

Alveolar Bone Loss Is Associated With Circulating Anti-Citrullinated Protein Antibody (ACPA) in Patients With Rheumatoid Arthritis

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Background: This study examines: 1) alveolar bone loss (ABL), a hallmark of periodontitis, in anti-citrullinated protein antibody (ACPA)-positive rheumatoid arthritis (RA) patients versus control patients with osteoarthritis (OA); and 2) the association of ABL with RA disease activity and ACPA concentrations, including multiple antigen-specific ACPA.

Methods: This multicenter case-control study includes 617 patients diagnosed with RA (n = 287) or OA (n = 330). Panoramic radiographs were taken; patients were categorized into low, moderate, or high tertiles based on mean percentage ABL. Serum ACPA was measured using second-generation anti-cyclic citrullinated peptide enzyme-linked immunosorbent assay and a multiplex platform to assess distinct antigen-specific ACPA. A generalized linear mixed model for binary data was used to compare stratified ABL in RA versus OA patients. Associations of moderate and high ABL (versus low) with RA disease activity and severity measures were examined using multivariate regression. Antigen-specific ACPA responses were compared among ABL tertiles using significance analysis of microarrays.

Results: ACPA-positive patients with RA had a significantly higher mean percentage of sites with ABL >20% compared with patients with OA ($P = 0.03$). After multivariate adjustment, greater ABL was significantly associated with higher serum ACPA concentration ($P = 0.004$), 28-joint Disease Activity Score ($P = 0.023$), health assessment questionnaire disability ($P = 0.05$), tender joint count ($P = 0.02$) and joint space narrowing scores ($P = 0.05$) among patients with RA. ACPAs targeting citrullinated vimentin and histone were significantly higher in moderate and high ABL groups versus low, regardless of smoking status ($q < 0.1\%$).

Conclusions: Greater ABL was associated with higher ACPA, consistent with findings at articular sites. ACPA targeting could provide novel insight into important linkages between RA and periodontitis. *J Periodontol* 2015;86:222-231.

KEY WORDS

Alveolar bone loss; arthritis, rheumatoid; histones; peptides, cyclic; periodontitis; vimentin.

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Periodontitis is an inflammatory disease involving the supporting tissues surrounding teeth and is accompanied by attachment loss (AL), alveolar (periodontal) bone loss (ABL), and ultimately, tooth loss in many patients. Rheumatoid arthritis (RA) is a systemic inflammatory disease that shares a similar pathogenesis with periodontitis^{1,2} and results in breakdown of joint cartilage and bone.³ Recent evidence suggests that RA may be a risk factor in the progression and severity of periodontal bone loss.^{4,5} Likewise, periodontitis has been implicated in RA pathogenesis through the formation of anti-citrullinated protein antibody (ACPA).⁶ With the oral cavity, and the periodontium in particular, acting as a potential reservoir of citrullinated protein, possibly via *Porphyromonas gingivalis*-derived peptidyl-arginine deiminase activity,⁷ it has been suggested that periodontitis may represent an extra-articular disease manifestation in ACPA-positive patients with RA.

Radiographic outcomes in RA joints are predicted by the presence of ACPA.⁸⁻¹⁰ Recent evidence suggests that ACPA specifically targeting citrullinated vimentin can directly mediate bone loss by enhancing the differentiation of osteoclast precursors into mature bone-resorbing cells.¹¹ Thus, the current authors hypothesized that ABL would be more severe in ACPA-positive participants with RA compared to control patients with osteoarthritis (OA) and that ABL would be associated with serum ACPA concentrations and antigen-specific ACPA. Therefore, the aims of this large case-control study are to compare ABL between ACPA-positive patients with RA and control patients with OA and to examine the associations of ABL with RA-related measures of disease activity and severity, including the expression of multiple antigen-specific ACPA. Previous studies have been much smaller,^{4,5} have not looked specifically at the relationship between ACPA and ABL, and have not examined antibody responses to specific citrullinated auto-antigens and their association with ABL.

MATERIALS AND METHODS

Study Participants

This study is a multicenter investigation from four U.S. Veterans Affairs Medical Centers (Omaha, Nebraska; Dallas, Texas; Salt Lake City, Utah; and Washington, DC) and a single academic coordinating center (University of Nebraska Medical Center, Omaha).¹² A total of 617 participants (380 males and 237 females; mean age: 59 years), 287 cases with RA and 330 controls with OA, were enrolled from rheumatology, orthopedic, and primary care clinics from these centers. Cases satisfied the 1987 American College of Rheumatology classification criteria for RA (age of disease onset >18 years).¹³ Patients with OA were enrolled as non-healthy controls based on

the expectation that they would have sociodemographic characteristics similar to patients with RA. Individuals were deemed to have OA based on appropriate medical documentation from a corresponding provider or previous imaging results consistent with degenerative arthritis. As previously described,¹² all patients underwent a standardized and calibrated periodontal evaluation with periodontitis defined based on criteria of Machtei et al.¹⁴

Inclusion criteria for the study are: 1) diagnosis of RA or OA based on the criteria above; 2) ≥ 19 years of age; 3) nine or more posterior teeth (excluding third molars), representing the presence of more than 50% of posterior teeth, an inclusion criterion in other studies examining the relationship between oral and systemic diseases;^{15,16} and 4) being willing and able to provide informed consent. Exclusion criteria were: 1) tetracycline or related antibiotic use in the previous 6 months; 2) antibiotic premedication required before dental probing (e.g., total joint replacement); 3) being pregnant or breastfeeding; and 4) prior use of cyclosporine or phenytoin or a history of concomitant systemic inflammatory disease (e.g., systemic lupus erythematosus, ankylosing spondylitis, polymyalgia rheumatica, inflammatory bowel disease, acute gout). The study was approved by the institutional review board at each center. All participants provided written informed consent before study enrollment.

Sociodemographic and Comorbidity Assessments

As previously described,¹² factors ascertained at enrollment included age, sex, body mass index (calculated using weight and height measured during the study visit), marital status, race/ethnicity (self-identified), highest level of education achieved, and smoking status (current, former, or never). Select comorbid conditions and/or symptoms were obtained from patient self-report and included diabetes mellitus, hypertension, cardiovascular disease, osteoporosis, and symptoms of dry mouth or dry eyes. For patients with RA, additional measures included date of diagnosis, disease duration, prednisone use, and the use of biologic and non-biologic disease-modifying antirheumatic drugs.

ABL Determination

The dental radiographic examination consisted of a digital panoramic radiograph. The radiographs were imported into radiographic viewing software.^{§§} Two trained examiners (PJ and MS), masked to patient disease status, measured ≤ 24 sites per individual. Mesial sites on second molars, first molars, and second premolars and distal sites on first molars, second premolars, and first premolars were evaluated.

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At each site, two measurements were made: from the cemento-enamel junction (CEJ) to the alveolar crest and from the CEJ to the root apex. The two examiners were calibrated by an oral and maxillofacial radiologist (SG), who also was masked to the diagnosis of RA versus OA. Interrater agreement was evaluated by having both examiners reread a subset of radiographs ($n = 69$) and was strong (0.85, 95% CI: 0.77 to 0.91). Mean percentage ABL was determined with a CEJ-to-alveolar-crest distance >2 mm representing ABL.¹⁷ The percentage of sites demonstrating ABL $>20\%$ was also evaluated; this threshold was determined a priori. Only sites in which the fixed reference point (CEJ) was visible were included in the data analyses. ABL data were unavailable for 12 participants with OA and 11 with RA (no panoramic radiograph was taken for two patients with RA, and the remaining patients with RA and OA were missing radiographic data due to restorations obscuring CEJs at all measurement sites or overlap of teeth, thereby also preventing CEJ detection). ACPA data were unavailable for two patients with RA; thus, these data were not included in the analysis.

RA Disease Activity and Severity Measures

ACPA was measured in patients with RA using a second-generation anti-cyclic citrullinated peptide (anti-CCP) enzyme-linked immunosorbent assay (ELISA) (positive test ≥ 5 U/mL).^{|||} Both rheumatoid factor (RF; positive ≥ 15 IU/mL) and high sensitivity C-reactive protein (hs-CRP; mg/L) were determined by nephelometry.^{¶¶} Serum samples were also evaluated in anti-CCP antibody-positive patients with RA for 19 specific ACPAs using a bead-based multiplex antigen array on a multiplex platform,^{##18} an array measuring disease-specific auto-antibody reactivity to a panel of putative citrullinated auto-antigens. Given the low anticipated frequency of reactivity, ACPAs were not measured, and therefore not available, for all controls.

Treatment data were collected for participants with RA. Disease measures collected included the following: 1) tender and swollen joint counts (0 to 28 joints); 2) health assessment questionnaire disability (0-to-3 range); 3) pain (0 to 10); and 4) a composite measure of RA disease activity, the 28-joint Disease Activity Score (DAS-28-CRP),¹⁹ which includes swollen joint count, tender joint count, patient global well-being score, and CRP as a measure of systemic inflammation.

Bacterial Serologies

Serum concentrations of immunoglobulin G antibody to outer membrane antigen of *P. gingivalis*, *Fusobacterium nucleatum*, and *Prevotella intermedia* were analyzed by ELISA, as previously described.²⁰ Serum concentrations of antibody responses to

P. gingivalis-specific lipopolysaccharide (LPS) were also measured.²¹

HLA-DRB1 Shared Epitope Status Determination

Single nucleotide polymorphism-based imputation of four-digit HLA-DRB1 alleles was used, as previously described.^{12,22,23} HLA-DRB1 shared epitope (SE) is a known genetic risk factor for RA and a possible genetic risk factor for aggressive periodontitis.²⁴ The single nucleotide-based imputation agreed well with direct HLA-DRB1 sequencing as part of a separate study of 116 patients with RA.¹² The two methods were concordant in defining SE status (positive versus negative) in 108 of 116 patients (93%; $\kappa = 0.79$). Levels of agreement were similar among white (96% concordance; $\kappa = 0.86$) and African American (89% concordance; $\kappa = 0.72$) patients with RA. Comparisons of SE status and multivariable models including HLA-DRB1 SE were, thus, limited to whites and African Americans.¹²

Hand and Wrist Radiographs in Patients With RA

Digitized posterior-anterior hand and wrist radiographs were obtained in patients with RA. A single examiner (AE), masked to ABL and periodontitis status, scored these radiographs using the modified Sharp scoring method.²⁵ Radiographic disease severity was examined as a total Sharp score in addition to erosion and joint space narrowing component scores.

Statistical Analyses

Sample size was determined using standard power calculations for the primary study¹² based on the power to detect differences in periodontitis prevalence between patients with RA and patients with OA. Descriptive statistics including relative frequency for categorical variables and mean and SD for continuous variables were calculated. χ^2 test and non-parametric Wilcoxon rank sum test were used separately to compare the categorical or continuous demographics and comorbidities between patients with RA and those with OA. The mean percentage of sites with ABL was calculated for each patient and compared among ACPA-positive RA or ACPA-negative patients with RA versus patients with OA using the Kruskal-Wallis test. In addition, the generalized linear mixed model for binary data was used to compare the average number of sites with $>20\%$ ABL in ACPA-positive or ACPA-negative patients with RA versus control patients with OA after adjusting for the correlation within the same individual.¹⁵ Both univariate and multivariate analyses were conducted to study the associations of ABL (independent variable) with RA-related measures

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(dependent variables) in participants with RA, which were categorized into low ($\leq 3.86\%$), moderate ($> 3.86\%$ and $\leq 8.80\%$), and high ($> 8.80\%$) ABL groups based on the tertiles of mean ABL values. Log transformation was applied when necessary on the continuous RA-related measures to render the data normally distributed. Specifically, in univariate analyses, the χ^2 test was used for categorical RA-related measures, and analysis of variance or Kruskal Wallis test, when appropriate, was used for continuous RA-related measures.

In multivariate analysis, the joint counts and hand/wrist radiographic scores were categorized into three levels based on their tertiles and associated with ABL and other covariates using ordinal logistic regression. Linear regression was used to examine the association of ABL and other covariates with continuous RA-related measures. Factors considered as potential covariates included: 1) periodontitis; 2) log-transformed serum anti-*P. gingivalis* outer membrane antigen and LPS; 3) log-transformed serum anti-*F. nucleatum*; 4) log-transformed serum anti-*P. intermedia*; 5) use of biologics; 6) use of methotrexate or prednisone; 7) smoking status (ever versus never); 8) age; 9) sex; 10) race (white versus non-white); 11) education (high school or less versus more than high school); 12) marital status (married versus other); 13) body mass index (< 25 , 25 to 29.9 , and ≥ 30 kg/m²); 14) RA duration; 15) dry

mouth; 16) dry eyes; 17) diabetes mellitus; 18) hypertension; 19) cardiovascular disease; and 20) osteoporosis. Two steps were used to identify the important confounding factors included in the multivariate models. First, the ABL-adjusted effects of different covariates on RA-related measures were calculated, and those variables with a *P* value ≤ 0.2 were then entered in the multivariate regression model with a step-wise variable selection procedure (*P* value for entry and removal < 0.05).

The significance analysis of microarrays was used to analyze the ACPA microarray data to identify different ACPA profiles associated with ABL among anti-CCP antibody-positive patients with RA. Microarray analyses were done separately among ever- and never-smokers given robust associations of smoking with both periodontitis^{16,26} and RA risk.²⁷⁻²⁹ Antigen reactivity with a *q* value of $< 0.1\%$ was considered to be statistically significant.

RESULTS

Participants

Patients were recruited from October 20, 2010, to July 30, 2012. A total of 2,339 patients were screened, 1,266 individuals in the OA group and 1,073 individuals in the RA group. A total of 330 patients with OA enrolled in the study, whereas 936 patients with OA did not enroll because they did not meet

Table 1.

Demographics and Comorbidities Among Patients With RA and OA

Characteristics	Total (N = 617)	RA Cases (n = 287)	OA Controls (n = 330)	<i>P</i>
Sociodemographics				
Age (years); mean (SD)	59 (11)	59 (12)	59 (11)	0.867
Male sex; number (%)	380 (62)	182 (63)	198 (60)	0.384
Race*; number (%)				0.174
White	462 (75)	223 (78)	239 (72)	
African American	123 (20)	48 (17)	75 (23)	
Other	32 (5)	16 (6)	16 (5)	
Comorbidities				
Body mass index, kg/m ² ; mean (SD)	31 (7)	30 (7)	32 (7)	0.001
Smoking status†; number (%)				<0.001
Never	285 (46)	108 (38)	177 (54)	
Former	241 (39)	124 (43)	117 (35)	
Current	91 (15)	55 (19)	36 (11)	
Diabetes mellitus; number (%)	133 (22)	52 (18)	81 (25)	0.053
Hypertension; number (%)	318 (52)	130 (45)	188 (57)	0.004
Cardiovascular disease; number (%)	70 (11)	37 (13)	33 (10)	0.259
Osteoporosis; number (%)	81 (13)	31 (11)	50 (15)	0.111

* *P* value for race represents comparison among white, African American, and other.

† *P* value for smoking status represents comparison among never, former, and current smokers; the *P* value is the same for the comparison of ever-smokers (former plus current) versus never-smokers.

the eligibility criteria ($n = 830$), were potentially eligible and were not interested ($n = 100$), or did not show up for the baseline visit ($n = 6$). A total of 287 patients with RA enrolled in the study, whereas 786 patients with RA did not enroll because they did not meet the eligibility criteria ($n = 693$), were potentially eligible and were not interested ($n = 83$), or did not show up for the baseline visit ($n = 10$).

Table 2.**ABL in Patients With RA and OA**

Group	Mean % ABL (SD)	Mean Percentage of Sites With >20% ABL
ACPA ⁺ RA ($n = 231$)	8.7 (7.5)*	11.2 [†]
ACPA ⁻ RA ($n = 43$)	6.7 (5.9)	6.9
OA ($n = 318$)	7.6 (7.4)	8.8

* ACPA⁺ patients with RA versus patients with OA ($P = 0.092$).

[†] ACPA⁺ patients with RA versus patients with OA ($P = 0.03$).

Sociodemographic and Comorbidity Assessments

Patients with RA and those with OA did not differ based on age, sex, or race, nor did they differ with respect to having cardiovascular disease or osteoporosis (Table 1). However, patients with OA were more likely to have diabetes mellitus ($P = 0.053$) and hypertension ($P = 0.004$) than patients with RA and had a higher body mass index ($P = 0.001$). On the other hand, participants with RA were more likely to have been ever-smokers ($P < 0.001$) than patients with OA.

ABL in ACPA-Positive RA Versus OA

Mean percentage ABL did not differ between ACPA-positive patients with RA and patients with OA ($P = 0.092$) (Table 2). However, ACPA-positive patients with RA had a statistically significantly higher mean percentage of sites with ABL >20% than

Table 3.**Univariate Analyses of the Associations of ABL With RA-Related Measures of Disease Activity and Severity***

RA-Related Measure	Low ABL ($n = 88$)	Moderate ABL ($n = 85$)	High ABL ($n = 101$)	<i>P</i>
Tender joint count (0 to 28); mean (SD)	2.6 (4.8)	3.0 (4.2)	4.0 (4.9)	0.002
Swollen joint count (0 to 28); mean (SD)	3.0 (3.5)	3.4 (4.7)	4.2 (4.3)	0.041
Total Sharp score; mean (SD)	13.2 (16.4)	18.1 (20.4)	25.8 (25.4)	<0.001
Joint space narrowing; mean (SD)	10.5 (13.3)	13.8 (14.5)	20.1 (19.3)	<0.001
Joint erosions; mean (SD)	2.7 (4.7)	4.4 (7.8)	5.7 (8.7)	0.041
DAS-28-CRP; mean (SD)	2.9 (1.4)	3.1 (1.3)	3.6 (1.2)	<0.001
HAQ disability (0 to 3) [†] ; mean (SD)	0.6 (0.7)	0.8 (0.7)	1.0 (0.7)	0.001
Pain (0 to 10); mean (SD)	2.7 (2.5)	3.0 (2.4)	3.9 (2.7)	0.007
Anti-CCP positivity (%)	80.7	84.7	87.1	0.474
Anti-CCP (U/mL) [‡] ; mean (SD)	144.9 (122.1)	162.3 (116.7)	203.3 (118.7)	0.007
hs-CRP (mg/L); mean (SD)	7.6 (12.0)	5.6 (8.5)	11.5 (32.6)	0.289
RF positivity (%)	65.9	77.7	85.2	0.008
RF (IU/mL); mean (SD)	200.3 (518.6)	273.4 (465.4)	280.7 (489.5)	0.002
Methotrexate (%)	65.9	60.0	57.4	0.480
Prednisone (%)	30.7	24.7	33.7	0.406
Biologic (%)	28.4	29.4	34.7	0.605
HLA-DRB1 SE (%)	75.0	73.8	80.4	0.540

* Mean (patient basis) percentage bone loss tertiles are as follows for low, moderate, and high ABL: $\leq 3.86\%$, $>3.86\%$ and $\leq 8.80\%$, and $>8.80\%$, respectively.

[†] Health assessment questionnaire.

[‡] Analysis was restricted to anti-CCP antibody-positive participants.

patients with OA ($P = 0.03$). There was no difference in ACPA-negative patients with RA versus patients with OA with respect to ABL ($P > 0.1$).

Univariate Associations Between ABL and Measures of Disease Activity in RA

Among individuals with RA, greater ABL was associated with higher tender and swollen joint counts ($P = 0.002$ and $P = 0.041$, respectively) (Table 3). Likewise, greater ABL was associated with greater radiographic disease progression based on hand and wrist radiographs with a higher total Sharp score ($P < 0.001$), joint space narrowing ($P < 0.001$), and joint erosions ($P = 0.041$). ABL also was associated with RA disease activity measures: DAS-28-CRP ($P < 0.001$), health assessment questionnaire disability ($P = 0.001$), and pain ($P = 0.007$).

Regarding RA serologies, greater ABL was associated with higher anti-CCP antibody ($P = 0.007$) and RF ($P = 0.002$) concentrations. Greater ABL also was associated with a higher likelihood of RF positivity ($P = 0.008$), but was not associated with anti-CCP antibody positivity ($P = 0.474$) or CRP concentration ($P = 0.289$). Use of methotrexate ($P = 0.480$), prednisone ($P = 0.406$), and biologics ($P = 0.605$) and HLA-DRB1 SE ($P = 0.540$) positivity did not differ among ABL tertiles.

Multivariate Associations Between ABL and Measures of Disease Activity in RA

For multivariate models, patients with RA with low ABL were considered as reference. After multivariate adjustment, high ABL remained significantly associated with higher values of the continuous variables anti-CCP antibody concentration ($P = 0.004$), DAS-28-CRP ($P = 0.023$), and health assessment questionnaire disability ($P = 0.05$) (Table 4), associations that were independent of periodontitis, bacterial serologies, RA treatments, smoking status, age, sex, race, education, marital status, body mass index, RA duration, dry mouth, dry eyes, diabetes mellitus, hypertension, cardiovascular disease, and osteoporosis.

After multivariate adjustment using ordinal logistic regression, higher levels of ABL in patients with RA remained significantly associated with higher tender joint counts ($P = 0.02$ for both moderate and high ABL versus low ABL) and joint space narrowing scores ($P = 0.05$ for high ABL versus low) (Fig. 1).

ACPA Microarray

Given ABL's independent association with anti-CCP antibody concentrations, the authors subsequently examined whether there were associations of ABL in patients with RA with antigen-specific ACPA. Microarray analyses, conducted in anti-CCP antibody-positive patients with RA only, showed that ACPAs targeting citrullinated vimentin and histone were significantly higher

Table 4.

Multivariate Analyses of the Associations of ABL (independent variable) With Continuous RA-Related Measures of Disease Activity and Severity (dependent variables) (β regression coefficient [P value])

Measure	ABL		
	Low	Moderate (P value)	High (P value)
Serologic			
ACPA (anti-CCP)*	Reference	0.12 (0.49)	0.52 (0.004)
CRP [†]	Reference	-0.30 (0.14)	-0.01 (0.96)
RF [‡]	Reference	0.44 (0.053)	0.34 (0.17)
Clinical disease activity			
DAS-28-CRP [§]	Reference	0.04 (0.49)	0.13 (0.023)
HAQ disability	Reference	0.07 (0.47)	0.21 (0.05)
Pain score [¶]	Reference	0.11 (0.76)	0.43 (0.24)

ACPA, CRP, RF, and DAS-28-CRP were log-transformed for analysis. Mean % bone loss tertiles (patient basis) are as follows: low = $\leq 3.86\%$; moderate = $> 3.86\%$ and $\leq 8.80\%$; and high = $> 8.80\%$ ABL. Covariates considered for the multivariate models included: periodontitis, log-transformed serum anti-*P. gingivalis* outer membrane antigen and LPS, log-transformed serum anti-*F. nucleatum*, log-transformed serum anti-*P. intermedia*, RA treatments, smoking status (ever versus never), age, sex, race (white versus non-white), education (high school or less versus more than high school), marital status (married versus other), body mass index (< 25 , 25 to 29.9 , and ≥ 30 kg/m²), RA duration, dry mouth, dry eyes, diabetes mellitus, hypertension, cardiovascular disease, and osteoporosis. Covariates in the final multivariate models included: * sex, education, dry mouth, cardiovascular disease; [†] biologic use, prednisone use, sex, marital status, body mass index; [‡] periodontitis, log-transformed serum anti-*P. gingivalis* outer membrane antigen, smoking status, race, body mass index; [§] prednisone use, smoking status, race, dry mouth; ^{||} health assessment questionnaire (HAQ) disability: log-transformed serum anti-*P. gingivalis* outer membrane antigen, prednisone use, smoking status, race, body mass index, dry mouth, osteoporosis; and [¶] log-transformed serum anti-*P. intermedia*, prednisone use, smoking status, race, dry mouth, diabetes mellitus.

in the moderate and high ABL groups versus the low ABL group, regardless of smoking status (Fig. 2).

DISCUSSION

This study shows for the first time that: 1) greater ABL among patients with RA was associated with higher serum ACPA concentrations; and 2) ACPAs targeting citrullinated vimentin and histone were significantly higher in the groups with higher ABL, regardless of smoking status. Furthermore, ACPA-positive participants with RA were more likely to have more severe ABL loss than patients with OA who were demographically similar to those with RA, but without inflammatory arthritis. Finally, greater ABL was associated with a number of RA disease activity measures, including DAS-28-CRP, health assessment questionnaire disability, tender joint counts, and joint space narrowing scores, after adjusting for multiple confounding factors including the presence of periodontitis. This is important, given the strong association of greater ABL with periodontitis observed in the study ($P < 0.001$; data not shown), suggesting

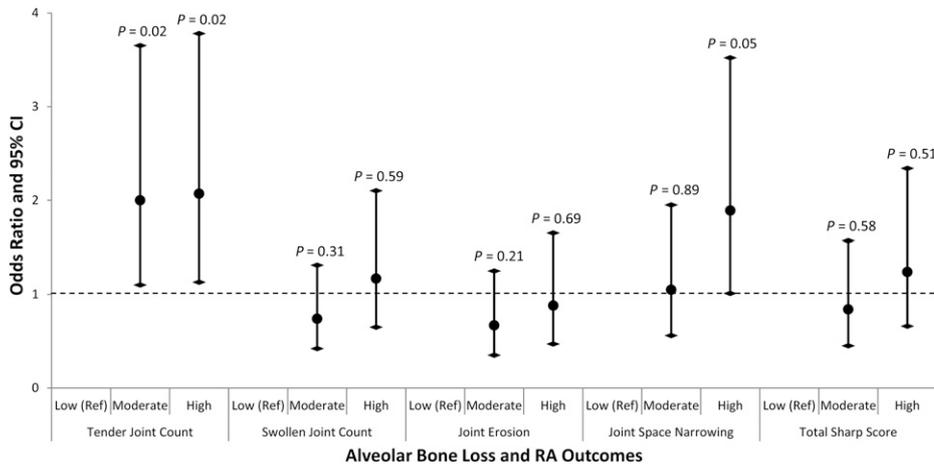


Figure 1.

Forest plot showing results of multivariate analyses of associations among ABL by tertile (low, moderate, and high; independent variable) and ordinal measures of RA disease activity and severity (dependent variables). Results are reported as odds ratio, 95% confidence interval (CI), and P value. Mean % bone loss tertiles (patient basis) are as follows: low = $\leq 3.86\%$; moderate = $> 3.86\%$ and $\leq 8.80\%$; and high = $> 8.80\%$ ABL. The following covariates were considered for the multivariate model: periodontitis, log-transformed serum anti-P. gingivalis outer membrane antigen and LPS, log-transformed serum anti-F. nucleatum, log-transformed serum anti-P. intermedia, RA treatments, smoking status (ever versus never), age, sex, race (white versus non-white), education (high school or less versus more than high school), marital status (married versus other), body mass index (< 25 , 25 to 29.9 , and ≥ 30 kg/m²), RA duration, dry mouth, dry eyes, diabetes mellitus, hypertension, cardiovascular disease, and osteoporosis. Covariates in the final multivariate models included: prednisone use, smoking status, race, and dry mouth for tender joint count; prednisone use and smoking status for swollen joint count; log-transformed serum anti-F. nucleatum, age, RA duration, and dry mouth for joint erosion; age and RA duration for joint space narrowing; and age, education, and RA duration for total Sharp score.

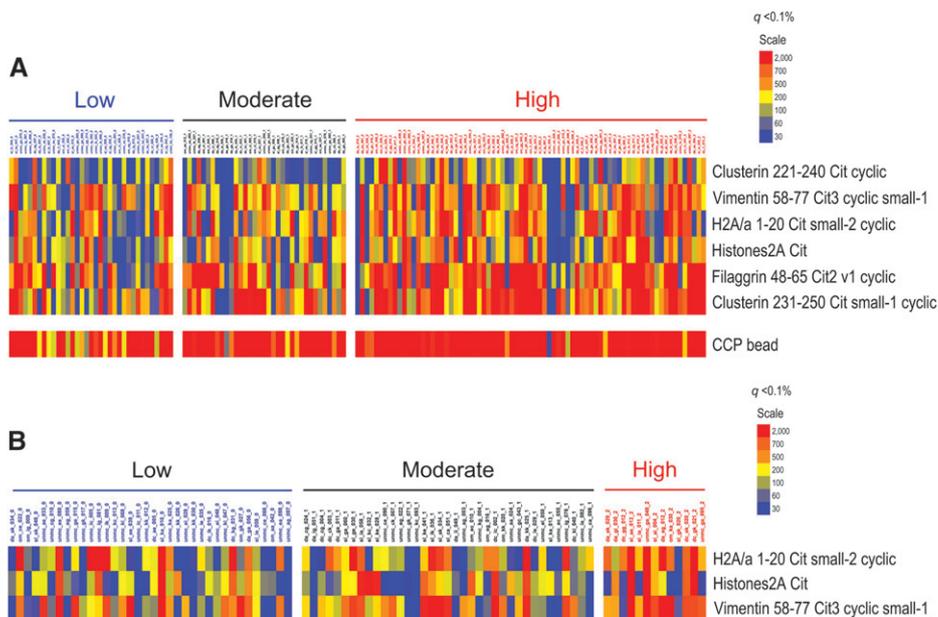


Figure 2.

Heat maps showing antigen-specific ACPAs based on stratified ABL in anti-CCP antibody-positive RA ever-smokers (A) and never-smokers (B). ACPAs targeting citrullinated (Cit) vimentin and histone were significantly higher in the moderate and high ABL groups ($q < 0.1\%$) in both ever- and never-smokers. Mean % bone loss tertiles (patient basis) are as follows: low = $\leq 3.86\%$; moderate = $> 3.86\%$ and $\leq 8.80\%$; and high = $> 8.80\%$ ABL. Cit2 and Cit3 refer to peptides citrullinated at 2 and 3 arginine sites, respectively.

that ABL, a hallmark and distinctive feature of periodontitis, is a reflection of a patient's periodontitis status. Indeed, the authors have previously shown that relative clinical attachment level (relative to the occlusal surface) and ABL were significantly and positively associated at baseline ($P < 0.0001$) in a study examining postmenopausal women with periodontitis and systemic osteopenia.³⁰ In the current study, for sites in patients with RA with high ABL ($> 8.80\%$), the average clinical AL (relative to the CEJ) was 3.75 mm.

Mercado et al.⁵ previously showed that ABL was associated with a number of indicators of RA disease activity, including swollen joints, health assessment questionnaire scores, CRP levels, and erythrocyte sedimentation scores. However, their paper did not report serum ACPA concentrations, as these measurements were not commonly made at the time of that study, nor did that prior effort appear to account for the many factors that might confound the relationship of ABL and RA disease activity. Dissick et al.⁴ reported that ACPA-positive patients with RA were more likely to have moderate-to-severe periodontitis than ACPA-negative patients. Periodontitis in that study was defined based on a combination of clinical and radiographic measurements, so the investigators did not specifically examine the association between ACPA positivity and ABL. Furthermore, the Dissick et al.⁴ study did not examine serum ACPA concentrations and only reported whether patients were ACPA-positive or -negative. Therefore, this prior effort did not analyze the association between periodontitis and serum ACPA concentrations. Finally, Dissick et al.⁴ did not find any

significant associations between periodontitis and RA disease activity measures, likely because of the smaller sample size (69 patients with RA and 35 patients with OA).

Periodontitis has emerged as a potential environmental risk factor associated with RA.^{4,12,31-36} The oral cavity, and the periodontium in particular, may serve as a source of citrullinated protein.⁶ Citrullination of proteins in the periodontium may result from microbially induced oral inflammation and potentially contribute to ACPA generation in a susceptible host.³⁷ Antibodies to citrullinated proteins appear years before the onset of clinical RA³⁸ and predict a worsened radiographic outcome. Thus, the finding in this current study that more severe bone loss in ACPA-positive patients with RA extends to the oral cavity is a unique and important observation. Harre et al.¹¹ recently directly linked ACPA to bone loss and reported that ACPA binding to citrullinated vimentin enhanced osteoclast differentiation into mature osteoclasts. Using ACPA microarray analyses, the present authors have shown for the first time that ACPAs targeting citrullinated vimentin were elevated in groups with higher ABL, suggesting a possible mechanism for ABL in ACPA-positive participants with RA.

The association of ABL with auto-antibody-targeting citrullinated forms of histone also is noteworthy. There is increasing appreciation that neutrophil activation plays an important role in the pathogenesis of not only RA,^{39,40} but also periodontitis.^{41,42} Vitkov et al. have demonstrated an abundance of activated phagocytic neutrophils in purulent crevicular exudate, suggesting a central role for neutrophils in host defense against invasive bacteria causing periodontitis.⁴¹ Neutrophils, however, also appear to serve as a source of citrullinated histone antigen,³⁹ suggesting that neutrophil activation in the context of periodontitis could direct ACPA-driven inflammation in the periodontium, leading to increased ABL.

A limitation of this study is its case-control design. Although associations were observed between ABL and ACPA concentrations, a cause-and-effect relationship between RA and ABL could not be ascertained. It is possible that ABL may be a manifestation of RA (i.e., with the alveolus serving as another “joint” with bone resorption) or conversely that periodontitis may be a risk factor in RA pathogenesis. Another limitation, which may have affected the findings, is that the majority of the patients were males, unlike most RA cohorts. Thus, these study results may not be generalizable to a predominantly female study population.

CONCLUSIONS

In summary, the authors have demonstrated an association between ABL and ACPA concentrations and RA disease activity measures. In addition, the authors have shown that ACPAs targeting citrullinated vimentin and histone were significantly higher with greater ABL regardless of smoking status. These results suggest that ACPA targeting, potentially of both vimentin and histones, could provide novel insights between RA and ABL.

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