Review

Markers of Systemic Bacterial Exposure in Periodontal Disease and Cardiovascular Disease Risk: A Systematic Review and Meta-Analysis

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Background: Recent meta-analyses reported a weak association between periodontal disease (PD) on clinical examination and cardiovascular disease (CVD). Systemic bacterial exposure from periodontitis, which correlates poorly with the clinical examination, has been proposed as the more biologically pertinent risk factor. The purpose of this study was to review and analyze the association between PD with elevated systemic bacterial exposure and CVD.

Methods: We searched in the PubMed, Cochrane Controlled Trials Register, EMBASE, and SCOPUS databases for all literature examining PD and CVD. From 10 selected publications, we extracted 12 cohort (N = 5) and cross-sectional (N = 7) studies and included 11 of these in a meta-analysis. With stratified analyses, this resulted in 14 analyses of coronary heart disease (CHD; N = 7), stroke (N = 4), and carotid intima-medial thickening (CIMT; N = 3) as a measure of early atherosclerosis. Systemic bacterial exposure was measured by periodontal bacterial burden (N = 1), periodontitis-specific serology (N = 12), or C-reactive protein (N = 1).

Results: Periodontal disease with elevated markers of systemic bacterial exposure was associated strongly with CHD compared to subjects without PD, with a summary odds ratio of 1.75 (95% confidence interval (CI): 1.32 to 2.34; $P < 0.001$). This group was not associated with CVD events or with stroke but was associated with a significant increase in mean CIMT (0.03 mm; 95% CI: 0.02 to 0.04).

Conclusion: Periodontal disease with elevated bacterial exposure is associated with CHD events and early atherogenesis (CIMT), suggesting that the level of systemic bacterial exposure from periodontitis is the biologically pertinent exposure with regard to atherosclerotic risk. *J Periodontol 2007;78:2289-2302.*

KEY WORDS

Cardiovascular disease; C-reactive protein; immunoglobulins; periodontal disease; periodontitis; stroke.

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Severe periodontal disease affects 10% to 15% of the general population and has been linked to cardiovascular disease (CVD) in cross-sectional and cohort studies.[1-4] These have been reviewed and meta-analyzed recently by several investigators.[2-4] They estimated a statistically significant but clinically modest risk conferred by periodontal disease, with a 13% to 19% increased relative risk of CVD, which includes stroke, or isolated coronary heart disease (CHD), independent of traditional cardiovascular risk factors.[2,3] However, these reviews[2-4] defined periodontal disease by clinical examination or radiologic criteria and did not include biologic markers of the systemic bacterial exposure associated with periodontal disease.

One such marker of the systemic bacterial exposure from periodontitis is C-reactive protein (CRP). CRP is a pentameric molecule produced mainly by the liver in response to macrophage-derived signals, primarily interleukin (IL)-6, as part of the innate immunity's response to bacterial infection. It has antibacterial properties as a soluble bacterial pattern recognition receptor, which can opsonize bacteria for use by CRP receptors on neutrophils and macrophages and can fix complement directly as part of the classical cascade to lyse bacteria.[5] Periodontal disease has been associated

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with elevations in CRP[6] as well as with a reduction in CRP after its treatment.[7]

Over the past decade, an individual's CRP level, as a marker of the systemic inflammatory response and measured years before known CVD, has been associated consistently with incident CHD in the general population.[8-12] This elevation in CRP may reflect the burden of subclinical atherosclerosis in a person because the atherosclerotic process is inflammatory in nature.[13] CRP is found within atheromas[14,15] and is generated by the atheromatous process. Endothelial cells in atheromatous lesions locally produce CRP, as measured by in situ mRNA, and macrophage-derived IL-6 originating in the atheroma can elicit further hepatic generation of CRP.[16,17] As such, CRP has been seen simply as a marker for an individual's total atheromatous burden.

CRP also has been documented to play a direct role in the progression of atheromatous disease.[18,19] In support of this, CRP was shown to opsonize oxidized or enzymatically modified low-density lipoprotein (LDL) cholesterol, allowing for complement activation as well as macrophage uptake, leading to the characteristic foam cell generation associated with atheroma formation.[18,19] In these studies, neither native nor modified LDL cholesterol induced foam cell generation, as a measure of atherogenic potential, in the absence of CRP. Thus, the epidemiologic association between CRP and CVD also may reflect an increased risk conferred by CRP itself. As such, it has been proposed[20] that CRP arising from the liver in response to distant sites of chronic infection, including periodontal disease, may become deposited in atheromas and contribute to their accelerated progression.

Independent of a systemic response with CRP, however, the direct deposit of periodontal-derived bacteria within atheromas also has been proposed as an inflammatory stimulus leading to atheromatous progression. Periodontal pathogens have been detected within atheromatous plaques[21,22] where they may activate endothelial cells and monocytes when taken up by bacterial pattern recognition Toll-like receptors. It was proposed[23] that by activating macrophages, these macrophages also are stimulated to recognize modified LDL cholesterol by molecular mimicry (thus, the Toll-like receptors also are known as cholesterol-scavenger receptors), leading to foam cell generation and atheroma progression. Because blood cultures seldom reveal bacteremia by periodontal pathogens in the absence of endocarditis, homologous serologic titers (immunoglobulin A [IgA] and G [IgG] antibodies) against periodontal pathogens have been used as a surrogate marker for the level of occult periodontal bacterial translocation and systemic exposure. The level of homologous serology correlates with the bacterial burden of that particular periodontal pathogen in gingival plaque.[24-27] There remains controversy on the use of serology to diagnose periodontal disease itself, in particular because the acquired immune system is better at detecting recurrent disease, i.e., exacerbations, than initial periodontitis.[28] Thus, although serology has been validated in the diagnosis of periodontal disease in large studies, with 90% specificity, its sensitivity is only 71%; 10% of those with antibody levels will not have clinical disease, consistent with a past exposure, and 29% with periodontal disease will not have mounted an acquired-immunity antibody response yet.[29] Nevertheless, serology can be used conservatively as a marker of the systemic bacterial exposure of periodontal disease, in particular when looking at CVD, where chronic, ongoing exposure is the hypothesized risk factor.[30] Persons with chronic bacterial exposure from periodontitis over many years, corresponding to the years of atheromatous progression, are likely to develop a systemic immune response.

Thus, the bacterial burden of gingival plaque, the innate immunity response by CRP, and the acquired immunity serologic response can be combined reasonably as measures of the bacterial systemic exposure of periodontitis. This also was suggested by one study[31] in mice, which showed that oral inoculations with *Porphyromonas gingivalis* (*Pg*), the most common periodontal pathogen in adult humans, resulted in the onset of periodontitis along with *Pg* DNA in aortic tissue, an increase in IL-6 (CRP was not measured), and an increase in homologous serum IgG response.

Several investigators[1,30,32-39] suggested that bacterial systemic exposure may be the more pertinent biologic risk factor for CVD compared to periodontal disease per se. For example, in the Atherosclerosis Risk in Communities (ARIC) Study, Beck et al.[24,40] demonstrated that IgG antibody levels against *P. gingivalis* had a stronger association with carotid intima-media thickening (CIMT ≥1 mm) than the clinical examination. In another ARIC study, Beck et al.[30] demonstrated that periodontitis, as determined by clinical examination, was not predictive of CHD, whereas the systemic antibody response against multiple periodontal organisms was associated with CHD in smokers and non-smokers.

Several recent studies[1,24,33-39,41] linked biologic markers of periodontal disease exposure with cardiovascular outcomes. Therefore, this review was undertaken to examine these recent studies and to perform a meta-analysis, if this could be done meaningfully. Among adults in the general population, we hypothesized that people with periodontal disease associated with high bacterial exposure are at higher risk for CVD compared to people without periodontal disease as defined by clinical examination and/or these markers of exposure.

MATERIALS AND METHODS

Literature Search

All four reviewers ran independent literature searches using PubMed, the Cochrane Controlled Trials Register (CCTR), EMBASE, and SCOPUS in March 2006. All publication years through March 2006 were included. The following medical subject headings (MeSH terms) were searched in PubMed: *periodontal diseases*, *periodontitis*, *periodontal attachment loss*, *oral hygiene*. These terms were combined with *cardiovascular diseases*, *atherosclerosis*, *carotid artery disease*, *myocardial infarction*, *cerebrovascular accident*, or *transient ischemic attack*. In addition, the following key words were searched: *periodontal disease*, *periodontitis*, *periodontal attachment loss*, *oral hygiene*, in combination with *cardiovascular disease*, *coronary disease*, *myocardial infarction*, *cerebrovascular accident*, *stroke* or *atherosclerosis*. The PubMed search was restricted to studies involving humans and to papers published in English. The CCTR database, SCOPUS, and EMBASE were searched similarly using the key words. Finally, references were hand searched from the identified papers and previous systematic reviews.[2-4]

Data Extraction

Titles of all articles obtained were screened by each reviewer, and all abstracts of possible articles were screened further. Upon identification of a possible abstract for inclusion, the full text of the article was assessed. There were no possible abstracts without full-text versions. There were no occasions of reviewer disagreement as to inclusion eligibility. In our initial PubMed search, 822 possible papers were found. After screening by title and then by abstract, 10 publications were selected for full-text review. One publication was excluded for not having necessary data[39] and another was included from the hand search of citations.[35] Thus, 10 papers were included in the systematic review[1,24,30,33-38,41] (Fig. 1). These 10 publications contained 12 separate studies, both cohort and cross-sectional, and, accounting for reporting on stratified analyses, 14 separate analyses that could be combined in meta-analysis. One study[24] was excluded in the meta-analysis because of the inability to combine the measure of association with other studies (Fig. 1; Table 1). No additional publications were discovered through the other searches. A

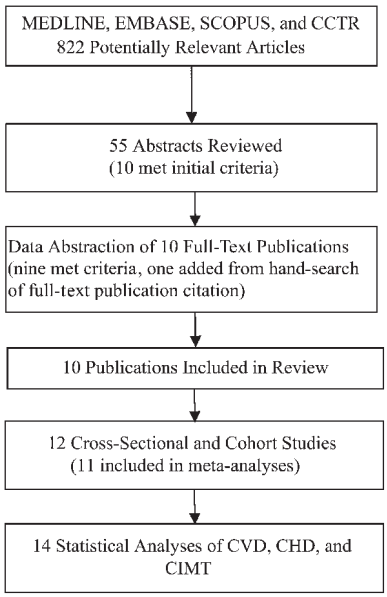

Figure 1.
Literature search results.

Table 1.

Examples of Reasons for Non-Inclusion of Studies in the Meta-Analysis

Reference	Database	Exposure	Outcome	Reason for Exclusion
Beck et al., 2001[40]	Dental ARIC Study	Periodontitis	CIMT ≥1 mm	No assessment of systemic inflammation
Pussinen et al., 2005[1]	KIHD Study	*Pg* IgA*	Prevalent CHD	Includes edentulous; same study participants as in dentate subgroup included in analysis
Pussinen et al., 2005[1]	KIHD Study	*Aa* IgG†	AMI, CHD death	Per algorithm, alternative antibody (*Pg* IgA) included in analysis
Beck et al., 2005[30]	Dental ARIC Study	*Pg* IgG	CIMT ≥1 mm	Unable to combine measure of association (odds ratio) with remaining studies, which measured differences in mean CIMT

KIHD = Kuopio Ischemic Heart Disease; AMI = acute myocardial infarction.
* Anti-*Pg* IgA.
† Anti-*Aa* IgG.

detailed examination of the search results from other databases found SCOPUS to contain ~20% of the literature on this topic compared to those found in PubMed, whereas EMBASE contained ~40% of those displayed in PubMed.

Study Designs

Among the eight studies used in the meta-analysis of cardiovascular events, five were cohort studies and three were cross-sectional studies (Table 2). All three studies of CIMT were cross-sectional. A publication could be used for multiple studies because of the presence of several study types, e.g., a cross-sectional component at the baseline visit comparing subjects with prevalent CVD to those without, followed by a cohort study of incident disease among subjects without baseline CVD.[1] A study could result in more than one analysis because of reporting results of stratified

Table 2.

General Study Characteristics

Reference	Study Design (n)	Outcome	Country	Database (duration)	Population	Analysis Includes Edentulous
Ajwani et al., 2003[34]	Cohort (175)	CV mortality	Finland	Helsinki Aging Study (5 years)	Men aged >75 years	No
Pussinen et al., 2004[33]	Cohort* (126)	Fatal, non-fatal MI	Finland	North Karelia Project (10 years)	Men aged 30 to 59 years	Presumed†
Pussinen et al., 2005[1]	Cohort (1,023)	Acute MICHD death	Finland	Kuopio Ischemic Heart Disease Study (10 years)	Men aged 46 to 64 years	Yes (54%)
Pussinen et al., 2004[36]	Cohort* (519)	Stroke	Finland	Mobile Clinic Health Survey (13 years)	Men aged 30 to 59 years	Presumed†
Johansson et al., 2005[37]	Cohort* (729)	Stroke	Sweden	Monica Project and Vasterbotten Intervention Program (12 years)	Age 26 to 74 years	Presumed†
Pussinen et al., 2005[1]	Cross-sectional (459)	Prevalent CHD	Finland	Kuopio Ischemic Heart Disease Study (10 years)	Men aged 46 to 64 years	Yes
Pussinen et al., 2003[38]	Cross-sectional (1,163)	Prevalent CHD	Finland	Platelet Aggregation and Inflammation Study	Age 45 to 74 years	No
Beck et al., 2005[30]	Cross-sectional (5,002)	Prevalent CHD	U.S.	ARIC Study	Age 45 to 64 years	No
Taniguchi et al., 2003[35]	Cross-sectional (134)	CIMT	Japan	Hospital based	Non-obese, with type 2 diabetes of 11 years mean duration, mean age 60	Unknown
Pussinen et al., 2005[1]	Cross-sectional (910)	CIMT	Finland	Kuopio Ischemic Heart Disease Study	Men aged 46 to 64 years	No
Desvarieux et al., 2005[41]	Cross-sectional (1,056)	CIMT	U.S.	Oral Infection and Vascular Disease Epidemiology Study	Manhattan residents, 57% Hispanic, mean age 69 years	Yes
Beck et al., 2005[24]	Cross-sectional (4,585)	CIMT	U.S.	ARIC Study	Age 45 to 64 years	No

CV = cardiovascular; MI = myocardial infarction.
* Nested case-control categorized as cohort study.
† Studies in which analyses were presumed to include edentulous subjects by the investigators.

analysis.[3,6,24,30,37] Each study was determined to result in analyses of independent subpopulations except for one study,[1] which included a cross-sectional assessment of prevalent CHD and CIMT on the same population. Nevertheless, both analyses were able to be used because they had different measured outcomes (CHD and CIMT) and, thus, were used in separate meta-analyses. Therefore, each meta-analysis combines analyses on independent populations.

Cohort studies included several case-control studies[33,36,37] that were deemed "nested" by the investigators. In these studies, the controls were not sampled at the time of the incident events. Therefore, they would be described more accurately as case-control studies of incident cases and prevalent controls sampled from a known cohort. Nevertheless, we know the cohort from which they came, that the exposure predated the outcome, and that controls were selected without knowledge of the exposure status. In addition, by collecting all incident cases, these studies avoid the prevalence-incidence ("survival") bias associated with cross-sectional and case-control designs, i.e., the bias toward observing a null effect if patients with CHD had a higher mortality risk (and not survive to be counted) if they also had periodontal disease. Thus, these nested studies were considered equivalent in quality to cohort studies. Cross-sectional studies were grouped in analysis with cohort studies because, although subject to survival bias, the quality of the information on periodontal exposure and the degree of adjustment for confounders was similar in both types of studies. Meta-regression was used to detect significant survival bias between cross-sectional and cohort studies.

Geographic Location/Source Population

The studies were performed across many countries, including the United States,[24,30,41] Scandinavia,[1,33,34,36-38] and Japan[35] (Table 2) and among differing subpopulations. Five of the 11 studies used in the meta-analysis were limited to men; one study[34] was limited to men >75 years of age; one study[37] was stratified on gender, one[41] between ever and never smokers, and one[36] between those with and without CVD at baseline; one study[41] was limited to residents of Manhattan, New York, with a majority of Hispanics; and one study[35] included only non-obese Japanese with type 2 diabetes.

Assessment of Periodontal Disease With Systemic Bacterial Exposure

We defined the exposure of interest as the presence of clinical periodontal disease with elevated systemic markers of periodontal-related bacterial exposure. Studies that included a clinical examination to establish the diagnosis of periodontal disease followed criteria based on Community Periodontal Index of Treatment Needs[34] or assessment of probing depth, bleeding upon probing, plaque scores, and attachment loss.[30,40,41] The edentulous were excluded by selecting analyses, whenever possible, stratified on people who were dentate. There were six studies[1,33,35-38] that lacked a clinical examination and, thus, knowledge of edentulous status, relying instead on antibody levels specific to periodontal pathogens for the diagnosis of periodontal disease. Thus, these studies were subject to including edentulous subjects, who may continue to have serum antibody levels to periodontal organisms. Therefore, of the 11 studies included in the meta-analysis, four studies included only the dentate, six studies were known or presumed by the investigators to include edentulous subjects, and information about one study could not be ascertained from the text (Table 2). Nevertheless, the selection of markers of high bacterial systemic exposure as the exposure of interest would favor selection of the dentate among those associated with the exposure of interest. Antiperiodontal serology level was shown to decrease following full dental extraction;[42] therefore, as Pussinen et al.[1] outlined, although the edentulous subjects may have persistent antibody titers, these likely represent a lingering response to recent periodontal disease, prior to dental extraction. The report by Pussinen et al.[38] suggested that the majority of edentulous subjects go on to lose these high antibody titers; the prevalence of periodontal disease was similar in the Finnish population whether diagnosed by clinical examination, which excluded the edentulous or antibody measurement. They also demonstrated that higher serological quartiles were associated with a greater number of teeth, which presumably were the source of the bacterial exposure.[38] Thus, these antibody levels are used in this meta-analysis as evidence of recent systemic exposure from periodontal pathogens and inherently include among the exposed those who are dentate.

Many of the studies examined the predictive power of antibodies against multiple potential periodontal pathogens. To limit the risk for multiple comparisons, i.e., data dredging, and to pool together similar serologies as much as possible, we followed a prespecified algorithm for the selection of systemic markers. In studies that reported periodontal-specific serologies, we selected antibodies against the two most common periodontal pathogens: *Pg* and *Actinobacillus actinomycetemcomitans* (*Aa*). Because the hypothesis was of chronic exposure over many years causing a progression of atherosclerosis, we chose *Pg*, which is most associated with chronic generalized periodontitis in adults, when available. When not available, we chose antibodies against *Aa*. If results from IgA and IgG levels were available, IgA was selected in primary analysis,

but replaced with IgG in repeat meta-analysis as part of model sensitivity analysis. Thus, if anti-*Pg* IgA was not available, we used, in order: *Pg* IgG, combined *Pg* and *Aa* IgG, and anti-*Aa* IgG. One study[37] used a measure of the antibody level directed against the *Aa*-derived leukotoxin. These leukotixin-neutralizing antibodies separated those subjects whose serum, when added to a cell culture of leukocytes, abrogated the leukocyte damage by >50%, as measured by lactate dehydrogenase (LDH) release, upon addition of *Aa* leukotoxin. One study[34] reported only CRP. One study[35] of CIMT presented the results of analysis based on various bacterial burdens on periodontal cultures; the "etiologic" bacterial burden was chosen because this was the group that contained *Pg* and *Aa*. Finally, dentition status was deemed more significant to control than selection of the most appropriate antibody exposure. Therefore, we selected one study[1] for inclusion with combined *Pg* and *Aa* IgG restricted to the dentate rather than *Pg* IgA because the latter included the edentulous.

The exposure in most studies was analyzed in a dose-response by quantiles, such as tertiles or quartiles. To limit strong confounding, it was decided a priori not to use the highest quantile, but rather the penultimate quantile, as compared to the reference population in the meta-analysis. It was believed that those with the very highest levels of markers of bacterial exposure were possibly a distinct population and would pose the highest risk for residual confounding, despite adjustment for traditional risk factors. Therefore, it was anticipated that the odds ratios (ORs) in this analysis, e.g., using second versus first tertile, or third versus first quartile, would be more conservative than those reported in the original studies.

Outcome Ascertainment

The effect of exposure to periodontal disease was examined with both hard endpoints (cardiovascular events) as well as surrogate endpoints (carotid intima-media thickness.) The studies on CIMT were included as secondary analysis to explore the relationship of periodontal disease to early atherosclerotic progression. Of the studies exploring periodontal disease and CIMT, one study[24] was excluded because it was analyzed as a categorical outcome and measured the association by an OR and could not be combined with the remaining studies, which analyzed intima-thickness as a continuous variable in linear regression. Among the studies of cardiovascular events, the diagnosis of CVD was determined in all but one of the studies[30] by *International Classification of Diseases, Ninth Revision* codes,[43] collected in national death registries, national registries of medication reimbursement, or hospitalizations. The remaining study, the ARIC Study,[30] was believed to be comparable in quality of outcome ascertainment with chart review.

Matching of Controls and Adjustment for Confounders

All except three studies[1,35,37] matched or adjusted for all the traditional cardiovascular risk factors. In one of these studies,[35] among Japanese diabetics, periodontal disease was not associated with any traditional CVD risk factor. In another study,[34] the text was unclear, but there was adjustment for at least some traditional risk factors, and perhaps all, depending on which model was being reported. There was only one study[1] of CIMT in which no attempt at adjustment was made. There was appropriate matching or restriction to subpopulations with strong confounders across multiple studies (Table 3).

Statistical Methods

Software‖ designed for the meta-analysis of observational studies was used. Primary outcomes were CVD, CHD, and CIMT. ORs were taken from the text when reported or read from the figures. Proportions of cases and non-cases were converted to ORs and 95% confidence intervals (CIs) were calculated when not otherwise reported. For one cohort study, data were collected from a 10-year report[39] that was unavailable in the 5-year report.[34] Publication bias was assessed by Begg rank correlation method. The effect size in log-odds was plotted against the standard error of the log-odds, and a statistical test for symmetry was performed (Fig. 2). Homogeneity across single studies was assessed by Q statistics and by the proportion of the total variance explained by heterogeneity (I^2). Fixed- or random-effect models were used to meta-analyze the data, as suggested by the results. In studies of CIMT, with a continuous outcome, heterogeneity in studies was tested by the tau-squared estimate of between-study variance. Meta-regression was performed to test whether study design, chosen outcomes, or evaluation methods of periodontal inflammation affected the summary result. Sensitivity analyses were performed by repeating the analyses while dropping a different study in each analysis with replacement. In additional sensitivity analyses, IgA was replaced by IgG-based serologies as available.[1,33,36]

RESULTS

Qualitative Analysis

The details of the 11 studies are shown in Tables 2 and 3. As indicated, the overall quality of the studies was very high. The definition and ascertainment of the exposure and outcome was clear and seemed to be unbiased. There was an attempt to control for strong confounders through restriction to certain populations or adjustment for traditional cardiovascular risk factors and socioeconomic status. This seems to be due, in part, to the limited number of investigators

‖ Intercooled Stata 9, Stata, College Station, TX.

Table 3.

Exposure and Outcome Summary by Study

Study	Exposure Versus Reference	CHD Outcome (95% CI)	Stroke Outcome (95% CI)	CIMT (mm; mean ± SD)	Adjustments
Ajwani et al, 2003[34]	**Periodontitis (CPITN ≥3), CRP ≥3 mg/l** versus no periodontitis (CPITN 0 to 2), CRP <3 mg/l	**OR 3.80 (2.84 to 15.95)***			Age, gender, CVD, smoking, cholesterol, BP, and BMI
Pussinen et al., 2004[33]	*Pg* IgA: 1st quartile *Pg* IgA: 2nd quartile ***Pg* IgA: 3rd quartile** *Pg* IgA: 4th quartile *Pg* IgG: 1st quartile *Pg* IgG: 2nd quartile *Pg* IgG: 3rd quartile *Pg* IgG: 4th quartile	OR 1 (Reference) OR 2.47 (0.75 to 8.11) **OR 3.3 (1.03 to 10.58)*** OR 3.99 (1.22 to 13.10) OR 1 (Reference) OR 0.41 (ns) OR 0.54 (ns) OR 0.54 (ns)			Smoking, cholesterol, BP, diabetes, and BMI
Pussinen et al., 2005[1]	*Pg* IgA: 1st tertile ***Pg* IgA: 2nd tertile** *Pg* IgA: 3rd tertile *Pg* IgG: 1st tertile *Pg* IgG: 2nd tertile *Pg* IgG: 3rd tertile	OR 1 (Reference) **OR 2.10 (1.26 to 3.37)** OR 1.50 (0.87 to 2.40) OR 1 (Reference) OR 1.3 (ns) OR 1.5 (ns)			Age, SES, smoking, cholesterol, BP, diabetes, and plasma fibrinogen
Pussinen et al., 2004[36]	Prevalent CVD at baseline: ***Pg* IgA** (≥2.0 EU versus <2.0 EU) *Pg* IgG (≥5.0 EU versus <5.0 EU) No CVD at baseline: ***Pg* IgA** (≥2.0 EU versus <2.0 EU) *Pg* IgG (≥5.0 EU versus <5.0 EU)		**OR 2.62 (0.98 to 7.01)** OR 0.43 (ns) **OR 0.65 (0.34 to 1.21)** OR 0.73 (ns)		Age, gender, residence, alcohol consumption, smoking, BP, diabetes, and BMI
Johansson et al., 2005[37]	***Aa* LNA among women** (≥50% reduction in LDH release from leukocytes when exposed to leukotoxin pretreated with subject's serum versus <50% reduction) ***Aa* LNA among men**		**OR 0.28 (0.13 to 0.59)** **OR 0.88 (0.52 to 1.52)**		Education, smoking, cholesterol, BP, diabetes, and BMI
Pussinen et al., 2005[1]	***Pg*/*Aa* combined IgG** (≥5.0 EU versus <5.0 EU)	**OR 2.43 (1.73 to 3.10)**			Age, SES, smoking, cholesterol, BP, diabetes, and plasma fibrinogen
Pussinen et al., 2003[38]	***Pg*/*Aa* combined IgG** (≥14.0 EU versus <14.0 EU)	**OR 1.50 (0.95 to 2.50)**			Age, education, smoking, cholesterol, and BMI

Table 3. (continued)

Exposure and Outcome Summary by Study

Study	Exposure Versus Reference	CHD Outcome (95% CI)	Stroke Outcome (95% CI)	CIMT (mm; mean ± SD)	Adjustments
Beck et al., 2005[30]	Among ever smokers **>median level *Pg* IgG**	**OR 1.3 (1.00 to 1.80)***			Age, gender, race, education, smoking, cholesterol, BP, diabetes, and waist/hip ratio
	Among never smokers **>median level *Pg* IgG**	**OR 1.2 (0.80 to 1.80)***			
Taniguchi et al., 2003[35]	***Pg* IgG: high titer (>310 EU)**			**0.73 ± 0.03***	No adjustment; high titer not statistically associated with age, gender, cholesterol, diabetes, or BMI
	***Pg* IgG: normal titer (<310 EU)**			0.68 ± 0.02*	
Pussinen et al., 2005[1]	*Pg* IgA: 1st tertile			~0.84 ± 0.01†	No adjustment
	2nd tertile			**~0.87 ± 0.01***	
	3rd tertile			~0.90 ± 0.01	
	Combined *Pg*/*Aa* IgG				
	1st tertile			~0.82 ± 0.01*	
	2nd tertile			~0.83 ± 0.01*	
	3rd tertile			~0.88 ± 0.02	
Desvarieux et al., 2005[41]	**Etiologic bacterial burden‡**			0.84 ± 0.01*	Age, gender, race, ethnicity, education, smoking, cholesterol, BP, diabetes, and BMI
	1st tertile			**0.86 ± 0.01***	
	2nd tertile			0.87 ± 0.01	
	3rd tertile				
Beck et al., 2005[24]	Among ever smokers **>median *Pg* IgG**			**OR 1.5 (1.20 to 2.10)**	Age, gender, race, education, smoking, cholesterol, BP, diabetes, and waist/hip ratio
	Among never smokers **>median *Pg* IgG**			**OR 1.4 (1.00 to 1.90)**	

Markers and measures of association selected for use in the meta-analysis are in **bold** type. They were selected by a prespecified algorithm for antibody type and (next-to-highest) quantile comparison. If IgA was used for analysis, the results for IgG in the same study also are presented. The alternate IgG markers may have significant qualitative differences in the ORs in individual studies. In sensitivity analysis, the use of these alternate IgG markers did not change the results of the meta-analysis.
CPITN = Community Periodontal Index of Treatment Needs; BP = hypertension; BMI = body mass index; ns = not significant; *Aa* = *Aa* IgG; SES = socioeconomic status; EU = enzyme-linked immunosorbent assay units; LNA = leukotoxin-neutralizing antibodies.
* Calculated from data presented in text.
† As derived from figure in article; data not presented in the text.
‡ Includes *Pg* and *Aa*.

who have produced multiple studies. There are only six primary investigators represented in all, and Pussinen et al.[1,33,36,38] account for six of 11 of the included analyses. This multiplicity of studies per investigator may be viewed as a strength because it lends itself to similar adjustment across studies and, thus, easier comparison. Each study also was carried out from an independent database or as a stratified analysis within a single database. For example, Pussinen et al. used four databases for the six studies. These investigators showed evidence of improving their control of confounders from one study to the next. Pussinen et al.[1,36] included more socioeconomic information in the later studies, and Beck et al.[24,30] used a stratified analysis by smoking status after previous studies of clinical periodontal disease in ARIC.[40]

However, this stratification introduces a potential weakness, which is to over- or underreport differences in the effect of periodontal disease in different populations. This weakness is of particular concern in studies of stroke because all four studies were stratified analyses. Each study was restricted to men or women or people with or without CVD. This design is very useful in controlling for strong confounders, but is underpowered in these studies to detect effect modification. Johansson et al.[37] reported that the association

between antibodies and stroke varied by gender, with an inverse association between *Aa* leukotoxin-neutralizing antibodies and stroke among women. In this analysis, however, the confidence intervals overlap, and the investigators did not test formally for statistical interaction in a combined model. Pussinen et al.[1,33,36] demonstrated an association among subjects with and without a history of CVD, selectively presenting the *Aa* IgA results. However, in our a priori selection of *Pg* IgA, we noted a possible similar modified effect with an association among subjects with CVD but not among those without prior CVD. However, whether there is a true difference or this resulted from chance sampling cannot be studied further in the absence of formal tests of interaction.[36]

Finally, at times, the weakness of many of these studies taken individually was the extreme use of multiple measures of the exposure being studied by antibody types and the targeted periodontal organisms. This also led to some selective reporting of positive results in the publication titles and abstracts. Nevertheless, the full data are presented well in the body of the text of these publications. Thus, this meta-analysis was able to control for this risk by the prespecified algorithm to select the marker of exposure, which often was different from the one highlighted, as well as sensitivity analysis, as has been outlined.

Quantitative Analysis

Funnel-plot analysis of the 11 cardiovascular event studies did not show evidence of publication bias using the Begg test of symmetry ($P = 0.755$) (Fig. 2). The results of the CVD analysis, in which the outcomes for CHD and stroke are combined, suggested evidence of heterogeneity across the studies (Q = 48.02; $P < 0.001$). This was confirmed by an I^2 of 79%. This suggests that the effect of periodontitis that is associated with systemic markers of exposure was different across different studies. Allowing for the presence of different effects using random-effect modeling, the higher level of exposure was not associated with CVD (average OR, 1.36; 95% CI: 0.96 to 1.93; $P = 0.089$) (Fig. 3). The Skein (also known as Empirical Bayes) plot, which adjusts for different size studies, did not alter the findings (data not shown).

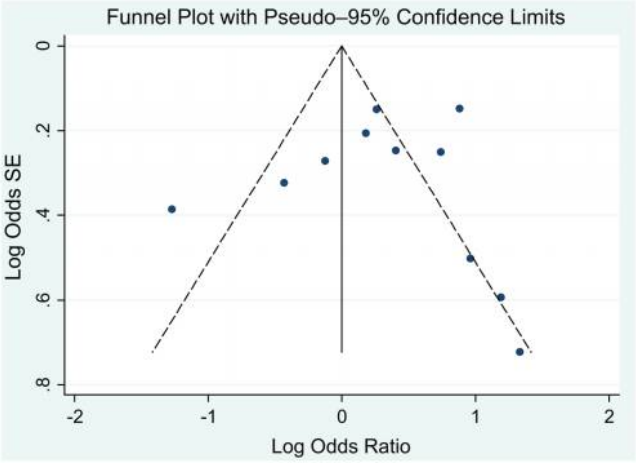

Figure 2.
Funnel-plot analysis of studies of cardiovascular events. There was no evidence of publication bias by the Begg test of symmetry (P = 0.76).

Exploring the source of heterogeneity, the forest plot and meta-regression suggested a difference in effect between studies of CHD and stroke. The effect estimate of periodontal disease was 2.39-fold higher in studies of CHD compared to studies of stroke, which was statistically significant ($P = 0.037$). When stratifying on study outcome, there remained significant heterogeneity among studies of CHD (Q = 15.12, $P = 0.019$; $I^2 = 67\%$). In random-effect models, periodontitis was associated significantly with CHD, with an average OR of 1.75 (95% CI: 1.32 to 2.34; $P < 0.001$) (Fig. 4).

In sensitivity analysis, using *Pg* IgG for *Pg* IgA did not alter the findings. Periodontal exposure again was heterogeneous in its association with CVD (Q = 45.64; $P < 0.001$), and the "average" effect in random-effect modeling was not significant (OR, 1.07; 95% CI: 0.75 to 1.52; $P = 0.709$). However, the effect estimate of periodontal pathogenic exposure was 2.57-fold higher in studies of CHD compared to studies of stroke ($P = 0.015$). In random-effect models, periodontitis was associated significantly with CHD, with an average OR of 1.47 (95% CI: 1.08 to 2.46; $P < 0.001$).

Further heterogeneity within studies of CHD was suggested by the difference between cross-sectional and cohort studies. Compared to cohort studies, cross-sectional studies of prevalent CHD underestimated the effect of exposure by 48%, suggestive of the survival bias to the null, although this difference did not reach statistical significance ($P = 0.223$).

Among studies of stroke, there remained significant heterogeneity (Q = 13.23; $P = 0.004$) and no significant average effect in random-effect modeling with an OR of 0.77 (95% CI: 0.37 to 1.61; $P = 0.49$).

Sensitivity analyses on the combined and separate CHD or stroke outcomes did not alter the findings, on reanalyzing after one study was excluded at a time. The study[34] that researched CRP had the greatest effect when withdrawn from the analysis of CHD, as its withdrawal removed the heterogeneity and resulted in a fixed-effect estimated OR of 1.47 (95% CI: 1.21 to 1.78; $P < 0.001$).

Among studies of CIMT, periodontitis increased mean thickness by means of 0.02, 0.03, and 0.05 mm across the three studies, which resulted in significant heterogeneity ($P = 0.0001$). Random-effect

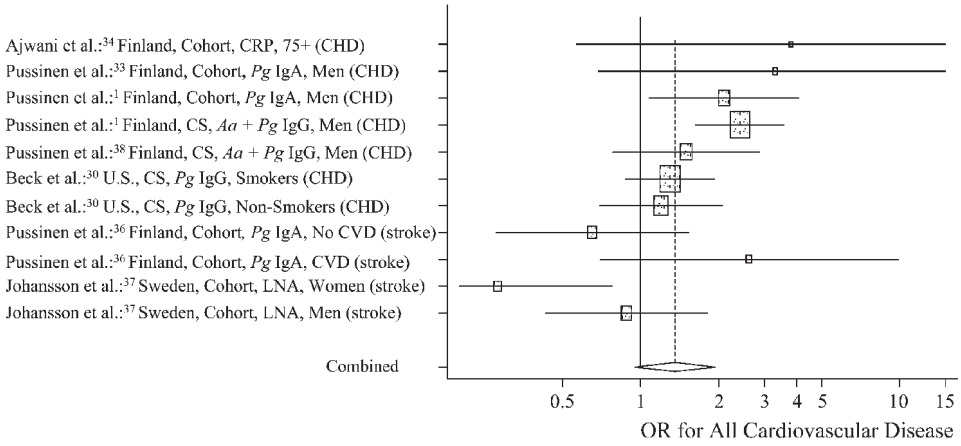

Figure 3.
Forest plot of studies using cardiovascular events (combined CHD and stroke) as outcomes. Investigators, location, inflammatory marker, cohort versus cross-sectional (CS) study, population restriction, and measured outcome as described. There was significant heterogeneity across studies. The association between systemic markers of periodontitis and CVD in meta-analysis by random-effect modeling was not statistically significant (OR 1.36; 95% CI: 0.96 to 1.93; P = 0.089). LNA = leukotoxin-neutralizing antibodies.

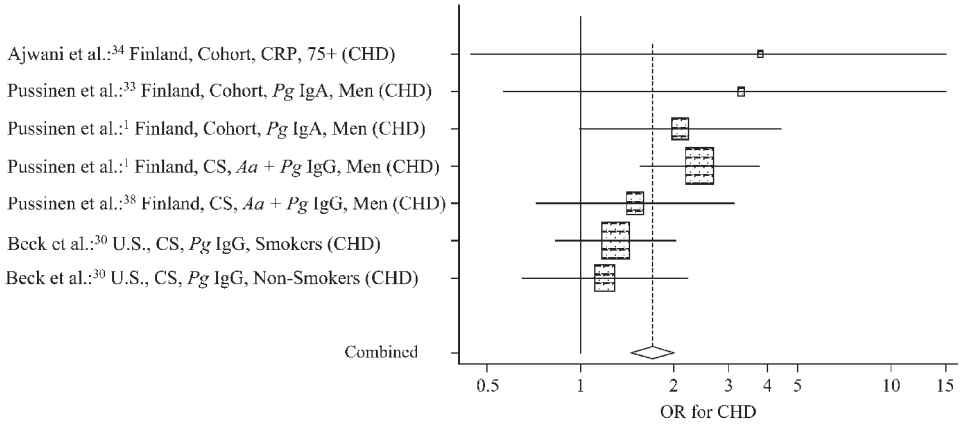

Figure 4.
Forest plot of studies using only CHD events as outcomes. Investigators, location, inflammatory marker, cohort versus cross-sectional (CS) study, population restriction, and measured outcome as described. There was significant heterogeneity across studies. There was a significant association between systemic markers of periodontitis and CHD in meta-analysis by random-effect modeling with combined odds ratio of 1.75 (95% CI: 1.32 to 2.34; P <0.001.) LNA = leukotoxin-neutralizing antibodies.

models estimated a significant average mean increase of CIMT of 0.03 mm (95% CI: 0.02 to 0.04) (Fig. 5). Sensitivity analyses did not alter the findings, on reanalyzing after one study was excluded at a time.[1,35,41]

DISCUSSION

Our results suggest that periodontal disease associated with elevated levels of systemic markers of bacterial exposure is associated with CHD, with an estimated effect that seems higher than previous meta-analyses have suggested for clinical periodontal disease alone (Table 4). A recent meta-analysis[3] in which the exposure was clinical periodontitis but without systemic markers of exposure suggested a statistically significant but clinically modest increased risk for CHD of roughly 15% in cohort studies conferred by periodontal disease (95% CI: 1.06 to 1.25). By comparison, our meta-analysis suggests a stronger association, with an OR of 1.75 (95% CI: 1.32 to 2.34; $P<0.001$), when periodontitis is defined as having not just clinical periodontal disease but elevated bacterial systemic exposure. These findings were not altered in sensitivity analysis, in which each study is sequentially left out of the analysis with replacement. One study[34] had the greatest effect when withdrawn, but still resulted in an estimated OR of 1.47 (95% CI: 1.21 to 1.78; $P<0.001$). The study findings were similarly robust in sensitivity analysis using different serologies. Repeating the analyses by using *Pg* IgG instead of IgA resulted in an estimated OR of 1.47 (95% CI: 1.08 to 2.46; $P<0.001$).

Therefore, we report an association between periodontal disease and CHD that builds a strong case for causality, to the extent that any observational study can lend evidence for this. The cohort studies demonstrated the proper temporal sequence, with the periodontal exposure preceding incident CHD. Furthermore, the association with CIMT suggests that this exposure plays a role in early atherosclerosis. The strength of the association also is moderately strong and, by using the second highest quantile as the exposure category, likely represents an underestimate of the effect. Furthermore, the use of cross-sectional analyses also may have underestimated the effect because of survival bias. Cross-sectional studies are known to be susceptible in design for the potential bias of

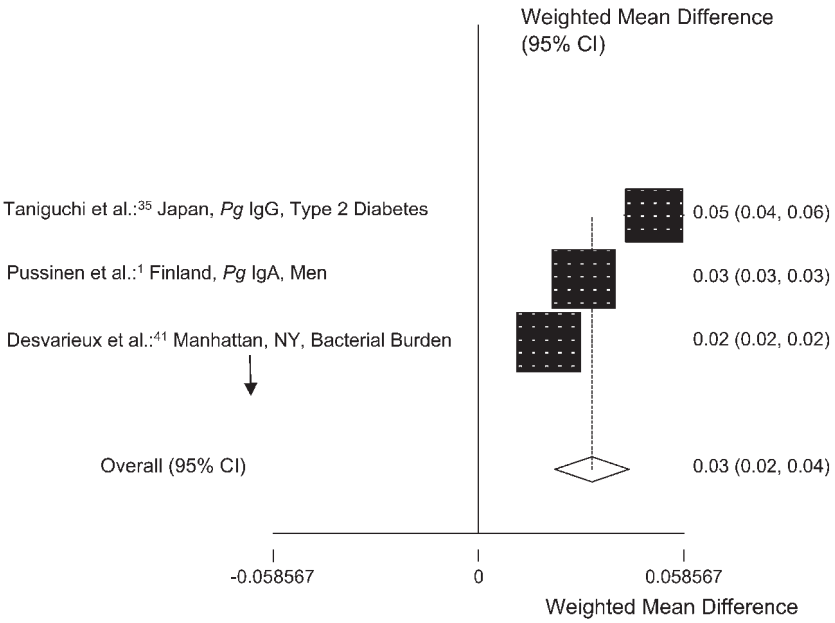

Figure 5.
Forest plot of studies using only CIMT as continuous outcome measured by ultrasound. Combined effect by random-effect model. (Note: Beck et al.[6] was not included in analysis because the reported ORs cannot be combined with continuous measure above.) Random-effect models estimated a significant average mean increase of CIMT of 0.03 (95% CI: 0.02 to 0.04).

Table 4.

Estimated Relative Risk of CVD Conferred by Clinical Periodontal Disease in Previous Meta-Analyses of Cohort Studies

Investigators	Systemic Inflammation Measured?	Outcome	Relative Risk	95% CI
Janket et al., 2003[2]	No	CVD	1.19	1.08 to 1.32
Janket et al., 2003[2]	No	Stroke	2.89	1.78 to 4.56
Khader et al., 2003[3]	No	CVD	1.13	1.01 to 1.27
Khader et al., 2003[3]	No	CHD	1.15	1.06 to 1.25

prevalence-incidence ("survival") bias. This is a bias toward a null effect and takes place whenever the exposure of interest leads to the studied outcome and confers a higher mortality to those who reach the outcome. Therefore, there is greater loss of follow-up among the exposed compared to the non-exposed, and they do not survive to be counted in a cross-sectional study. That this is a concern in the study of periodontal disease with regard to CVD is suggested by a study that showed that the degree of CRP elevation in the setting of a myocardial infarction, as a marker for the extent of the infarction, was significantly greater for patients with periodontal disease than for those without. This underestimate in prevalence studies also was suggested by one study by Pussinen et al.,[1] which was not used in this meta-analysis in preference to a group of only dentate (edentulous data was excluded), in which the association between *Pg* and CHD in this mixed group was greater in the cohort study (OR, 2.10; 95% CI: 1.26 to 3.37) than that found in the cross-sectional study (OR, 1.58; 95% CI: 1.16 to 2.00) using the same serology. In our study, cross-sectional studies underestimated the association seen in cohort studies by almost half, although this did not achieve statistical significance in meta-regression (Fig. 4). A third aspect of this meta-analysis that would have biased the results toward an underestimate of the true association relates to the inclusion of edentulous subjects in the majority of the studies. Pussinen et al.[38] found that elevated serology was associated among the edentulous with current CHD, consistent with the effect of long-lasting exposure until recently; however, it was not associated with incident CHD, consistent with a resolution of ongoing bacterial exposure and, thus, risk. To the degree that the groups included the edentulous, they were most likely to be in the lowest, and thus, reference, quantile of exposure and become misclassified as without evidence of periodontal disease. Because the edentulous have a higher risk for CHD than persons who have never had periodontitis,[34] this increases the risk among the reference group and causes a bias toward the null. To the degree that persons with elevated bacterial exposure titers are just recently edentulous, this also would lead to misclassification of the risk and a bias toward the null.

The link between periodontal bacterial systemic exposure and atherosclerosis also is biologically plausible. CRP and periodontal pathogens have been detected within inflamed atheromatous plaques and have clear molecular pathways to induce macrophage uptake of LDL cholesterol, leading to foam cell generation and atheroma progression.[44] In addition, our selection of the organism (*Pg*) represents the most likely chronic exposure to plausibly contribute

to persistent atherosclerosis progression. The use of serology in all but two of the studies further emphasizes the selection for chronicity of bacterial systemic exposure because acquiring specific immunity is associated with recurrent disease.[28]

The association between periodontal exposure and CHD also is consistent across studies, despite some heterogeneity as to its strength. In addition, it often is associated in individual studies with a dose response across tertiles and quartiles (Table 3). This feature is particularly strong in arguing against confounding because it suggests that if it is confounded by another risk factor, this other risk factor would need to increase in exposure in tandem with the increasing levels of the periodontal exposure. It could be argued that tobacco use may represent such a confounder, causing step-wise increases in periodontal disease and atherosclerosis. Against this as an explanation for the observed association, however, we note the study by Beck et al.,[30] which showed the same association among never smokers. Finally, the fact that periodontal disease is not specific for atherosclerosis does not detract significantly from suggesting a causal role because atherosclerosis is well established to be multifactorial, with lipids as the sine qua non at the core of the atheroma, but many other risk factors contribute to the endothelial damage and inflammation. The results of systemic bacterial periodontal exposure, be they the organisms themselves, their antigens, or the CRP generated in response, can be seen as one such contributing risk factor. Conversely, the specificity of the exposure of periodontal disease is strengthened greatly by the use of periodontal pathogen–specific serologic titers in this meta-analysis.

Our results do not suggest a similar relationship between periodontitis and stroke. These results mirror the epidemiology of stroke, in which atherosclerosis has a far weaker association than it has with CHD. One study estimated that only 18% of first-time strokes involved large-vessel cervical or intracranial atherosclerosis with >50% stenosis.[45] That stroke often is not atherosclerotic in nature is suggested further by the lack of risk associated with elevated lipid levels. In the large ARIC study, lipids had only weak and inconsistent associations with ischemic stroke in multivariate analyses.[46] Similarly, a nested case-control study[47] in the Physician's Health Study did not reveal any benefit from statin therapy.

Arguing against a non-association with stroke is the strong and significant association found in a previous meta-analysis of clinical periodontal disease (relative risk = 2.89, 95% CI; 1.78 to 4.56).[2] In addition, our results suggested that there is an association between systemic markers from exposure to periodontitis and early progression of atherosclerosis, as measured by increases in mean carotid intima-media thickness. This raises the possibility of a true association with atherosclerotic, ischemic stroke, which becomes lost when one looks at strokes of all types as the outcome. Indeed, true differences may exist between people with or without CVD, as reported by Pussinen et al.[36] Stratifying on CVD may be selecting those people who are more likely to have ischemic and atherosclerotic disease, accounting for the observed difference by cardiovascular status.

Limitations to our meta-analysis include the combination of study types, exposures, and outcomes, which makes the appropriate merging of studies a challenge. The mix of studies adds to heterogeneity in the effect size. Nevertheless, although merging studies may pool together grossly heterogeneous studies in some cases, combining these analyses may bolster each other in the assumptions they make because each study design has strengths and weaknesses. The cohort studies lack a longitudinal component of repeated dental examinations, so that they assume that periodontal disease at the baseline visit is chronic or predicts recurrent periodontal inflammation. The cross-sectional studies lend their evidence of ongoing exposure. Conversely, cohort studies avoid the potential for reverse causality and survival bias inherent in the cross-sectional studies. The diverse populations included in this meta-analysis similarly were viewed as a strength because they controlled for strong confounders, such as gender, age, CVD, tobacco use, and socioeconomic status. They also served to look for evidence of a differing effect of periodontal disease in different populations. In addition, although the stratified analyses and population restrictions help with avoiding strong confounders, they limit generalizability. Indeed, four of the studies[1,33,34,36] of CHD were restricted to men. Nevertheless, there is no strong evidence that inflammation affects women differently from men. A study by Ridker et al.[9] showed that CRP predicts CVD in women.

The studies also used a combination of exposures, including CRP,[34] the microbiotic burden,[41] and various serologies or combinations of serologies.[1,24,30,33,38] These exposures were combined as evidence of systemic bacterial exposure to periodontitis. Nevertheless, 10 of the 12 studies used antiperiodontal pathogen antibody titers, and dropping CRP or microbiota in sensitivity analysis did not alter the findings. In addition, sensitivity analysis using IgG instead of IgA antibody class did not alter the findings.

Finally, there is a limitation to the implications of these results. These results suggest that more aggressive treatment of periodontal disease in those with evidence of higher levels of bacterial systemic exposure to periodontitis would translate into a lower risk for atherosclerotic complications. This is suggested by the study by Pussinen et al.,[38] in which elevated

serologies among the edentulous no longer conferred increased cardiovascular risk. This has been supported by two recent randomized clinical trials of intensive periodontal therapy, which used short-term surrogate markers of cardiovascular risk. One trial[48] showed that intensive intervention would elevate inflammatory mediators, such as IL-6 and CRP in the short-term, but with an improvement in endothelial function at 6 months. A second trial[49] found that periodontal therapy, which included scaling and root planing and adjunctive localized antibiotic therapy, reduced inflammatory mediators, such as IL-6 and CRP, and improved lipid profiles, such as total cholesterol, over 6 months. However, it is not known whether there is any benefit that might be expected from full dental extraction because complications from the use of dentures has been associated with even higher CRP levels than periodontal disease.[34]

Future studies should focus on clarifying the effect of inflammatory periodontal disease among women and on the change in risk following intervention, with a particular view to the potential risks associated with edentulousness. Furthermore, more studies using carotid intima-media thickness as a surrogate for cardiovascular event outcomes may strengthen the evidence linking periodontitis and stroke. Such studies will have shorter duration than those that have used mortality outcomes and may lead to decreases in morbidity and mortality associated with both diseases.

CONCLUSIONS

Periodontal disease associated with elevated markers of bacterial systemic exposure is associated with CHD with a stronger association than clinical periodontal disease. This suggests that markers of bacterial systemic exposure are the biologically pertinent exposure in periodontal disease as regards atherosclerosis and supports inflammation at a distance as playing a role in the progression of atherosclerosis. This evidence is strong among dentate men with CHD but less so for women. There is no consistent relationship to stroke, although there is, in a very limited group of studies, to early carotid atherosclerosis. A longitudinal analysis with repeated dental examinations and serologies would strengthen the evidence significantly and would clarify the effect of full dental extraction and the use of dentures. Our analysis suggests that future studies of CVD should include serum markers specific to periodontal pathogens to assess for recent systemic exposure to periodontitis.

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