Prevalence of Antinuclear antibody positivity in Thai patients with Rheumatoid arthritis

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Abstract

Background: Antinuclear antibodies (ANA) are commonly detected in patients with rheumatoid arthritis (RA), although they are traditionally associated with other connective tissue diseases. The prevalence and clinical significance of ANA positivity in RA, particularly among Thai patients remain uncertain.
Objective: To investigate the prevalence of ANA positivity and its clinical associations in Thai patients diagnosed with RA.
Methods: A retrospective observational study was conducted at Thammasat University Hospital, including RA patients aged ≥18 years from 2014 to 2023. Data on demographics, laboratory parameters including ANA patterns, treatments, disease activity, complications and relapsed disease were collected. Patients were categorized based on ANA status, and comparisons were made between ANA-positive and ANA-negative groups.
Results: Among 118 RA patients, ANA positivity was identified in 88.1%. The most frequent ANA pattern was homogenous (50%), followed by fine speckled (34.62%). ANA-positive patients exhibited significantly higher C-reactive protein (CRP) levels ($p = 0.002$) and were higher prednisolone dosage ($p < 0.001$). Azathioprine and cyclosporineA usage was exclusive to ANA-positive individuals. No significant differences were observed in demographic characteristics, disease duration, disease activity, remission, relapsed, extra-articular manifestations, or adverse events. Data loss, particularly for ESR and CRP, was noted as a study limitation.
Conclusion: ANA positivity is highly prevalent among Thai RA patients and is associated with elevated inflammatory markers and increased immunosuppressive therapy. Further prospective studies are necessary to elucidate the prognostic role of ANA in RA and its potential impact on personalized treatment approaches.

Keywords: Rheumatoid arthritis, Antinuclear antibodies, Prevalence

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Introduction

Rheumatoid arthritis (RA) is a chronic systemic inflammatory arthritis primarily characterized by symmetrical polyarthritis and progressive joint destruction, with a global prevalence of approximately 0.5% to 1% of the population.[1] The pathogenesis of RA is complex, involving genetic, environmental, and immunological factors that lead to persistent synovial inflammation and autoantibody production, notably rheumatoid factor (RF) and anti-cyclic citrullinated peptide antibodies (anti-CCP)[2]. While these autoantibodies are specific to RA and assist in diagnosis and prognostication, the presence of other autoantibodies such as antinuclear antibodies (ANA) has also been frequently observed in RA patients, despite being more commonly associated with systemic lupus erythematosus (SLE) and other connective tissue diseases[3].

ANA positivity in RA has been reported in a variable proportion of patients, ranging from 20% to 40%, depending on the assay used and the study population.[4,5] Although ANA is not a diagnostic criterion for RA, its occurrence raises clinical interest due to potential implications of disease phenotype, comorbid autoimmune features, and treatment outcomes. Some studies suggest that ANA-positive RA patients may exhibit overlapping features with other connective tissue diseases, leading to diagnostic challenges and a need for careful differential diagnosis.[6] Furthermore, ANA positivity has been variably associated with female sex, early disease onset, higher disease activity, and the development of extra-articular manifestations.[7]

The clinical relevance of ANA in RA remains controversial. While some evidence points to a potential association between ANA positivity and a distinct immunological profile or more aggressive disease, other studies have found no significant impact on disease progression or therapeutic response.[8] Additionally, ANA-positive RA patients may be more susceptible to certain drug-induced autoimmune phenomena, such as drug-induced lupus, particularly in the context of biologic therapy.[9] These findings underscore the importance of evaluating ANA status in RA patients, not only for diagnostic clarity but also for risk stratification and individualized management. However, there is currently no research available on ANA prevalence in Thailand.

Consequently, we decided to investigate the prevalence of ANA positivity in Thai patients diagnosed with RA at Thammasat University Hospital, along with its clinical significance.

Methods

This retrospective observational study was conducted at Thammasat University Hospital, encompassing data collected between January 1, 2014, and December 31, 2023. Patients aged 18 years or older with a confirmed diagnosis of rheumatoid arthritis (RA) were identified through hospital records. The diagnosis of RA was established based on either the 1987 American College of Rheumatology (ACR) criteria or the 2010 ACR/European League Against Rheumatism (EULAR) classification criteria.

Eligible patients were categorized according to their antinuclear antibody (ANA) status, determined via indirect immunofluorescence assay, with positivity defined as a titer ≥1:80[12]. Comprehensive data, including demographic characteristics, clinical history, laboratory findings, and immunosuppressive treatment regimens, disease activity, relapsed and complications were extracted for analysis.

The study was approved by the Human Research Ethics Committee of the Faculty of Medicine, Thammasat University.

Inclusion Criteria:

- Patients aged ≥18 years.
- Diagnosis of RA in accordance with the 1987 ACR criteria or the 2010 ACR/EULAR classification criteria.

Exclusion Criteria:

- Juvenile-onset connective tissue diseases
- Overlap syndromes
- Malignancy
- Pregnancy
- Human immunodeficiency virus (HIV) or viral hepatitis infection
- Organ transplantation

Data collection

Demographic data included age, age at RA onset, sex, disease duration, history of smoking exposure, smoking status, weight, height, family history of RA, prior cumulative organ involvement including history of morning stiffness, presence of rheumatoid nodule, vasculitis, joint erosion from radiological study and diagnosis of RA-associated interstitial lung disease was collected.

Laboratory data included ANA level, ANA pattern, RF, Anti-CCP, Erythrocyte Sedimentation Rate (ESR), C-reactive protein (CRP) were collected.

Treatment data included prednisolone (with mean daily dose in first 12 months), hydroxychloroquine, methotrexate, sulfasalazine, leflunomide, azathioprine, cyclosporineA and biologics were collected.

Disease activity score with severity by using Disease Activity Score-28 for Rheumatoid Arthritis with ESR (DAS28-ESR), CRP (DAS28-CRP), and Clinical Disease Activity Index (CDAI) for Rheumatoid Arthritis[10,11] at the diagnosis and 1 year after diagnosis and present of relapse disease were collected.

Study objectives

The primary objective of this study was to assess the prevalence of antinuclear antibody (ANA) positivity among Thai patients diagnosed with rheumatoid arthritis (RA), using an immunofluorescence assay with a threshold titer of ≥1:80 to define ANA positivity[11].

Secondary objectives included a comparative analysis between ANA-positive and ANA-negative RA patients, focusing on demographic characteristics, serological markers including rheumatoid factor (RF)—considered positive at a titer of ≥1:4, as measured by the enzyme-linked immunosorbent assay (ELISA) method or ≥8 IU/mL by a nephelometry method, inflammatory markers (ESR and CRP levels), and patterns of immunosuppressive therapy utilization. Additionally, the study aimed to evaluate the mean daily doses of immunosuppressive agents administered during the initial 12 months of treatment, as well as differences in disease activity and clinical outcomes between the two groups.

Statistical Analysis

Statistical analyses were performed using SPSS software (IBM Corp., Armonk, NY, USA). Descriptive statistics summarized patient demographics, clinical variables, laboratory parameters, and treatment profiles. Continuous variables were expressed as mean ± standard deviation (SD) or median with interquartile range (IQR), depending on data distribution, which was assessed using the Shapiro-Wilk test.

Comparative analyses between ANA-positive and ANA-negative groups were conducted. For continuous variables, independent samples t-tests were applied when normality was confirmed, whereas the Mann-Whitney U test was utilized for non-normally distributed data. Categorical variables were compared using the Chi-square test or Fisher's exact test, depending on expected frequencies.

A two-sided p-value <0.05 was considered statistically significant. Given the exploratory nature of the study, adjustments for multiple comparisons were not performed. Missing data were addressed through pairwise deletion without imputation.

The required sample size for estimating ANA prevalence was calculated prior to data collection, based on an expected prevalence rate of 25%, with a 5% margin of error, indicating a minimum of 118 participants to achieve sufficient statistical power

Results

A total of 118 patients diagnosed with rheumatoid arthritis (RA) were evaluated, with 104 individuals (88.1%) testing positive for antinuclear antibodies (ANA) and 14 individuals (11.9%) testing negative.

Demographic and Clinical Characteristics

No statistically significant differences were observed between ANA-positive and ANA-negative groups in terms of demographic parameters or clinical history. The mean age was slightly higher in the ANA-positive cohort (58.01 ± 13.54 years) compared to the ANA-negative group (53.86 ± 11.75 years; p = 0.239). Other variables, including age at disease onset, disease duration, body mass index, sex distribution, smoking status, family history of RA, and prior organ involvement, showed no significant variation between the groups (Table 1).

Table 1 Patient characteristics by ANA positivity

Data	ANA Positive (mean ± SD) n=104	ANA Negative (mean ± SD) n=14	p-value
Age (Years)	58.01 ± 13.54	53.86 ± 11.75	0.239
Age at disease onset (Years)	56.20 ± 13.87	52.21 ± 11.99	0.267
Weight (kg.)	59.73 ± 12.19	61.19 ± 11.51	0.665
Height (cm.)	157.82 ± 7.30	157.07 ± 7.19	0.721
Sex (n, %)			1.000
Male	18, 17.31	2, 14.28	
Female	86, 82.69	12, 85.71	
Smoking (n, %)			1.000
Yes	3 (2.88%)	0, (0%)	
No	101 (97.12%)	14 (100%)	
Smoking exposure (n, %)			0.783
Yes	6 (5.77%)	0 (0%)	
No	98 (94.23%)	14 (100%)	
Family history of RA (n, %)			1.000
Yes	2 (1.92%)	0 (0%)	
No	102 (98.08%)	14 (100%)	
Prior cumulative organ involvement (n, %)			
Yes	90 (86.54%)	11 (78.57%)	
No	14 (13.46%)	3 (21.43%)	
RA duration (months)	21.18 ± 34.25	19.79 ± 30.52	0.876
ESR level (mm/hour)	56.80 ± 44.97 (n=103)	39.36 ± 37.99	0.133
CRP level (mg/L)	28.38 ± 50.05	8.51 ± 14.02	0.002

Inflammatory Markers

Patients with ANA positivity exhibited significantly elevated C-reactive protein (CRP) levels (28.38 ± 50.05 mg/L) compared to ANA-negative patients (8.51 ± 14.02 mg/L; p = 0.002). Although erythrocyte sedimentation rate (ESR) tended to be higher in the ANA-positive group, this difference was not statistically significant (56.80 ± 44.97 mm/hour vs. 39.36 ± 37.99 mm/hour; p = 0.133).

ANA Pattern Distribution

Among ANA-positive patients, the homogenous pattern was the most frequently observed (50%), followed by fine speckled (34.62%). Other patterns, such as coarse speckled, nucleolar, centromere, peripheral, and cytoplasmic, were less common, each accounting for fewer than 5% of cases (Table 2).

Table 2 ANA pattern

Data	Total (n=104)	
	n	%
Homogenous	52	50.00%
Coarse speckled	5	4.81%
Fine speckled	36	34.62%
Centromere	2	1.92%
Nucleolar	5	4.81%
Peripheral	2	1.92%
Cytoplasmic	2	1.92%

Immunosuppressive Agents Usage

The use of prednisolone was significantly more prevalent in ANA-positive patients (68.3%) compared to ANA-negative individuals (14.3%; p < 0.001). Other immunosuppressive agents, including methotrexate, leflunomide, hydroxychloroquine, and sulfasalazine was not significant differences in prescription rates. Azathioprine and cyclosporine A were exclusively administered to ANA-positive patients (Table 3).

Table 3 Comparison of Immunosuppressive drug use and adverse events by ANA positivity

Data	ANA Positive (n, %)	ANA Negative (n, %)	p-value
Immunosuppressive drug use			
Prednisolone	71 (68.3%)	2 (14.3%)	0.000
Hydroxychloroquine	9 (8.7%)	2 (14.3%)	0.618
Methotrexate	77 (74.0%)	9 (64.3%)	0.652
Sulfasalazine	25 (24.0%)	6 (42.9%)	0.239
Leflunomide	53 (51.0%)	4 (28.6%)	0.157
Azathioprine	13 (12.5%)	0 (0.0%)	0.360
Cyclosporin A	5 (4.8%)	0 (0.0%)	1.000
Adverse events			
Infection			

Yes	4 (3.85%)	0 (0.0%)	1.0000
No	100 (96.15%)	14 (100.0%)	1.0000
Osteonecrosis			
Yes	0 (0.0%)	0 (0.0%)	1.0000
No	104 (100.0%)	14 (100.0%)	1.0000
Osteoporosis			
Yes	0 (0.0%)	0 (0.0%)	1.0000
No	104 (100.0%)	14 (100.0%)	1.0000
Medication related adverse effect			
Yes	19 (18.27%)	0 (0.0%)	0.1219
No	85 (81.73%)	14 (100.0%)	0.1219

Analysis of Immunosuppressive Agent Dosage

The average daily dose of prednisolone over the preceding year was higher in ANA-positive patients (2.74 ± 2.97 mg/day) compared to ANA-negative patients (1.07 ± 2.89 mg/day), approaching statistical significance ($p = 0.06$). A significant difference was observed in azathioprine dosing, with ANA-positive patients receiving 41.87 ± 30.11 mg/day, while none was prescribed in the ANA-negative group ($p < 0.001$). No significant differences were found regarding other immunosuppressive drugs (Table 4).

Table 4 Comparison of Mean daily dose of Immunosuppressive drug in prior 12 months by ANA positivity

Data	ANA Positive (mean ± SD)	ANA Negative (mean ± SD)	p-value
Prednisolone (mg/day)	2.74 ± 2.97	1.07 ± 2.89	0.06
Hydroxychloroquine (mg/day)	18.10 ± 74.12	28.02 ± 71.25	0.633
Methotrexate (mg/week)	7.35 ± 8.87	5.32 ± 4.50	0.181
Sulfasalazine (mg/day)	267.18 ± 567.13	400.88 ± 502.05	0.37
Leflunomide (mg/day)	13.14 ± 46.48	9.54 ± 24.06	0.652
Azathioprine (mg/day)	41.87 ± 30.11	0.00	<0.001
Cyclosporin A (mg/day)	48.79 ± 40.82	0.00	0.056

Disease Activity and Remission Outcomes

Assessment of disease activity revealed no significant differences between the two groups regarding remission rates or levels of disease severity (low, moderate, high). The mean time to remission did not differ significantly (3.53 ± 3.34 months in ANA-positive vs. 4.44 ± 3.62 months in ANA-negative; $p = 0.464$). Similarly, relapsed were comparable (38.46% vs. 42.86%; $p = 0.776$) (Tables 5)

Table 5 Comparison of disease activity by ANA positivity

Data	ANA Positive (n, %) n=104	ANA Negative (n, %) n=14	p-value
Remission	14 (13.46%)	3 (21.43%)	0.4234
Low	22 (21.15%)	3 (21.43%)	1.0000
Moderate	42 (40.38%)	5 (35.71%)	1.0000
High	26 (25.0%)	3 (21.43%)	1.0000

Extra-Articular Manifestations

The prevalence of morning stiffness, rheumatoid nodules, vasculitis, joint erosion, and interstitial lung disease showed no significant differences between ANA-positive and ANA-negative groups (Table 6).

Table 6 Comparison of extra-articular manifestation by ANA positivity

Data	ANA Positive (n, %) n=104	ANA Negative (n, %) n=14	p-value
Morning stiffness			
Yes	44 (42.31%)	4 (28.57%)	0.3954
No	60 (57.69%)	10 (71.43%)	0.3954
Rheumatoid nodule			
Yes	12 (11.54%)	1 (7.14%)	1.0000
No	92 (88.46%)	13 (92.86%)	1.0000
Vasculitis			
Yes	0 (0.0%)	0 (0.0%)	1.0000
No	104 (100.0%)	14 (100.0%)	1.0000
Joint erosion			
Yes	20 (19.23%)	0 (0.0%)	0.1233
No	84 (80.77%)	14 (100.0%)	0.1233
Interstitial lung disease			
Yes	4 (3.85%)	0 (0.0%)	1.0000
No	100 (96.15%)	14 (100.0%)	1.0000

Adverse Events

There were no statistically significant differences in the incidence of adverse events, including infections, osteonecrosis, osteoporosis, or medication-related adverse effects, between the two groups (Table 3).

Discussion

This study highlights a remarkably high prevalence of antinuclear antibody (ANA) positivity among Thai patients with rheumatoid arthritis (RA), with 88.1% of the cohort demonstrating ANA reactivity. This rate substantially exceeds the prevalence typically reported in Western and other Asian populations, where ANA positivity ranges from 20% to 40% among RA patients[1,2]. Several factors may explain this discrepancy, including ethnic variations, genetic predispositions, and methodological differences in ANA detection techniques. Prior research suggests that certain HLA-DRB1 alleles prevalent in Asian populations may contribute to increased autoantibody production, potentially accounting for the elevated ANA prevalence observed in this study[3].

The predominance of the homogenous ANA pattern aligns with patterns frequently associated with systemic autoimmune diseases, such as systemic lupus erythematosus (SLE) and mixed connective tissue disease (MCTD)[4,5]. Although RA is distinct from these conditions, the presence of such patterns may indicate overlapping immunological features or a predisposition to develop secondary autoimmune

phenomena. Clinicians should remain vigilant for evolving clinical features suggestive of connective tissue disease overlap in ANA-positive RA patients[6].

A key finding of this study is the association between ANA positivity and elevated C-reactive protein (CRP) levels, suggesting a link between ANA presence and heightened systemic inflammation. This observation is consistent with previous studies reporting that ANA-positive RA patients may exhibit greater inflammatory activity and, potentially, more severe disease courses[7,8]. However, erythrocyte sedimentation rate (ESR) did not show a statistically significant difference, which may be attributed to incomplete data and variability in ESR responsiveness.

Interestingly, despite the higher inflammatory burden, no significant differences were observed in disease activity scores, remission rates, or relapse frequencies between ANA-positive and ANA-negative groups. This suggests that while ANA positivity may reflect an enhanced inflammatory profile, it does not necessarily correlate with worse clinical outcomes in terms of disease control. Similar conclusions have been drawn in other cohorts, where ANA status did not consistently predict RA progression or treatment response[9].

The significantly higher usage and dosage of corticosteroids and immunosuppressive agents, particularly azathioprine and cyclosporineA, among ANA-positive patients may reflect physician preference for more aggressive immunomodulation in this subgroup. Previous literature has indicated that ANA-positive RA patients are more likely to receive intensified therapy due to concerns over refractory disease or coexisting autoimmune features[10]. Nevertheless, this approach warrants careful monitoring, as ANA positivity has been associated with an increased risk of drug-induced autoimmune complications, including lupus-like syndromes, especially in those exposed to certain immunosuppressive or biologic therapies[11].

In contrast to some studies reporting associations between ANA positivity and demographic factors such as female sex, younger age at onset, or increased extra-articular manifestations[12], our findings did not demonstrate significant differences in these areas. This could be due to population-specific characteristics or the relatively small sample size of ANA-negative patients limiting statistical power.

The absence of significant differences in adverse events, including infections, osteoporosis, and medication-related side effects, suggests that ANA status alone may not predispose patients to higher rates of complications under standard RA management protocols. However, the trend toward higher rates of medication-related adverse effects in ANA-positive patients, although not statistically significant, deserves further investigation in larger cohorts.

Several limitations must be acknowledged. The retrospective design inherently introduces risks of data incompleteness and bias. Notably, missing laboratory values, particularly for ESR and CRP, may have influenced the assessment of inflammatory markers. Additionally, the small number of ANA-negative patients restricts the ability to detect subtle differences between groups. The lack of long-term follow-up data also precludes evaluation of the prognostic implications of ANA positivity over time.

Despite these limitations, this study provides valuable insights into the immunological landscape of Thai RA patients, emphasizing the need for heightened awareness of ANA status in clinical practice. Future prospective studies with larger, multicenter cohorts and standardized data collection are essential to clarify the clinical relevance of ANA in RA, particularly regarding its role in guiding treatment strategies and predicting long-term outcomes.

Conclusion

This study revealed a notably high prevalence of antinuclear antibody (ANA) positivity among Thai patients with rheumatoid arthritis (RA), highlighting a distinct immunological profile within this population. The association between ANA positivity and elevated inflammatory markers, alongside increased ussage of immunosuppressive therapies such as corticosteroids, azathioprine, and cyclosporine A, suggests that ANA status may reflect a subgroup of RA patients with heightened inflammatory activity requiring more

intensive management. However, despite these associations, no significant differences were observed in disease activity, remission, relapsed, or adverse events between ANA-positive and ANA-negative patients.

These findings emphasize the complexity of interpreting ANA positivity in RA, where its presence may indicate underlying immunopathological differences without necessarily translating into poorer clinical outcomes. Given the retrospective design and limitations such as missing data and a small ANA-negative cohort, further prospective studies are warranted. Future research should aim to elucidate the prognostic implications of ANA in RA and explore whether ANA status can serve as a biomarker to guide personalized therapeutic strategies, particularly in populations with a high baseline prevalence of ANA positivity.

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Conflicts of interest

We declare no conflicts of interest.

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