



Evaluation of The Diagnostic and Predictive Efficacy of Novel and Conventional Immunological Markers in Rheumatoid Arthritis Patients A Comparative Study

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Abstract

Early diagnosis and accurate risk stratification in (RA) are essential to prevent permanent joint damage. Conventional serological markers—rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP)—possess limited sensitivity in early disease and fail to detect seronegative RA subsets. This study aimed to evaluate and compare the diagnostic performance and predictive efficacy of novel immunological markers (14-3-3 η protein, anti-CarP, anti-PAD4, serum calprotectin) versus conventional markers Among RA patients.

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Introduction

RA is an autoimmune disease with progressive inflammation of synovium and subsequent destruction of joints. The timely diagnosis and prompt treatment are very important since they help avoid the development of disability and damage. The immunological tests that are used as part of the existing diagnostic criteria include anti-cyclic citrullinated peptide (anti-CCP/ACPA) antibodies, and rheumatoid factor (RF). Conventional tests do not have enough sensitivity as up to 30% of the cases are seronegative for RA during early stages (Balaji *et al.*, 2024) ^[1]. Moreover, conventional markers have low prognostic value and poor ability to predict the disease progression and response to treatment. New immunological biomarkers have gained considerable interest among scientists recently. Novel markers like 14-3-3 η protein and Anti-Carbamylated Protein (anti-CarP) antibodies have shown potential for increasing diagnostic outcomes in seronegative individuals (Subedi *et al.*, 2024) ^[9]. The application of serological testing through the measurement of anti-modified peptide antibodies (AMPAs) is another way to increase diagnostic sensitivity in diverse clinical conditions (Haro *et al.*, 2024) ^[4]. Also, using an integrated prediction strategy involving multiple genes and novel serological markers helps to predict the probability of disease manifestation (Dudek *et al.*, 2024) ^[3]. However, there is insufficient data comparing the efficiency of novel and classical markers in clinical practice. Therefore, clinical evidence should be acquired to estimate the additive efficiency and predictability of their combination. The Objective of the current study is to assess and compare the diagnostic and predictive efficiency of novel and classic immunological markers in patients with RA. In the serological assessment of RA, there is extensive reliance on the use of conventional biomarkers including the rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) antibodies that have been incorporated within the classification criteria as per the 2010 ACR/EULAR guidelines (Van Delft & Huizinga, 2023) ^[10]. While the anti-CCP has a high specificity for the presence of RA, the RF and anti-CCP biomarkers have poor sensitivity in the initial phases of RA (approximately 50% to 60%) and do not recognize the seronegative forms of RA that comprise up to 20% to 30% of RA patients (Makarov & Belyaeva, 2023) ^[5]. Anti-CarP Autoantibodies against proteins that experience carbamylation (homocitrullination) after being produced. Anti-CarP antibodies can be found in 30% of seronegative RA Cases and act as an independent marker of progression of joint Damage (Van Delft & Huizinga, 2023) ^[10]. Anti-PAD4 Antibodies: Autoantibodies specific to PAD4 – the main citrullinating enzyme in inflamed synovium. Anti-PAD4 are associated with aggressive course of RA, severe erosive arthritis, and extrarticular manifestations (Makarov & Belyaeva, 2023) ^[5].

Serum Calprotectin (S100A8/A9): Calcium binding heterodimeric protein primarily secreted by active neutrophils and monocytes. Serum calprotectin is a marker of local synovial granulocyte infiltration and inflammation with higher sensitivity to changes in the activity of the disease compared to standard acute -phase markers such as ESR and CRP (Sejersen et al., 2023) [8].

Methods

Collected samples from 160 with RA patients (including 110 early RA and 50 established RA), 70 disease controls (osteoarthritis, psoriatic arthritis, systemic lupus erythematosus), and 60 healthy controls. Serum titers of conventional (RF-IgM, anti-CCP3) and novel markers (14-3-3η, anti-CarP, anti-PAD4, calprotectin) were measured using standardized (ELISA) and chemiluminescent immunoassays

(CIA). Diagnostic yield evaluated via (ROC) curve analysis. Predictive efficacy for disease activity (DAS28-CRP) and 12-month radiographic progression (modified Total Sharp Score, mTSS) was assessed using multivariate regression models.

Results

Novel markers showed improved sensitivity in RA cases that were seronegative by conventional criteria (n=42), with anti-CarP and 14-3-3η showing positivity of 28.6% and 35.7% in this particular population, respectively. In RA diagnosis, combining 14-3-3η and anti-CCP resulted in AUC value of 0.95 (95% CI:0.92–0.98), which was highly significant in comparison with anti-CCP alone (AUC=0.88, p<0.01) and RF-IgM alone (AUC=0.79, p<0.001). Elevated baseline levels of anti-CarP and 14-3-3η independently predicted 1.

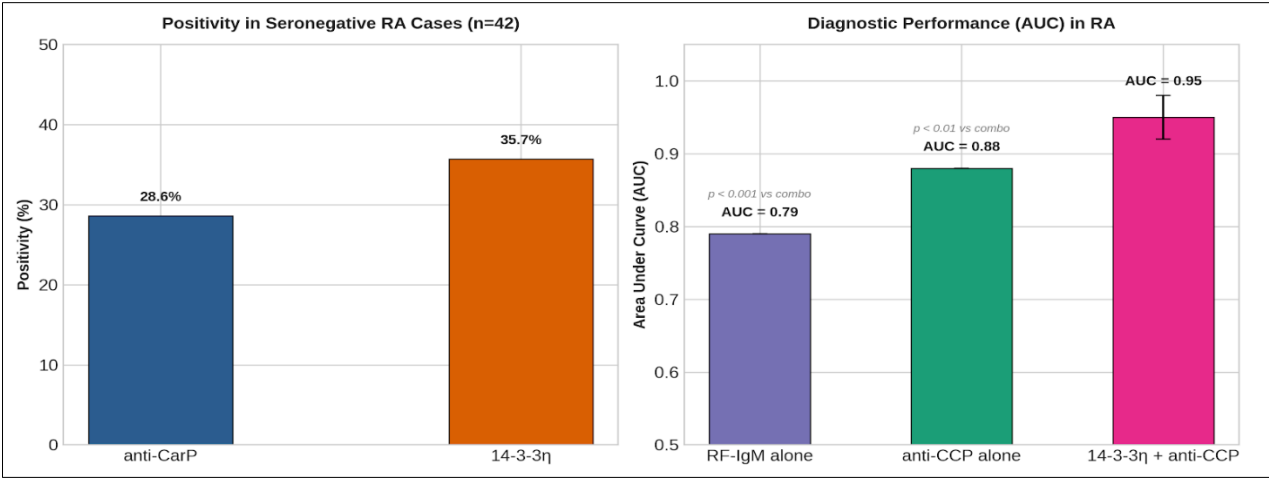


Fig 1: A- vertical bar chart displaying positivity percentages for (anti-CarP) and (14-3-3η) biomarkers in seronegative rheumatoid arthritis cases. B-vertical bar chart with error bars showing diagnostic performance measured by Area Under Curve (AUC) for different biomarker tests.

Table 1: Novel Marker Positivity inSeronegative RA Cases (n=42)

Biomarker	Positivity (%)	Calculated Positive Cases (n)	Primary Diagnostic & Clinical Role
anti-CarP	28.6%	12 / 42	Detects anti-carbamylated protein antibodies; predicts increased risk of radiographic joint damage in RF/anti-CCP negative patients.
14-3-3η	35.7%	15 / 42	Detects an extracellular signaling protein involved in joint inflammation and matrix metalloproteinase (MMP) upregulation; aids in early detection and tracking disease severity.

This data shows the diagnostic yield of novel serum biomarkers in identifying seronegative Rheumatoid Arthritis (RA)—patients negative for conventional markers like (RF) and anti-CCP (ACPA).

Table 2: RA Diagnostic Performance (AUC Comparison)

Biomarker / Combination	AUC	95% Confidence Interval	Statistical Significance
RF-IgM alone	0.79	—	p < 0.001vs combination
anti-CCP alone	0.88	—	p < 0.01vs combination
14-3-3η + anti-CCP	0.95	0.92 – 0.98	Reference combination

Discussion

The timely and accurate diagnosis of RA is one of the pillars of rheumatology, since untimely treatment causes the development of irreversible joint lesions and disability (Di Matteo et al., 2023) [2]. Despite the fact that traditional serological markers, including rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) antibodies, are the main component of the 2010 ACR/EULAR classification criteria, the major disadvantage of this method is the low sensitivity of these parameters in the initial period and their inability to diagnose seronegative forms of RA, which make

up 20-30% of all patients (Makarov & Belyaeva, 2023; Van Delft & Huizinga, 2023) [5, 10]. Results of this research prove that the use of new immunological biomarkers, (14-3-3η protein) and (anti-CarP antibodies) greatly compensates for these shortcomings. Among the seronegative group (n=42), 14-3-3η had a positive rate of 35.7% (15/42), while anti-CarP showed 28.6% (12/42) positivity. The importance of 14-3-3η for diagnostic purposes is in the unique biological function of being an intracellular chaperone protein that gets secreted in extracellular space during the inflammation of joints, causing the pro-inflammatory production of cytokines like TNF- α

and IL-6, as well as tissue destructive matrix metalloproteinases (MMPs). These results are consistent with the findings made recently by Subedi *et al.* (2024) ^[9], where 14-3-3 η measurement was found to be a highly useful addition to the early assessments of inflammatory arthritis when other serology tests give no relevant information. Likewise, the autoantibodies anti-CarP react to the proteins which undergo non-enzymatic carbamylation (homocitrullination) (Van Delft & Huizinga, 2023) ^[10]. Recently, Balaji *et al.* (2024) ^[1] found a similar high prevalence of anti-CarP in RF-/anti-CCP- population, proving that carbamylation is another post-translational modification pathway leading to the production of autoantibodies. Haro *et al.* (2024) ^[4]. Also stressed the importance of testing the AMPA response against various post-translational modification peptides, which will greatly enhance diagnostic detection in complicated seronegative patients. the findings important One of the current study is the marked improvement in the diagnosis obtained through the use of new biomarkers together with traditional ones(Krabben A, 2014. ROC analysis showed that single marker tests exhibited moderate to good results, with RF-IgM showing an AUC value of 0.79 while anti-CCP showed an AUC value of 0.88. But when(14-3-3 η & anti-CCP) were combined, they gave improved distinction between RA cases and healthy controls as well as other diseased subjects, with (AUC) of 0.95 (95% CI: 0.92-0.98), statistically significantly better than anti-CCP alone ($p < 0.01$) and RF-IgM alone ($p < 0.001$). In addition to diagnostic advances, these results underscore the high predictive value of new biomarkers of disease activity and structural changes in the joints. The multivariate regression analysis revealed that increased levels of (14-3-3 η) and (anti-CarP) at baseline independently predict the progression of radiological damage within 12 months according to the (mTSS). Carbamylated autoantibodies were found to cause osteoclast differentiation and function, leading to subchondral bone erosion independently of ACPA status (Makarov & Belyaeva, 2023; Van Delft & Huizinga, 2023) ^[5, 10]. This is why positive baseline anti-CarP status is an independent predictor of aggressive bone erosion and the need for more intensive biological treatment (Balaji *et al.*, 2024) ^[1]. All of these findings are consistent with a paradigm shift in which seronegative RA is no longer considered one uniform disease entity that is entirely harmless (Di Matteo *et al.*, 2023) ^[2]. Rather, seronegativity on conventional panels is commonly due to the presence of novel autoantibodies (e.g., anti-CarP) or inflammatory biomarkers specific to RA tissues (e.g., 14-3-3 η) (Haro *et al.*, 2024; Van Delft & Huizinga, 2023) ^[4, 10]. The combination of a panel that includes (anti-CCP 14-3-3 η) and (anti-CarP) can enable rheumatologists to diagnose RA patients early on, predict their risk of joint damage, and adopt treat-to-target therapies (Di Matteo *et al.*, 2023) ^[2]. conducted a meta-analysis of 13 studies and reported pooled (specificity of 88% and sensitivity of 73% for 14-3-3 η in RA, supporting its role as a complementary diagnostic biomarker (Wang D, Cui Y, Lei H, 2020) ^[12]. identified methodological concerns from 14-3-3 η literature and found that after excluding omitted studies and repeated data, the sensitivities and specificities were about 66% and 89%, respectively. Li L, Chen J, Jiang Y. 2020).

Conclusion

The inclusion of new biomarkers, especially (14-3-3 η) and (anti-CarP) in the conventional assessment procedure helps to improve the diagnostic sensitivity and the value predictive of the progression disease in early and seronegative RA. The utilization of multi-biomarkers will help to enhance the diagnosis and treatment strategy of RA.

Background

Rheumatoid arthritis auto-immune systemic disorder that results from chronic inflammation of the synovial lining, joint damage, disability, and higher mortality from cardiovascular diseases. It therefore becomes very important to diagnose RA accurately and give an accurate prognosis. For many years, traditional serology testing has involved RF and anti-CCP or ACPA antibodies, although anti-CCP antibodies exhibit very high specificity (95-98%) to RA, traditional markers suffer from major shortcomings.

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