

ANTIBODIES AGAINST CITRULLINATED PROTEINS IN PATIENTS WITH EARLY RHEUMATOID ARTHRITIS: RELEVANCE OF LIKELIHOOD RATIOS

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ABSTRACT

Background: Antibodies against citrullinated proteins (ACPA) and rheumatoid factor (RF) are part of the classification criteria for the diagnosis of rheumatoid arthritis (RA) and are useful in the diagnosis and prognosis of RA. **Objective:** To measure serum ACPA and RF levels in patients with early RA (up to 12 months)—including very early RA (<3 months)—who had not received treatment with disease-modifying antirheumatic drugs. Ninety patients with other autoimmune rheumatic diseases served as controls. **Materials and Methods:** There were 269 patients (226 women and 43 men) with a median age of 50 years seen at our hospital between 2012 and 2022. ACPA levels were measured using chemiluminescence (cut-off >17 U/mL) and RF levels were primarily measured using latex agglutination tests (cut-off ≥ 160). Sensitivity, specificity, likelihood ratios (LRs) and CI95% were calculated. **Results:** 215 (80%) patients tested positive for ACPA; specificity was 93% and the positive LR was 11.9 (excellent clinical utility). The sensitivity of the ACPA+RF combination was 43%, with 93% specificity and an LR of 6.3 (good clinical utility). RF sensitivity and specificity were 62% and 67%, respectively, with a positive LR of 1.8 (poor clinical utility). Among the 90 controls, 6 (7%) tested positive for both ACPA and RF ("rhus"), and 33 tested positive for RF. **Conclusion:** ACPA levels in early RA demonstrated high sensitivity, specificity, and LR >10. The combination of ACPA and RF offers good clinical utility compared to testing for RF alone.

KEYWORDS: Early rheumatoid arthritis, anti-citrullinated protein antibodies, evaluation.

INTRODUCTION

The 2010 ACR/EULAR rheumatoid arthritis classification criteria include four weighted variables: symmetric arthritis predominantly of small joints, ≥ 6 weeks of disease duration, acute phase reactants (CRP or ESR) and anti-citrullinated protein antibody (ACPA) and rheumatoid factor (RF).^[1] ACPA positive-rheumatoid arthritis (RA) is more closely related to HLA-DRB1 alleles with a shared epitope than RF positive RA.^[2,3] ACPA-negative RA are considered a different subclass

from ACPA positive RA.^[4] Both serological markers precede the onset of disease symptoms by many years, named subclinical autoimmunity^[5,6] indicating that citrullination and RF production occur in very early stages of RA.

The oldest and best-known autoantibody is RF, but it is not very specific for RA. Healthy individuals, those over 65 years of age, those with chronic infections, or those with other autoimmune inflammatory diseases can have

positive RF. In 1964, a new antibody, anti-perinuclear factor, was described, which targets citrulline residues, but for many reasons, it is not routinely used as a serological marker. Autoantibodies reactive with synthetic peptides containing the amino acid citrulline, a post-translational modified arginine residue, are specifically present in the serum of patients with RA. Using peptide variants containing deaminated arginine (citrulline) as an antigenic substrate, Schellekens et al.^[7,8] developed an ELISA test for the detection of ACPA. The study revealed a sensitivity of 68% and a specificity of 98%. In a group of patients from the Early Arthritis Clinic, Schellekens' group achieved a specificity of 96%, compared to 91% for RF IgM by ELISA. The sensitivity of both tests was 48% for ACPA and 54% for RF. The combination of ACPA and RF showed a positive predictive value (PPV) of 91% and a negative predictive value (NPV) of 78%.

Due to a lack of data on ACPA diagnostic tests in early RA and very early RA in the Mexican population, the author conducted a study to evaluate sensitivity, specificity and likelihood ratios (LRs).^[9-10] The terms early RA and very early RA are variable. It is not well established what constitutes early.^[11]

METHODS

269 patients with early RA (≤ 12 months) including 59 patients with very early RA (≤ 3 months) were evaluated. Serum level of ACPA was measured by chemiluminescence (cut-off >17 U/ml, COBAS 6000) outside the hospital and in the same laboratory, and RF was measured by the latex agglutination test (cut-off point ≥ 160). The likelihood ratio (LR) was used as part of the diagnostic test parameters. A LR > 10 has excellent clinical utility, 5-10 good, 3-5 fair, and <2 poor. Ninety patients with autoimmune diseases (30 with lupus, 44 with psoriasis and psoriatic arthritis, 2 with scleroderma, 4 with Sjogren's disease, 4 with myositis, and 6 with rhus) served as control.

Statistical analysis

Diagnostic performance was assessed by sensitivity, specificity, positive-negative likelihood ratio (LR) and CI95%. Statistics were performed using Murcia+Salud Calculadora clínica. Parámetros de una prueba diagnóstica. <https://www.murciasalud.es/web/recursos-humanos>.

RESULTS

Figure 1 and 2 show the results and the impact on the diagnostic utility of ACPA, RF and the combination ACPA+RF in individuals with total RA (≤ 12 months), including 59 with very early RA ≤ 3 months. Based on the cut-off point proposed by the manufacturer, the sensitivity of the ACPA assay was of 80% and specificity of 93% with positive LR of 12 (excellent utility). The diagnostic sensitivity and specificity of RF assay was 62% and 64% with LR 1.7. The sensitivity and specificity of the combination of positive ACPA and RF

was 43% and 93% with positive LR of 6.4. Of the 90 patients with other autoimmune diseases, 6 had rhus with elevated ACPA and RF levels, and RF test was positive in 33 patients.

DISCUSSION

In 2007, Nishimura et al.^[12] synthesized the sensitivities, specificities, and positive and negative LR of 37 ACPA studies and 50 RF studies. The sensitivity and specificity for ACPA were 67% and 95%, and positive and negative LR were 12.4 and 0.36, and for RF they were 69%, 85%, 4.8, and 0.38, respectively. According to the authors, many studies had methodological limitations. The RF analysis was heterogeneous and had wide ranges of sensitivity and specificity. In summary, the meta-analysis found a specificity for ACPA of 95% versus 85% for RF. A study published in the Colombian Journal of Rheumatology^[13] evaluated the clinical utility of ACPA using electrochemiluminescence and the detection of RF by immunoturbimetry. The researchers included 136 subjects (68 with RA with symptom duration of 16 months, range 2–168, $57\% \leq 12$ months) and 68 controls. The cut-off point was 11.3 U/ml obtained by ROC curve analysis. The sensitivity, specificity, and PPV of ACPA were 81%, 92%, and 90%, respectively, similar to the results with RF, except for the specificity of 90%. The positive LR was 6.3 (good utility).

In this study, early RA, including 59 patients with very early RA showed a diagnostic sensitivity and specificity of ACPA of 80% and 93% with a positive LR of 12. The predictive values depend on prevalence, while LR depend on sensitivity and specificity and how many times more likely a subject with a positive test is to have the disease. The positive LR for ACPA was >10 (excellent utility). In contrast, the diagnostic sensitivity and specificity for RF was 62% and 64% and positive LR of 1.7 (bad utility). The combination ACPA and RF showed LR of 6.4 (good utility).

CONCLUSION

RA is an autoimmune disease characterized by the formation of autoantibodies that function as disease markers. Their pathogenicity has not been demonstrated. ACPA measurement in RA has high specificity and an excellent positive LR. The LR is an alternative to PPV and NPV for estimating likelihood after diagnostic testing. The higher the LR, the more likely a patient with a positive test has the disease, and the lower the negative LR, the more likely a patient with a negative test does not have the disease. A positive ACPA and rheumatoid factor (RF) combination may be more useful for diagnosis than RF determination alone.

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