

Breaking Through Diagnostic Barriers

**Advancing Precision Diagnosis of
Seronegative Rheumatoid Arthritis**

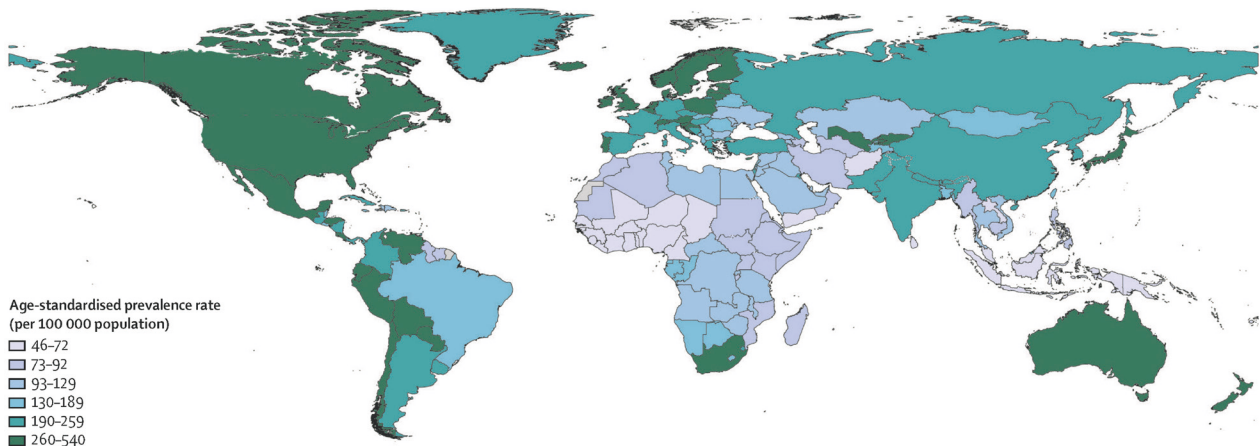


01

Global Public Health Challenge: High Disability Burden and Low Remission Rates in Rheumatoid Arthritis



Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic, destructive inflammatory arthritis. According to the Global Burden of Disease (GBD) study, approximately 17.6 million individuals worldwide were living with RA in 2020, and this number is projected to increase to 31.7 million by 2050. In 2020, RA accounted for approximately 3.06 million disability-adjusted life years (DALYs) globally, with more than 75% attributable to long-term functional impairment. RA is not a rare disease affecting only a minority of individuals, characterized by high disability burden, chronic progression, and substantial socioeconomic costs, RA has emerged as a major public health challenge worldwide¹.



02

The Diagnostic Gap: Why Conventional Serology Misses Nearly One-Third of RA Patients



The Hidden Blind Spot of Conventional Serology in Early RA Diagnosis

Up to one-third of RA patients remain seronegative, causing delayed diagnosis and irreversible joint damage.



Currently, serological assessment of RA primarily relies on rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA, detected as anti-CCP). Both biomarkers have been incorporated into the 2010 ACR/EULAR classification criteria and serve as important components of clinical evaluation and diagnosis². However, approximately one-third of RA patients lack detectable RF and ACPA, and are classified as having seronegative rheumatoid arthritis (SNRA)³. These patients do not have a milder disease phenotype, they experience persistent inflammatory activity and joint damage, yet face delayed diagnosis due to the lack of specific serological evidence, potentially missing the optimal window for early intervention⁴.

Delayed Diagnosis: A Risk Factor for Irreversible Joint Damage in Seronegative RA

Joint damage caused by RA is irreversible. Up to 50% of patients develop bone erosion within two years of disease onset, and the disability rate reaches as high as 75% within five years. For patients with seronegative RA, the absence of serological evidence further increases the risk of delayed diagnosis. Missing the window for early intervention may mark the transition from a manageable disease state to irreversible outcomes—joint deformity, functional impairment, reduced work capacity, and ultimately long-term disability. The challenge is not that the disease cannot be detected, but that it may be identified too late⁵.

Therefore, the discovery of novel biomarkers and improvement of detection rates in seronegative RA have become critical needs for precision diagnosis of RA.

03

Four Breakthrough Strategies Beyond Conventional Serology

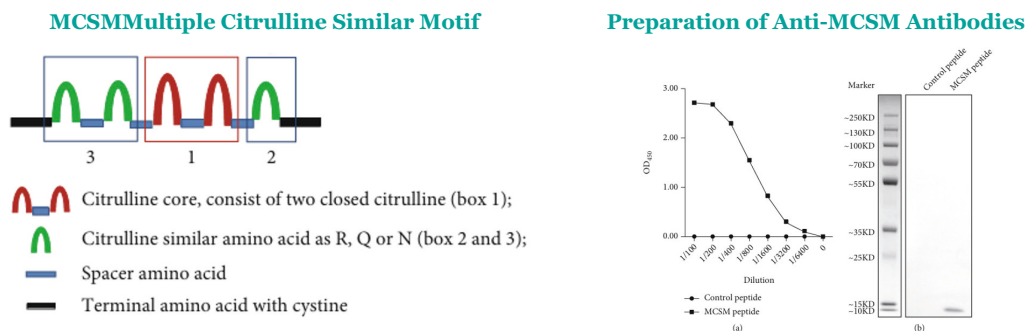
High-Density Peptide Microarray and Multi-Epitope Strategy: Overcoming the Limitations of Anti-CCP2 Detection

Table 2. Comparison of the diagnostic performance of the CCP2 assay and an 8-epitope biomarker for the initial and validation cohorts*

	No. of controls	No. of RA patients	Specificity(TN/N)*	Sensitivity(TP/P)†
Initial cohort				
CCP2 assay	29	63	0.966 (28/29)	0.683 (43/63)
8-epitope biomarker	29	63	0.966 (28/29)	0.921 (58/63)
Validation cohort				
CCP2 assay	54	127	0.963 (52/54)	0.535 (68/127)
8-epitope biomarker	54	127	0.944 (51/54)	0.850 (108/127)

Ken C. Lo et al.⁶ developed a high-density peptide array covering all 20246 human proteins and comprising more than 4.6 million peptide sequences. By incorporating both citrullinated and homocitrullinated peptides, the platform enabled unbiased profiling of the serum autoantibody repertoire in RA. Using an initial cohort of 92 patients and an independent validation cohort of 181 patients, the study established a novel diagnostic model based on an 8- epitope biomarker. The model achieved a sensitivity and specificity of 92.1% and 96.6%, respectively, in the initial cohort, and 85.0% and 94.4%, respectively, in the validation cohort, demonstrating higher sensitivity than the anti-CCP2 assay. Notably, the model successfully identified 40 anti-CCP2-negative RA patients, suggesting that diagnostic strategies based on multi-epitope combination can substantially improve the sensitivity of conventional serological testing. This approach provides an important technological framework for the discovery of novel biomarkers for RA.

◆ A Paradigm Shift in RA Detection: Direct Antigen Targeting with MCSM Achieves 95.3% Sensitivity

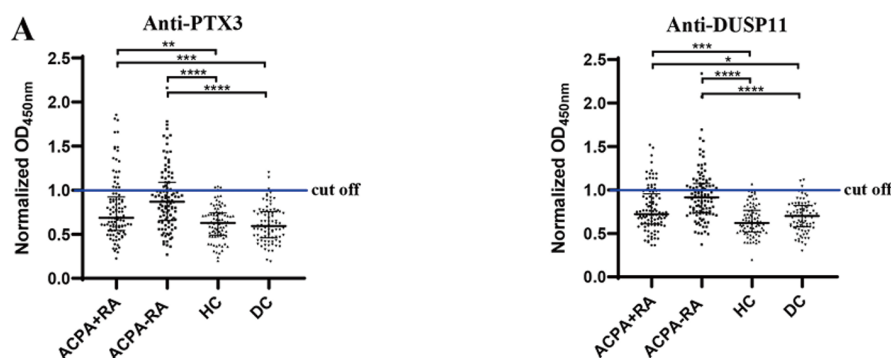


Zhu Chen et al.⁷ shifted the conventional RA diagnostic paradigm from detecting autoantibodies to directly identifying disease-associated antigens. The study developed synthetic peptides containing multiple citrulline-like motifs (MCSM) and generated anti-MCSM antibodies for the direct detection of citrullinated antigens carrying MCSM motifs in serum. A total of 106 RA patients, 48 patients with other arthritic diseases, and 41 healthy controls were enrolled in the study.

The results demonstrated that MCSM achieved a sensitivity of 95.3%, the area under the curve (AUC) reached 0.948, which further increased to 0.971 when combined with anti-CCP. Among anti-CCP-negative and RF-negative RA patients, 15/20 and 19/24 cases, respectively, tested positive for MCSM. This study represents the first attempt to extend the diagnostic target of RA from autoantibodies to citrullinated antigens, providing a novel detection strategy for the adjunctive diagnosis of seronegative RA.

◆ Protein Microarray Profiling: Anti-PTX3 + Anti-DUSP11 Combination for Identifying 38% of ACPA-Negative RA Patients

Positive Rate in ACPA-Negative RA: 27.56% **Positive Rate in ACPA-Negative RA: 31.80%**



Xuan Zhang et al.⁸ developed a three-stage protein microarray strategy to discover and validate novel autoantibodies. In the first stage, a high-density protein microarray containing 21827 recombinant human proteins was used for comprehensive profiling of serum samples from 120 participants. In the second stage, candidate autoantibodies were validated using a focused protein microarray in an independent cohort of 487 participants. In the third stage, further confirmation was performed using ELISA and Western blot assays in 404 additional samples. The study identified anti-PTX3 and anti-DUSP11 autoantibodies as significantly enriched in ACPA-negative RA patients, with positive rates of 27.56% and 31.80%, respectively. Both markers demonstrated significantly higher positive rate compared with healthy controls and disease controls ($P < 0.0001$). The combination of anti-PTX3 and anti-DUSP11 antibodies enabled the identification of 38.0% of ACPA-negative RA patients, with a specificity of 88.72%. This study represents the first systematic identification of non-modified protein autoantibodies in ACPA-negative RA, providing a novel biomarker combination for diagnosis and suggesting that innate immune-related pathways may contribute to disease pathogenesis in this patient population.

◆ **Novel Citrullinated Autoantigen CSP Addresses the Diagnostic Gap in Anti-CCP-Negative RA**

Supplementary Table 2. The diagnostic value of emerging biomarkers alone or in combination with anti-CCP in RA.

Biomarkers	Alone			In combination with anti-CCP			References
	Sensitivity (%)	Specificity (%)	Positive rate in anti-CCP-RA(%)	Sensitivity (%)	Specificity (%)	Positive rate in anti-CCP-RA(%)	
Anti-CSP	58.98	93.96	33.06	84.83	92.43	33.06	-
Anti-CarP	44.00	88.00	10.40	76.00	82.42	10.40	(23)
Anti-CEP-1	61.40	90.19	20.80	81.19	85.43	20.80	(26)
Anti-MCV	62.00	93.90	15.79	68.00	-	15.79	(25)
Anti-AAPA	36.60	86.29	13.20	-	-	-	(24)
Anti-PAD4	35.00	95.00	19.00	-	-	-	(27)
Anti-GPI	75.00	64.30	-	85.70	64.30	-	(28)
14-3-3n	63.00	90.00	-	83.00	87.00	-	(29)
SR-A	66.40	91.50	42.60	86.70	90.10	42.60	(16,18)

Based on their previous studies of SR-A⁹, the teams led by Zhan-Guo Li and Fanlei Hu¹⁰ from Peking University People’ s Hospital further identified potential autoantigenic epitopes within the SR-A protein through B-cell autoantigenic epitopes prediction and molecular simulation. Following citrullination modification, these epitopes were developed into a novel antigen, CSP. A subsequent multicenter study enrolled a total of 1954 participants.

Among 646 RA patients and 1308 controls, anti-CSP demonstrated a diagnostic sensitivity of 58.98% and specificity of 93.96%. Notably, anti-CSP positivity rate reached 37.97% (60/158) among anti-CCP-negative RA patients. Furthermore, combined detection of anti-CSP and anti-CCP achieved an area under the curve (AUC) of 0.908 and increased diagnostic sensitivity to 84.83%, significantly improving the identification capability of conventional anti-CCP testing. This study established a complete translational pipeline from epitope discovery → diagnostic validation → functional evaluation, providing a novel strategy to overcome the diagnostic limitations of seronegative RA.

04

DiYaLab Collaborates with Peking University People’s Hospital: Building a Translational Pathway for Precision RA Diagnostics

DiYaLab focuses on the development of autoantibody detection assays for autoimmune diseases, and has established an academia-industry collaboration with the team of Professor ZhanGuo Li and Professor Fanlei Hu at Peking University People’ s Hospital to accelerate the development and clinical translation of novel RA biomarkers. Leveraging CLIA and TRFIA technology platforms, DiYaLab is advancing the discovery, validation, and clinical implementation of emerging diagnostic markers for RA.

Building on dual technology platforms of chemiluminescence immunoassay (CLIA) and time-resolved fluorescence immunoassay (TRFIA), Diyalab has developed a comprehensive diagnostic portfolio covering key autoantibody profiles associated with RA diagnosis.

DiYaLab CLIA Platform		
RF	Anti-CCP	RF-IgM*
RF-IgG	Anti-MCV	
RF-IgA	Anti-RA33*	

* Registration in process

DiYaLab TRFIA Platform	
RF	ASO
RF-IgG/IgM/IgA	Anti-MCV
Anti-CCP	

Reference

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