

Exploring the Role of Antinuclear Antibody Positivity in the Diagnosis, Treatment, and Health Outcomes of Patients With Rheumatoid Arthritis

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Objective. The objective of this study was to describe differences in the clinical course of patients with rheumatoid arthritis (RA) who are antinuclear antibody (ANA)-positive compared with those who are ANA-negative.

Methods. This was a retrospective population-based cohort study of residents in Olmsted County, Minnesota, who first fulfilled 1987 American College of Rheumatology criteria for RA in 2009–2014. Data were collected on first documentation of joint swelling. Data on rheumatoid factor or anti-cyclic citrullinated peptide antibody testing and the ANA level were also collected. Comparisons between groups were performed by using χ^2 and rank sum tests.

Results. In this cohort, 64% of patients were tested for ANA within ± 90 days of RA criteria fulfillment. In the 161 patients with ANA testing, 25% were ANA-positive. Patients who were ANA-positive were younger, female, and less likely to be current smokers. ANA positivity did not differ between patients with RA who were seropositive and seronegative. In seropositive patients who were ANA-positive, there was an increased time to fulfillment of RA criteria, increased time to treatment with disease-modifying antirheumatic drugs (DMARDs), and increased likelihood of being treated with hydroxychloroquine as opposed to methotrexate. Other outcomes, including disease activity and mortality, did not differ significantly between groups.

Conclusion. In patients with RA, important differences exist between those who are ANA-positive and ANA-negative in terms of time to fulfillment of RA criteria and time to DMARD initiation as well as choice of initial pharmacotherapy. These findings could indicate a difference in clinical presentation or perception of patients with RA who are ANA-positive. Further research is needed to study the long-term outcomes of patients with RA who are ANA-positive.

INTRODUCTION

Antinuclear antibody (ANA) testing is an important screening tool for autoimmune conditions, such as systemic lupus erythematosus (SLE) and scleroderma. Large population analyses, such as the Dallas Regional Autoimmune Disease Registry, have estimated that the ANA positivity rate is between 20% and 30% of the healthy general population (1). Dinse et al (2) suggest that there was an increased prevalence of ANA positivity in the United States between 1988 and 2012 and that this mirrored an increase in autoimmune pathology. Other work has shown that patients in the general population with ANA positivity have increased rates

of all-cause mortality and cardiovascular events (3). It remains unclear what drives these observed differences.

In the realm of rheumatology, there is a gap in the understanding of the utility of ANA positivity in the diagnosis and management of rheumatoid arthritis (RA). Current practice for the diagnosis of RA does not use ANA positivity as a screening tool. However, ANA positivity is often gleaned during the laboratory workup for many patients who are ultimately diagnosed with RA. There has not been a large population-based cohort study examining ANA positivity specifically in patients with RA. As such, it remains unclear how this information may inform clinical management of RA.

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There is existing research into patients who are ANA-positive and taking tumor necrosis factor α inhibitors (TNFi) for the treatment of their RA (4–6). The impetus to study ANA positivity in this cohort comes from multiple observations of autoimmune complications, such as a lupus-like syndrome being enriched in patients with RA treated with TNFi who are ANA-positive (4,5). A single-center cohort study of patients with RA showed that there was not a clear ANA pattern that could help predict which patients would develop lupus-like syndrome (6). However, there was an association between development of ANA positivity during treatment and secondary nonresponse to TNFi (6). It has not yet been established how ANA positivity may influence treatment of RA in clinical practice. Therefore, the goal of this study was to describe what, if any, differences exist in the diagnosis, treatment, and clinical course of patients with RA on the basis of ANA positivity.

MATERIALS AND METHODS

The study design was a retrospective population-based cohort study of residents of Olmsted County, Minnesota. The cohort was assembled by using the resources of the Rochester Epidemiology Project (REP), a collaboration between local health care facilities that enables sharing of medical records across institutions (7). Individuals were included in the cohort on the basis of the following eligibility criteria: age 18 years or older; residency in Olmsted County, Minnesota; and earliest fulfillment of 1987 or 2010 American College of Rheumatology criteria for RA between January 1, 2009, and December 31, 2014. Patients were excluded if they were documented to have diagnoses of other connective tissue diseases, including SLE. Data were collected until the last medical visit, death, or conclusion of data abstraction (December 31, 2017). The study was approved by the Institutional Review Boards of the Mayo Clinic (17-002593) and Olmsted Medical Center (017-OMC-17).

Data collection and study variables. Demographic data were collected on patients, including age, sex, presence of obesity (body mass index greater than or equal to 30), and smoking status at the time of fulfillment of RA criteria. Diagnostic data were collected on the components of the 1987 and 2010 RA criteria for each patient. Seropositivity was determined by presence of either rheumatoid factor (RF) or anti-cyclic citrullinated peptide (CCP) antibodies. ANA positivity was determined by ANA levels greater than or equal to 1 U by enzyme-linked immunosorbent assay (Bio-Rad Laboratories) or greater than or equal to a titer of 1:80 by immunofluorescence. ANA tests performed within ± 90 days from the time of fulfillment of RA criteria were considered to be baseline assessments. The first documentation of clinical synovitis, as defined by joint swelling documented by a medical provider, was determined. Start dates for disease-modifying antirheumatic drug (DMARD) and corticosteroid use were also collected. Choice of

first pharmacotherapy was noted; therapies included, but were not limited to, hydroxychloroquine (HCQ), methotrexate (MTX), leflunomide, sulfasalazine, and azathioprine.

Disease activity measures included the following: the Clinical Disease Activity Index (CDAI), the Simplified Disease Activity Index (SDAI), and a functional assessment tool, the Health Assessment Questionnaire (HAQ). Additional measurements collected included the HAQ, patient and provider global assessments (0-100) of disease activity, self-reported pain on a visual analog scale, tender joint count (TJC; 0-28) and swollen joint count (SJC; 0-28), and the C-reactive protein level (CRP), as available in the electronic health record.

Statistical analysis. Descriptive statistics were used to summarize the data. Comparisons of patient characteristics, disease activity measures, and medications initiated at the time of fulfillment of RA criteria between patients who were ANA-positive and ANA-negative were made by using χ^2 and rank sum tests. Cox proportional hazards models adjusted for age and sex were used to examine outcomes, including mortality in patients who were ANA-positive versus ANA-negative. Patients who met the outcome (eg, erosions, corticosteroid use) at the time of fulfillment of RA criteria were excluded from these models because they were not at risk of developing the outcome after fulfilling criteria for RA. Generalized linear models with random subject effects to account for multiple measurements per patient were used to examine trends in RA disease activity measures over the first 5 years of the disease course. *P* values less than 0.05 were considered to be statistically significant. Analyses were performed by using SAS version 9.4 (SAS Institute Inc.) and R 3.6.1 (R Foundation for Statistical Computing).

RESULTS

The study included 252 patients with RA. Of these, 161 (64%) were tested for ANA within ± 90 days of RA criteria fulfillment. Patients tested for ANA were more likely to be younger (mean age: 54 vs 58 years; *P* = 0.03) and to be CCP-positive (65% vs 49%; *P* = 0.01; Table 1). There were no significant differences between the groups in terms of sex, smoking status, presence of obesity, or severity of disease activity on the basis of available data.

In the 161 patients with ANA testing, 25% were ANA-positive. Patients who were ANA-positive were less likely to be male (20% vs 37%; *P* = 0.04). Patients who were ANA-positive were also slightly younger (mean age: 51 vs 55 years; *P* = 0.08) and somewhat less likely to be current smokers (5% vs 17%; *P* = 0.10; Table 2), but these associations did not reach statistical significance. In terms of diagnostics, there were no differences in RF or CCP seropositivity (66% in patients who were ANA-positive vs 65% in patients who were ANA-negative; *P* = 0.92). Furthermore, there was no difference in ANA positivity between patients who were RF- and/or CCP-positive versus RF- and/or CCP-negative (median: 0.6 vs 0.5 U; *P* = 0.48). There was no significant

Table 1. Patient characteristics by presence or absence of ANA testing

	ANA tested (n = 161)	ANA not tested (n = 91)	Total (n = 252)	P
Age at fulfillment of 1987 or 2010 criteria, mean (SD), years	54.0 (14.1)	58.5 (15.1)	55.6 (14.6)	0.03
Sex, female, n (%)	109 (68)	61 (67)	170 (67)	0.91
Smoker, n (%)				
Never	95 (59)	44 (48)	139 (55)	0.19
Current	22 (14)	19 (21)	41 (16)	–
Former	44 (27)	28 (31)	72 (29)	–
Obesity (BMI ≥ 30), n (%)	61 (38)	39 (43)	100 (40)	0.44
RF-positive, n/n tested (%)	84/154 (55)	35/91 (38)	119/252 (53)	0.01
CCP-positive, n/n tested (%)	87/145 (60)	36/85 (42)	123/230 (53)	0.01
RF- and/or CCP-positive, n (%)	105 (65)	45 (49)	150 (60)	0.01
Presence of morning stiffness, n (%)	90 (56)	58 (64)	148 (59)	0.23
Presence of rheumatoid nodule, n (%)	2 (1)	4 (4)	6 (2)	0.12
Presence of joint erosions, n (%)	27 (17)	20 (22)	47 (19)	0.31
Abnormal ESR/CRP level, n (%)	111 (69)	73 (80)	184 (73)	0.05

Abbreviations: ANA, antinuclear antibody; BMI, body mass index; CCP, anti-cyclic citrullinated peptide; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; RF, rheumatoid factor.

difference in meeting RA criteria on the basis of ANA positivity. Disease activity (eg, CDAI and SDAI) and other measures (eg, HAQ, patient and provider global assessments of disease activity, self-reported pain on a visual analog scale, TJC, SJC, and CRP) were compared on the basis of ANA positivity. Over a 5-year period following the patient meeting the 2010 diagnostic criteria for RA, no significant differences were found between the ANA-positive and ANA-negative groups (data not shown).

In this cohort, a further stratification was made between patients who were seropositive and seronegative. Of the 161 patients who received ANA testing, 105 were seropositive and 56 were seronegative. Among patients who were seropositive, those who were ANA-positive took longer to fulfill RA criteria than those who were ANA-negative (Table 3). In terms of therapy in patients who were seropositive, the length of time to the first DMARD initiation was somewhat increased in the patients who

were ANA-positive (median: 41 vs 13 days; $P = 0.08$), but this association did not reach statistical significance. Patients who were ANA-positive were more likely to receive HCQ as their first treatment (44% vs 23%; $P = 0.03$) and less likely to receive MTX as a first treatment (48% vs 71%; $P = 0.03$). Results were similar when we repeated analyses, considering only CCP positivity without regard to RF. Among patients who were CCP-positive, the length of time to the first DMARD initiation was significantly increased in the patients who were ANA-positive (median: 50 vs 10 days; $P = 0.043$); patients who were ANA-positive were somewhat more likely to receive HCQ as their first treatment (35% vs 18%; $P = 0.10$) and somewhat less likely to receive MTX as a first treatment (55% vs 75%; $P = 0.09$).

Time to event analyses among patients with RF and/or CCP positivity revealed that patients with ANA positivity were less likely to be escalated to an MTX dose of 20 mg (Table 4). There was no significant difference in groups for development of first joint erosion or first use of corticosteroid therapy. Comparison based on first use of biologic therapy trended toward significance, with fewer patients who were ANA-positive receiving biologic therapy. Seven patients died during follow-up.

DISCUSSION

This study found that there are important differences in patients with RA who are seropositive with respect to ANA positivity. The time to fulfillment of RA criteria is increased in patients who are ANA-positive, although there were no differences in how the RA criteria were met. There was no significant difference in disease activity on the basis of ANA positivity over the short-term follow-up of 5 years. However, the time to first DMARD therapy was increased in patients who were ANA-positive, and these patients were more likely to be started on HCQ than MTX as the initial therapy. This observation is noteworthy given that for most patients

Table 2. Patient characteristics by ANA positivity

	Positive (n = 41)	Negative (n = 120)	P
Age at fulfillment of 1987 or 2010 criteria, mean (SD), years	51.0 (12.5)	55.0 (14.6)	0.08
Sex, female, n (%)	33 (80)	76 (63)	0.04
Smoker, n (%)			0.10
Never	29 (71)	66 (55)	–
Current	2 (5)	20 (17)	–
Former	10 (24)	34 (28)	–
Obesity (BMI ≥ 30), n (%)	17 (41)	44 (37)	0.58
RF-positive, n/n tested (%)	23/40 (58)	61/114 (54)	0.66
CCP-positive, n/n tested (%)	20/36 (56)	67/109 (61)	0.53
RF- and/or CCP-positive, n (%)	27 (66)	78 (65)	0.92
Presence of morning stiffness, n (%)	24 (59)	66 (55)	0.69
Presence of rheumatoid nodule, n (%)	0 (0)	2 (2)	0.41
Presence of joint erosions, n (%)	7 (17)	20 (17)	0.95
Abnormal ESR/CRP level, n (%)	28 (68)	83 (69)	0.92

Abbreviations: ANA, antinuclear antibody; BMI, body mass index; CCP, anti-cyclic citrullinated peptide; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; RF, rheumatoid factor.

Table 3. Patient characteristics of RF- and/or CCP-positive and RF- and/or CCP-negative patients by ANA positivity

	ANA+, RF/CCP+ (n = 27)	ANA-, RF/CCP+ (n = 78)	P	ANA+, RF/CCP- (n = 14)	ANA-, RF/CCP- (n = 42)	P
Age at earlier fulfillment of 1987 or 2010 criteria, mean (SD), years	48.9 (12.3)	55.1 (14.5)	0.05	54.9 (12.5)	54.9 (14.8)	0.90
First DMARD used, n (%)			0.19			0.48
Methotrexate	13 (48)	55 (71)	...	8 (57)	15 (36)	...
Hydroxychloroquine	12 (44)	18 (23)	...	5 (36)	22 (52)	...
Leflunomide	0 (0)	2 (3)	...	0 (0)	0 (0)	...
Sulfasalazine	1 (4)	1 (1)	...	1 (7)	3 (7)	...
Other	1 (4)	2 (3)	...	0 (0)	2 (5)	...
Methotrexate used first, n (%)	13 (48)	55 (71)	0.04	5 (36)	22 (52)	0.28
Hydroxychloroquine used first, n (%)	12 (44)	18 (23)	0.03	8 (57)	15 (36)	0.16
Days from first joint swelling to fulfillment of 1987 criteria, median (IQR)	16 (0–120)	0 (0–12)	0.02	2 (0–354)	21 (0–121)	0.26
Days from first joint swelling to fulfillment of 2010 criteria, median (IQR)	3 (0–59)	0 (0–10)	0.07	4 (0–196)	0 (0–91)	0.56
Days from first joint swelling to first DMARD, median (IQR)	41 (5–160)	13 (0–73)	0.08	98 (14–283)	16 (3–125)	0.20
Days from first joint swelling to first steroid, median (IQR)	50 (4–567)	7 (0–53)	0.03	1 (0–47)	0 (0–12)	0.43
Presence of morning stiffness, n (%)	13 (48)	33 (42)	0.60	11 (79)	33 (79)	1.00
Presence of rheumatoid nodules, n (%)	0 (0)	1 (1)	0.55	0 (0)	1 (2)	0.56
Presence of joint erosions, n (%)	4 (15)	12 (15)	0.94	3 (21)	8 (19)	0.85
Abnormal ESR or CRP level, n (%)	18 (67)	57 (73)	0.53	10 (71)	26 (62)	0.52

Abbreviations: ANA, antinuclear antibody; CRP, C-reactive protein; DMARD, disease-modifying antirheumatic drug; ESR, erythrocyte sedimentation rate; IQR, interquartile range; +, positive; -, negative.

with RA, MTX remains the first-line therapy of choice (8–12). Moreover, HCQ is a common drug in the treatment of patients with SLE who are ANA-positive. Because patients with a diagnosis of SLE were excluded from this cohort study, there are several possible explanations for this observation in treatment preference, including diagnostic ambiguity due to disease phenotype or patient factors such as age or fertility planning.

Going beyond initial treatment, there appears to be a decreased likelihood to use biologic therapy in patients with RA who are ANA-positive. This observation is not statistically significant because of limitations of cohort size. However, it could represent a clinical practice preference to reconsider biologic treatment in patients with RA who are ANA-positive because of prior observations of decreased treatment efficacy (6,13,14).

The strengths of the study include the comprehensive and longitudinal nature of this population-based data collection and the systematic and standardized approach to data analysis. Limitations of this study include generalizability of the REP cohort

because of the homogenous demographics of the study population. ANA laboratory tests were not obtained for all patients with RA in this study. However, patient factors were similar between patients, irrespective of whether an ANA level was obtained (Table 1). There was a limited cohort size of patients who were both ANA-positive and seronegative, which constrained comparison of seropositive and seronegative patients who were ANA-positive. In this cohort, analysis of mortality differences in patients with RA who were ANA-positive was limited by the small number of deaths (n = 7).

In conclusion, this study was able to establish that there is an association between the diagnosis and management of RA in patients and presence of ANA positivity, specifically with respect to the delayed timing and choice of pharmacological agent: HCQ over MTX. At this point, it is unclear what the driver for these differences is. It certainly warrants further study if there is a difference in clinical presentation or perception of patients with RA who are ANA-positive. There is existing evidence of increased

Table 4. Association between ANA positivity and each outcome among patients with RF- and/or CCP-positive rheumatoid arthritis

Outcome of interest	Number of patients with outcome	Hazard ratio (95% CI)	P
First joint erosion	13	0.56 (0.12–2.57)	0.46
First biologic therapy	19	0.47 (0.13–1.66)	0.24
MTX dose ≥20 mg	43	0.50 (0.23–1.10)	0.087
First corticosteroid treatment	84	1.04 (0.63–1.73)	0.87
Mortality	7	0.96 (0.10–8.90)	0.97

Abbreviations: ANA, antinuclear antibody; CCP, anti-cyclic citrullinated peptide; CI, confidence interval; MTX, methotrexate; RF, rheumatoid factor.

cardiovascular and all-cause mortality among patients who are ANA-positive in the general population. Follow-up work with this cohort or with a pooled cohort could increase sample size, which could deepen our understanding of health-outcome differences based on ANA positivity in patients with RA.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Paknikar had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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