Changes in Clinical and Microbiological Periodontal Profiles Relate to Progression of Carotid Intima–Media Thickness: The Oral Infections and Vascular Disease Epidemiology Study

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ORIGINAL RESEARCH

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Changes in Clinical and Microbiological Periodontal Profiles Relate to Progression of Carotid Intima-Media Thickness: The Oral Infections and Vascular Disease Epidemiology Study

Moïse Desvarieux, MD, PhD; Ryan T. Demmer, PhD, MPH; David R. Jacobs, Jr, PhD; Panos N. Papapanou, DDS, PhD; Ralph L. Sacco, MD, MS; Tatjana Rundek, MD, PhD

***Background*—**No prospective studies exist on the relationship between change in periodontal clinical and microbiological status and progression of carotid atherosclerosis.

***Methods and Results*—**The Oral Infections and Vascular Disease Epidemiology Study examined 420 participants at baseline (68±8 years old) and follow-up. Over a 3-year median follow-up time, clinical probing depth (PD) measurements were made at 75 766 periodontal sites, and 5008 subgingival samples were collected from dentate participants (average of 7 samples/subject per visit over 2 visits) and quantitatively assessed for 11 known periodontal bacterial species by DNA-DNA checkerboard hybridization. Common carotid artery intima-medial thickness (CCA-IMT) was measured using high-resolution ultrasound. In 2 separate analyses, change in periodontal status (follow-up to baseline), defined as (1) longitudinal change in the extent of sites with a ≥3-mm probing depth (Δ% PD≥3) and (2) longitudinal change in the relative predominance of bacteria causative of periodontal disease over other bacteria in the subgingival plaque (Δetiologic dominance), was regressed on longitudinal CCA-IMT progression adjusting for age, sex, race/ethnicity, diabetes, smoking status, education, body mass index, systolic blood pressure, and low-density lipoprotein cholesterol and high-density lipoprotein cholesterol. Mean (SE) CCA-IMT increased during follow-up by 0.139±0.008 mm. Longitudinal IMT progression attenuated with improvement in clinical or microbial periodontal status. Mean CCA-IMT progression varied inversely across quartiles of longitudinal improvement in clinical periodontal status (Δ%PD≥3) by 0.18 (0.02), 0.16 (0.01), 0.14 (0.01), and 0.07 (0.01) mm (*P* for trend<0.0001). Likewise, mean CCA-IMT increased by 0.20 (0.02), 0.18 (0.02), 0.15 (0.02), and 0.12 (0.02) mm (*P*<0.0001) across quartiles of longitudinal improvement in periodontal microbial status (Δetiologic dominance).

***Conclusion*—**Longitudinal improvement in clinical and microbial periodontal status is related to a decreased rate of carotid artery IMT progression at 3-year average follow-up. **(*J Am Heart Assoc.* 2013;2:e000254 doi: 10.1161/JAHA.113.000254)**

Key Words: atherosclerosis • infection • inflammation • periodontal • progression

Studies have linked periodontal disease (clinical manifestation of chronic periodontal infections and inflammation) to both cardiovascular disease (CVD) and atherosclerosis.[1–5] The clinical evidence was extended to serological studies linking elevated periodontal bacteria antibody titers to atherosclerotic vascular disease,[6–10] and we have reported cross-sectional evidence of greater carotid intima-media thickness with increasing proportion of "etiologic" periodontal bacteria in the subgingival plaque.[11] One unanswered question is evaluating the relationship between temporal change in chronic periodontal infections levels and subclinical atherosclerosis progression. No prospective studies exist on the parallel evolution of chronic low-grade infections, including periodontal infections, and subclinical vascular disease. Prospective studies of this nature are important for establishing or refuting causality, thus, filling a critical gap, as recently summarized in an American Heart Association statement regarding the association between periodontal disease and atherosclerotic vascular disease.[12]

The Oral Infections and Vascular Disease Epidemiology Study (INVEST) was specifically designed to study the hypothesis that periodontal infections predispose to accelerated

From the Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, NY (M.D., R.T.D.); INSERM U738 Paris (M.D.); École des Hautes Études en Santé Publique, Rennes, France (M.D.); Division of Epidemiology and Community Health, School of Public Health, University of Minnesota, Minneapolis, MN (D.R.J.); Department of Nutrition, University of Oslo, Oslo, Norway (D.R.J.); Division of Periodontics, Section of Oral and Diagnostic Sciences, College of Dental Medicine, Columbia University, New York, NY (P.N.P.); Department of Neurology, Miller School of Medicine, University of Miami, Miami, FL (R.L.S., T.R.).

Correspondence to: Moïse Desvarieux, MD, PhD, Department of Epidemiology, Mailman School of Public Health, Columbia University, 722 W 168th Street, Room 525, New York, NY 10032. E-mail: mdesvarieux@columbia.edu

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progression of carotid atherosclerosis and subsequent CVD. In addition to clinical assessments of periodontal status, the study examined the level of a number of bacterial species in the subgingival environment adjacent to selected teeth, namely, those considered to be etiologically related to periodontitis and a selection of others as controls. In this report, we investigated whether changes in periodontal status, assessed clinically and microbiologically, were associated with progression of carotid atherosclerosis longitudinally. Our a priori hypothesis was that improvement in periodontal status and reduction in the proportion of "etiologic" periodontal bacteria in the subgingival plaque would be related to slower intima-medial thickness (IMT) progression, whereas worsening periodontal infections would increase IMT progression.

Methods

INVEST is a randomly sampled prospective cohort study investigating the relationship between oral infections, carotid atherosclerosis, and CVD in participants residing between 145th Street and 218th Street in Manhattan.[11,13] Hispanics, blacks, and whites live together in this area with similar access to medical care. The selection process was derived from the Northern Manhattan Study.[14]

INVEST baseline eligibility criteria were: (1) resident >3 months of zip codes 10031, 10032, 10033, 10034, or 10040; (2) contacted by random-digit dialing; (3) age ≥55 years; (4) no history of stroke, myocardial infarction, or chronic inflammatory conditions; and (5) ambulatory. This protocol was approved by the Columbia University and University of Miami institutional review boards. All participants provided written informed consent.

Of 1056 participants enrolled at baseline, 842 were dentate, and 626 were alive and attended a follow-up visit (81% of surviving dentate participants). Two hundred six subjects were excluded from the present analysis for antibiotic prophylaxis requirements (n=81), missing baseline cardiovascular data (n=63), logistical reasons (n=47), or no longer being dentate (n=15), a necessary condition for periodontal and microbiological assessments. Thus, we include 420 for analyses of longitudinal change in clinical periodontal status and progression of IMT, representing 79% of dentate subjects not requiring premedication, in accordance with American Heart Association criteria.[15] Among these 420, 364 (87%) also had longitudinal bacteriological data.

Oral Examination

Participants were examined by calibrated dental examiners.[16] Briefly, full-mouth periodontal assessment for all teeth present included probing depth (PD) and attachment loss (AL) measurements (both in millimeters) at 6 sites/tooth (mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual, and distolingual) using a UNC-15 manual probe (HuFriedy, Chicago, IL). Intraexaminer reliability studies on 14 participants yielded intraclass correlations of 0.97 and 0.94 between repeat measures of mean PD or AL, respectively. Mean absolute differences between repeat examinations for mean PD or AL were 0.09 and 0.17 mm.

Subgingival Plaque Collection and Processing

Up to 8 subgingival bacterial plaque samples were collected per participant (65% contributed 8 biofilms and 91% contributed ≥4 biofilms) from the 2 most posterior teeth (mesiolingual in maxilla and mesiobuccal in mandible) in each quadrant to avoid biased collection of samples from the most diseased sites. Thus, we report on 5008 plaque samples over 2 visits. At baseline and follow-up, digoxigenin-labeled whole-genomic probes were used to assess microbial levels for 11 bacterial strains selected to include species considered to be (1) etiologically linked to periodontal disease or frequently encountered in pathological periodontal conditions[17,18] (*Aggregatibacter actinomycetemcomitans* [ATCC 43718], *Porphyromonas gingivalis* [ATCC 33277], *Tannerella forsythia* [ATCC 43037], *Treponema denticola* [ATCC 35404]); (2) putative periodontal pathogens[17] (*Prevotella intermedia* [ATCC 25611], *Fusobacterium nucleatum* [ATCC 10953], and *Micromonas micros* [ATCC 33270], *Campylobacter rectus* [ATCC 33238], and *Eikenella corrodens* [ATCC 23834]); and (3) primarily associated with gingivitis or healthy periodontal conditions (*Veillonella parvula* [ATCC 10790] and *Actinomyces naeslundii* [ATCC 49340]), using checkerboard DNA–DNA hybridization[11,17] in all 5008 subgingival plaque samples.

Gingival Crevicular Fluid Interleukin-1β

Methylcellulose filter paper strips (IDE, Amityville, New York) were inserted into the mesiobuccal crevice/pocket of each quadrant's most posterior tooth until reaching its base, and kept in place for 30 seconds. Gingival crevicular fluid (GCF) volume determinations were performed using a calibrated Periotron 6000. Interleukin-1β (IL-1β) levels (in picograms per milliliter) were determined by ELISA (Multikine Kit; Cistron Biotechnology, Pine Brook, NJ.

Carotid Ultrasound

Bilateral carotid arteries were scanned longitudinally in the common carotid artery (CCA), internal carotid artery and bifurcation, as described.[11] The optimal angle of insonation was used to measure the CCA-IMT in the near and far walls extending from 10 mm distal to the flow divider and stopping

at 20 mm below the flow divider. In addition, to minimize measurement error, a rigorous follow-up protocol involved (1) identification of internal landmarks, (2) utilization of a carotid mask to maximize superimposable visualizations at both visits, and (3) utilization of the same ultrasound machine at both visits.

Carotid IMT measurements were performed off-line using IMAGE-Pro V.5.1 (Microsoft) image analysis software, outside of plaque by 1 reader.[11] In accordance with the third Manheim consensus,[19] we selected digital frames corresponding to the minimum observed arterial diameter during the cardiac cycle, which represents diastolic phase. The reader visualized blood-intima and media-adventitia boundaries with a mouse-controlled tracer (caliper) within the carotid segment. The measurement algorithm takes a minimum of 100 measurements within the 10-mm segment. Bilateral IMT values were averaged across the near and far walls of either (1) the maximal CCA-IMT or (2) all 12 sites (C-IMT), that is, the maximal CCA-IMT, the maximal bifurcation IMT, and the maximal internal carotid artery IMT.

Interultrasonographer reliability studies yielded a mean absolute difference between repeat measures of 0.04 and 0.05 mm for C-IMT and CCA-IMT, respectively; intraclass correlation coefficients were 0.77 and 0.75 and consistent with previous findings from longitudinal studies of IMT change.[20] Ultrasound examiners were unaware of periodontal status at IMT scanning and reading. Similarly, periodontal laboratory technicians were blinded to risk factors and IMT results.

Risk Factor Assessment

Subjects were interviewed regarding sociodemographic characteristics, cardiovascular risk factors, and other medical conditions using standardized questions.[16] Research assistants measured height and weight with calibrated scales, blood pressure (2 measurements average) using a calibrated sphygmomanometer (Omron).[13] Fasting glucose and lipids were measured.[21] Diabetes mellitus was defined by a fasting glucose ≥126 mg/dL (7.7 mmol/L) or self-report of physician-diagnosed diabetes. Smoking was assessed both categorically (current/former/never) and continuously (pack-years).[16]

Statistical Analysis

Analyses were performed using SAS 9.2. Periodontal status was defined on the basis of clinical and bacterial periodontal measures as follows.

Clinical periodontal status was defined by either (1) full-mouth mean probing depth (MPD) values or (2) the percentage of measured sites with PD≥3 mm (%PD≥3). These variables were selected a priori on the basis of previous research demonstrating their strong correlation with underlying bacterial profiles[22] and clinical signs of inflammation.[23] We also considered mean attachment loss and the percentage of sites with AL≥5 mm (%AL≥5) to assess the sensitivity of associations to commonly used epidemiological definitions of periodontitis.[24]

For each microbial species, bacterial counts were natural log–transformed, averaged within the mouth, and standardized by dividing these values by the natural log–transformed population standard deviation. As a result, 1 standard deviation on the natural log scale was equivalent across microbes.[11] Thus, for each person and both evaluations, and based on a priori evidence at baseline (ie, the consensus of the 1996 World Workshop in Periodontics[25] and Socransky's Red Complex,[26]) we operationalized periodontal bacterial data in 3 ways according to the cumulative sum of colonization levels as follows: (1) combined levels of *P gingivalis, T forsythia*, *A actinomycetemcomitans,* and *T denticola* were defined as "etiologic bacterial burden" (EB); (2) combined levels of *C rectus*, *E corodens*, *F nucleatum*, *M micros*, and *P intermedia* were defined as "putative bacterial burden"; and (3) combined levels of *A naeslundii* and *V parvula* were defined as "health-associated" bacterial burden as previously,[11,22] with the last 2 groups being used as controls. Finally, etiologic dominance (ED) was defined as the relative predominance of the etiologic bacterial group in the studied ecological niche by dividing EB by the cumulative sum of all 3 aforementioned bacterial burdens (EB, putative bacterial burden, and health-associated bacterial burden). Etiologic dominance informs the specific effect of etiologic species on IMT change via division, rather than statistical adjustment.

Both baseline and longitudinal change in the aforementioned periodontal variables were considered independent variables in statistical models. The dependent variable was defined as change in either the CCA-IMT (ΔCCA-IMT) or the C-IMT (ΔC-IMT). With both measurements providing similar conclusions, CCA-IMT results are reported.

Change in either independent or dependent variables were calculated as follow-up−baseline (positive value indicates worsening, negative value indicates improvement). The Δ symbol signifies longitudinal change.

Multivariable linear regression models assessed the association between quartiles of either baseline or change in periodontal status and IMT change, adjusting for age, sex, race/ethnicity, diabetes, smoking status, education, body mass index, systolic blood pressure, low-density lipoprotein cholesterol and high-density lipoprotein cholesterol. We opted to not include results arising from models adjusted for baseline IMT to avoid bias[27,28] in the regression estimates that might exaggerate any observed associations. Analyses adjusting for baseline IMT did not change results. Tests for

linear trend were conducted by modeling the 3-level ordinal categorical exposure variable as a linear term.

Results

The mean±SD age of participants was 68±8 years at baseline; 62% were female, 60% Hispanic, 20% black, 19% white, and 1% other (Table 1). The median follow-up time was 3.1 years (range, 1.8 to 7.2 years), and 94% were reexamined within 2 to 4 years.

Participants with greater levels of clinical periodontal disease at baseline generally experienced more improvement in periodontal health longitudinally, reflecting a strong inverse correlation between baseline MPD and ΔMPD ($r=-0.57$, $P<0.0001$) or between baseline %PD≥3 and Δ%PD≥3 ($r=-0.64$, $P<0.0001$). Baseline bacterial EB and ΔEB were also

Table 1. General Characteristics According to Quartiles of Progression in Periodontal Pockets (Δ%PD≥3)

Variable	All Participants	Worsening Periodontal Status ←			Improving Periodontal Status →	*P* Value for Linear Trend Across PD
		ΔPD IV, N=105	ΔPD III, N=105	ΔPD II, N=105	ΔPD I, N=105	
Age, y	68±8	69±8	69±8	67±8	65±8	<0.0001
Male, %	37	32	35	38	42	0.12
Race/Ethnicity, %						
Hispanic	61	59	58	58	68	0.10
Black	20	28	18	20	13	
White	18	11	24	19	18	
Other	1	2	0	3	1	
Completed high school, %	50	44	50	55	51	0.83
Never smoker, %	52	54	53	51	49	0.13
Former smoker, %	38	33	44	38	37	
Current smoker, %	10	12	3	11	14	
Pack-years	10±20	12±1.9	11±1.9	11±1.9	7±1.9	0.13
Body mass index, kg/m^2	28.4±5.5	29.3±0.5	27.8±0.5	27.9±0.5	28.5±0.5	0.33
Diabetes, %	15	19	10	14	15	0.62
Hypertension, %	62	64	55	59	69	0.36
Systolic blood pressure, mm Hg	138±19	137±1.8	135±1.8	137±1.8	142±1.8	0.03
Diastolic blood pressure, mm Hg	79±11	77±1.1	78±1.1	79±1.1	81±1.1	<0.0001
LDL-cholesterol	125±35	121±3.4	127±3.4	123±3.4	127±3.4	0.38
HDL-cholesterol	52±16	53±1.5	51±1.5	51±1.5	51±1.5	0.55
CCA-IMT	0.847±0.15	0.827±0.01	0.834±0.01	0.849±0.01	0.878±0.01	0.01
%PD≥3, %	43	27±0.02	31±0.02	46±0.02	67±0.02	<0.0001
Δ%PD≥3, %	−0.17	12±0.01	−8±0.01	−23±0.01	−50±0.01	<0.0001
Mean PD	2.47±0.67	2.1±0.06	2.3±0.06	2.5±0.06	3.0±0.06	<0.0001
ΔMean PD	−0.39±0.65	0.3±0.04	−0.3±0.04	−0.5±0.04	−1.1±0.04	<0.0001
Mean AL	2.88±1.41	2.6±0.1	2.4±0.1	3.1±0.1	3.4±0.1	<0.0001
ΔMean AL	0.20±1.44	1.2±0.1	0.6±0.1	0.05±0.1	−1.0±0.1	<0.0001
Number of missing teeth	13±8	16±0.8	12±0.8	14±0.8	12±0.8	<0.01
Etiologic burden	31.3±3.5	30±0.3	30±0.3	32±0.3	34±0.3	<0.0001
ΔEtiologic burden	1.1±4.3	3.2±0.4	2.4±0.4	0.6±0.4	−2.4±0.4	<0.0001
Etiologic dominance, %	29±2	28±0.1	28±0.1	29±0.2	30±0.2	<0.0001
ΔEtiologic dominance, %	0±2	0.15±0.1	−0.31±0.2	−1.0±0.2	−2.0±0.2	<0.0001

Results are mean±SD for all participants and mean±SE across categories of periodontal change. Categories of ΔPD are defined by longitudinal change in percentage of periodontal sites with probing depth ≥3 mm. Average and median follow-up times were 3.2 and 3.1 years. AL indicates attachment loss; CCA-IMT, common carotid artery intima-medial thickness; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PD, probing depth; SD, standard deviation; SE, standard error of the mean.

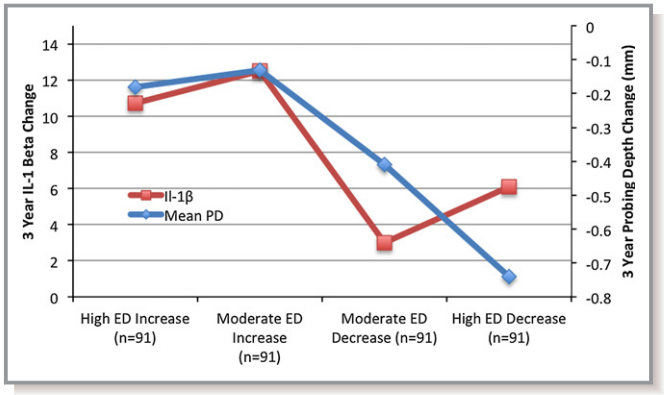

Figure 1. Association between 3-year bacterial etiologic dominance change and concurrent change in gingival IL-1β and periodontal probing depth adjusted for age, sex, race/ethnicity, diabetes, smoking status, education, body mass index, systolic blood pressure, LDL-cholesterol, and HDL-cholesterol. Mean probing depth *P* for linear trend<0.0001; IL-1β *P* for linear trend=0.12. ED indicates etiologic dominance; HDL, high-density lipoprotein; IL-1β, interleukin-1β; LDL, low-density lipoprotein; PD, probing depth.

strongly inversely correlated (r=−0.86, P<0.0001). Change in MPD was correlated with change in EB (r=0.37, P<0.0001).

Mean baseline CCA-IMT was 0.847±0.154 mm (range, 0.52 to 1.45 mm). Mean CCA-IMT increased by 0.139±0.170 mm (range, −0.533 to 0.516 mm). The annualized rate of CCA-IMT change was 0.044±0.054 mm/year. Baseline CCA-IMT was positively correlated with follow-up CCA-IMT (0.34, P<0.0001) but inversely correlated with ΔCCA-IMT (−0.62, P<0.0001).

General characteristics according to categories of periodontal change are in Table 1. Primary correlates of prospective decline in periodontal status were greater age, lower blood pressure, and corresponding changes in other periodontal measures. Correlates of ΔED were similar (data not shown).

Validation of A Priori Concepts Regarding Periodontal Bacteria

Periodontal bacterial levels were related to clinical periodontal status at baseline.[11] Further, Figure 1 shows that change in etiologic dominance (ΔED) was directly related to changes in both MPD and IL-1β in the GCF. Similarly, mean change values (Δ%PD≥3) across quartiles of change of ΔED bacterial values were −33%, −17%, −6%, and −5% (P for trend<0.0001).

Change in Clinical Periodontal Status and Atherosclerotic Progression

Longitudinal change in either mean PD or AL was positively associated with IMT change; the difference in IMT between participants with the greatest worsening in PD versus those with the greatest improvement was 0.072 mm, whereas this difference was 0.049 mm when comparing worsening versus improving AL (Table 2). Results were stronger and more linear

Table 2. Longitudinal Change in Common Carotid Artery Intima-Media Thickness Across Quartiles of Longitudinal Change in Clinical Periodontal Probing Depth or Attachment Loss

Worsening Periodontal Status ←				→ Improving Periodontal Status		
Model	Worsening PD* (0.38±0.5)† n=105	Stable PD* (−0.23±0.1)† n=105	Moderate PD* Improvement (−0.57±0.1)† n=105	High PD* Improvement (−1.1±0.5)† n=105	*P* Value for Fourth vs First Quartile Comparison	*P* Value for Linear Trend
Category of Periodontal Probing Depth Change (PD)						
1	0.181±0.02	0.120±0.02	0.142±0.02	0.102±0.02	0.003	0.008
2	0.180±0.02	0.120±0.02	0.136±0.02	0.108±0.02	0.01	0.01
	Worsening AL‡ (1.94±0.5)§ n=103	Stable AL‡ (0.85±0.3)§ n=104	Moderate AL‡ Improvement (−0.22±0.4)§ n=104	High AL‡ Improvement (−1.75±0.7)§ n=103		
Category of Clinical Attachment Level Change (AL)						
1	0.142±0.02	0.180±0.02	0.150±0.02	0.071±0.02	0.007	0.0008
2	0.132±0.02	0.179±0.02	0.150±0.02	0.083±0.02	0.08	0.02

Periodontal measurements were performed at 75 766 periodontal sites. Model 1 was adjusted for baseline periodontal status (ie, mean probing depth or mean attachment loss). Model 2 was also adjusted for age, sex, race/ethnicity, education, diabetes, smoking status, body mass index, systolic blood pressure, hypertension, LDL-cholesterol, and HDL-cholesterol. HDL indicates high-density lipoprotein; INVEST, Oral Infections and Vascular Disease Epidemiology Study; LDL, low-density lipoprotein; SD, standard deviation.

*PD, longitudinal change in mean full-mouth probing depth (mm).

†Category-specific mean±SD of longitudinal change in mean full-mouth probing depth (follow-up−baseline in millimeters).

‡AL, longitudinal change in mean full-mouth attachment loss (mm).

§Category-specific mean±SD of longitudinal change in mean full-mouth attachment loss (follow-up−baseline in millimeters).

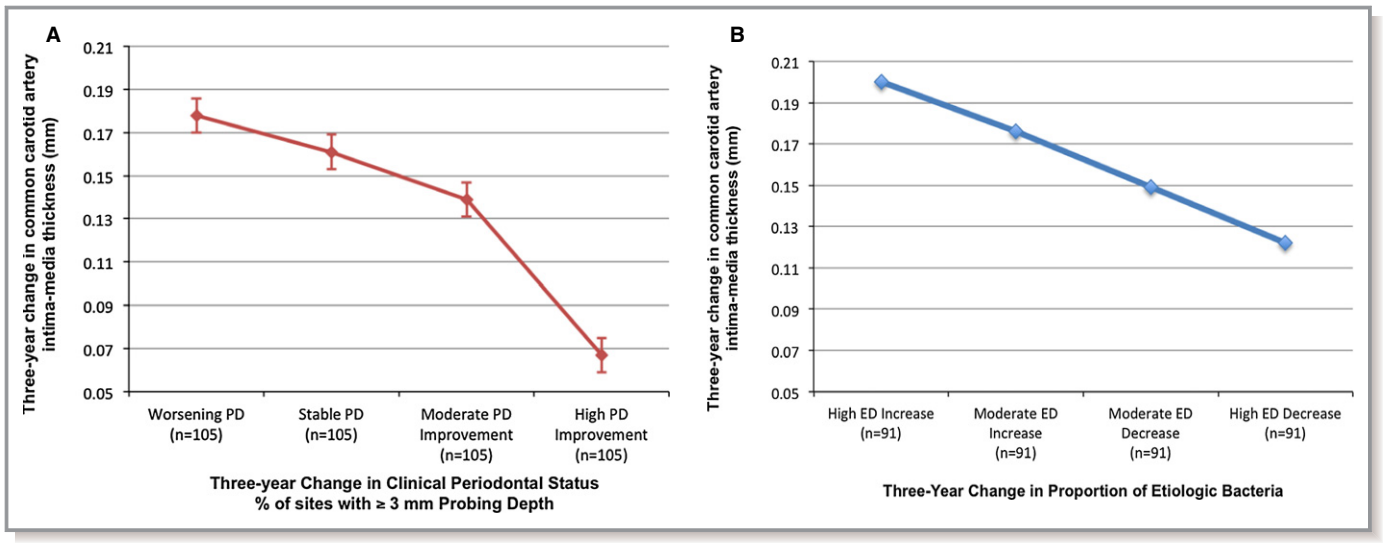

Figure 2. Relationship between longitudinal change in periodontal status and change in common carotid artery intima-media thickness. Periodontal status (A) defined clinically, according to percentage of sites with ≥3-mm probing depth (*P* for linear trend<0.0001); or (B) defined microbiologically—etiologic dominance (ED)—as the proportion of oral bacteria believed to be periodontally etiologic (*P* for linear trend<0.01). Both were adjusted for age, sex, race/ethnicity, diabetes, smoking status, education, body mass index, systolic blood pressure, LDL-cholesterol, and HDL-cholesterol. HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; PD, probing depth.

when modeling Δ%PD≥3 as the exposure (Figure 2A). The extent of longitudinal attachment loss was also related, although not statistically, to IMT progression: multivariable-adjusted mean CCA-IMT change across quartiles of Δ%AL≥5 was 0.094, 0.146, 0.158, and 0.145 mm (*P* for linear trend=0.09).

Adjusting for change in periodontal status, baseline levels of clinical periodontal disease were unrelated to IMT change. Mean±SE ΔCCA-IMT values across quartiles of increasing baseline MPD were 0.124±0.02, 0.169±0.02, 0.124±0.02, and 0.128±0.02 (*P* for linear trend=0.64). Mean ΔCCA-IMT changes across quartiles of increasing baseline mean AL were 0.147, 0.122, 0.117, and 0.158 (*P* for linear trend=0.61). ΔCCA-IMT values across baseline quartiles of %AL≥5 were: 0.142, 0.101, 0.144, and 0.169 mm (*P* for linear trend=0.07).

Change in Periodontal Bacteria and Atherosclerotic Progression

Compared with participants who experienced decreases in etiologic bacterial levels, those who experienced little change or increased burden of etiologic bacteria during follow-up also experienced greater progression of CCA-IMT (Table 3). This association was not observed for changes in levels of either putative or health-associated burden, adjusting for changes in etiologic burden (Table 3). In analyses assessing the association between change in etiologic dominance and IMT progression, mean carotid IMT progressed in a direct and dose-responsive manner, with increasing dominance of the etiologic bacterial group during follow-up (Figure 2B). The results for ΔED were unchanged when further adjusting for GCF IL-1β or MPD. Interestingly, in the model mutually adjusting for microbiological (ΔED) and clinical (ΔMPD) status, the ΔCCA-IMT trends across quartiles of ΔMPD were attenuated: 0.190, 0.159, 0.174, and 0.152 mm (*P* for trend=0.47), with only the bacterial exposure (ΔED) remaining statistically significant. Adjustment for follow-up time did not change results in either the clinical or bacteriological periodontal models related to atherosclerotic progression.

Baseline hypertension was the only traditional CVD risk factor that predicted longitudinal IMT change but in the direction opposite that expected; ΔCCA-IMT was 0.040 mm greater among normotensive participants compared with hypertensive participants (*P*=0.03). However, adjustment for longitudinal change in systolic blood pressure removed this association, consistent with the observation that systolic blood pressure decreased by an average of 11 mm Hg among baseline hypertensives but increased by 4 mm Hg among normotensives (*P*<0.0001). Longitudinal change in systolic blood pressure was positively, though not statistically, associated with ΔCCA-IMT.

Discussion

We had previously reported that higher levels of etiologic bacteria were cross sectionally associated with thicker carotid IMT at baseline.[11] We now report longitudinal change in periodontal status to be concurrent with longitudinal carotid artery IMT progression over an average period of 3 years:

Table 3. Longitudinal Change in Common Carotid Artery Intima-Media Thickness Across Quartiles of Longitudinal Change in Etiologic, Putative, and Health-Associated Periodontal Bacterial Colonization Patterns

Worsening Periodontal Status ←				→ Improving Periodontal Status		
Model	Greatest Bacterial Burden Increase (Change Category I), n=90	Bacterial Burden Change (Category Intermediate II), n=92	Bacterial Burden Change (Category Intermediate III), n=92	Greatest Bacterial Burden Decrease (Change Category IV), n=90	*P* Value for Fourth vs First Quartile Comparison	*P* Value for Linear Trend
Etiologic*						
†	6.2±1.9	2.9±0.8	−0.2±1.0	−4.7±1.7		
1	0.184±0.020	0.186±0.016	0.188±0.016	0.088±0.021	0.005	0.01
2	0.182±0.021	0.182±0.016	0.188±0.017	0.095±0.021	0.01	0.004
Putative*						
†	10.7±6.5	5.9±0.8	3.0±0.9	−1.8±2.1		
1	0.170±0.02	0.142±0.02	0.163±0.02	0.173±0.02	0.93	0.98
2	0.169±0.02	0.143±0.02	0.164±0.02	0.172±0.02	095	0.61
Health Associated*						
†	4.3±1.0	2.1±0.5	0.7±0.4	−1.3±1.0		
1	0.178±0.02	0.153±0.02	0.178±0.02	0.138±0.02	0.15	0.39
2	0.182±0.02	0.153±0.02	0.174±0.02	0.138±0.02	0.76	0.29

The Oral Infections and Vascular Disease Epidemiology Study (INVEST) with 364 subjects and 5008 periodontal biofilms. Model 1: change in etiologic, putative, and health-associated periodontal bacterial burdens simultaneously modeled. Model 2: model 1+age, sex, race/ethnicity, education, diabetes, smoking status, body mass index, systolic blood pressure, hypertension, LDL-cholesterol, and HDL-cholesterol. HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; SD, standard deviation.

*Etiologic bacterial burden is defined by the cumulative colonization levels of the 4 periodontal bacterial species: *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia,* and *Treponema denticola.* Putative bacterial burden is defined by the cumulative colonization levels of 5 species: *Fusobacterium nucleatum, Prevotella intermedia, Campylobacter rectus, Micromonas micros,* and *Eikenella corrodens.* Health-associated bacterial burden is defined by the cumulative colonization levels of *Veillonella parvula* and *Actinomyces naeslundii.*

†Category-specific mean±SD of bacterial burden (follow-up−baseline).

relative to participants who experienced deterioration in their periodontal status, those who experienced improvements in periodontal status (clinical and bacteriological) also realized slower CCA-IMT progression during follow-up. These findings held after multivariable CVD risk factor adjustments and across multiple definitions of periodontal status.

The observed associations were strongest for periodontal exposures defined using either (1) etiologic periodontal bacterial levels or (2) clinical periodontal measures previously demonstrated to have strong associations with etiologic bacterial colonization in this population.[22] We found the relative predominance of bacteria traditionally considered causally related to periodontal disease to be most closely linked to atherosclerotic progression. These bacterial species, strongly associated with baseline clinical periodontal disease,[23] are now associated with change in both clinical periodontal status and subgingival inflammation (GCF interleukin-1β).

In addition to measuring established etiologic bacterial species, we also measured 7 species serving as internal controls to minimize the potential for confounding by healthy behaviors. Because participants who brush and floss more regularly have lower absolute colonization levels of all species[11] (etiologic or otherwise) and these participants are also more likely to engage in CVD health-promoting behaviors,[11] confounding would be possible. The specificity of the relationship to the periodontal etiologic fraction of bacteria significantly alleviates that concern, adding credence to our findings.

The clinical periodontal definitions that demonstrated the strongest associations with atherosclerotic changes were consistent with our a priori expectations and methodological investigations showing low-threshold periodontal measures to correlate more strongly with etiologic periodontal bacteria[22] and inflammation.[23] Of particular note is the dose-responsive relationship between IMT progression and change in the extent of ≥3-mm periodontal pockets, a probing depth threshold generally regarded as compatible with periodontal health. This suggests that future intervention studies might consider defining periodontal eligibility criteria using low-threshold ("preclinical") characteristics. Our results are consistent with Pussinen et al's[29] report that serum antibody levels to periodontal bacteria (and thus systemic translation of local infection) are more related to bacterial levels than overt clinical disease. Hence, as an exposure of significance for systemic disease, "preclinical" periodontal disease cannot be ignored.[30]

In analyses jointly modeling bacterial exposures and either clinical exposures or GCF IL-1β levels, only bacterial exposures remained statistically significantly associated with IMT progression, identifying bacterial exposure constructs as more systemically relevant.

The potential for oral microbes to contribute to atherogenesis is biologically plausible (for reviews, see references 31,32). Oral bacterial species can induce immune system activation characterized by chronic elevations in systemic inflammatory markers,[11,33,34] possibly resulting from bacteremias of oral origin,[35] which may in turn initiate or exacerbate the inflammatory aspect of atherogenesis,[36] as in animal models.[37–39] In humans, anti-infective periodontal therapy improves endothelial function in as little as 2 months.[40]

We have found an ≈0.1 mm difference in IMT change among participants with deteriorating versus improving periodontal health during a relatively short time (ie, 3 years). A baseline difference of this magnitude has been found relevant by Salonen and Salonen,[41] O'Leary et al,[42] and Polak and colleagues[43] in predicting CVD. In progression studies, Hodis et al[44] reported that a 0.03 mm/year increase in carotid IMT (equivalent to a ≈0.1 mm progression over 3 years, as here) is associated with a 2.3-fold increased risk for coronary events. Interventional studies with statins reported as clinically significant a difference of 0.0082 mm/year in carotid IMT between annualized progression rates with placebo (0.067 mm) and pravastatin (0.059).[45] Thus, our findings of ≈0.1-mm difference in progression during an average follow-up time of 3 years appear to meet the threshold of clinical significance.

The lack of any strong associations between baseline periodontal status or CVD risk factors and IMT change may reflect that periodontal status changed substantially for many (Table 1). Similar trends were observed for blood pressure (see Results). It is likely that as a consequence of receiving examination results during baseline enrollment, participants sought advice from health professionals (dentists for periodontal findings and physicians for hypertension findings) who could have then intervened on risk factors differentially according to baseline indication (ie, levels of periodontal disease or blood pressure).

We measured only 11 bacterial species, whereas hundreds of species are known to colonize the subgingival space.[46] The 4 species presently defined as "etiologic" were selected a priori at the time of baseline enrollment on the basis of contemporary scientific evidence.[25,26] We believe that from an epidemiological standpoint, the combined sum of these species remains a robust etiologic meter of periodontal pathology, either as directly causal or correlate of yet-unmeasured pathogenic bacterial species.[22,23] Nevertheless, additional pathogens could conceivably modify the overall relationship.

Our results should be interpreted within the context and methodology of our randomly selected cohort and the timing of our exposure and outcome assessments. Our IMT progression rates are slightly higher than what has been previously reported.[47] This is likely explained by the older age (≈10 years older) and higher prevalence of diabetes (≈10% to 15% higher prevalence) in our population relative to studies included in a previous meta-analysis.[47] Although we did not use explicit cardiac gating, we identified the end-diastole frames for measurement based on lumen diameter in accordance with the Mannheim consensus,[19] and we only present results from the CCA as previously recommended.[47] Our high follow-up rate (80%) for an observational cohort with a mean age of 69 years at baseline is a strength, although the possibility for bias induced by loss to follow-up cannot be completely dismissed. However, it is most likely that, if anything, loss to follow-up would bias our findings toward the null because it is usually the least healthy individuals (ie, greater baseline periodontal disease levels and higher IMT progression rates) who do not return for in-person exams. Finally, as longitudinal change in exposure and outcome were assessed concurrently, the potential issue of lag time remains unresolved, and we must await further follow-up of INVEST for clarification and translation to clinical events.

In summary, we report the first evidence that improvement in periodontal status—defined both clinically and microbiologically—is associated with less progression in carotid atherosclerosis in a randomly selected population-based sample of men and women. These findings were observed during a relatively short period, strengthening the hypothesis that accelerated atherosclerotic progression is a mechanistic explanation for previous reports linking periodontal disease and clinical CVD. Because they were observed in a population setting, they also emphasize the importance of primary periodontal care as a possible preventive health measure. Future randomized clinical trials are required to definitively determine whether anti-infective periodontal interventions can reduce atherosclerotic progression and prevent subsequent clinical cardiovascular events, and we provide a potential target and timeline for so doing. In INVEST, carotid atherosclerosis progression paralleled progression in both bacterial periodontal profiles and clinical periodontal measures, in the dual directions of worsening and improvement, providing change-on-change population-based results equivalent to a quasi-experimental design.[48]

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Disclosures

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Unraveling the Link Between Periodontitis and Cardiovascular Disease

Thomas E. Van Dyke and Jacqueline R. Starr

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EDITORIAL

Unraveling the Link Between Periodontitis and Cardiovascular Disease

Thomas E. Van Dyke, DDS, PhD; Jacqueline R. Starr, PhD

This report of recent data from the Oral Infections and Vascular Disease Epidemiology Study (INVEST) study adds to an already substantial body of epidemiologic evidence for an association between cardiovascular disease (CVD) and periodontal disease.[1] Important questions regarding this association remain unanswered: What is the nature of the association? Does one disease influence the pathogenesis of the other? How might the association influence treatment strategies?

In what ways do Desvarieux and colleagues provide answers to any of these questions? They examine the relationship between longitudinal changes in intimal media thickness and changes over the same time period in periodontal disease severity, observing a correlation between the two. The report thus provides information about an as yet unexplored facet of the periodontitis-CVD association. Yet several factors mitigate the study's ability to answer the most important questions outlined above.

First, among the various measures of periodontal disease intensity, the authors focus attention on the "etiologic burden": the summed counts of 3 periodontitis-associated species divided by the summed counts of 11 species total, of which the "nonetiologic" species are possible periodontal pathogens or have been associated with a healthy periodontium. This confusing definition assumes that it is known exactly which bacteria cause periodontitis. However, the specific role of pathogens previously associated with periodontitis is not known. Indeed, the gram-negative bacteria of interest in this study have been strongly associated with periodontitis in cross-sectional studies, but longitudinal studies implicating these specific organisms as causative are lacking. In fact, evidence from several recent studies suggests that these pathogens emerge after initiation of the disease, possibly because the inflamed environment and tissue destruction provide an ecological niche for their overgrowth.[2,3] Thus it is potentially misleading to group them in an "etiologic burden" measure. Imbuing them with this meaning may even obscure their true relevance.

Second, as the authors point out, the experimental design precludes the assessment of the temporal relationship between periodontal disease and CVD because the 2 clinical aspects are shown only to covary over time. The chosen analysis therefore cannot discern among the following (or any other) possibilities: whether the observed association is likely to reflect a causal relationship in which periodontitis leads to CVD; whether it is due to a shared risk factor between periodontitis and CVD, such as host behaviors or immune responses; or whether it results from another source of confounding. If the association is causal, one possible explanatory mechanism is that specific periodontal pathogens directly influence the pathogenesis of cardiovascular lesions, presumably through direct interactions with the vessel wall. An alternative argument focuses on the inflammatory burden, which is now generally accepted as a major determinant in the pathogenesis of cardiovascular disease *and* periodontitis (for review, see Schenkein and Loos[4]). However, because very few inflammatory markers were included in the analysis, the work does little to distinguish between these 2 possibilities.

Animal experiments have allowed testing of hypotheses about whether periodontitis directly or indirectly influences the pathogenesis of cardiovascular lesions, and the resulting data support both mechanisms. Numerous reports implicate *Porphyromonas gingivalis* in the pathogenesis of CVD in mice (for review, see Reyes et al[5]), including evidence of direct invasion of vascular endothelium. An equally substantial literature demonstrates a role for periodontitis-induced inflammation. In a rabbit model of early atherogenesis, the presence of periodontitis significantly increased the extent of atherosclerotic lesions,[6] but no periodontal bacteria could be identified in the vessel wall lesions. A similar experiment in

From The Forsyth Institute, Boston, MA.

Correspondence to: Thomas E. Van Dyke, DDS, PhD, Department of Applied Oral Sciences, Center for Periodontology, 245 First Street, Cambridge, MA 02142. E-mail: tvandyke@forsyth.org

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mice provided the same increase in atherosclerotic lesions, but in this case, periodontal pathogens were isolated from the lesion in the vessel wall.[7]

In humans as well, it is clear that increased inflammatory burden, such as that caused by metabolic syndrome or type-2 diabetes, is associated with increased risk for atherosclerosis.[8] The inflammatory burden of periodontitis is significant (for review, see Schenkein and Loos[4]), but it is not clear what role periodontal bacteria play in directly stimulating the inflammatory response in the vessel wall. Nevertheless, the plausibility of such a role was strengthened recently when viable *Porphyromonas gingivalis* were isolated from human atheromatous plaques.[9] Further prospective intervention studies that target inflammation and/or bacteria are required, and experimental design will be critical. For instance, interventions that reduce inflammation systemically, such as statins, do not clarify this question because successful interventions will improve both diseases simultaneously.[10] Rather, antibacterial or antiinflammatory treatment interventions must be applied locally to periodontitis-affected oral sites, with subsequent assessment of intermediate periodontal, bacterial, and immune markers as well as the degree of change in cardiovascular-related endpoints.

Periodontitis is recognized as an inflammatory disease of bacterial origin by the American Academy of Periodontology. Taken together, the data suggest that both in animals and humans periodontitis is also associated with the progression of atherosclerosis.[11] We applaud the authors' achievement in following a population-based cohort and making systematic measurements of both periodontal and CVD indicators over time, none of which are trivial undertakings. Their current report describes a novel way of examining the relationship between manifestations of periodontitis and CVD. Future work in the INVEST or other cohorts would benefit by including a greater number of time points and by incorporating assessment of an expanded number of bacterial taxa and host biomarkers. Such a systems biology approach may be needed to begin to tease out the complex relationship among these factors and between the 2 diseases. And ultimately, to distinguish among confounded or causal associations, intervention studies are likely to be needed.

Disclosures

None.

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