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Association between serum antibodies to periodontal bacteria and rheumatoid factor in NHANES III

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Abstract

Objective—Alterations in the microbiome, including the periodontal microbiome, may be a risk factor for rheumatoid arthritis (RA). Most studies that have analyzed this association are relatively small, focus primarily on a single periodontal pathogen (*Porphyromonas gingivalis*), and are not population-based. We investigated the association between elevated serum IgG antibodies to 19 periodontal species and the prevalence of rheumatoid factor (RF) in a large nationally representative sample of adults.

Methods—The Third National Health and Nutrition Examination Survey is a cross-sectional sample of the non-institutionalized US population (n=33,994). Our study population included all dentate participants ≥60 years, who did not have RA as defined by a modified version of the American College of Rheumatology 1987 criteria, and had complete data for both serum IgG antibodies against periodontal bacteria and serum RF antibody titer (n=2461).

Results—Adjusted odds ratios (ORs) (95% CI) summarizing the relationship between the 19 periodontal serum IgGs and RF seropositivity ranged from 0.53 (0.29, 0.97) to 1.27 (0.79, 2.06), and 17 of the 19 observed ORs were < 1.0. The ORs for RF seropositivity among participants with elevated *Prevotella intermedia* [0.53 (0.29, 0.97)] and *Capnocytophaga ochracea* [0.54 (0.31, 0.95)] IgG were statistically significant.

Conclusion—We have found elevated periodontal IgGs to be mostly unassociated with RF seropositivity in the nationally representative NHANES III. Elevated antibody levels to *P. intermedia* and *C. ochracea* were associated with lower odds of RF seropositivity.

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by inflammation and pain in the joints, and varying degrees of systemic involvement. It affects 0.5–1.0% of the U.S population, and results in disability and increased mortality (1). While genetic factors are the best understood risk factors for RA, they explain only 50–60% of the total risk for RA (2–4). Other known environmental risk factors, of which tobacco use has shown the strongest evidence (1), have been identified but they do not fully explain the remaining risk (4).

Proposed environmental risks for RA include adverse microbial exposures common in periodontitis. Periodontal pathogens might contribute to the development of RA in susceptible individuals, possibly by triggering a loss of immune tolerance (5). Accordingly, several studies have demonstrated higher rates of periodontitis in patients with RA (6–8). Periodontitis is also associated with increased incidence of RA longitudinally (9–11), and anti-infective periodontal therapy might reduce RA severity (12). A small number of studies have also explored the periodontitis-RA link by examining the associations between antibody levels to periodontal pathogens – a marker of chronic adverse microbial exposure – and RA, with mixed results. Antibodies to *Porphyromonas gingivalis,* a well-studied periodontal pathogen, are elevated in RA cases compared to controls in some studies (13–15), while others found no difference between RA cases and controls (16–18).

Previous studies have helped identify important considerations for unbiased investigation of the relationship between periodontitis and RA. While periodontitis is an attractive candidate environmental risk factor for rheumatoid arthritis, untangling whether periodontitis represents a risk for RA or is simply a comorbid condition has been problematic because periodontitis and RA share common risk factors. For example, the strongest known genetic risk for RA, the HLA-DRB1 ‘shared epitope’ alleles, has also been shown to be a risk factor for periodontal disease (19, 20). Furthermore, tobacco use is also a strong risk factor for RA and periodontitis.

Most previous investigations into the relationship between periodontitis and RA have been cross-sectional and studied only existing RA, increasing the potential for reverse causation through RA-induced host changes due to medications and behavioral modifications. It is therefore of interest to study these relationships prior to the onset of clinically manifested RA when the risk of reverse causality is minimized. Moreover, as early treatment of RA reduces the severity of the disease course (21), identification of risk factors for preclinical RA could spur interventions that improve the trajectory of disease outcomes or potentially allow prevention of disease.

Two serum autoantibodies associated with preclinical RA are anti-citrullinated peptide antibodies (ACPA) and rheumatoid factor (RF). ACPA and RF appear to develop prior to the onset of clinically apparent synovitis (22–25) and most RA patients are characterized by the presence of serum antibodies of at least these two distinct types, and are thus denoted as being seropositive. ACPAs are highly specific biomarkers of preclinical RA and are associated with severity of RA (22–25). Similarly, RF has long been known to be present

prior to clinical RA and to predict future RA development, particularly when present in high titers (22, 26). Both RF and ACPA however, have been shown to be present in very low titers for long periods preclinically prior to the onset of RA, at which point titers of both types of antibodies tend to rise dramatically (23–25).

The biological rationale supporting periodontal pathogens as a trigger for the development of ACPAs is particularly noteworthy as *P. gingivalis*, a well-studied periodontal pathogen, is the only bacteria currently known to contain an enzyme peptidyl arginine deiminase (PAD) able to citrullinate arginine residues in human proteins common to both periodontal and joint tissues (27). Previous studies report conflicting results with some studies observing a positive relationship between *P. gingivalis* antibodies and only ACPA (13, 16), or RF (28, 29), while others show a positive relationship between *P. gingivalis* antibodies and both ACPA and RF (17, 30), or a combined outcome measure of either ACPA or RF (31). One recent study found no relationship between *P. gingivalis* antibodies and ACPA or RF (18).

There are several possible explanations for previous inconsistent findings. First, the bacterial etiology of periodontitis is polymicrobial but the majority of previous serological studies only measure *P. gingivalis* antibodies (13, 16, 18, 28, 30); and more robust microbial exposure assessments might be important as other periodontal pathogens have been linked with RA (32, 33). Furthermore, many prior studies examining the relationship between antibodies to periodontal bacteria and RA relevant outcomes are small, conducted in samples of fewer than 200 participants (13–15, 28, 30), and none are population-based. To our knowledge, no study has investigated whether the levels of serum antibodies to several periodontal bacteria, measured in a population-based sample, are related to preclinical RA risk.

The Third National Health and Nutrition Examination survey (NHANES III) provides an opportunity to investigate the association between IgG antibody levels to periodontal bacteria and RF seropositivity in a large, population-based sample. We hypothesized that elevated serum antibodies would be associated with increased risk of RF seropositivity. In order to establish consistency with previous research, we also evaluated the association between clinical periodontal measures and RF seropositivity.

MATERIALS & METHODS

Study population

Details on the design and methodology of NHANES III have been previously published (34). Briefly, the Third National Health and Nutritional Examination Survey (NHANES III) is a nationally representative cross-sectional sample of the non-institutionalized US population (n=33,994) aged 2 months and older. The NHANES III is a multi-stage, stratified and clustered sample and was conducted in two phases, Phase 1 from 1988–1990 and Phase 2 from 1991–1994. Survey interviews and examinations were conducted, and the data contains information on sociodemographic characteristics, health behaviors, clinical periodontal measures, physical examinations of the joints of the upper and lower extremities, as well as serum analysis for rheumatoid factor (RF) antibody and antibodies to periodontal pathogens.

The current analysis includes all dentate participants without ACR-defined rheumatoid arthritis, with a non-missing value for the outcome rheumatoid factor antibody titer, and who had complete serum IgG periodontal antibody data. Because musculoskeletal examinations and serum analysis for RF antibody were only carried out for those 60 years and older, our study only includes participants ≥60 years. Individuals were excluded if they did not have a periodontal examination or coronal caries examination, or were missing important sociodemographic or health behavior variables related to periodontal disease and rheumatoid factor seropositivity such as age, sex, race, education, smoking status, alcohol use and body mass index (BMI). The final sample size was n=2461.

Immunoglobulin G (IgG) Antibody Assessment

The presence and level of IgG antibodies against 19 periodontal bacteria were measured from serum using a checkerboard immunoblotting technique and the results were reported in gravimetric units (35, 36). Optimal antibody IgG thresholds corresponding to moderate/severe periodontitis (according to the CDC/AAP definition) have previously been published for the 19 bacteria in the same NHANES III dataset (37). Because differing lots of protein A preparations were used in the laboratory analysis of Phase 1 and Phase 2 samples, there was a systematic difference in antibody levels between Phase 1 and 2 (35, 37). To take into account the differences, the antibody titers thresholds for elevated titer status were calculated separately for Phase 1 and 2. In the present analysis, “elevated” antibody thresholds are defined using these a priori Dye et al. cutoffs that maximized the association of antibody levels and clinical periodontal status.

For our sensitivity analysis in the Supplemental Results, we also created bacteria antibody clusters in an a priori fashion utilizing previous groupings derived from hierarchical cluster analysis (an unsupervised method) of these same antibody levels from the NHANES III dataset (38). The authors identified the following four mutually exclusive clusters and named them using the commonly used color coding for periodontal bacteria (39): i) orange-blue (*Eubacterium nodatum, Actinomyces naeslundii*), ii) yellow-orange *(Streptococcus intermedius, Streptococcus oralis, Streptococcus mutans, Fusobacterium nucleatum, Parvimonas micros (previously Micromonas micros), Capnocytophaga ochracea*), iii) red-green (*Tannerella forsythia, Treponema denticola, Aggregatibacter actinomycetemcomitans mix, Eikenella corrodens, Selenomonas noxia, Veillonella parvula, Campylobacter rectus*), and iv) orange-red (*Prevotella melaninogenica, Prevotella intermedia, Prevotella nigrescens, Porphyromonas gingivalis mix*) clusters (38). Cluster scores were created from the sum of the natural log transformed values of antibody levels for each species in the cluster to form continuous measures. We then dichotomized the scores of each cluster by the ≥ 75th percentile versus the < 75th percentile to indicate “high” versus “low” cluster scores, creating a binary measure of cluster burden.

Rheumatoid factor antibody positivity and rheumatoid arthritis assessment

The main outcome in the present analysis was rheumatoid factor seropositivity. Blood samples were first screened using latex-enhanced nephelometry (Behring Nephelometer Analyzer System) and the presence and levels of serum RF antibodies then determined using the Singer-Plotz latex agglutination test (40). A negative measurement was defined in

NHANES III as ≤ 13 IU/ml, and the lowest non-zero recorded RF titer level in the NHANES III dataset was 20 IU/ml. For our analysis we classified all non-zero, non-missing values for RF as being RF seropositive, and zero values were coded as being RF negative, creating a dichotomous outcome.

We used clinical RA status as a secondary outcome for our sensitivity analyses. We defined clinical RA according to a modified version of the American College of Rheumatology (formerly the American Rheumatoid Association) (ACR) 1987 revised criteria for the classification of rheumatoid arthritis (41). The original ACR criteria required 4 out of 7 criteria to be met for the diagnosis of RA, but hand radiographs were not available in NHANES III. Hence in the modified version, participants who met 3 or more of the 6 available criteria were classified as having RA. These modified criteria have been used previously by other authors (6), and have shown good agreement with other classification methods used to classify RA in the NHANES III dataset (42).

Data drawing from the questionnaire, physical examination and laboratory files were used to fulfill our modified ACR criteria as follows: 1) self-reported presence of morning stiffness in the hands or knees for at least 1 hour for at least 6 weeks; 2) physical examination findings of the presence of soft tissue swelling in 3 or more of the following joint areas: left and right proximal interphalangeal joint (PIP), metacarpophalangeal (MCP), wrist, knee and first metatarsophalangeal (MTP) (physical examination of elbow and ankle soft tissue swelling, one of the joint areas included in the original ACR criteria was not available in NHANES III); 3) examination findings of soft tissue swelling in at least one hand joint (PIP, MCP or wrist); 4) symmetric arthritis as defined as simultaneous soft tissue swelling of the same joint (PIP or MCP or wrist or knee or first MTP) on both sides of the body; 5) presence of subcutaneous nodules observed on the shaft of either forearm; 6) presence of serum RF. Participants who had missing values on some criteria but still met 3 or more criteria were classified as having RA.

Risk factor assessment

Confounding variables associated with rheumatoid arthritis and periodontitis were also collected. Age, gender, race ethnicity (Non Hispanic White, Non Hispanic Black, Mexican American, Other), education level (< 12^{th} grade, completed 12^{th} grade, > 12^{th} grade), self-reported smoking status (never, current, former), alcohol use (never, former, current drinker), and body mass index (BMI, measured weight in kilograms divided by height in meters squared) were measured.

Periodontal disease assessment

The oral health examination of the NHANES III has been described previously (43). It consisted of a visual and tactile oral and dental examination performed by a licensed dentist specially trained in the use of specific epidemiologic indices for oral health.

Periodontal measures were done in two randomly assigned quadrants. The mid-buccal and mesial-buccal sites of each fully erupted tooth, excluding the third molars, were assessed for gingival bleeding on probing (BOP), calculus, gingival recession, and pocket depth. Loss of

attachment was calculated from measurements of the free gingival margin to the cemento-enamel junction and the probing depth (free gingival margin to base of the sulcus).

Statistical Analysis

Analysis of variance and categorical analysis methods were used for descriptive statistics, accounting for the weights used in the complex survey design. Multivariable logistic regression models were used to analyze the relationship between clinical periodontal measures and RF seropositivity. We also examined the association between elevated periodontal bacteria antibody levels and RF seropositivity, controlling for confounders such as age, gender, race, ethnicity, education, smoking status, alcohol use and BMI. As the main outcome of interest is preclinical RA, defined by RF seropositivity, the sample for the main analysis excluded those with ACR-defined RA (n=40). In sensitivity analyses, we explored the associations between elevated periodontal bacteria antibody levels with ACR-defined RA as the outcome so comparisons could be made with other publications. We also examined the association between the high versus low periodontal bacteria antibody clusters scores with RF seropositivity in the Supplemental Results. NHANES III uses a complex multi-stage probability sample survey design and oversamples young children, older adults, Blacks and Mexican Americans. The NHANES III Analytic and Reporting Guidelines recommends appropriate sample weights and the sample design to be taken into account in all analyses to avoid misinterpretation (44). Therefore the SURVEYLOGISTIC (SAS) procedure in SAS was used to account for the complex survey design and used with sample weights (WTPFEX6), cluster and strata variables (SDPPSU6 and SDPSTRA6 respectively) provided by the Center for Disease Control and Prevention (45). All statistical analyses were carried out using SAS 9.3 software (SAS, Cary, North Carolina, USA). Based on our final analysis sample of N=2461, we had only 57% power for detecting an odds ratio (OR) of either 0.7 or lower or 1.4 or higher.

RESULTS

Study participants

The mean age of the participants was 70 years; 19% of the participants were Non-Hispanic Black and 23% were Mexican-American. Approximately 50% had less than a 12^{th} grade education, and 49% reported never smoking. Table 1 summarizes additional participant characteristics. The prevalence of rheumatoid factor seropositivity (RF+) was 4.9% (n=143). Having rheumatoid factor seropositivity was associated with having higher self-reported RA, lower diastolic blood pressure, lower white blood cell counts and lower BMI. The mean ± SD probing depth was 1.6 ± 0.6, and the mean ± SD attachment loss was 2.2 ± 1.5. The percent of individuals with elevated serum IgG to specific periodontal microbiota ranged from 28% – 40% across the different bacteria species. The highest prevalence of elevated serum IgG was for *V. parvula* while the lowest was for *E. nodatum* (Table 3).

Clinical Periodontal Measures and prevalent RF seropositivity

After multivariable adjustment for age, sex, education, smoking, alcohol use and BMI, the OR [95% CI] for RF seropositivity for every 1 mm increase in either mean probing depth or mean attachment loss were 1.34 [0.92, 1.97]; p=0.13 and 1.09 [0.93, 1.28]; p=0.29,

respectively (Table 2). Findings were weaker for bleeding on probing and tooth loss (Table 2). Sensitivity analyses controlling for the presence of both probing depth and attachment loss also show similar results. In supplemental analyses, results for the ACR-defined RA outcome (prevalence 1.6%) were similar (Table S1).

Association between elevated serum IgG to periodontal bacteria and prevalent RF seropositivity

The IgG levels to the 19 periodontal bacteria were all positively correlated ($p < 0.001$) with moderate to strong correlations (Figure S1). Odds ratios summarizing the relationship between 19 serum IgG levels to periodontal bacteria and RF seropositivity are shown in Table 3. Adjusted odds ratios ranged from 0.53 to 1.27, controlling for the age, gender, race, education, smoking status, alcohol use and BMI, and 17 of the 19 observed ORs were < 1.0 suggesting inverse relationships, although only two ORs were statistically significant. *P.intermedia* antibody was inversely associated with RF+ : OR [95% CI] =0.53 [0.29, 0.97]; p=0.04. Similarly, the OR [95% CI] for *C. ochracea* was 0.54 [0.31, 0.95]; p=0.03. The observed ORs summarizing the relationship between antibody levels and ACR-defined RA were weaker and none were statistically significant (Table S2). The aforementioned findings were also unchanged in sensitivity analyses stratified by either smoking status or clinical periodontal status (data not shown).

In further sensitivity analyses using a priori antibody clusters as the main exposure and mutually adjusting for the clusters, the results were not meaningfully changed from the species specific analyses, and none of the observed ORs were statistically significant (Table S3). When considering RA as the outcome in the sensitivity analysis, there were no clear patterns observed for the analyses based on the cluster groupings (Table S4). Results for the association between antibody titers to periodontal bacteria and RF+ when using different RF titer thresholds for positivity (>25th percentile, >50th percentile, 75th percentile) also demonstrate no meaningful difference in the pattern of findings (Table S5).

DISCUSSION

We have found elevated serum IgG antibodies to *P. intermedia* and *C. ochracea* to be associated with lower prevalence of RF seropositivity. While the remaining antibody levels were not associated with prevalent RF seropositivity, it was noteworthy that ~75% of the antibodies to periodontal bacteria considered, including the two statistically significant findings, demonstrated ORs <1.0. In contrast, the ORs observed for clinical measures of periodontal disease (probing depth, attachment loss and bleeding) with RF seropositivity were >1.0. The trend for positive relationships (i.e., ORs >1.0) between periodontal disease and both RF seropositivity and ACR-defined clinical RA were consistent with several previous reports (6, 17). This observed consistency, particularly when using the same data as previous findings (6), gives support to the internal validity of our current unexpected results for the overall inverse relationships between IgG antibody levels to periodontal microbiota and RF seropositivity.

In a study utilizing the same NHANES III dataset (37), serum IgG levels to periodontal bacteria were found to be moderately accurate proxies for clinical periodontal status. Thus

we expected that the serological markers of periodontitis would show a similar pattern to the positive relationship between periodontitis and prevalent RA previously found in other studies. For example, Mikuls and colleagues found a significant association (OR 1.56) between IgG antibodies to *P. gingivalis* and the presence of RA-related autoantibody positivity (31). However the outcome "autoantibody positivity" in their study was defined as the presence of at least one of the autoantibodies measured (ACPA, RF measured by nephelometry, or ELISA for RF isotypes), and it thus cannot be determined if the observed associations were due to a stronger positive relationship between *P.gingivalis* antibodies and ACPA. Furthermore, their study population consisted of individuals at high risk of RA (subjects with HLA-DR4 and first-degree relatives of RA patients), whereas our study was population-based, had higher mean age, lower extent and severity of periodontal disease, but included more ever-smokers. Lastly, our methods of assessing RF positivity were different.

Interestingly, while non-significant the same study by Mikuls et al. (2012) also found an inverse association similar to our study of OR 0.76 (0.47, 1.23) for *P.intermedia* with autoantibody positivity, controlling for confounders and *P.gingivalis* antibodies (31). Inverse associations between serum antibodies to periodontal bacteria and RA have also been observed in other studies. Scher et al. observed a higher percentage of healthy participants testing positive for antibodies against *P.gingivalis* when compared to patients with new onset RA and chronic RA (32). They also identified *Prevotella* as one of the only two organisms found with increased prevalence in new-onset RA, regardless of periodontal disease status, versus healthy controls (32), and called for further exploration into the role of *Prevotella*.

Thus while our findings of inverse associations have some precedence and may warrant further investigation, they may also reflect the poor correlation and mixed findings between antibodies to periodontal bacteria and RF seropositivity seen in the literature. A recent study on a cohort of patients with early arthritis found no significant difference in *P.gingivalis* antibody levels by RF positivity status (18). The authors proposed that the strong effect of smoking on RA could have obscured the association between *P.gingivalis* and early RA. As with RA risk, smoking has also been associated with the development of both rheumatoid factor (46, 47) and anti-citrullinated peptide antibodies (48). Therefore, the potential for smoking–related confounding or causal preemption exists even in preclinical studies. Nevertheless, in our sample of older adults with no clinical diagnosis of RA, we found no meaningful difference in results even among never smokers.

The interrelationship between RF, ACPA and periodontal bacteria is complex and the nature of the interaction between ACPA and RF in the development of RA is unresolved. Furthermore, RF has been associated with a number of other chronic infectious disease states in individuals who do not appear to be developing RA, and its use as a prodromal indicator of incipient RA may be problematic. ACPAs are more sensitive and more specific for the presence of rheumatoid arthritis than rheumatoid factor (49). Synergy between the development of ACPA and RF has also been observed (50), indicating that development of both of these types of antibodies is the strongest predictor for incipient rheumatoid arthritis. Whether antibodies to periodontal bacteria are related to the presence of ACPA would thus be of interest. Unfortunately, ACPAs were not measured in the NHANES III cohort and cannot be analyzed in our study.

Rather than indicating a null association between periodontitis and RA, we believe that our findings draw attention to the potentially problematic use of serum antibodies to periodontal pathogens as proxies of bacterial exposure in cross-sectional studies of RA risk, particularly in population-based samples. The use of serological markers to periodontal bacteria exposure has some important advantages, including the ability to be measured from stored or fresh serum, allowing the study of large retrospective cohorts, and requiring much less participant burden and examiner time than a clinical periodontal examination or microbial sampling. However, the dual connotation of high antibody levels complicates the interpretation of results from studies using serum antibodies to mark exposure to adverse microbial exposures. While elevated antibodies likely reflect increased bacterial burden, they simultaneously signify a presumably protective immune response to the microbial challenge that may in turn control or reduce lifetime bacterial exposures.

Due to the cross-sectional nature of our data, we cannot establish temporality and the presence of RF antibodies could be concomitant with a reduced immune response to periodontal bacteria, secondary to a dysregulated immune response. This raises the potential for misclassification of exposure status (i.e., classifying individuals with high levels of bacterial exposure as having low levels of exposure and vice-versa) that is differential by outcome status. If the misclassification rate is high enough, the bias induced can be substantial and might even produce measures of association that are opposite to the true direction. This may have been especially important due to an increasingly senescent immune response in the individuals included in our sample, who were all over age 60 (younger individuals were not included in this analysis because of the lack of musculoskeletal exams and RF serum analysis). Similarly, age-related exclusions might have created a selection bias due to the exclusion of individuals with HLA-DRB1 shared epitope rheumatoid arthritis risk alleles who tend to experience rapid progression of periodontal disease and rheumatoid arthritis at younger ages.

We did not exclude patients based on the use of immunosuppressive or disease-modifying RA medications, and our study sample could have included a number of participants using such medications with conceivably altered immune responses to periodontal bacteria, resulting in the inverse association seen. Because of our exclusion of edentulous individuals and those without complete data on all periodontal antibodies, we had very few RF seropositive participants with RA (n=8) and were unable to perform further subgroup analyses on seropositive RA subjects only as previous studies have done. An alternative explanation for our inverse findings could be incidence-prevalence bias. If individuals with elevated (vs. low) serum antibodies to periodontal bacteria are more likely to die or progress rapidly from RF+ asymptomatic status to overt RA, this can induce an association that appears inverse because the frequency of the exposure is underestimated among the cases studied (51).

Finally, we were only able to consider serum antibodies to a small number of microorganisms available in the NHANES III dataset, among the hundreds of known periodontal bacteria. Recent literature suggests a complex periodontal microbiome with synergistic effects on health and disease that this study cannot address (52). We also did not have data on RA risk genotypes, such as the *HLA-DRB1* shared epitope strongly associated

with RA. It is possible that periodontal antibody levels might demonstrate stronger associations in genetically susceptible individuals, as previously seen in the gene-environment interactions between the HLA genotype and smoking (48).

This study adds to the periodontitis-RA literature by exploring the associations between serological markers of exposure to periodontal bacteria and a preclinical marker of RA, RF seropositivity. We found ORs to be predominantly inverse though largely not statistically significant between antibodies to periodontal bacteria and RF seropositivity in the nationally representative NHANES III sample. The strengths of our study include extending the investigation beyond the commonly studied *P.gingivalis* association to the antibodies to 19 common periodontal bacteria. To the best of our knowledge, our study is the first to examine the relationship between antibodies to periodontal bacteria and RF seropositivity in a population-based sample. Our findings were consistent after controlling for important confounders such as smoking and alcohol. Future studies examining the association between periodontal infection and RA would be improved by including assessments of the periodontal microbiota and measuring genetic susceptibility. In addition, the use of prospective cohort designs starting at ages younger than those of the known peak incidence of RA, and data on the future development of RA in these subjects and the subsequent associations between RA, RF and antibodies to periodontal pathogens would be of great interest.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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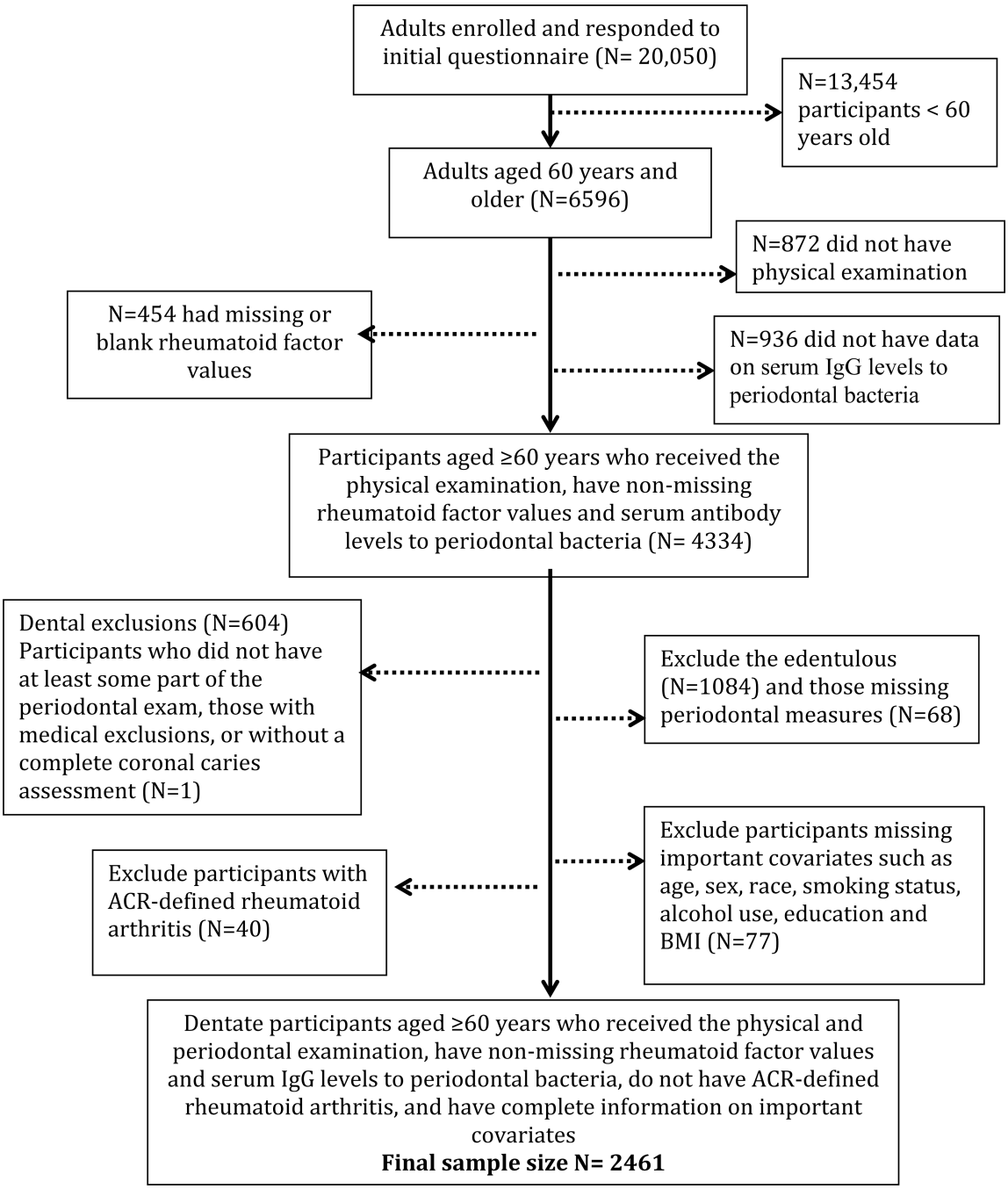

Figure 1.

Flowchart of sample population derivation for this study of the association between serum IgG levels to 19 periodontal bacteria and rheumatoid factor (RF) in dentate participants aged ≥ 60 years from the NHANES III (1988–1994) dataset.

Table 1

General Characteristics of Participants according to prevalent rheumatoid factor seropositivity (RF+): Dentate participants aged ≥ 60 years, without ACR-defined rheumatoid arthritis, enrolled in NHANES III 1988–1992. (N=2461)

Characteristic	Unweighted All (n=2461)	Weighted All	RF+ n= 143 (4.9%)	RF− n= 2318 (95.1%)	P value
Self Report RA	7	6	16	5	0.01
Age (years)	70.2 ± 7.5	69.4 ± 0.3	70.2 ± 0.7	69.4 ± 0.4	0.24
Female (%)	51	57	61	57	0.53
Race/Ethnicity (%)					0.25
Non-Hispanic White	56	85	79	85	
Non-Hispanic Black	19	7	12	7	
Mexican-American	23	3	3	3	
Other	3	5	6	5	
Education (%)					0.64
< 12^{th} grade	50	32	33	32	
12^{th} grade	26	34	37	33	
>12^{th} grade	24	34	30	35	
Smoking Status (%)					0.54
Never	49	49	45	49	
Former	37	39	38	39	
Current	14	12	17	12	
Pack Years of Smoking	15.9 ±29.4	17.5 ± 1.0	18.8 ± 3.8	17.5 ± 1.0	0.73
Alcohol use (%)					0.38
Never	21	18	17	18	
Former	44	41	49	41	
Current	35	40	34	41	
Systolic Blood Pressure (mmHg)	137.9 ± 22.5	134.8 ± 0.7	131.5 ± 2.4	134.8 ± 0.7	0.17
Diastolic Blood Pressure (mmHg)	72.8 ± 12.0	72.4 ± 0.4	68.1 ± 1.7	72.6 ± 0.4	0.02
Hypertension	42	37	36	37	0.77
White Blood Cell Count	7.1 ± 2.6	7.0 ± 0.1	6.6 ± 0.2	7.0 ± 0.1	0.02

Characteristic	Unweighted All (n=2461)	Weighted All	RF+ n= 143 (4.9%)	RF− n= 2318 (95.1%)	P value
(cells/mm^3)					
Mean probing depth (mm)	1.6 ± 0.6	1.5 ± 0.03	1.6 ± 0.06	1.5 ± 0.02	0.16
Percentage of probing depths ≥ 4mm (%)	4.5 ± 11.0	2.9 ± 0.3	3.7 ± 0.9	2.8 ± 0.3	0.38
Percentage of sites with bleeding on probing (%)	14.2 ± 21.1	11.3 ± 0.7	12.0 ± 1.7	11.1 ± 0.7	0.55
Mean attachment loss (mm)	2.2 ±1.5	1.9 ± 0.1	2.1 ± 0.2	1.9 ± 0.1	0.15
Percentage of site with attachment loss ≥ 5mm (%)	11.7 ± 21.3	8.4 ± 0.7	10.9 ± 2.1	8.2 ± 0.7	0.22
C-reactive Protein (mg/dl)	0.55 ± 1.1	0.49 ± 0.02	0.51 ± 0.06	0.48 ± 0.02	0.69
LDL- cholesterol	140.9 ± 37.6	142.5 ± 1.4	141.7 ± 6.0	142.4 ± 1.5	0.91
HDL- cholesterol	51.3 ± 16.1	51.8 ± 0.5	49.7 ± 2.3	51.9 ± 0.5	0.34
Body Mass Index BMI (kg/m^2)	27.4 ± 5.0	27.0 ± 0.1	26.0 ± 0.5	27.1 ± 0.1	0.02
Lymphocytes (percent of 100 cells)	31.7 ±11.3	31.6 ± 0.5	32.8 ± 2.0	31.5 ± 0.5	0.53
Monocytes (percent of 100 cells)	5.8 ± 3.3	5.9 ± 0.2	6.3 ± 0.6	5.9 ± 0.2	0.46
Log-transformed *P.gingivalis* antibody level (fg/ml)	6.2 ± 2.2	5.7 ± 0.08	5.7 ± 0.2	5.7 ± 0.09	0.82

Note: Values shown are either percent or mean (SD). Weighted results incorporate survey weights making the results generalizable to the civilian non-institutionalized US population aged ≥ 60 years and RA free. Results presented among RF subgroups are weighted including the prevalence of RF+ and Rao-Scott Modified Chi square and T-tests were used as appropriate to test for differences across RF positivity.

Subsamples due to missing data: n = 2393 with pack years data; n = 2392 with systolic blood pressure, diastolic blood pressure and hypertension; n = 2434 with white blood cell count; n = 1020 with LDL counts; n = 2441 with HDL counts; n = 747 with lymphocytes and monocytes counts.

Self Report RA represents patients who self-reported a doctor diagnosis of RA in an interviewer administered questionnaire regardless of physical exam results or serology.

Table 2

Odds ratios for prevalent rheumatoid factor seropositivity (RF+) by clinical periodontal measures: Dentate participants aged ≥ 60 years and without ACR-defined rheumatoid arthritis, enrolled in NHANES III 1988–1992. (N=2461)

	Crude RF+ prevalence= 5.8 % (143/2461)	***p*-value**	**Adjusted* RF+ prevalence= 5.8 % (143/2461)**	***p*-value**
Mean probing depth	1.34 (0.97, 1.87)	0.08	1.34 (0.92, 1.97)	0.13
Mean attachment loss	1.14 (0.98, 1.33)	0.10	1.09 (0.93, 1.28)	0.29
Total missing teeth	1.01 (0.98, 1.04)	0.64	1.00 (0.97, 1.03)	0.80
Percent of sites bleeding on probing	1.03 (0.94, 1.11)	0.53	1.02 (0.93, 1.12)	0.63

Mean probing depths, attachment loss and percent of bleeding on probing sites are based on the mesiobuccal and midbuccal sites of all fully erupted teeth, excluding 3rd molars, in 2 randomly selected quadrants.

Odds ratios are for either a 1 mm increase in probing depth or attachment loss; 1 additional missing tooth; or a 10% increase in BOP.

*Adjusted for age, sex, race, education, smoking, BMI, alcohol.

Table 3

Odds ratios for prevalent rheumatoid factor seropositivity (RF+) by Serum Antibody Levels for 19 Periodontal Bacteria: Dentate participants aged ≥ 60 years and without ACR-defined rheumatoid arthritis, enrolled in NHANES III 1988–1992. (N=2461)

Periodontal bacteria antibody levels	Phase 1* cut point	Phase 2* cut point	% Elevated Antibody levels based on cut points	Crude OR	*p*-value	Adjusted OR	*p*-value
P. gingivalis (Pg) mix antibody	713	1149	39%	0.89 (0.55, 1.45)	0.65	0.80 (0.47, 1.33)	0.38
P. intermedia (Pi) antibody	457	502	38%	0.57 (0.33, 1.00)	0.05	0.53 (0.29, 0.97)	0.04
P. nigrescens (Pn) antibody	310	392	38%	0.70 (0.41, 1.20)	0.20	0.70 (0.41, 1.21)	0.20
T. forsythia (Tf) antibody	128	276	38%	0.81 (0.49, 1.34)	0.41	0.82 (0.51, 1.34)	0.44
A. actinomycetemcomitans (Aa) mix	766	2612	33%	0.84 (0.45, 1.58)	0.59	0.82 (0.43, 1.55)	0.54
F. nucleatum (Fn) antibody	154	164	34%	0.62 (0.37, 1.05)	0.07	0.61 (0.35, 1.05)	0.08
S. oralis (So) antibody	83	189	35%	0.85 (0.51, 1.41)	0.52	0.80 (0.48, 1.35)	0.40
P. micra (Pmi) antibody	235	337	33%	0.92 (0.58, 1.46)	0.73	0.92 (0.59, 1.44)	0.73
C. rectus (Cr) antibody	112	219	38%	0.91 (0.60 1.39)	0.68	0.87 (0.55, 1.39)	0.56
E. corrodens (Ec) antibody	198	450	38%	0.65 (0.36, 1.17)	0.12	0.62 (0.35, 1.12)	0.11
E. nodatum (En) antibody	1755	3897	28%	1.08 (0.58, 2.00)	0.82	1.15 (0.61, 2.17)	0.67
S. intermedius (Si) antibody	201	459	33%	0.87 (0.48, 1.57)	0.77	0.88 (0.48, 1.61)	0.67
C.ochracea (Co) antibody	285	206	30%	0.56 (0.32, 0.97)	0.04	0.54 (0.31, 0.95)	0.03
V. Parvula (Vp) antibody	51	74	40%	0.93 (0.54, 1.60)	0.79	0.98 (0.57, 1.69)	0.95
A. naeslundii (An) antibody	1097	640	30%	1.14 (0.70, 1.87)	0.60	1.27 (0.79, 2.06)	0.32
P. melaninogenica (Pm) antibody	290	354	38%	0.97 (0.56, 1.70)	0.91	0.97 (0.56, 1.65)	0.90
S. noxia (Sn) antibody	38	83	35%	0.92 (0.51, 1.65)	0.78	0.91 (0.50, 1.66)	0.76
T. denticola (Td) antibody	136	403	33%	0.64 (0.35, 1.18)	0.15	0.63 (0.35, 1.15)	0.13
S. mutans (Sm) antibody	117	201	35%	0.88 (0.54, 1.46)	0.63	0.84 (0.51, 1.39)	0.50

*Based on calculated threshold values for elevated antibody status for the CDC/AAP definition of moderate to severe periodontitis in Dye et al. “Serum Antibodies to Periodontal Bacteria as Diagnostic Markers of Periodontitis” (2009)

Multivariable model adjusted for age, sex, race, education, smoking, BMI, alcohol