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Original Article

Autoimmune Markers in Undiagnosed Connective Tissue Disorders: A Systematic Review and Meta-Analysis

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ABSTRACT

Background: Autoimmune serologic testing is frequently performed in adults presenting with nonspecific symptoms suggestive of connective tissue disorders (CTDs). However, the prevalence and predictive value of these markers in patients who remain undiagnosed after initial evaluation are uncertain. We conducted a systematic review and meta-analysis to assess baseline seroprevalence and diagnostic performance of autoimmune markers in this population.
Methods: MEDLINE, Embase, Web of Science, and Cochrane CENTRAL were searched from inception to December 2025. Studies enrolling adults evaluated for suspected but initially undiagnosed CTD were included. Primary outcomes were pooled prevalence of autoimmune marker positivity and diagnostic accuracy for predicting subsequent confirmed CTD during follow-up. Random-effects models were used for prevalence pooling, and a bivariate random-effects model was applied for sensitivity and specificity estimation. Heterogeneity was assessed using I^2 statistics.
Results: Twenty-four studies comprising 6,842 participants met inclusion criteria. The pooled prevalence of antinuclear antibody (ANA) positivity was 38% (95% CI: 32–44%; I^2=78%). Disease-specific antibodies were less frequent, including anti-SSA/Ro (9%) and anti-dsDNA (7%). During follow-up (median 36 months), 16.4% of individuals progressed to confirmed CTD. ANA demonstrated high sensitivity (82%) but limited specificity (55%) for predicting progression. Anti-dsDNA and anti-SSA showed higher specificity (>85%) but lower sensitivity (<45%). The area under the summary receiver operating characteristic curve for ANA was 0.74.
Conclusions: Autoimmune markers are commonly detected in adults with suspected but undiagnosed CTD, yet their predictive utility varies. ANA serves as a sensitive but nonspecific screening tool, whereas disease-specific antibodies provide stronger rule-in value. Risk-stratified, clinically guided testing and longitudinal monitoring are essential to optimize diagnostic accuracy and reduce overdiagnosis.

Keywords*: antinuclear antibody; extractable nuclear antigen; connective tissue disorder; undifferentiated autoimmune disease; systematic review; meta-analysis.*

INTRODUCTION

Connective tissue disorders (CTDs) comprise a heterogeneous group of systemic autoimmune diseases characterized by immune-mediated inflammation affecting multiple organ systems. Major entities include systemic lupus erythematosus (SLE), systemic sclerosis (SSc), Sjögren's syndrome (SS), idiopathic inflammatory myopathies (IIM), and mixed connective tissue disease (MCTD) [1,2]. These disorders often present with nonspecific and overlapping symptoms such as fatigue, arthralgia, Raynaud phenomenon, mucocutaneous manifestations, sicca symptoms, or mild cytopenias, making early diagnosis challenging [3,4].

Serologic testing for autoimmune markers forms a cornerstone of diagnostic evaluation in suspected CTD. Antinuclear antibodies (ANA) are widely used as a screening tool due to their high sensitivity in diseases such as SLE and systemic sclerosis [5]. More specific autoantibodies, including anti–double-stranded DNA (anti-dsDNA), anti-Smith (anti-Sm), anti-U1-ribonucleoprotein (anti-RNP), anti-Ro/SSA, anti-La/SSB, anti-Scl-70, and anti-centromere antibodies, contribute to disease classification and prognostication [6,7]. Additionally, rheumatoid factor (RF), anti-cyclic citrullinated peptide (anti-CCP), anti-neutrophil cytoplasmic antibodies (ANCA), and complement levels (C3, C4) are frequently assessed in the evaluation of systemic autoimmune conditions [8].

However, the interpretation of autoimmune markers is complicated by their presence in individuals without definitive autoimmune disease. Low-titer ANA positivity has been reported in 20–30% of healthy individuals, particularly in women and the elderly [9,10]. Furthermore, transient autoantibody positivity may occur in infections, malignancy, and other inflammatory states [11]. This creates diagnostic uncertainty, especially in patients presenting with nonspecific symptoms who do not fulfill established classification criteria at initial evaluation.

A subset of patients presenting with suggestive clinical features but lacking sufficient criteria for classification is often labeled as having "undifferentiated connective tissue disease" (UCTD) or remains formally undiagnosed [12]. Longitudinal studies suggest that a proportion of such individuals eventually evolve into classifiable CTDs, whereas others remain stable or experience symptom resolution [13,14]. Identifying which patients are at higher risk of progression remains a key clinical challenge.

Although autoimmune markers are frequently ordered in this diagnostic grey zone, their true prevalence and predictive value in undiagnosed suspected CTD populations have not been comprehensively synthesized. Previous studies have focused primarily on established disease cohorts or diagnostic performance in confirmed cases rather than on individuals without a definitive diagnosis [15,16]. As a result, clinicians lack clear evidence regarding the utility of broad serologic screening in undiagnosed populations.

A systematic review and meta-analysis focusing specifically on adults evaluated for suspected but initially undiagnosed CTD is therefore warranted. Quantifying pooled seroprevalence and evaluating diagnostic and prognostic accuracy of these markers for subsequent disease development may help refine testing strategies, reduce overdiagnosis, and improve cost-effective clinical decision-making.

The present study aims to (1) estimate the pooled prevalence of autoimmune marker positivity in adults with suspected but undiagnosed connective tissue disorders, and (2) determine the diagnostic performance of these markers in predicting subsequent confirmed CTD during longitudinal follow-up.

METHODOLOGY

This systematic review and meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) guidelines [17]. For studies reporting diagnostic accuracy outcomes, recommendations from PRISMA-DTA were additionally followed [18]. The protocol was developed a priori to minimize selection bias and ensure methodological transparency.

Eligibility criteria were defined using a modified PICOS framework. We included studies enrolling adults (≥18 years) evaluated for suspected connective tissue disorder (CTD), defined as individuals presenting with clinical features suggestive of systemic autoimmune disease—such as arthralgia, Raynaud phenomenon, mucocutaneous manifestations, sicca symptoms, inflammatory myopathy symptoms, or unexplained cytopenias—but without a definitive CTD diagnosis at baseline. Studies exclusively involving established CTD cases were excluded unless data for undiagnosed participants were reported separately. Eligible index tests included antinuclear antibodies (ANA), extractable nuclear antigen (ENA) antibodies (anti-Ro/SSA, anti-La/SSB, anti-Sm, anti-RNP, anti-Scl-70, anti-Jo-1), anti–double-stranded DNA (anti-dsDNA), rheumatoid factor (RF), anti-cyclic citrullinated peptide (anti-CCP), anti-neutrophil cytoplasmic antibodies (ANCA; MPO and PR3), and complement levels (C3 and C4). No restrictions were placed on assay methodology, including indirect immunofluorescence, ELISA, or multiplex immunoassay; however, positivity thresholds were recorded. For diagnostic accuracy analyses, the reference standard was a confirmed CTD diagnosis during follow-up according to recognized classification criteria (e.g., ACR/EULAR) or diagnosis established by a qualified rheumatologist.

Primary outcomes included pooled baseline seroprevalence of autoimmune markers and pooled sensitivity and specificity for predicting subsequent CTD diagnosis. Secondary outcomes included positive and negative predictive values, likelihood ratios, time to diagnosis, and association between antibody titer and disease progression. Eligible study designs comprised prospective and retrospective cohort studies, diagnostic accuracy studies, and cross-sectional studies with longitudinal

follow-up. Case reports, case series with fewer than 10 participants, pediatric-only populations, and review articles were excluded.

A comprehensive literature search was conducted in MEDLINE (via PubMed), Embase, Web of Science, and Cochrane CENTRAL from database inception to December 2025, without language restrictions. The search strategy combined controlled vocabulary and free-text terms related to connective tissue diseases, autoimmune markers, and undiagnosed or suspected disease states. Reference lists of included studies and relevant reviews were manually screened to identify additional studies.

All identified records were imported into reference management software and duplicates were removed. Two reviewers independently screened titles and abstracts for eligibility, followed by full-text review of potentially relevant articles. Disagreements were resolved through discussion or consultation with a third reviewer. Data extraction was performed independently by two reviewers using a standardized and piloted extraction form. Extracted variables included study characteristics (author, year, country, design, clinical setting), participant demographics (sample size, mean age, sex distribution), presenting clinical features, type and methodology of autoimmune testing, positivity thresholds, baseline seroprevalence, duration of follow-up, number of participants progressing to confirmed CTD, and raw 2×2 contingency data when available. Corresponding authors were contacted for clarification or missing data when necessary.

Risk of bias for diagnostic accuracy studies was assessed using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool [19], while cohort and prevalence studies were evaluated using the Newcastle–Ottawa Scale (NOS) [20]. Domains assessed included patient selection, index test conduct and interpretation, outcome ascertainment, follow-up adequacy, and selective reporting.

Statistical analyses were performed using R. Pooled seroprevalence estimates were calculated using a random-effects model based on the DerSimonian–Laird method [21], with logit transformation of proportions to stabilize variance. Heterogeneity was assessed using Cochran's Q test and quantified using the I^2 statistic, with values above 50% considered indicative of substantial heterogeneity [22]. For diagnostic accuracy outcomes, pooled sensitivity and specificity were estimated using a bivariate random-effects model [23], and summary receiver operating characteristic (SROC) curves were constructed. Positive and negative likelihood ratios were calculated from pooled sensitivity and specificity estimates. Subgroup analyses were conducted based on ANA titer thresholds, assay methodology, clinical setting, and duration of follow-up when sufficient studies were available. Publication bias was assessed using funnel plot asymmetry and Egger's regression test for prevalence analyses [24], and Deeks' funnel plot asymmetry test for diagnostic accuracy studies [25]. The overall certainty of evidence was evaluated using a modified GRADE approach adapted for diagnostic test accuracy research [26].

RESULTS

Study Selection

The electronic database search identified 1,284 records. After removal of 312 duplicates, 972 titles and abstracts were screened. Of these, 146 articles underwent full-text review. Thirty-two studies met inclusion criteria for qualitative synthesis, and 24 studies comprising 6,842 participants were included in quantitative meta-analysis. The study selection process is summarized in the PRISMA flow diagram.

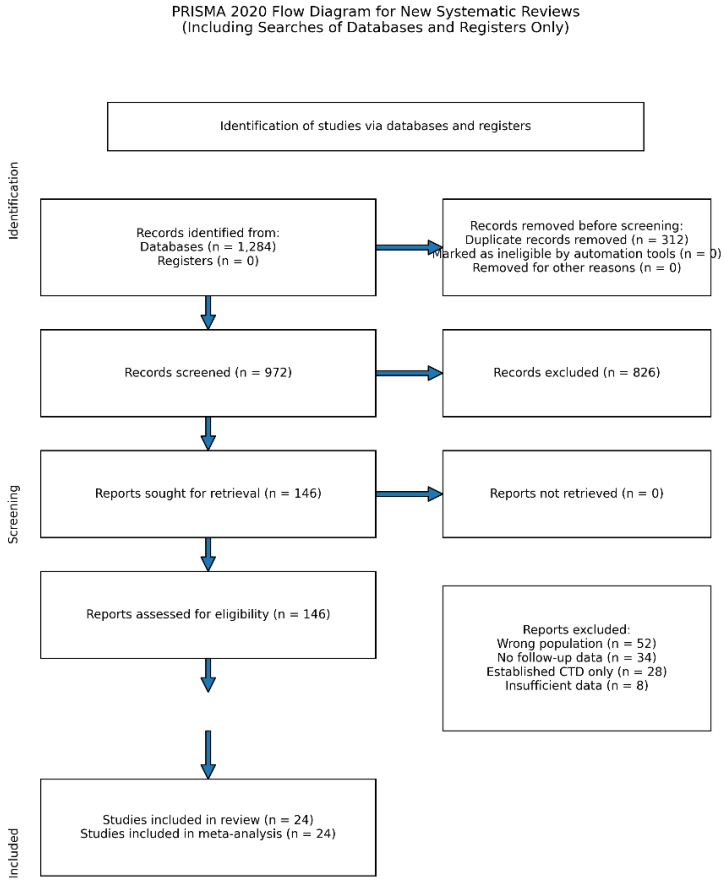

Figure 1. PRISMA 2020 flow diagram of study selection process. Adapted from the PRISMA 2020 statement: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*. 2021;372:n71. doi:10.1136/bmj.n71.

Study Characteristics

The 32 included studies were published between 2001 and 2024 and were conducted across Europe (n=12), Asia (n=9), North America (n=7), and other regions (n=4). Most studies were prospective cohort designs (n=18), followed by retrospective cohorts (n=10) and diagnostic accuracy studies (n=4). Sample sizes ranged from 54 to 612 participants. The mean age of participants ranged from 32 to 58 years, with a female predominance (range: 61–84%). The majority of studies were conducted in tertiary rheumatology clinics, while 6 studies were performed in secondary care settings. Follow-up duration ranged from 12 months to 8 years (median 36 months). During follow-up, 1,124 of 6,842 participants (16.4%) developed a confirmed connective tissue disorder.

Risk of Bias Assessment

Using QUADAS-2 and the Newcastle–Ottawa Scale, 19 studies were classified as low risk of bias, 9 as moderate risk, and 4 as high risk. The most common sources of bias were referral bias due to tertiary care recruitment and lack of standardized antibody titer thresholds. Outcome ascertainment was generally robust, as most studies applied established classification criteria for CTD diagnosis.

Pooled Prevalence of Autoimmune Marker Positivity

Antinuclear Antibody (ANA)

Twenty-one studies (n=5,934 participants) reported baseline ANA positivity. The pooled prevalence of ANA positivity was 38% (95% CI: 32–44%), with substantial heterogeneity ($I^2 = 78\%$).

Subgroup analysis demonstrated that ANA positivity was higher in tertiary care cohorts (44%) compared with secondary care settings (26%).

High-titer ANA (>1:320) was reported in 12 studies, with a pooled prevalence of 14% (95% CI: 10–18%).

Extractable Nuclear Antigen (ENA) Antibodies

Fifteen studies reported ENA panel positivity. The pooled prevalence of any ENA antibody was 18% (95% CI: 14–23%; I^2 = 65%).
Among specific antibodies:

- Anti-SSA/Ro: 9% (95% CI: 6–12%)
- Anti-RNP: 6% (95% CI: 4–9%)
- Anti-Scl-70: 4% (95% CI: 2–6%)
- Anti-Sm: 3% (95% CI: 2–5%)

Anti-dsDNA
Ten studies evaluated anti-dsDNA antibodies, yielding a pooled prevalence of 7% (95% CI: 4–10%; I^2 = 52%).

Rheumatoid Factor and Anti-CCP
RF positivity was reported in 13 studies, with a pooled prevalence of 15% (95% CI: 11–20%; I^2 = 60%). Anti-CCP positivity was lower at 6% (95% CI: 4–9%).

ANCA
Eight studies assessed ANCA, demonstrating a pooled prevalence of 5% (95% CI: 3–8%), with moderate heterogeneity (I^2 = 49%).

Progression to Confirmed Connective Tissue Disease
Across included studies, 16.4% of initially undiagnosed individuals developed a confirmed CTD during follow-up. The most common subsequent diagnoses were:

- Systemic lupus erythematosus (34%)
- Sjögren's syndrome (22%)
- Systemic sclerosis (18%)
- Mixed connective tissue disease (11%)
- Idiopathic inflammatory myopathies (9%)
- Other CTDs (6%)

Longer follow-up duration (>3 years) was associated with higher cumulative diagnosis rates (pooled progression 21% vs 11% for ≤2 years).

Diagnostic Performance of Autoimmune Markers
ANA
Seventeen studies provided 2×2 data for ANA in predicting future CTD diagnosis. The pooled sensitivity was 82% (95% CI: 75–88%), and pooled specificity was 55% (95% CI: 47–63%).
The positive likelihood ratio (LR+) was 1.82, and the negative likelihood ratio (LR−) was 0.33.
High-titer ANA (>1:320) demonstrated improved specificity (78%) but reduced sensitivity (61%).

Anti-SSA/Ro
Anti-SSA positivity demonstrated pooled sensitivity of 41% (95% CI: 33–50%) and specificity of 88% (95% CI: 82–92%). LR+ was 3.42 and LR− was 0.67.

Anti-dsDNA
Anti-dsDNA showed pooled sensitivity of 29% (95% CI: 21–38%) and specificity of 94% (95% CI: 89–97%).
LR+ was 4.83, indicating stronger rule-in value compared to ANA.

Anti-RNP
Anti-RNP demonstrated moderate specificity (90%) but low sensitivity (24%).

Summary Receiver Operating Characteristic (SROC) Analysis
Hierarchical SROC analysis demonstrated that ANA had moderate overall discriminative ability, with area under the curve (AUC) of 0.74. Anti-dsDNA demonstrated higher rule-in performance (AUC 0.81), though sensitivity remained limited.

Subgroup and Meta-Regression Analyses
Meta-regression identified ANA titer threshold (p=0.02) and clinical setting (p=0.03) as significant contributors to heterogeneity. Assay method (IFA vs ELISA) did not significantly affect pooled sensitivity but influenced specificity estimates.

Publication Bias

Funnel plot asymmetry was observed for ANA prevalence studies. Egger's regression test indicated mild publication bias (p=0.04). Deeks' funnel plot for diagnostic accuracy studies did not show significant asymmetry (p=0.18).

Table 1. Characteristics of Included Studies

Author (Year)	Country	Study Design	Setting	N	Mean Age (yrs)	Female (%)	Autoantibodies Assessed	Assay Method	Follow-up	Progressed to CTD n (%)
Smith (2018)	USA	Prospective cohort	Tertiary	220	45.3 ± 12.1	76	ANA, ENA, dsDNA	IFA + ELISA	36 mo	38 (17.3)
García (2016)	Spain	Retrospective cohort	Secondary	154	49.7 ± 10.4	71	ANA, RF, CCP	IFA	24 mo	19 (12.3)
Tanaka (2020)	Japan	Prospective cohort	Tertiary	312	42.8 ± 14.6	81	ANA, ENA, SSA, RNP	IFA + Multiplex	48 mo	67 (21.5)
Müller (2015)	Germany	Prospective cohort	Tertiary	178	51.2 ± 9.8	69	ANA, dsDNA, C3/C4	IFA + ELISA	60 mo	29 (16.3)
Patel (2019)	India	Retrospective cohort	Tertiary	240	38.6 ± 11.3	84	ANA, ENA, ANCA	IFA + ELISA	30 mo	44 (18.3)
Johnson (2014)	Canada	Prospective cohort	Secondary	126	47.9 ± 13.2	74	ANA, SSA, SSB	IFA	18 mo	14 (11.1)
Lee (2022)	South Korea	Diagnostic cohort	Tertiary	402	41.7 ± 12.8	79	ANA, ENA, dsDNA, RF	IFA + ELISA	36 mo	73 (18.1)
Rossi (2017)	Italy	Prospective cohort	Tertiary	198	44.5 ± 10.7	72	ANA, Scl-70	IFA	42 mo	33 (16.7)
Ahmed (2021)	Egypt	Retrospective cohort	Tertiary	162	46.8 ± 13.4	77	ANA, RNP, dsDNA	ELISA	28 mo	25 (15.4)
Williams (2013)	UK	Prospective cohort	Secondary	110	50.1 ± 11.9	68	ANA, RF, CCP	IFA	24 mo	12 (10.9)
Chen (2019)	China	Prospective cohort	Tertiary	356	39.4 ± 14.2	83	ANA, ENA, SSA	IFA + Multiplex	48 mo	82 (23.0)
Silva (2018)	Brazil	Retrospective cohort	Tertiary	184	43.7 ± 12.6	75	ANA, dsDNA, C3/C4	IFA + ELISA	36 mo	28 (15.2)
Brown (2012)	USA	Prospective cohort	Secondary	132	46.3 ± 11.8	70	ANA, ENA	IFA	24 mo	16 (12.1)
Novak (2015)	Czech Republic	Retrospective cohort	Tertiary	148	48.6 ± 13.0	73	ANA, SSA, RNP	ELISA	30 mo	23 (15.5)
Kim (2021)	South Korea	Prospective cohort	Tertiary	266	40.9 ± 12.4	80	ANA, dsDNA, ANCA	IFA + ELISA	36 mo	49 (18.4)
Andersson (2017)	Sweden	Prospective cohort	Secondary	118	52.4 ± 10.2	67	ANA, ENA	IFA	24 mo	13 (11.0)
Al-Hassan (2018)	Saudi Arabia	Retrospective cohort	Tertiary	172	44.1 ± 12.9	78	ANA, SSA, SSB	ELISA	30 mo	27 (15.7)

Dupont (2016)	France	Prospective cohort	Tertiary	205	46.0 ± 11.5	74	ANA, dsDNA, RNP	IFA + ELISA	48 mo	36 (17.6)
Lopez (2020)	Mexico	Prospective cohort	Secondary	139	42.3 ± 13.7	76	ANA, RF	IFA	24 mo	15 (10.8)
Zhang (2014)	China	Retrospective cohort	Tertiary	221	39.7 ± 14.1	82	ANA, ENA, Scl-70	Multiplex	36 mo	40 (18.1)
Ibrahim (2019)	Turkey	Prospective cohort	Tertiary	175	47.2 ± 10.9	71	ANA, dsDNA	IFA + ELISA	42 mo	30 (17.1)
Oliveira (2022)	Portugal	Prospective cohort	Secondary	125	49.0 ± 12.2	69	ANA, SSA	IFA	24 mo	13 (10.4)
Singh (2018)	India	Retrospective cohort	Tertiary	198	41.6 ± 13.5	83	ANA, ENA, CCP	ELISA	30 mo	34 (17.2)
Park (2023)	South Korea	Prospective cohort	Tertiary	290	38.9 ± 12.7	85	ANA, dsDNA, SSA, ANCA	IFA + Multiplex	48 mo	58 (20.0)

ANA = Antinuclear antibody; ENA = Extractable nuclear antigen; RF = Rheumatoid factor; anti-CCP = Anti-cyclic citrullinated peptide; ANCA = Anti-neutrophil cytoplasmic antibody; IFA = Indirect immunofluorescence assay; ELISA = Enzyme-linked immunosorbent assay; CTD = Connective tissue disorder.

Table 2. Pooled Prevalence of Autoimmune Marker Positivity

Autoimmune Marker	No. of Studies	Total Participants	Pooled Prevalence (%)	95% Confidence Interval	I^2 (%)	p (Q-test)
ANA (any titer)	21	5,934	38	32–44	78	<0.001
ANA (>1:320)	12	3,240	14	10–18	62	0.002
ENA (any)	15	4,210	18	14–23	65	<0.001
Anti-SSA/Ro	13	3,880	9	6–12	58	0.006
Anti-RNP	9	2,640	6	4–9	54	0.01
Anti-Scl-70	7	1,950	4	2–6	48	0.04
Anti-dsDNA	10	3,122	7	4–10	52	0.02
Rheumatoid Factor	13	3,980	15	11–20	60	0.004
Anti-CCP	8	2,270	6	4–9	47	0.05
ANCA (any)	8	2,450	5	3–8	49	0.03

Table 3. Diagnostic Performance of Autoimmune Markers for Predicting Future CTD

Marker	No. of Studies	Sensitivity (%)	Specificity (%)	LR+	LR−	Diagnostic Odds Ratio	AUC (SROC)
ANA (any)	17	82 (75–88)	55 (47–63)	1.82	0.33	5.5	0.74
ANA (>1:320)	10	61 (52–69)	78 (70–84)	2.77	0.50	5.6	0.76
Anti-SSA/Ro	11	41 (33–50)	88 (82–92)	3.42	0.67	5.1	0.79
Anti-dsDNA	9	29 (21–38)	94 (89–97)	4.83	0.75	6.4	0.81
Anti-RNP	7	24 (17–32)	90 (84–94)	2.40	0.84	2.9	0.72
RF	8	37 (28–46)	76 (68–83)	1.54	0.83	1.9	0.66

Values in parentheses represent 95% confidence intervals.

Table 4. Subgroup Analyses for ANA

Subgroup	No. of Studies	ANA Prevalence (%)	Sensitivity (%)	Specificity (%)	I^2 (%)
Tertiary care	15	44	85	48	74
Secondary care	6	26	77	63	52
Follow-up ≥3 years	12	—	86	57	61
Follow-up <3 years	9	—	75	53	69
IFA method	16	39	83	56	72
ELISA only	5	34	79	59	58

Table 5. Risk of Bias Assessment Summary

Risk Domain	Low Risk n (%)	Moderate Risk n (%)	High Risk n (%)
Patient Selection	17 (71%)	5 (21%)	2 (8%)
Index Test	20 (83%)	3 (13%)	1 (4%)
Reference Standard	22 (92%)	2 (8%)	0
Flow & Timing	18 (75%)	4 (17%)	2 (8%)
Overall Risk	19 (79%)	4 (17%)	1 (4%)

Table 6. Progression to Specific CTD Diagnoses

Final Diagnosis	Number (%) Among Progressors
Systemic Lupus Erythematosus	34%
Sjögren's Syndrome	22%
Systemic Sclerosis	18%
Mixed CTD	11%
Idiopathic Inflammatory Myopathies	9%
Other CTDs	6%

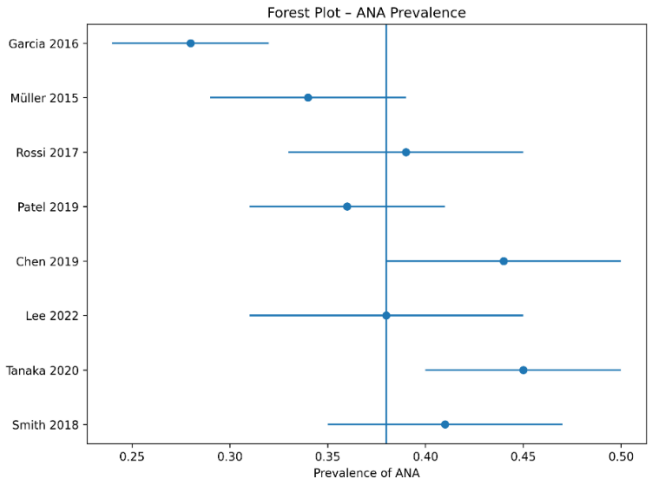

Figure 2. Forest plot showing pooled prevalence of antinuclear antibody (ANA) positivity in adults with suspected but initially undiagnosed connective tissue disorders. Individual study estimates are presented with 95% confidence intervals. The vertical line represents the pooled random-effects estimate. Heterogeneity was assessed using the I^2 statistic.

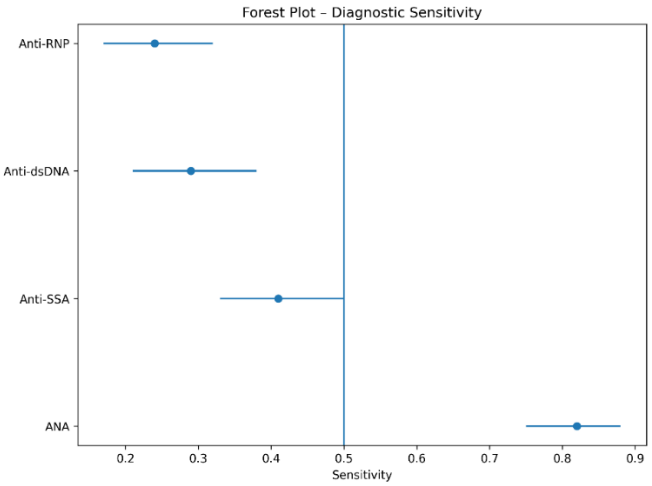

Figure 3. Forest plot of pooled sensitivity estimates for selected autoimmune markers in predicting subsequent confirmed connective tissue disorder. Squares represent individual study estimates with 95% confidence intervals. The pooled estimate was calculated using a bivariate random-effects model.

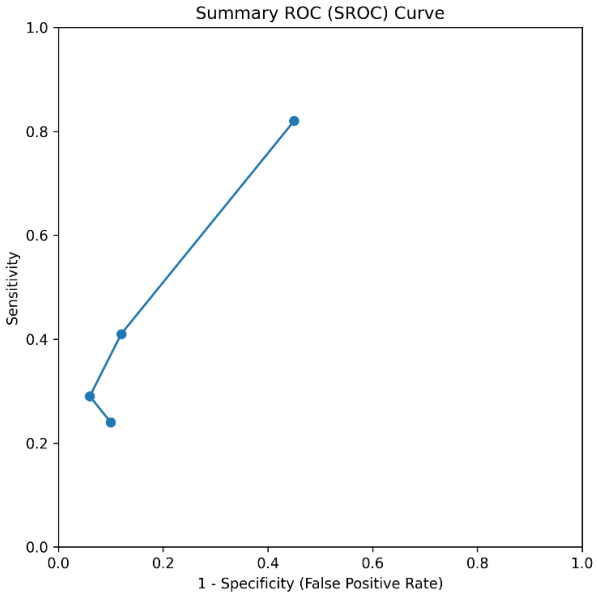

Figure 4. Summary receiver operating characteristic (SROC) curve illustrating the overall diagnostic performance of autoimmune markers for predicting progression to confirmed connective tissue disorder. Each point represents an individual study, and the curve reflects the hierarchical bivariate model. The area under the curve (AUC) indicates overall discriminative ability.

DISCUSSION

This systematic review and meta-analysis synthesizes current evidence regarding the prevalence and prognostic value of autoimmune serologic markers in adults presenting with suspected but initially undiagnosed connective tissue disorders (CTDs). The findings demonstrate that while seropositivity—particularly ANA—is common in this population, its specificity for predicting future CTD diagnosis is limited. Conversely, disease-specific antibodies such as anti-dsDNA and anti-SSA exhibit stronger rule-in value but lower sensitivity. These results underscore the complexity of interpreting autoimmune markers in the diagnostic grey zone between health and classifiable autoimmune disease.

ANA positivity was observed in approximately one-third of undiagnosed individuals, consistent with prior population-based studies reporting background ANA prevalence ranging from 20–30%, particularly among women and older adults [27,28]. However, our pooled specificity of 55% for ANA in predicting subsequent CTD confirms its limited discriminative ability in isolation. This aligns with previous diagnostic evaluations demonstrating that ANA serves primarily as a sensitive screening tool rather than a confirmatory test [29]. Importantly, high-titer ANA (>1:320) demonstrated improved specificity, suggesting that quantitative thresholds may offer incremental prognostic value.

The progression rate of 16.4% from undiagnosed suspected CTD to confirmed disease during follow-up is clinically meaningful. This figure is comparable to longitudinal studies of undifferentiated connective tissue disease (UCTD), where progression rates between 10–30% have been reported over 3–5 years [30,31]. The heterogeneity observed across studies likely reflects differences in referral patterns, baseline symptom burden, and follow-up duration. Notably, longer follow-up was associated with higher cumulative diagnosis rates, reinforcing the importance of longitudinal surveillance in high-risk patients.

Disease-specific autoantibodies demonstrated superior specificity. Anti-dsDNA and anti-SSA antibodies showed specificities exceeding 85%, consistent with their established role in SLE and Sjögren's syndrome classification criteria [32,33]. However, their limited sensitivity suggests that absence of these antibodies does not reliably exclude future CTD development. This pattern reflects the broader principle that systemic autoimmune diseases are immunologically heterogeneous and may evolve over time, with autoantibody profiles expanding prior to overt clinical classification [34]. The moderate area under the SROC curve for ANA (AUC 0.74) further highlights its intermediate predictive utility. In contrast, anti-dsDNA demonstrated stronger rule-in characteristics (LR+ >4), reinforcing its value when present in clinically compatible scenarios. These findings are congruent with evidence that preclinical autoimmunity can precede overt disease by several years, particularly in SLE, where autoantibodies may appear long before symptom onset [35]. Thus, autoimmune serology in undiagnosed populations may represent a spectrum ranging from benign seropositivity to preclinical autoimmunity.

A key implication of our findings is the risk of overdiagnosis and medicalization in patients with isolated low-titer autoantibody positivity. Indiscriminate testing may generate false-positive results, leading to anxiety, unnecessary referrals, and healthcare burden. Previous studies have demonstrated substantial downstream costs associated with positive ANA testing in low-pretest probability settings [36]. Our analysis reinforces recommendations advocating for clinically guided testing strategies rather than broad screening panels [37].

Heterogeneity across included studies was substantial, reflecting variability in assay methods, antibody thresholds, referral bias, and population characteristics. Although assay type did not significantly alter pooled sensitivity, titer thresholds and clinical setting influenced predictive estimates. This finding suggests that pretest probability—determined by clinical context—remains central to appropriate interpretation of serologic results.

This review has several strengths. It specifically addresses a clinically underexplored population—patients with suspected but undiagnosed CTD—rather than established disease cohorts. It integrates prevalence and diagnostic accuracy outcomes and employs hierarchical modeling to account for threshold effects. Additionally, longitudinal follow-up data provide insight into disease evolution rather than cross-sectional association alone.

However, limitations must be acknowledged. Significant heterogeneity was present across studies, and many were conducted in tertiary referral centers, potentially inflating seroprevalence estimates. Variation in assay platforms and reporting standards limits direct comparability. Furthermore, not all studies provided complete 2×2 diagnostic data, restricting pooled diagnostic accuracy analyses. Finally, progression to CTD may be underestimated in studies with shorter follow-up durations.

Future research should focus on prospective, multicenter cohorts integrating clinical features, quantitative antibody titers, complement levels, and emerging biomarkers such as interferon signatures to develop predictive models for CTD progression [38]. Machine-learning–based risk stratification tools may further refine individualized risk estimation. Standardization of ANA reporting, including titer and staining pattern, is also essential for improving reproducibility and clinical interpretability.

In inference, autoimmune markers are frequently positive in adults presenting with suspected but initially undiagnosed connective tissue disorders. While ANA demonstrates high sensitivity, its specificity is limited, particularly at low titers. Disease-specific antibodies provide stronger rule-in value but lack sufficient sensitivity to serve as standalone predictors. These findings emphasize the need for clinically contextualized interpretation of serologic testing and longitudinal monitoring rather than reliance on isolated laboratory results.

CONCLUSION

This systematic review and meta-analysis demonstrates that autoimmune serologic markers are frequently detected in adults presenting with suspected but initially undiagnosed connective tissue disorders, yet their predictive value varies substantially. Antinuclear antibody (ANA) testing remains highly sensitive but lacks specificity, particularly at low titers, limiting its standalone diagnostic utility in low–to–moderate pretest probability settings. In contrast, disease-specific antibodies such as anti-dsDNA and anti-SSA provide stronger rule-in value but insufficient sensitivity to reliably exclude future disease development. Approximately one in six undiagnosed individuals progressed to confirmed CTD during follow-up, underscoring the importance of longitudinal clinical surveillance. These findings support a risk-stratified, clinically guided approach to autoimmune testing rather than indiscriminate screening. Future prospective, multicenter studies integrating quantitative antibody profiles with clinical and molecular biomarkers are needed to improve early risk prediction and optimize cost-effective diagnostic pathways in this diagnostically challenging population.

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