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Research Article

Increased prevalence of ANA and Anti-SSA in African American rheumatoid arthritis patients is not associated with increased serum chemokine concentrations

Tyler J. Sevco*[1], Doug P. Landsittel[2], Chengli Shen[2] & Larry W. Moreland[3]

[1]Department of Medicine, Division of General Internal Medicine, University of Pittsburgh Medical Center, Pittsburgh, PA
[2]Department of Biomedical Informatics, University of Pittsburgh School of Medicine, Pittsburgh, PA
[3]Department of Medicine, Division of Rheumatology and Clinical Immunology, University of Pittsburgh School of Medicine, Pittsburgh, PA
*Author for correspondence: tjs61@pitt.edu

Background: African American (AA) rheumatoid arthritis (RA) patients have increased disease activity compared to Caucasian (CAU) RA patients. Serum chemokines, such as CXCL10, are increased in RA patients and may function as markers of disease activity. The aim of this study was to compare autoantibody seropositivity in AA and CAU RA patients and analyze the link between antibody positivity and serum chemokine concentration.

Methods and Findings: 93 AA patients and 93 CAU patients from the University of Pittsburgh cohort of RA patients were matched using a propensity model with race as the outcome and age, gender, body mass index (BMI), disease duration, anti-tumor necrosis factor (TNF) use, rheumatoid factor (RF) positivity, and cyclic citrullinated peptide (CCP) positivity as the predictor variables in a logistic regression. Plasma from the matched subjects was analyzed for autoantibodies including antinuclear antibody (ANA), anti-Sjogren's syndrome-related Antigen A (SSA), and anti-Sjogren's syndrome-related Antigen B (SSB). To evaluate differences in serum chemokine concentrations, anti-SSA positive samples were matched with anti-SSA negative samples for age, gender, disease duration, RF positivity, and CCP positivity using a propensity model. CXCL10 was measured using an ELISA assay. Anti-SSA was more prevalent in AA RA patients compared to CAU RA patients (11.70% vs. 3.23%; p=0.02). ANA was more prevalent in AA RA patients (21.28% vs. 10.75%; p=0.04). A total of 14 patients (7.57%) were anti-SSA positive. Anti-SSA positivity was not associated with increased serum CXCL10 levels compared to the anti-SSA negative group. RF positivity was associated with an increased serum CXCL10 concentration compared to the RF negative group (253.14 vs 153.46 pg/ml).

Conclusions: AA RA patients have an increased prevalence of anti-SSA and ANA compared to CAU RA patients, which may be contributing to the increased disease activity seen in this population via a mechanism outside of the CXCL10 chemokine pathway.

Keywords: anti-SSA • anti-nuclear antibody-ANA • African American • CXCL10 • chemokine

Abbreviations: African American (AA) • Body Mass Index (BMI) • Caucasian (CAU) • Clinical Disease Activity Index (CDAI) • Disease Activity Score 28 (DAS28) • Health Assessment Questionnaire (HAQ) • Multiplex Bead Assay (MBA) • Rheumatoid Arthritis (RA)

Introduction

Rheumatoid arthritis (RA) has wide variability in both its clinical presentation and its autoantibody profile. Two well-known autoantibodies that are found in between 60-90% of RA patients are rheumatoid factor (RF) and cyclic citrullinated peptide (CCP) antibody [1]. Seropositivity for these antibodies is associated with more destructive joint pathology and radiographic progression of RA [2]. Anti-Sjogren's Sydrome-related Antigen A (SSA) is associated with numerous autoimmune conditions, including most notably Sjogren's Syndrome. Anti-SSA is also found in between 3-16% of RA patients and it is believed to be a clinical indicator of poor prognosis in RA [3]. Several studies have shown that RA patients with this antibody have a lesser clinical response to infliximab [4,5]. Anti-SSA seropositivity is also associated with secondary Sjogren's Syndrome. RA with secondary Sjogren's Syndrome is associated with worse clinical manifestations and increased antinuclear antibody (ANA) positivity [6,7].

The prevalence of ANA and anti-SSA has been shown to be higher in African American (AA)

RA patients compared to Caucasian (CAU) RA patients in two established RA cohorts [8]. Additionally, AA patients with RA have worse Health Assessment Questionnaire (HAQ) scores and increased disease activity clinically compared to CAU RA patients [9]. Despite differences in autoantibody prevalence, it is currently unknown if there is a higher occurrence of clinically diagnosed Sjogren's Syndrome in AA RA patients. Additionally, no studies have examined the link between increased autoantibody prevalence in African American RA patients and their increased disease activity.

In RA, inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) are the primary inducers of chemokine production [10]. Chemokines then lead to increased numbers of inflammatory cells, such as macrophages, lymphocytes, and fibroblast-like synoviocytes, in inflamed synovial tissue [11,12]. Chemokines also contribute to cartilage degradation and pannus formation by stimulating the release of various inflammatory cytokines [13]. Several studies have shown that serum chemokines including CX3CL1, CCL5, CXCL9, and CXCL10 are increased in active RA patients compared to healthy controls [14-16]. Particularly, several studies have found that CXCL10 could serve as a disease activity marker in RA [17,18]. Elevated CXCL10 and CXCL13 levels have been shown to be predictive of a favorable response to TNF inhibitor therapy [19]. Studies have also shown that serum chemokine levels, including CXCL9, CXCL10 and CXCL16 decrease after treatment with disease-modifying antirheumatic drugs or biologic agents [19,20]. There is also an association between reduction of serum chemokine levels and improved clinical activity in RA patients [14]. Therefore, serum concentrations of chemokines such as CXCL10 may function as useful biomarkers for disease activity in RA patients that are capable of predicting treatment response.

The aims of this study were to examine the prevalence of autoantibodies in matched AA and CAU RA patients and subsequently evaluate the association between autoantibody positivity and serum chemokine concentration. We hypothesized that the increased disease activity of RA in AAs may be linked to an increased frequency of ANA and anti-SSA antibodies leading to more severe RA in these patients. Specifically, the clinically worse disease activity in anti-SSA and/or ANA positive RA patients may be mediated by an increased concentration of chemokines, such as CXCL10.

Methods

- Cohort: The RA registry at the University of Pittsburgh Medical Center consists of approximately 1000 RA patients. Arthritis-related pain assessments were obtained at each clinic visit via self-administered questionnaires collected from subjects. Serum samples were also obtained at each visit and analyzed for RF and anti-CCP. Approximately, 10% of this cohort identified as African American. AA RA patients from our cohort (n=93) were matched with CAU RA patients (n=93) in a 1:1 fashion using a propensity model with race as the outcome and age, body mass index (BMI), gender, RF positivity, anti-CCP positivity, former anti-TNF use, current anti-TNF use, and disease duration as the predictor variables in a logistic regression model [21].

  Our study was approved by the University of Pittsburgh Institutional Review Board. At the time of enrollment in the study, all patients had previously met the revised 1987 American College of Rheumatology classification criteria for RA.

- Autoantibody Assays: Plasma samples from initial patient visits upon enrollment into the cohort were assayed from matched AA and CAU samples. All samples underwent standard clinical testing for RF (Beckman image) and anti-CCP (BioRad Bioplex) as part of being enrolled in the RA registry. ANA testing was performed using indirect immunofluorescence with a HEp-2 cell substrate at the University of Pittsburgh Medical Center Immunopathology Laboratory. ANA titers of ≥ 1:80 were classified as positive. All samples also underwent specific autoantibody testing using the Bioplex 2200 Multiplex Bead Assay (MBA) (Bio-Rad Laboratories, Inc. Hercules, CA). MBA detected antibodies including SSA, SSB, DS DNA, Smith, Sm/RNP, ribonucleoprotein (RNP), Chromatin, Ribosomal P, Centromere B, Scl-70, and Jo-1. For all antibodies assessed with MBA, titers ≥ 1:1.9 were considered positive.

- CXCL10 Assay: Serum samples from the 14 anti-SSA positive patients (including both AA and CAU samples) identified during the autoantibody assay were matched with 28 anti-SSA negative patients in a 1:2 fashion using a propensity model with anti-SSA as the primary outcome and age, gender, disease duration, RF positivity, CCP positivity as the predictor variables in a logistic regression. Serum samples were then analyzed in duplicate for CXCL10 using a Quantikine ELISA assay (R&D Systems, Minneapolis, MN). CXCL10 concentrations were calculated using a standard curve.
- Statistical Analysis: Continuous variables were presented as mean ± standard deviation and discrete variables were presented as percentages. Differences in autoantibody prevalence between AA and CAU RA patients were assessed using unadjusted chi-square and Fisher exact tests. The null hypothesis of this study was that there would be no difference in autoantibody prevalence between the AA and CAU groups. Differences in CXCL10 concentrations were assessed using a nonparametric rank sum test to compare the two groups. Other analyses of categorical variables were performed using unadjusted chi-square and Fisher's exact tests for discrete variables and nonparametric rank sum tests for continuous variables. P values <0.05 were considered to be statistically significant.

Results

- Demographics: Plasma samples of 92 AA RA patients and 93 CAU RA patients from our RA cohort were included in the study. One AA patient was excluded due to inadequate serum to complete the autoantibody assays. The mean age for AA subjects and CAU subjects was similar at 60.4 and 58.7, respectively. The mean BMI was slightly higher in the AA subjects at 31.0 *vs.* 29.5. Both sets of patients were primarily female at 93.5% for African Americans and 95.6% for the Caucasian subjects. RF positivity (77.2% for AA and 82.8% for CAU) and anti-CCP positivity (75.0% for AA and 76.3% for CAU) were similar between the two groups. Disease duration at the time of enrollment into the cohort was also similar at 14.0 years for AA subjects and 13.0 years for CAU subjects. Modified HAQ scores were 0.57 for AA subjects and 0.52 for CAU subjects. No statistically significant differences were seen in the above variables when comparing AA and CAU subjects, which confirmed the success of the matching process Table 1.
- Autoantibodies: Anti-SSA was significantly more prevalent in the AA RA patients compared to CAU RA patients (11.70% *vs.* 3.23%; p=0.02). ANA was detected approximately twice as often in AA RA patients (21.28% *vs.* 10.75%; p=0.04). Other antibodies that were assessed, including DS DNA, Sm/RNP, RNP, anti-chromatin, Centromere B, and Scl-70, were similar between the two groups Table 2. Smith, Ribosomal P, and Jo-1 antibodies were not positive in any of the plasma samples from either group. There were a total of 14 anti-SSA+ subjects and 171 anti-SSA- subjects after combining the AA and CAU groups. When comparing the anti-SSA positive and anti-SSA negative groups, both groups had similar age, disease duration, RF positivity, CCP positivity, HAQ score, Disease Activity Score 28 (DAS28), and Clinical Disease Activity Index (CDAI) scores Table 3. However, 50% of the anti-SSA positive subjects were also ANA positive compared to 13.45% of the anti-SSA negative subjects (p=0.002). Additionally, 21.40% of the anti-SSA positive subjects were also anti-SSB positive compared to 1.17% of the anti-SSA negative subjects (p=0.003). There were a total of 30 ANA+ subjects and 155 ANA- subjects when combining all samples. When comparing the ANA positive and ANA negative RA subjects, the two groups had similar age, BMI, RF positivity, CCP positivity, HAQ, CDAI, and DAS28 scores. ANA positive subjects were significantly more likely to be anti-SSA positive (p=0.002) and were also more likely to be DS DNA positive (p=0.01). Anti-SSB positivity was also seen more frequently in the ANA positive group (6.67% *vs.* 1.93%).
- CXCL10 Assay: Of the 185 patients included in the study, 14 (7.57%) were

Table 1. AA and CAU RA patients were matched for the above variables and there were no significant differences between any of these demographic variables, confirming a successful matching process.

	African American (n=92)	Caucasian (n=93)	P-value
Mean Age (years)	60.39 ± 13.72	58.72 ± 12.96	0.39
Mean BMI (kg/m²)	31.04 ± 6.83	29.54 ± 6.61	0.24
Female (%)	93.50%	95.60%	0.75
RF Positive	77.20%	82.80%	0.36
Anti-CCP Positive	75.00%	76.30%	0.86
Mean Disease Duration (years)	14.00 ± 13.01	13.02 ± 12.11	0.52
Ever TNF Use	51.09%	60.22%	0.21
Current TNF Use	8.70%	6.45%	0.56
Modified HAQ Score	0.59 ± 0.52	0.52 ± 0.55	0.23

Table 2. There was a significant difference in the prevalence of anti-SSA and ANA between AA and CAU subjects. There were no significant differences in the other autoantibodies studied.

	AA % Positive (n=92)	CAU % Positive (n=93)	P-value
ANA IIF (≥1:80)	21.73% (20/92)	10.75% (10/93)	0.04
SSA	11.96% (11/92)	3.23% (3/93)	0.02
SSB	4.35% (4/92)	1.08% (1/93)	0.18
DS DNA	4.35% (4/92)	6.45% (6/93)	0.52
Sm	0.00%	0.00%	1
Sm/RNP	3.26% (3/92)	1.08% (1/93)	0.32
RNP	6.52% (6/92)	4.30% (4/93)	0.48
Chromatin	4.35% (4/92)	2.15% (2/93)	0.1
Ribosomal P	0.00%	0.00%	1
Centromere B	0.00%	2.15% (2/93)	0.16
SCL-70	1.09% (1/92)	0.00%	0.32
Jo-1	0.00%	0.00%	1

Table 3. SSA positive subjects were significantly more likely to be positive for other autoantibodies including ANA, SSB, and DS DNA compared to SSA negative subjects. ANA positive subjects were more likely to be SSA or DS DNA positive compared to ANA negative subjects. There were no significant differences in age, BMI, gender, RF positivity, CCP positivity, disease duration, CDAI, or DAS28 within the SSA and ANA groups.

	SSA+ (n=14)	SSA– (n=171)	P-value	ANA+ (n=30)	ANA- (n=155)	P-value
Age (years)	57.32 ± 15.56	59.69 ± 13.24	0.8	61.41 ± 15.41	59.29 ± 13.02	0.53
BMI (kg/m²)	27.01 ± 6.68	30.48 ± 6.75	0.21	28.96 ± 6.072	30.46 ± 6.84	0.52
Female	92.86% (13/14)	95.32% (163/171)	0.48	96.67% (29/30)	94.20% (146/155)	0.58
RF	78.57% (11/14)	78.94% (135/171)	0.97	83.33% (25/30)	81.29% (126/155)	0.79
CCP	64.29% (9/14)	74.85% (128/171)	0.36	73.33% (22/30)	75.4% (117/155)	0.82
Disease Duration (years)	12.27 ± 13.69	13.69 ± 12.54	0.52	14.21 ± 13.65	13.17 ± 12.19	0.62
Ever TNF Use	64.29% (9/14)	54.39% (93/171)	0.58	56.67% (17/30)	53.55% (83/155)	0.75
Current TNF Use	7.14% (1/14)	7.60 % (13/171)	0.95	3.33% (1/30)	8.38% (13/155)	0.47
HAQ	0.46 ± 0.61	0.55 ± 0.53	0.39	0.56 ± 0.52	0.54 ± 0.54	0.73
DAS 28	3.30 ± 1.05	3.57 ± 1.41	0.62	3.63 ± 1.49	3.56 ± 1.36	0.92
CDAI	14.93 ± 8.89	16.17 ± 13.77	0.87	18.40 ± 16.01	15.66 ± 13.02	0.6
SSB	21.4% (3/14)	1.17% (2/171)	0.003	6.67% (2/30)	1.93% (3/155)	0.19
DS DNA	21.4% (3/14)	4.09% (7/171)	0.03	16.67% (5/30)	3.22 % (5/155)	0.01
SSA +	-	-	--	23.33% (7/30)	4.52% (7/155)	0.002
ANA +	50.0% (7/14)	13.45% (23/171)	0.002	-	-	--

anti-SSA positive (11 AA, 3 CAU). The remaining 171 patients (92.43%) were anti-SSA negative. Serum CXCL10 concentrations for the 14 anti-SSA positive samples averaged 247.07 pg/ml (range 54.59-519.17). Serum concentrations for the 28 matched anti-SSA negative samples averaged 221.91 pg/ml (range 91.18-531.00). There was no significant difference between these two groups. Of the patients included in the CXCL10 assay, 12 were ANA positive and 30 were ANA negative. ANA positive samples had an increased average serum

concentration of CXCL10 compared to ANA negative samples; however, this difference was not statistically different Table 4. Of the samples that underwent CXCL10 testing, 31 were RF positive and 11 were RF negative. The RF positive group had an average CXCL10 concentration of 253.14 pg/ml versus 153.46 pg/ml for the RF negative group (p=0.04). When comparing the RF positive and RF negative groups, the RF positive group had significantly elevated BMI and a higher DAS28 score Table 5. However, these two groups had no significant differences in age, gender, disease duration, HAQ score, or CDAI.

Discussion

This study demonstrates that AA RA patients have an increased prevalence of anti-SSA and ANA compared to CAU RA patients when controlling for age, gender, BMI, disease duration, previous anti-TNF use, RF positivity, and anti-CCP positivity.

Additionally, the prevalence of ANA, anti-SSA, and anti-SSB in AA and CAU RA subjects in this study was consistent with previously reported values in separate RA cohorts.

Previous studies have shown wide variation in anti-SSA prevalence across different RA populations. In this study, the prevalence of anti-SSA in AAs (11.96%) was lower than those previously reported in Japanese RA patients (16.8%) or those reported in Greek RA patients (14.3%) [4,22]. However, AA RA subjects had nearly three times the prevalence of anti-SSA compared to matched CAU subjects. It is possible that the increased frequency of anti-SSA in AA subjects may be due to an increased frequency of secondary Sjogren's Syndrome. Co-existent RA and SS may then partially explain the increased disease activity and worse clinical outcomes seen in AA RA patients. However, it was not possible to determine the prevalence of Sjogren's Syndrome in our cohort with the available data. The AA group also had a higher prevalence of anti-SSB than the CAU group (4.26% *vs.* 1.08%). This was not a statistically significant difference; however there were only 5 total patients that were anti-SSB positive. Further studies with a larger sample size may provide further insight into the anti-SSB differences seen in this study.

The biological and clinical implications of the increased prevalence of anti-SSA and ANA in AA RA patients are currently unknown. However, several studies have suggested that autoantibody profiles may be clinically significant. Specifically, anti-SSA has been shown to be associated with more severe disease in multiple connective tissue disease and it is also involved in the molecular pathogenesis of immune dysregulation in Sjogren's Syndrome [23]. ANA positivity in Japanese RA patients has been associated with

Table 4. There was no significant difference in serum CXCL10 concentration between SSA positive and SSA negative subjects. There was a significantly higher concentration of CXCL10 in RF positive subjects compared to RF negative subjects.

	Antibody Positive	Antibody Negative	P-value
SSA	247.07 ± 146.30 (n=14)	221.91 ± 135.50 (n=28)	0.78
ANA	247.56 ± 139.76 (n=12)	223.49 ± 139.22 (n=30)	0.56
RF	253.14 ± 134.46 (n=31)	153.46 ± 128.09 (n=11)	0.04
CCP	244.02 ± 130.55 (n=26)	206.01 ± 152.86 (n=16)	0.22

Table 5. RF positive subjects that underwent CXCL10 assay had significantly increased BMI and DAS28 scores compared to RF negative subjects.

	RF Positive (n=31)	RF Negative (n=11)	P-Value
Age (years)	59.32 ± 12.36	47.93 ± 19.11	0.17
BMI (kg/m²)	33.18 ± 5.75	27.22 ± 5.66	0.05
Female	90.32%	100%	0.55
Disease Duration (years)	12.79 ± 13.22	12.34 ± 10.66	0.98
Ever TNF Use	58.06%	54.54%	0.99
Current TNF Use	9.68%	0%	0.55
HAQ	0.52 ± 0.57	0.26 ± 0.27	0.34
DAS28	3.81 ± 1.42	2.72 ± 0.91	0.02
CDAI	19.4 ± 16.66	10.39 ± 7.36	0.1

decreased treatment response to infliximab, suggesting that ANA-positive RA may also be a clinically important subset [24]. Further studies analyzing the functional consequences of anti-SSA and ANA positivity are needed to elucidate the biological implications and the clinical significance of this subset of RA patients.

This study aimed to examine the relationship between autoantibody seropositivity and serum chemokine concentration. In this study, AA and CAU anti-SSA positive subjects were combined (n=14) and compared to anti-SSA negative samples in a 1:2 fashion because it was impractical to compare AA versus CAU anti-SSA positive samples due to the small sample size (11 AA, 3 CAU). There was no significant difference between serum CXCL10 concentration when comparing the anti-SSA positive and anti-SSA negative groups. However, the CXCL10 concentration from both groups was approximately twice that of previously reported values in healthy subjects [25]. This is consistent with previous studies, which have shown an increase in synovial CXCL9 and CXCL10 in RA patients [11-13,26]. The RF positive group of patients that underwent the CXCL10 assay did have a significantly increased CXCL10 concentration compared to the RF negative group. Previous studies analyzing baseline chemokine levels in seropositive and seronegative RA patients have shown similar results with increased CXCL10 in CCP positive patients and increased CXCL13 in RF positive patients [19]. While the RF group was unmatched, the RF positive and RF negative groups had similar age, gender, disease duration, anti-TNF use, HAQ score, and DAS28 score. Therefore, future studies analyzing the effect of RF on serum chemokine concentrations may be warranted.

In RA, a predominance of Th17 cytokines, including IFN-γ and TNF have been suggested to be of pathological importance [27,28]. IFN-γ induces several chemokines including CXCL9, CXCL10, and CXCL11. Increased CXCL10 has been detected in the serum and synovial fluid of RA patients and in the saliva of Sjogren's Syndrome patients compared to healthy controls [25,29]. Additionally, this chemokine may have clinical significance as a human phase II clinical trial using an anti-CXCL10 monoclonal antibody (MDX-1100) showed a significantly increased response rate in RA patients who had an inadequate response to methotrexate therapy [30]. Our study found an association between RF seropositivity and increased CXCL10 levels but it found no association between anti-SSA positivity and CXCL10. Therefore, while the increased clinical severity seen in AA RA patients may be associated with a higher prevalence of anti-SSA, the presence of this autoantibody does not appear to directly affect expression of CXCL10. This implies that the worsening disease severity seen in AA RA patients may be mediated by a mechanism outside this cytokine-chemokine pathway. This is consistent with previous studies that have shown a lesser clinical response to anti-TNF therapy in anti-SSA positive RA patients [5]. However, since the only chemokine examined in this study was CXCL10, further studies may be warranted to examine the association between autoantibody seropositivity and other inflammatory mediators.

This study had several limitations. First, the sample sizes were small, particularly for the subsets of patients who were seropositive for the various autoantibodies. This was limited by the size of the cohort and the percentage of the cohort that identified as African American. As a result, there were only 14 patients in the study that were anti-SSA positive. Future studies on a larger number of patients could help further characterize the differences in autoantibody prevalence and serum chemokine concentration.

Another limitation of our cohort was that it included limited information regarding previous anti-TNF use. We had data regarding whether the patients had ever taken anti-TNF therapy and whether they were on anti-TNF therapy at the time of enrollment. However, our registry did not include information relating to the timing of previous anti-TNF therapy prior to enrollment. Because the production of autoantibodies such as ANA and dsDNA are commonly seen in patients after treatment with TNF inhibitors [31,32], it is unknown how this affected the prevalence in each group. However, the groups were matched for previous and current anti-TNF use to help mitigate this. Studies have also shown that disease modifying antirheumatic drugs including leflunomide and infliximab can lead to significant reductions in serum CXCL9 and CXCL10 concentrations [13,17-18,33]. However, it is unlikely that this significantly altered the results as the two groups were controlled for anti-TNF use.

Finally, there are a variety of chemokines that are upregulated in the synovial tissue of RA

patients. This study preliminarily examined CXCL10; however, other chemokines including CX3CL1, CXCL9, and CXCL13 could contribute to the pathogenesis of worsening RA [16,18]. Therefore, further studies examining the association between chemokine concentration and autoantibody positivity may help elucidate the link between the worsening disease severity of RA seen in AA patients and their increased prevalence of anti-SSA.

In conclusion, this study demonstrates that AA RA patients have an increased prevalence of anti-SSA and ANA compared to CAU RA patients, which may be contributing to the increased disease activity seen in this population via a mechanism outside of the CXCL10 chemokine pathway.

Conflict of interest

The authors report no declarations for conflict of interest.

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