterial pathogenic consortium in periodontitis. Periodontology 2000;2005(38):72–122.

[2] Dahlén G, Fiehn N-E, Olsen I, et al. Oral microbiology and immunology. Denmark: Munksgaard; 2012.
[3] Socransky SS, Haffajee AD. Evidence of bacterial etiology: a historical perspective. Periodontol 2000;1994(5):7–25.
[4] Bah A, Vergne I. Macrophage autophagy and bacterial Infections. Front Immunol 2017;6(8):1483.
[5] Olsen I, Lambris JD, Hajishengallis G. *Porphyromonas gingivalis* disturbs host-commensal by changing complement function. J Oral Microbiol 2017;30(9):1340085.
[6] Endo A, Watanabe T, Ogata N, et al. Comparative genome analysis and identification of competitive and cooperative interactions in a polymicrobial disease. ISME J 2015;9(3):629–42.
[7] Chhibber-Goel J, Singhal V, Bhowmik D, et al. Linkages between oral commensal bacteria and atherosclerotic plaques in coronary artery disease patients. NPJ Biofilms Microbiomes 2016;2:7.
[8] Hajishengallis G, Maekawa T, Abe T, et al. Complement involvement in periodontitis: molecular mechanisms and rational therapeutic approaches. Adv Exp Med Biol 2015;865:57–74.
[9] Ennibi OK, Benrachadi L, Bouziane A, et al. The highly leukotoxic JP2 clone of *Aggregatibacter actinomycetemcomitans* in localized and generalized forms of aggressive periodontitis. Acta Odontol Scand 2012;70:318–22.
[10] Haubek D, Johansson A. Pathogenicity of the highly leukotoxic JP2 clone of *Aggregatibacter actinomycetemcomitans* and its geographic dissemination and role in aggressive periodontitis. J Oral Microbiol 2014;6..
[11] DeStefano F, Anda RF, Kahn HS, et al. Dental disease and risk of coronary heart disease and mortality. BMJ 1993;306:688–91.
[12] Mattila KJ, Valtonen VV, Nieminen MS, et al. Role of infection as a risk factor for atherosclerosis, myocardial infarction, and stroke. Clin Inf Dis 1998;26:719–34.
[13] Darveau RP. Periodontitis: a polymicrobial disruption of host homeostasis. Nat Rev Microbiol 2010;8:481–90.
[14] Andriankaja O, Trevisan M, Falkner K, et al. Association between periodontal pathogens and risk of nonfatal myocardial infarction. Community Dent Oral Epidemiol 2011;2:177–85.
[15] Kumar PS. Oral microbiota and systemic disease. Anaerobe 2013;24:90–3.
[16] Kozarov E. Bacterial invasion of vascular cell types: vascular infectology and atherogenesis [Review]. Future Cardiol 2012;8:123–38.
[17] Pussinen PJ, Jauhiainen M, Vilkuna-Rautiainen T, et al. Periodontitis decreases the antiatherogenic potency of high density lipoprotein. J Lipid Res 2004;45:139–47.
[18] Griffiths R, Barbour S. Lipoproteins and lipoprotein metabolism in periodontal disease. Clin Lipidol 2010;5(3):397–411.
[19] Oral Infections and Cardiovascular disease (Editor Lise Lund Håheim). Bentham e-books.
[20] Caton JG, Gary Armitage G, Berglundh T, et al. A new classification scheme for periodontal and peri-implant diseases and conditions – Introduction and key changes from the 1999 classification. 2017 WORLD WORKSHOP. J Clin Periodontol 2018;45(Suppl 20):S1–8.
[21] World Health Organization. Oral Health: What is the burden of oral disease? Geneva: WHO. http://www.who.int/oral_health/disease_burden/global/en/ Accessed 06.10.2019.
[22] Aas JA, Paster BJ, Stokes LN, et al. Defining the normal bacterial flora of the oral cavity. J Clin Microbiol 2005;43:5721–32.
[23] Olsen I. Update on bacteraemia related to dental procedures. Transfus Apher Sci 2008;39:173–8.
[24] Bahrani-Mougeot FK, Paster BJ, Coleman S, et al. Diverse and novel oral bacterial species in blood following dental procedures. J Clin Microbiol 2008;46:2129–32.
[25] Kowara M, Kasarełło K, Czarzasta K, Opolski G, Cudnoch-Jędrzejewska A. Early-life inflammation pathways trigger a cascade leading to development of atherosclerotic plaque through the “butterfly effect” – An hypothesis. Med Hypotheses 2019;122:106–10.
[26] Libby P, Ridker PM, Hansson GK. Inflammation in atherosclerosis. J Am Coll Cardiol 2009;54:2129–38.
[27] Schenkein HA, Loos BG. Inflammatory mechanisms linking periodontal diseases to cardiovascular diseases. J Periodontol 2013;84(4 Suppl):S51–69.
[28] Kerrigan S, Cox Dermot. Biological mechanisms: Platelets and bacteria – current scientific evidence and methods of analyses Chapter 6 Oral Infections and Cardiovascular DiseaseNew York: Bentham Science Publishers Ltd; 2011. p. 45–66.
[29] Van Dyke TE, van Winkelhoff AJ. Infection and inflammatory mechanisms. J Clin Periodontol 2013;40(Suppl 14):S1–7.
[30] Fiehn NE, Gutschik E, Larsen T, Bangsborg JM. Identity of streptococcal blood isolates and oral isolates from two patients with infective endocarditis. J Clin Microbiol 1995;33:1399–401.
[31] Haraszthy VI, Zambon JJ, Trevisan M, Zeid M, Genco RJ. Identification of periodontal pathogens in atheromatous plaques. J Periodontol 2000;71:1554–60.
[32] Kolltveit KM, Geiran O, Tronstad L, et al. Multiple bacteria in calcific aortic valve stenosis. Microbial Health Dis 2002;14:110–7.
[33] Kuramitsu HK, Kang IC, Qi M. Interactions of Porphyromonas gingivalis with host cells: implications for cardiovascular diseases. J Periodontol 2003;74:85–9.
[34] Fiehn NE, Larsen T, Christiansen N, Holmstrup P, Schroeder TV. Identification of periodontal pathogens in atherosclerotic vessels. J Periodontol 2005;76:731–6.
[35] Beck JD, Eke P, Heiss G, et al. Periodontal disease and coronary heart disease: a reappraisal of the exposure. Circulation 2005;112:19–24.
[36] Olsen I. Oral bacteria in cardiovascular diseases Chapter 5 Oral Infections and Cardiovascular DiseaseNew York: Bentham Science Publishers Ltd; 2011. p. 35–44.
[37] Reyes L, Herrera D, Kozarov E, Roldá S, Progulske-Fox A. Periodontal bacterial invasion and infection: contribution to atherosclerotic pathology. J Periodontol 2013;84(4 Suppl):S30–50.
[38] Desvarieux M, Demmer RT, Jacobs DR, Papapanou PN, Sacco RL, Rundek T. Changes in clinical and microbiological periodontal profiles relate to progression of carotid intima-media thickness: the oral infections and vascular disease epidemiology study. J Am Heart Assoc 2013;2:e000254.
[39] Lund Håheim L. The infection hypothesis revisited: Oral infections and cardiovascular diseases. Epidem Research Int 2014. https://doi.org/10.1155/2014/75378. Article ID 735378.
[40] Leren P, et al. The Oslo Study. Cardiovascular disease in middle-aged and young Oslo men. Acta Med Scand 1975;Suppl 588:1–38.
[41] Lund Haheim L, Lund Larsen PG, Sogaard AJ, Holme I. Risk factors associated with body mass index increase in men at 28 years follow-up. Q J Med 2006;99:665–71.
[42] Lund Håheim L, Olsen I, Nafstad P, et al. Antibody levels to single bacteria or in combination evaluated against myocardial infarction. J Clin Periodontol 2008;35:473–8.
[43] Pussinen PJ, Vilkuna-Rautiainen T, Alfthan G, et al. Severe periodontitis enhances macrophage activation via increased serum lipopolysaccharide. Arterioscler Thromb Vasc Biol 2004;24:2174–80.
[44] Pussinen PJ, Jousilahti P, Alfthan G, Palosuo T, Asikainen S, Salomaa V. Antibodies to periodontal pathogens are associated with coronary heart disease. Arterioscler Thromb Vasc Biol 2003;23(7):1250–4.
[45] Aoyama N, Suzuki J-I, Kobayashi N, et al. Associations among tooth loss, systemic inflammation and antibody titers to periodontal pathogens in Japanese patients with cardiovascular disease. J Periodontal Res 2018;53(1):117–22.
[46] Pussinen PJ, Alfthan G, Rissanen H, Reunanen A, Asikainen S, Knekt P. Antibodies to periodontal pathogens and stroke risk. Stroke 2004;35:2020–3.
[47] Yamazaki K, Honda T, Domon H, et al. Relationship of periodontal infection to serum antibody levels to periodontopathic bacteria and inflammatory markers in periodontitis patients with coronary heart disease. Clin Exp Immunol 2007;149:445–52.
[48] D'Aiuto F, Parkar M, Nibali L, Suvan J, Lessem J, Tonetti MS. Periodontal infections cause changes in traditional and novel cardiovascular risk factors: results from a randomized controlled clinical trial. Am Heart J 2006;151(5):977–84.
[49] D'Aiuto F, Gkranias N, Bhowruth D, Khan T, Orlandi M, Suvan J, et al. Deanfield JE; TASTE Group. Systemic effects of periodontitis treatment in patients with type 2 diabetes: a 12 month, single-centre, investigator-masked, randomised trial. Lancet Diabetes Endocrinol 2018;12:954–65.
[50] Ramírez JH, Parra B, Gutierrez S, Arce RM, Jaramillo A, Ariza Y, et al. Biomarkers of cardiovascular disease are increased in untreated chronic periodontitis: a case control study. Aust Dent J 2014;59:29–36.
[51] Hansen GM, Egeberg A, Holmstrup P, Hansen PR. Relation of periodontitis to risk of cardiovascular and all-cause mortality (from a Danish Nationwide Cohort Study). Am J Cardiol 2016;118:489–93.
[52] Amano A, Chen C, Honma K, Li C, Settem RP, Sharma A. Genetic Characteristics and Pathogenic Mechanisms of Periodontal Pathogens. Adv Dent Res 2014:15–22.
[53] Dashper SG, Seers CA, Tan KH, Reynolds EC. Virulence factors of the oral spirochete *Treponema denticola*. J Dent Res 2011;90:691–703.
[54] Sekot G, Posch G, Oh YJ, et al. Analysis of the cell surface layer ultrastructure of the oral pathogen *Tannerella forsythia*. Arch Microbiol 2012;194:525–39.
[55] Sekot G, Posch G, Messner P, et al. Potential of the *Tannerella forsythia* S-layer to delay the immune response. J Dent ResJ Dent Res. 2011;90:109–14.
[56] Settem RP, Honma K, Nakajima T, et al. A bacterial glycan core linked to surface (S)-layer proteins modulated host immunity through Th17 suppression. Mucosal Immunol 2013;6:415–26.