HHS Public Access

Author manuscript

Arthritis Rheumatol. Author manuscript; available in PMC 2022 April 01.

Published in final edited form as:

Arthritis Rheumatol. 2021 April ; 73(4): 568–575. doi:10.1002/art.41572.

Associations of Antibodies Targeting Periodontal Pathogens with Subclinical Coronary, Carotid, and Peripheral Arterial Atherosclerosis in Rheumatoid Arthritis

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Abstract

Objective—Both periodontal disease and cardiovascular disease (CVD) are over-represented in rheumatoid arthritis (RA). However, the contribution of periodontal pathogens to CVD in RA is unknown.

Methods—RA patients underwent assessments of coronary artery calcification (CAC), carotid intima media thickness and plaque, and ankle-brachial index via computed tomography, ultrasound, and Doppler ultrasound, respectively. Sera were assayed for antibodies targeting *Porphyromonas gingivalis* (anti-*Pg*), *Aggregatibacter actinomycetemcomitans* serotype b (anti-*Aa*), and *Aa* leukotoxin A (anti-LtxA). Associations of antibodies against these periodontal pathogens with measures of atherosclerosis were explored using generalized linear models.

Results—Among 197 RA patients, anti-*Pg* was detected in 72 (37%), anti-*Aa* in 41 (21%), and anti-LtxA in 84 (43%). Adjusting for relevant confounders and reported tooth loss, the mean CAC score was 90% higher in those with anti-*Aa* and/or anti-LtxA vs. those without either antibody [19 vs. 10 units, respectively (p=0.033)]. The adjusted odds of CAC≥100 units was 2.23-fold higher for those with anti-*Aa* and/or anti-LtxA vs. those without either antibody (p=0.040). Anti-*Aa* and/or anti-LtxA seropositivity was associated significantly with all other assessed measures of atherosclerosis except carotid plaque. Anti-*Pg* was not associated with any measure of atherosclerosis. Higher swollen joint count was associated with CAC exclusively in the group with anti-*Aa* and/or anti-LtxA.

Conclusions—Immunoreactivity against *Aa* and/or its major virulence factor LtxA was associated with atherosclerosis in multiple vascular beds of RA patients, and amplified the effect of swollen joints on coronary atherosclerosis, suggesting a role for treatment/prevention of periodontal disease in the prevention of CVD in RA.

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Keywords

cardiovascular; periodontal disease; atherosclerosis; antibodies

INTRODUCTION

The burden of atherosclerotic cardiovascular disease (CVD) is greater in individuals with rheumatoid arthritis (RA) compared with those without RA.(1) In addition to traditional CVD risk factors, a number of RA-associated factors, including seropositivity, RA duration, and measures of articular and systemic inflammation have been associated with atherosclerotic burden.(2–4) However, these factors do not account for all of the excess, suggesting that other mechanisms may contribute.

Periodontal disease, and the bacterial pathogens that cause periodontitis, are potential contributors to atherosclerotic CVD in RA. Periodontal disease is associated with both RA(5) and atherosclerosis(6), and links between the two have been proposed. In particular, pathogenic microbiota in the subgingival biofilm in periodontitis have been associated with systemic inflammation and immunoactivation.(7) One such bacterial pathogen, *Porphyromonas gingivalis* (*Pg*), expresses numerous virulence factors that modulate host immune defenses, leading to overgrowth of oral commensal bacteria leading to inflammatory destruction of periodontal tissue.(8) Gingival ulceration enables translocation of *Pg* into systemic circulation, which invade endothelial cells leading to dysfunction. Moreover, locally produced pro-inflammatory cytokines and bacterial products (e.g. *Pg* lipopolysaccharide, LPS) can circulate and induce an acute-phase response.(8, 9) A second periodontitis-associated pathogen, *Aggregatibacter actinomycetemcomitans* (*Aa*), produces a highly virulent exotoxin with leukotoxic potential (Leukotoxin A; LtxA). Binding of LtxA to lymphocytes, neutrophils, and macrophages causes pyroptosis and activation of the inflammasome, releasing inflammatory cytokines and inducing secondary immunoactivation.(10) *Aa*-derived LtxA is also a potent inducer of leukotoxic hypercitrullination (LTH) in neutrophils which is not caused by other periodontal pathogens. (11, 12) Moreover, RA sera contain autoantibodies directed against citrullinated autoantigens generated during LtxA-induced LTH.(11)

Both pathogens have been implicated in atherogenesis. Microbial nucleic acids from both have been isolated from atheroma samples.(13) *Aa*-associated LtxA induces the upregulation of vascular adhesion molecules on endothelial cells(14) and atherosclerosis prone mice infected with *Aa* demonstrated upregulation of vascular adhesion molecules, higher expression of inflammatory cytokines and chemokines in the wall of the aorta and higher atherosclerotic plaque burden compared with non-infected mice.(15) Multiple observational studies have linked periodontal disease, and its associated pathogens, independently with measures of subclinical atherosclerosis and CVD events,(16) although causality remains questioned.

Considering these potential associations, we measured immunoreactivity to *Pg*, *Aa* serotype b, and *Aa*-associated LtxA and explored their associations with measures of atherosclerosis in the coronary, carotid, and peripheral arterial circulation of RA patients. We hypothesized

that RA patients with immunoreactivity against periodontal pathogens would demonstrate a greater burden of atherosclerosis in multiple vascular beds compared with those without such immunoreactivity.

METHODS

Participants were enrolled in ESCAPE RA (Evaluation of Subclinical Cardiovascular disease And Predictors of Events in Rheumatoid Arthritis), a prospective cohort study investigating subclinical CVD in RA described in detail previously.(17, 18) Participants met 1987 RA classification criteria(19) and were 45-84 years of age without known prior CVD events. Enrollment occurred between 2004 and 2006. Written consent was signed prior to participating. The study was approved by the Institutional Review Boards (IRB) of the Johns Hopkins Medical Center and Columbia University Medical Center. Patient input was not directly involved in the design or conduct of the study.

Imaging of Subclinical Atherosclerosis

CAC was measured using multidetector row computed tomography (MDCT), as described previously,(17) using the method described by Agatston.(20) Scoring was blinded to group allocation and clinical characteristics. Carotid imaging was performed as previously described(18) and involved measures in the common carotid artery (CCA), internal carotid artery (ICA) and the carotid bulb. Carotid plaques were localized to the ICA and bulb and were defined as maximal focal protrusion into the lumen with reduction in the lumen diameter>25%. Baseline and follow-up scans were re-analyzed concurrently by a single reader aware of the temporal ordering but unaware of clinical characteristics. The ankle brachial index (ABI) was calculated as the ratio of the highest Doppler ultrasound-detected blood pressure of either the dorsalis pedis or posterior tibial arteries divided by the highest arm blood pressure, as previously described.(21)

Sociodemographic Characteristics and CVD Risk Factors

Demographics and smoking history were assessed by self-report. Current use and dosage of medications were ascertained from prescription bottles. Body mass index (BMI) was calculated as body weight (kg) divided by height (meters^2). Patients self-reported the current number of missing teeth.

Insulin resistance was assessed using the HOMA-IR index from the HOMA2 model.(22) Hypertension was defined as systolic BP≥140 mmHg, diastolic BP≥90, or antihypertensive medication use. Diabetes was defined as a fasting serum glucose≥126 mg/dL or use of anti-diabetic medications.

RA Disease Characteristics

Forty-four joints were examined by a single trained assessor. RA disease duration was assessed from the self-reported date of diagnosis. RA disease activity was calculated using the Disease Activity Score for 28 joints with CRP (DAS28-CRP).(23) Current and past use of glucocorticoids and DMARDs was queried by detailed examiner-administered questionnaires. The Stanford Health Assessment Questionnaire (HAQ)(24) was used to

assess disability related to common activities. Single view, anterior-posterior radiographs of the hands and feet were scored using the Sharp-van der Heijde (SHS) method(25) by a single trained radiologist.

Laboratory Assessments

IgG antibodies to *Aa* strain HK1651 (serotype b), *Pg* strain W83, and purified LtxA were previously determined in serum by ELISA. Anti-LtxA antibody positivity was also confirmed by immunoprecipitation. (11) High sensitivity C-reactive protein (CRP) and IL-6 were measured as previously described.(26) Plasma lipids and glucose were measured by standard assays; LDL-cholesterol was estimated using the Friedewald equation. Rheumatoid factor (RF) was assessed by ELISA, with seropositivity≥40 units. Anti-CCP antibody was assessed by ELISA, with seropositivity≥60 units. HLA alleles bearing the “shared epitope” were investigated by DRB1 sequencing as previously described.(18)

Statistical Analysis

Exposure to leukotoxic *Aa* strains was defined as having either anti-*Aa* or anti-LtxA antibodies. Exposure to leukotoxic *Aa* strains other than serotype b strains was captured in those who were seropositive for anti-LtxA but seronegative for Anti-*Aa* HK1651 (ATCC 700685). Variables were examined according to the presence/absence of immunoreactivity to anti-*Aa* and/or anti-LtxA and anti-*Pg* using t-tests for normally distributed continuous variables, the Kruskal-Wallis test for non-normally distributed variables, and the chi-square goodness-of-fit test or Fisher’s exact test, as appropriate, for categorical variables. The association of anti-*Aa* and/or anti-LtxA seropositivity with CAC, normally transformed as natural log (CAC+1), was explored using multivariable linear regression, first in a crude model with anti-*Aa* and/or anti-LtxA positivity as the only covariate. Next, variables associated with CAC at the $p<0.20$ level from univariate models were modeled. A reduced model was derived by excluding non-contributory covariates using Akaike’s Information Criterion for nested models. An additional sensitivity analysis included the number of reported missing teeth in order to ensure that observed associations of anti-*Aa* and/or anti-LtxA and CAC from prior models were not confounded by the presence of oral diseases, including periodontal disease and other causes of tooth loss. The normality assumption required for linear regression was tested using the Shapiro-Wilk test on the studentized residuals. Similar modeling was used for the other atherosclerosis outcomes, except for logistic regression for CAC >0 units, CAC >100 units, and carotid plaque presence. CCA-IMT and ICA-IMT also required log transformation. The same models were repeated with anti-*Pg* as the covariate of interest. From all models, adjusted means and frequencies, and their associated 95% confidence intervals, were derived and graphed according to immunoreactivity to periodontal pathogens, with back transformation as appropriate. Next, we explored whether anti-*Aa* and/or anti-LtxA status modified the associations of other covariates with atherosclerosis outcomes by introducing anti-*Aa* and/or anti-LtxA x covariate interaction terms into the models, with p-values for interaction terms derived using analysis of covariance (ANCOVA). STATA SE 16 (StataCorp, College Station, TX) was used. A two-tailed $\alpha=0.05$ was used throughout.

RESULTS

Among the 197 RA patients tested, anti-*Aa* serotype b was detected in 41 (21%) while anti-LtxA was detected in 84 (43%). Only eight patients with anti-*Aa* were seronegative for anti-LtxA. Anti-*Pg* was detected in 72 patients (37%). Baseline characteristics according to anti-*Aa* and/or anti-LtxA are summarized in Table 1. Those with anti-*Aa* and/or anti-LtxA were an average of 3 years older than those without anti-*Aa* and/or anti-LtxA and were significantly less likely to be White race. Those with anti-*Aa* and/or anti-LtxA did not differ significantly on other lifestyle characteristics, CVD risk factors, or RA disease features with the exception of a significantly higher prevalence of RF seropositivity. Those with anti-*Aa* and/or anti-LtxA were significantly more likely to be anti-*Pg* seropositive and have more reported tooth loss.

Atherosclerosis was Associated with Antibodies to Aa and LtxA but not against Pg in RA

The univariate associations of *Aa*-directed, anti-LtxA-directed and *Pg*-directed serologic status with measures of coronary, carotid, and peripheral arterial atherosclerosis are summarized in Table 2. The median CAC score was 30 units higher among those with anti-*Aa* and/or anti-LtxA compared with those without these antibodies (p=0.046). Likewise, the prevalence of any CAC or having a CAC ≥100 units was higher among those with anti-*Aa* and/or anti-LtxA. Similarly, both median CCA and ICA-IMT, but not the frequency of carotid plaque, was significantly higher for those with anti-*Aa* and/or anti-LtxA. For the peripheral arteries, median ABI was significantly lower among RA patients with anti-*Aa* and/or anti-LtxA. In contrast, anti-*Pg* was not significantly associated with any measure of atherosclerosis.

Atherosclerosis was Associated with Anti-Aa and Anti-LtxA Antibodies After Adjustment for Potential Confounders

Seropositivity for anti-*Aa* and/or anti-LtxA remained significantly associated with CAC in models adjusted for all of the characteristics associated with CAC in univariate models (Table 3, Model 2) and in a reduced model (Table 3, Model 3). Here, the magnitude of the independent association of anti-Aa/or anti-LtxA seropositivity with CAC was equivalent to about a five years increment in age or having ever smoked. In this model, longer RA duration and higher swollen joint counts were both significantly associated with CAC. After adjustment for age, sex, ever smoking, BMI, triglyceride level, statin use, RA duration, and swollen joint count, the adjusted mean CAC score was 90% higher in the group with vs. without anti-*Aa* and/or anti-LtxA [19 vs. 10 units, respectively (p=0.033); Figure 1A]. Adjusting for these same covariates, anti-*Aa* and/or anti-LtxA seropositivity was significantly associated with CAC>0 units (Figure 1B), CAC ≥100 units (Figure 1C), CCA-IMT (Figure 2A), and ABI (Figure 2C). The association of anti-*Aa* and/or anti-LtxA with ICA-IMT was higher, but not quite statistically significant, after adjustment (Figure 2B). Reported tooth loss was not associated with CAC and did not modify the association of anti-*Aa* and/or anti-LtxA with CAC when co-modeled (Table 3, Model 4).

The Association of Swollen Joints with Coronary Atherosclerosis was Restricted to RA Patients Positive for Antibodies to Aa and/or LtxA.

We next studied whether seropositivity for anti-*Aa* and/or anti-LtxA modified the associations of any of the other characteristics associated with atherosclerosis (summarized in the Supplemental Table). Among the characteristics associated with measures with atherosclerosis, only the association of swollen joint count with CAC differed according to anti-*Aa* and/or anti-LtxA status, such that higher swollen joint count was strongly associated with higher CAC score (Figure 3A) and a higher frequency of CAC ≥100 units (Figure 3B) among those seropositive for anti-*Aa* and/or anti-LtxA. The association was not linear, as the inflection of the association of swollen joints with CAC occurred at around 7 or 8 swollen joints (Figure 3). In contrast, a higher number of swollen joints was not associated with CAC among those without anti-*Aa* or anti-LtxA. A significant interaction was also observed when DAS28 was modeled instead of swollen joint count; however, substitution of tender joint count or CRP did not show an interaction with anti-*Aa* and/or anti-LtxA status (data not shown), suggesting that the association of DAS28 with measures of CAC among those with anti-*Aa* and/or anti-LtxA was due to swollen joint count and not the other components of the DAS28 score. This modification was only observed for CAC and not for measures of carotid or peripheral arterial atherosclerosis (data not shown). The associations of anti-*Aa* and/or anti-LtxA with the atherosclerosis outcomes did not differ according to ACPA or shared epitope status (data not shown).

DISCUSSION

To our knowledge, ours is the first report linking immunoreactivity to common periodontal pathogens with atherosclerosis burden in RA. RA patients with serological evidence of *Aa* exposure by either anti-*Aa* or its leukotoxin (LtxA) had significantly higher levels of CAC, a surrogate for coronary atherosclerosis, thicker carotid IMT, a surrogate for carotid atherosclerosis, and lower ABI, a surrogate for peripheral arterial atherosclerosis, even after adjusting for relevant confounders. Importantly, seropositivity for anti-*Pg* was not associated with any measure of atherosclerosis. Interestingly, an association of higher swollen joint count with CAC was only observed in the subgroup seropositive for anti-*Aa* and/or anti-LtxA.

Multiple studies have established a link between periodontal disease and RA. In a recent meta-analysis,(5) periodontitis was 13% higher in RA patients than non-RA controls. Both anti-*Aa* and anti-*Pg* were more prevalent in RA compared with controls in some studies(11, 27, 28) with some, but not all, demonstrating correlations with ACPA and disease activity. (27) Anti-*Pg* levels were higher among RA patients at-risk of developing RA compared with those not at risk,(29) and levels correlated with RA-associated antibodies. In a smaller study that included periodontal sampling, the abundance of *Pg* at both healthy and inflamed periodontal sites was significantly higher among ACPA positive individuals at risk for developing RA compared with healthy controls.(30) However, *Aa* abundance was not significantly higher, although differences may have been affected by the small sample size of the study (n=48 at-risk and n=32 controls), ethnicity,(31) and restriction of *Aa* to non-periodontal oral reservoirs.(31) Taken together, these studies provide some argument for an

increased prevalence of periodontitis and periodontitis-associated pathogens in RA and circumstantial links to disease risk and severity.

Periodontitis has been linked to atherosclerosis in the general population.(16) CVD events were higher among those with periodontitis(32), and carotid atherosclerosis was linked to severe periodontitis.(33) DNA from periodontal pathogens, including *Aa* and *Pg*, was isolated from atheroma in several,(13) but not all,(34) studies, particularly among those with chronic advanced periodontitis. Across several studies of atherosclerosis-prone mice,(15) intravenous inoculation with *Aa* was associated with endothelial invasion and activation, LDL oxidation, toll-like receptor activation, upregulation of inflammatory cytokines and chemokines, and promotion of macrophage foam cell formation. In one study, *Pg* was associated with promotion of plaque rupture through upregulation of metalloproteinases.(35) Calhoun et al reported that high levels of circulating anti-*Aa* and anti-*Pg* were associated with higher CAC scores,(36) an association primarily observed among the subgroup with diabetes, suggesting interaction with other CVD risk factors. RA has been described as a diabetes-equivalent for CVD, and may represent a population in which the effect of periodontal pathogens on atherogenesis is heightened. In our study, we did not observe an association of anti-*Pg* with any measure of atherosclerosis, which could indicate specificity in the effect related to only *Aa* in RA. This finding could also be unique to the cohort studied and thus deserves to be confirmed in other cohorts. However, anti-*Pg* was as prevalent as anti-*Aa* in our sample, and did not confound any of the associations of anti-*Aa* and/or anti-LtxA with measures of atherosclerosis when co-modeled.

We also observed that higher swollen joint counts were associated with CAC only among RA patients with reactivity to *Aa* or LtxA. This interaction suggests that *Aa* infection may create a permissive environment for the inflammatory features of RA to contribute to atherogenesis and raises the possibility that anti-*Aa* status could be used to identify a subgroup of RA patients for whom aggressive control of synovitis may lead to a lower rate of atherosclerosis progression. Importantly, the interaction was specific to swollen joints and did not extend to tender joint count or circulating CRP. The mechanism underlying this interaction is unclear and warrants additional study. However, other types of effect modification in which anti-*Aa* status appeared to create a permissive environment for RA features has been noted in prior studies, where *HLA-DRB1* shared epitope alleles were only associated with higher levels of ACPA among RA patients with anti-*Aa*, (11, 37) although such conditional associations have not been observed in all studies.(28)

Our study has several notable strengths and weaknesses. Among the strengths, it is the first study of the association of periodontal pathogens with CVD in RA. Additionally, we measured atherosclerosis in multiple vascular beds, with confirmation of associations across vascular territories. Among weaknesses, the cross-sectional design does not allow firm conclusions regarding causality to be made. Since the point of seroconversion to reactivity against the periodontal pathogens was unknown, cumulative exposure could not be assessed. Because other periodontal pathogens may also contribute to atherogenesis, it is not certain that any of the observed associations were specific to *Aa* or to the confounding effects of an unmeasured correlated causal factor. However, that anti-*Pg* was not associated with atherosclerosis suggests that the associations of *Aa* with measures of atherosclerosis were

not strongly confounded by other periodontal pathogens, but the possibility of confounding by other periodontal pathogens is not fully excluded. Finally, since we did not compare these associations in a group without RA, we cannot assert that our findings are specific to RA.

In summary, RA patients with evidence of exposure to *Aa*, but not *Pg*, had higher levels of atherosclerosis across multiple vascular beds independent of other CVD risk factors. The association of swollen joints with coronary atherosclerosis was restricted to RA patients with seroreactivity to anti-*Aa* and/or anti-LtxA. Although speculative, these findings may suggest that assessing immunity against *Aa* may predict CVD in RA patients and that *Aa*-exposed patients may be appropriate for heightened CVD screening and primary prevention. However, confirmation in additional cohorts and studies demonstrating prediction and clinical utility are required before *Aa* immunoreactivity is appropriate for clinical practice. At minimum, our study provides evidence for mechanistic studies assessing the links between periodontitis and CVD in RA.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

Special thanks to Dr. Joan Bathon for access to data and samples from the ESCAPE RA cohort.

FUNDING

This work was supported by the Jerome L. Greene Foundation and the National Institutes of Health, National Institute of Arthritis and Musculoskeletal and Skin Diseases Grant Numbers AR050026-01, T32 AR048522-16A1 and R01 AR069569. The content of this paper is solely the responsibility of the authors and does not represent the official views of the NIH.

REFERENCES

1. Avina-Zubieta JA, Thomas J, Sadatsafavi M, Lehman AJ, Lacaille D. Risk of incident cardiovascular events in patients with rheumatoid arthritis: a meta-analysis of observational studies. Ann Rheum Dis 2012;71:1524–1529. [PubMed: 22425941]
2. Chung CP, Oeser A, Raggi P, Gebretsadik T, Shintani AK, Sokka T, et al. Increased coronary-artery atherosclerosis in rheumatoid arthritis: relationship to disease duration and cardiovascular risk factors. Arthritis and Rheumatism 2005;52:3045–3053. [PubMed: 16200609]
3. Karpouzas GA, Malpeso J, Choi TY, Li D, Munoz S, Budoff MJ. Prevalence, extent and composition of coronary plaque in patients with rheumatoid arthritis without symptoms or prior diagnosis of coronary artery disease. Ann Rheum Dis 2014;73:1797–1804. [PubMed: 23887286]
4. Giles JT, Post WS, Blumenthal RS, Polak J, Petri M, Gelber AC, et al. Longitudinal predictors of progression of carotid atherosclerosis in rheumatoid arthritis. Arthritis Rheum 2011;63:3216–3225. [PubMed: 21965129]
5. Fuggle NR, Smith TO, Kaul A, Sofat N. Hand to Mouth: A Systematic Review and Meta-Analysis of the Association between Rheumatoid Arthritis and Periodontitis. Front Immunol 2016;7:80. [PubMed: 26973655]
6. Mustapha IZ, Debrey S, Oladubu M, Ugarte R. Markers of systemic bacterial exposure in periodontal disease and cardiovascular disease risk: a systematic review and meta-analysis. J Periodontol 2007;78:2289–2302. [PubMed: 18052701]
7. Silva N, Abusleme L, Bravo D, Dutzan N, Garcia-Sesnich J, Vernal R, et al. Host response mechanisms in periodontal diseases. J Appl Oral Sci 2015;23:329–355. [PubMed: 26221929]

8. How KY, Song KP, Chan KG. Porphyromonas gingivalis: An Overview of Periodontopathic Pathogen below the Gum Line. Frontiers in Microbiology 2016;7. [PubMed: 26858696]
9. Amar S, Engelke M. Periodontal innate immune mechanisms relevant to atherosclerosis. Molecular Oral Microbiology 2015;30:171–185. [PubMed: 25388989]
10. Shenker BJ, Ojcius DM, Walker LP, Zekavat A, Scuron MD, Boesze-Battaglia K. Aggregatibacter actinomycetemcomitans cytolethal distending toxin activates the NLRP3 inflammasome in human macrophages, leading to the release of proinflammatory cytokines. Infect Immun 2015;83:1487–1496. [PubMed: 25644004]
11. Konig MF, Abusleme L, Reinholdt J, Palmer RJ, Teles RP, Sampson K, et al. Aggregatibacter actinomycetemcomitans-induced hypercitrullination links periodontal infection to autoimmunity in rheumatoid arthritis. Sci Transl Med 2016;8:369ra176.
12. Konig MF, Andrade F. A Critical Reappraisal of Neutrophil Extracellular Traps and NETosis Mimics Based on Differential Requirements for Protein Citrullination. Frontiers in Immunology 2016;7. [PubMed: 26834748]
13. Haraszthy VI, Zambon JJ, Trevisan M, Zeid M, Genco RJ. Identification of periodontal pathogens in atheromatous plaques. J Periodontol 2000;71:1554–1560. [PubMed: 11063387]
14. Dietmann A, Millonig A, Combes V, Couraud PO, Kachlany SC, Grau GE. Effects of Aggregatibacter actinomycetemcomitans leukotoxin on endothelial cells. Microb Pathog 2013;61-62:43–50. [PubMed: 23665198]
15. Zhang T, Kurita-Ochiai T, Hashizume T, Du Y, Oguchi S, Yamamoto M. Aggregatibacter actinomycetemcomitans accelerates atherosclerosis with an increase in atherogenic factors in spontaneously hyperlipidemic mice. FEMS Immunol Med Microbiol 2010;59:143–151. [PubMed: 20482627]
16. Lockhart PB, Bolger AF, Papapanou PN, Osinbowale O, Trevisan M, Levison ME, et al. Periodontal disease and atherosclerotic vascular disease: does the evidence support an independent association?: a scientific statement from the American Heart Association. Circulation 2012;125:2520–2544. [PubMed: 22514251]
17. Giles JT, Szklo M, Post W, Petri M, Blumenthal RS, Lam G, et al. Coronary arterial calcification in rheumatoid arthritis: comparison to the multi-ethnic study of atherosclerosis. Arthritis research & therapy 2009;11:R36. [PubMed: 19284547]
18. Kobayashi H, Giles JT, Polak JF, Blumenthal RS, Leffell MS, Szklo M, et al. Increased prevalence of carotid artery atherosclerosis in rheumatoid arthritis is artery-specific. The Journal of rheumatology 2010;37:730–739. [PubMed: 20110515]
19. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American-Rheumatism-Association 1987 Revised Criteria for the Classification of Rheumatoid-Arthritis. Arthritis and Rheumatism 1988;31:315–324. [PubMed: 3358796]
20. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M, Detrano R. Quantification of Coronary-Artery Calcium Using Ultrafast Computed-Tomography. Journal of the American College of Cardiology 1990;15:827–832. [PubMed: 2407762]
21. Allison MA, Criqui MH, McClelland RL, Scott JM, McDermott MM, Liu K, et al. The effect of novel cardiovascular risk factors on the ethnic-specific odds for peripheral arterial disease in the Multi-Ethnic Study of Atherosclerosis (MESA). J Am Coll Cardiol 2006;48:1190–1197. [PubMed: 16979004]
22. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. Diabetes care 2004;27:1487–1495. [PubMed: 15161807]
23. Prevoo ML, van ‘t Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis and Rheumatism 1995;38:44–48. [PubMed: 7818570]
24. Wolfe F, Kleinheksel SM, Cathey MA, Hawley DJ, Spitz PW, Fries JF. The clinical value of the Stanford Health Assessment Questionnaire Functional Disability Index in patients with rheumatoid arthritis. The Journal of rheumatology 1988;15:1480–1488. [PubMed: 3204597]
25. van der Heijde D How to read radiographs according to the Sharp/van der Heijde method. The Journal of rheumatology 2000;27:261–263. [PubMed: 10648051]

26. Nettleton JA, Steffen LM, Mayer-Davis EJ, Jenny NS, Jiang R, Herrington DM, et al. Dietary patterns are associated with biochemical markers of inflammation and endothelial activation in the Multi-Ethnic Study of Atherosclerosis (MESA). The American Journal of Clinical Nutrition 2006;83:1369–1379. [PubMed: 16762949]
27. Mikuls TR, Payne JB, Yu F, Thiele GM, Reynolds RJ, Cannon GW, et al. Periodontitis and Porphyromonas gingivalis in patients with rheumatoid arthritis. Arthritis Rheumatol 2014;66:1090–1100. [PubMed: 24782175]
28. Volkov M, Dekkers J, Loos BG, Bizzarro S, Huizinga TWJ, Praetorius HA, et al. Comment on "Aggregatibacter actinomycetemcomitans-induced hypercitrullination links periodontal infection to autoimmunity in rheumatoid arthritis". Sci Transl Med 2018;10.
29. Mikuls TR, Thiele GM, Deane KD, Payne JB, O'Dell JR, Yu F, et al. Porphyromonas gingivalis and disease-related autoantibodies in individuals at increased risk of rheumatoid arthritis. Arthritis Rheum 2012;64:3522–3530. [PubMed: 22736291]
30. Mankia K, Cheng Z, Do T, Hunt L, Meade J, Kang J, et al. Prevalence of Periodontal Disease and Periodontopathic Bacteria in Anti-Cyclic Citrullinated Protein Antibody-Positive At-Risk Adults Without Arthritis. JAMA Netw Open 2019;2:e195394. [PubMed: 31173126]
31. Fine DH, Markowitz K, Furgang D, Fairlie K, Ferrandiz J, Nasri C, et al. Aggregatibacter actinomycetemcomitans and Its Relationship to Initiation of Localized Aggressive Periodontitis: Longitudinal Cohort Study of Initially Healthy Adolescents. Journal of Clinical Microbiology 2007;45:3859–3869. [PubMed: 17942658]
32. Fedele S, Sabbah W, Donos N, Porter S, D'Aiuto F. Common oral mucosal diseases, systemic inflammation, and cardiovascular diseases in a large cross-sectional US survey. Am Heart J 2011;161:344–350. [PubMed: 21315218]
33. Beck JD, Elter JR, Heiss G, Couper D, Mauriello SM, Offenbacher S. Relationship of periodontal disease to carotid artery intima-media wall thickness: the atherosclerosis risk in communities (ARIC) study. Arterioscler Thromb Vasc Biol 2001;21:1816–1822. [PubMed: 11701471]
34. Ott SJ, El Mokhtari NE, Musfeldt M, Hellmig S, Freitag S, Rehman A, et al. Detection of diverse bacterial signatures in atherosclerotic lesions of patients with coronary heart disease. Circulation 2006;113:929–937. [PubMed: 16490835]
35. Kuramitsu HK, Qi M, Kang IC, Chen W. Role for periodontal bacteria in cardiovascular diseases. Ann Periodontol 2001;6:41–47. [PubMed: 11887470]
36. Colhoun HM, Slaney JM, Rubens MB, Fuller JH, Sheiham A, Curtis MA. Antibodies to periodontal pathogens and coronary artery calcification in type 1 diabetic and nondiabetic subjects. J Periodontal Res 2008;43:103–110. [PubMed: 18230111]
37. Konig MF, Giles JT, Teles RP, Moutsopoulos NM, Andrade F. Response to comment on "Aggregatibacter actinomycetemcomitans-induced hypercitrullination links periodontal infection to autoimmunity in rheumatoid arthritis". Sci Transl Med 2018;10.

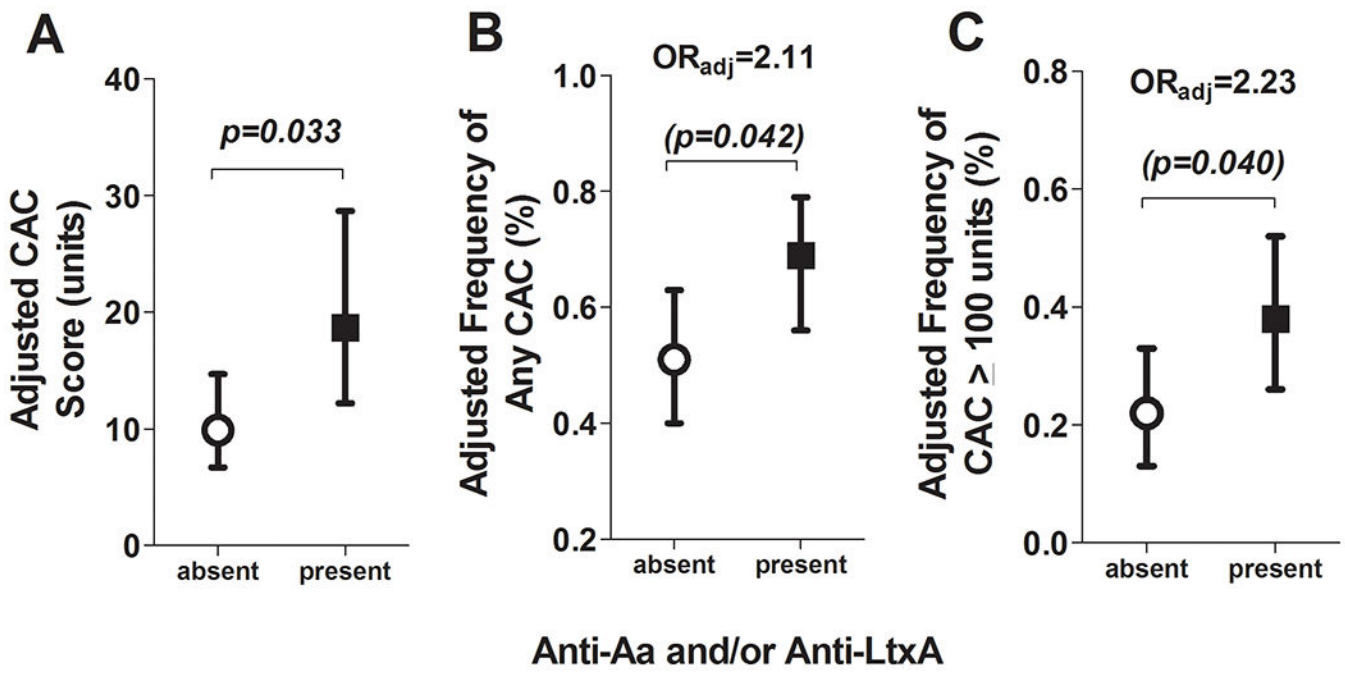

Figure 1. Adjusted Associations of Anti-Aa and/or Anti-LtxA with Measures of Coronary Atherosclerosis.

Means and 95% Confidence Intervals are depicted. Adjusted for age, gender, smoking history, body mass index, triglycerides, statin use, RA duration, and swollen joint count. *Aa= Aggregatibacter actinomycetemcomitans*; LtxA=leukotoxin A; OR_{adj}=adjusted odds ratio

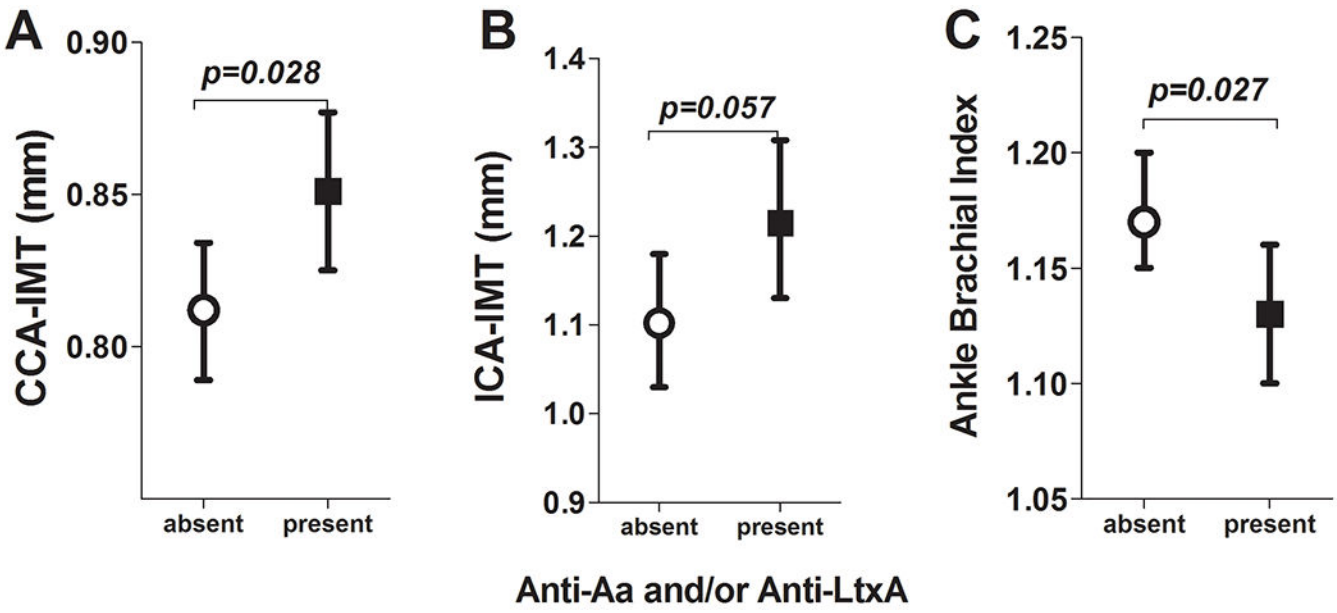

Figure 2. Adjusted Associations of Anti-Aa and/or Anti-LtxA with Measures of Carotid and Peripheral Arterial Atherosclerosis.

Means and 95% Confidence Intervals are depicted. Adjusted for age, gender, smoking history, body mass index, triglycerides, statin use, RA duration, and swollen joint count. *Aa= Aggregatibacter actinomycetemcomitans*; LtxA=leukotoxin A; OR_{adj}=adjusted odds ratio; CCA=common carotid artery; IMT=intima-media thickness; ICA=internal carotid artery

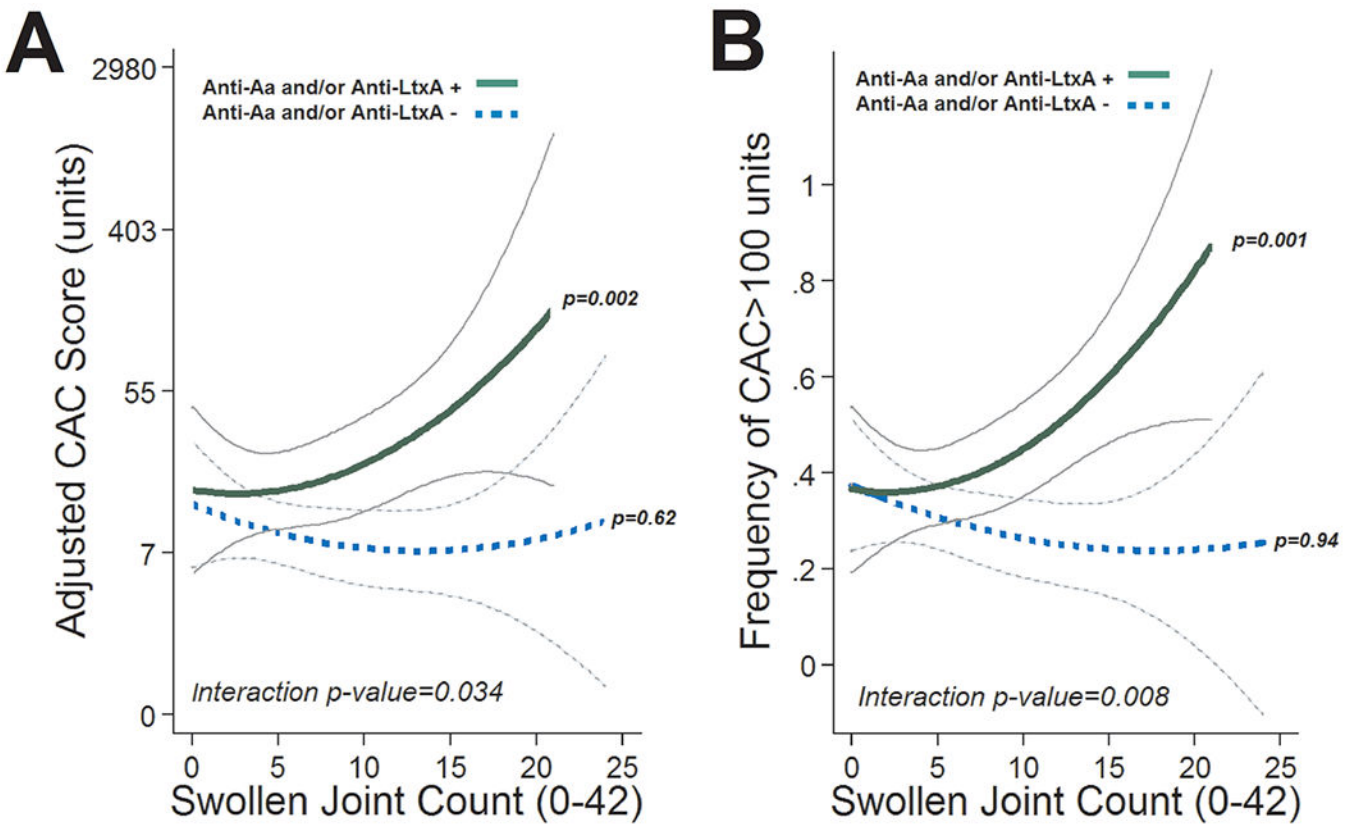

Figure 3. Differential Association of Swollen Joint Count with Measures of Coronary Atherosclerosis Conditioned on Anti-Aa and/or anti-LtxA Status

Quadratic fit line and 95% confidence intervals depicted. Adjusted for age, gender, smoking history, body mass index, triglycerides, statin use, and RA duration. CAC=coronary artery calcium; *Aa= Aggregatibacter actinomycetemcomitans*; LtxA=leukotoxin

Table 1.

Patient Characteristics According to the Presence of anti-*Aa* and/or anti-LtxA

	Total (n=197)	**No anti-*Aa* or anti-LtxA (n=105)**	**Anti-*Aa* and/or anti-LtxA (n=92)**	**p-value**
Age, years	59 ± 9	58 ± 8	61 ± 9	0.059
Male, n (%)	79 (42)	40 (38)	39 (42)	0.54
White, n (%)	168 (85)	96 (91)	72 (78)	0.009
Any College, n (%)	148 (76)	78 (74)	70 (77)	0.67
BMI, kg/m^2	28.5 ± 5.3	28.6 ± 5.0	28.3 ± 5.7	0.78
Ever smoking, n (%)	116 (59)	64 (61)	52 (57)	0.59
Current smoking, n (%)	23 (12)	9 (9)	14 (15)	0.14
Diabetes, n (%)	12 (6)	6 (6)	6 (6)	0.81
Hypertension, n (%)	105 (54)	55 (53)	50 (54)	0.84
Total cholesterol, mg/dL	194 ± 37	196 ± 34	192 ± 40	0.44
LDL-C, mg/dL	116 ± 30	115 ± 29	116 ± 32	0.79
HDL-C, mg/dL	54 ± 18	56 ± 20	52 ± 17	0.11
Triglycerides, mg/dL	104 (68-149)	98 (74-151)	109 (66-144)	0.98
Lipid lowering meds, n (%)	35 (15)	21 (20)	14 (15)	0.38
HOMA-IR	0.8 (0.5-1.4)	0.70 (0.50-1.35)	0.95 (0.55-1.40)	0.18
GFR	88 ± 22	89 ± 22	87 ± 23	0.61
Homocysteine	9.0 (7.5-10.6)	8.9 (7.5-10.4)	9.2 (7.5-11.1)	0.41
RA duration, years	9 (5-17)	10 (4-17)	8 (5-18)	0.73
RF>40 units, n (%)	130 (66)	60 (57)	70 (76)	0.005
anti-CCP>60 units, n (%)	140 (71)	72 (69)	68 (74)	0.41
Any shared epitope alleles, n (%)	136 (70)	74 (70)	62 (69)	0.81
DAS28-CRP	3.6 (2.9-4.3)	3.6 (2.9-4.4)	3.7 (2.9-4.3)	0.98
Swollen joints, (0-42)	7 (3-10)	7 (3-11)	6 (3-10)	0.59
Tender joints, (0-44)	6 (2-13)	6 (2-13)	6 (2-12)	0.88
CRP, mg/dL	2.6 (1.1-7.6)	2.8 (1.1-7.0)	2.4 (1.2-7.9)	0.68
IL-6, pg/mL	3.9 (1.8-8.2)	3.7 (1.6-8.1)	4.3 (1.8-8.4)	0.33
HAQ, units	0.62 (0.12-1.25)	0.62 (0.12-1.25)	0.75 (0.12-1.50)	0.54
Total mSvdH Score	8 (0-42)	7 (0-36)	11 (1-52)	0.28
Current prednisone, n (%)	76 (39)	44 (42)	32 (35)	0.31
Cumulative prednisone, grams	3.1 (0-9.5)	3.2 (0-10.1)	2.9 (0-8.7)	0.52
Non-biologic DMARDs, n (%)	165 (84)	90 (86)	75 (82)	0.53
Methotrexate, n (%)	125 (63)	64 (61)	61 (66)	0.44
Hydroxychloroquine, n (%)	47 (24)	29 (28)	18 (20)	0.19
Biologics, n (%)	90 (46)	54 (51)	36 (40)	0.096
TNF-inhibitors, n (%)	86 (44)	52 (50)	34 (37)	0.087
Anti-Pg positive, n (%)	72 (37)	31 (30)	41 (45)	0.029
Reported Tooth Loss[*]				0.042
None	34 (18)	24 (24)	10 (12)	

	Total (n=197)	No anti-*Aa* or anti-LtxA (n=105)	Anti-*Aa* and/or anti-LtxA (n=92)	p-value
1-9	119 (65)	62 (61)	57 (70)	
10-31	17 (9)	6 (6)	11 (13)	
32	14 (8)	9 (9)	4 (5)	

* available in n=184

Aa= Aggregatibacter actinomycetemcomitans; LtxA=leukotoxin A; BMI=body mass index; LDL=loe density lipoprotein; HDL=high density lipoprotein; HOMA-IR=Homeostatic Model Assessment Insulin Resistance Index; GFR=glomerular filtration rate, s-ICAM=soluble intercellular adhesion molecule; RA=rheumatoid arthritis; RF=rheumatoid factor; CCP=cyclic citrullinated peptide; DAS=disease activity score; IL=interleukin; HAQ=Health Assessment Questionnaire; mSvdH=modified Sharp-van der Heijde; TNF=tumor necrosis factor; *Pg= Porphyromonas gingivalis*

Table 2.

Crude Associations of Antibodies Against Periodontal Pathogens with Measures of Coronary, Carotid, and Peripheral Atherosclerosis

	No anti-*Aa* or anti-LtxA (n=105)	**Anti-*Aa* and/or anti-LtxA (n=92)**	***p-value***	**Anti-*Pg* Negative (n=125)**	**Anti-*Pg* Positive (n=71)**	***p-value***
Coronary Artery Calcium						
CAC Score, units	0 (0-134)	30 (0-215)	0.046	3 (0-161)	5 (0-202)	0.71
CAC>0	50 (48)	56 (62)	0.041	66 (53)	40 (57)	0.56
CAC≥100 units	31 (30)	38 (42)	0.064	43 (34)	26 (37)	0.70
Carotid Ultrasound						
CCA-IMT, mm	0.80 (0.74-0.88)	0.85 (0.76-0.94)	0.020	0.81 (0.73-0.90)	0.85 (0.76-0.94)	0.11
ICA-IMT, mm	1.02 (0.81-1.41)	1.22 (0.86-1.61)	0.042	1.13 (0.84-1.51)	1.07 (0.84-1.65)	0.74
Plaque, n (%)	19 (18)	23 (26)	0.21	26 (21)	16 (23)	0.74
Ankle:Brachial Index	1.18 (1.10-1.26)	1.13 (1.05-1.21)	0.035	114 (1.07-1.24)	1.16 (1.06-1.22)	0.93

Aa= Aggregatibacter actinomycetemcomitans; LtxA=leukotoxin A; CAC=coronary artery calcium; CCA=common carotid artery; IMT=intima-media thickness; ICA=internal carotid artery; *Pg= Porphyromonas gingivalis*

Table 3.

Multivariable Indicators of log (Coronary Artery Calcium Score + 1)

	Model 1		Model 2		Model 3		Model 4	
	β	**p-value**	**β**	**p-value**	**β**	**p-value**	**β**	**p-value**
anti-Aa and/or anti-LtxA positive	0.76	0.049	0.68	0.036	0.64	0.033	0.72	0.023
Age, per year			0.12	<0.001	0.12	<0.001	0.11	<0.001
Male gender			1.76	<0.001	1.86	<0.001	1.85	<0.001
White			0.17	0.72				
Ever smoking			0.68	0.044	0.68	0.035	0.83	0.019
Current smoking			−0.33	0.52				
BMI, per kg/m^2			0.058	0.11	0.049	0.092	0.038	0.23
Diabetes			0.57	0.44				
SBP, per mm/Hg			−0.0075	0.44				
Anti-hypertensive use			0.49	0.16				
HDL-C, per mg/dL			−0.0020	0.87				
log Triglycerides			0.62	0.057	0.53	0.056	0.69	0.020
Statin use			1.11	0.011	1.14	0.005	1.21	0.004
GFR			0.0062	0.56				
log Homocysteine			0.46	0.50				
RA duration, per year			0.042	0.009	0.037	0.011	0.041	0.010
RF>40 units			0.19	0.61				
Swollen joint count, per joint			0.052	0.11	0.068	0.024	0.074	0.021
log CRP			0.039	0.76				
Hydroxychloroquine use			−0.16	0.68				
Biologic use			0.20	0.53				
Number of missing teeth								
0							referent	---
1-9							0.12	0.78
10-31							0.085	0.90
32							−0.13	0.85

Aa= Aggregatibacter actinomycetemcomitans; LtxA=leukotoxin A; BMI=body mass index; SBP=systolic blood pressure; HDL=high density lipoprotein; GFR=glomerular filtration rate, RA=rheumatoid arthritis; RF=rheumatoid factor; CRP=C-reactive protein