Diagnostic Value of Anti-Sa and Anticitrullinated Protein Antibodies in Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** To determine the diagnostic value of anticitrullinated protein antibodies, second generation (ACPA2), by electrochemiluminescent immunoassay (ECLIA) and anti-Sa by ELISA in a large cohort of Chinese patients with early rheumatoid arthritis (RA).

Methods. One hundred ninety-eight patients with early RA (< 1 yr duration), 112 with other rheumatic diseases, and 60 healthy individuals were studied.

Results. The combination of anti-Sa and ACPA2 positivity had the highest specificity (99.42%), but it had a rather low sensitivity (50.0%). The combination of anti-rheumatoid factor (RF) and ACPA2 showed the highest sensitivity (80.30%), with specificity of 95.93%. The mean titer of ACPA2 and RF was significantly higher in the anti-Sa-positive group compared to the negative group (ACPA2, $p < 0.001$; RF, $p = 0.007$). The 28-joint Disease Activity Scores of the anti-Sa-positive patients were significantly higher than those of the negative group ($p = 0.01$). The anti-Sa had no significant correlation with age, sex, antinuclear antibody, SSA, SSB, erythrocyte sedimentation rate, C-reactive protein, immunoglobulin A (IgA), IgG, IgM, C3, and C4.

Conclusion. Our results come from a newly developed ECLIA for detection of ACPA2 and the anti-Sa-antibody-based ELISA system. The combined application of ACPA2 and anti-Sa tests can improve the laboratory diagnosis of early RA. (J Rheumatol First Release July 15 2012; doi:10.3899/jrheum.111523)

Key Indexing Terms:
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Rheumatoid arthritis (RA) is characterized by the development of a persistent, destructive synovitis that targets multiple joints. Vimentin is secreted and citrullinated by macrophages depending on the proinflammatory signals[1,2]. Several studies have shown that antibodies targeting the citrullinated antigens anti-Sa and anticitrullinated protein antibodies (ACPA) are very specific for diagnosis of RA[3,4,5,6], occurring at very early stages of the disease. Our aim was to determine the diagnostic value of ACPA2 (second generation) by electrochemiluminescent immunoassay (ECLIA) and anti-Sa by ELISA in patients with early RA.

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MATERIALS AND METHODS

Study population. Serum samples were obtained from 198 patients with RA and 112 patients with other rheumatic diseases. All patients with RA fulfilled the 2010 American College of Rheumatology/European League Against Rheumatism criteria[7]. The 112 patients with other rheumatic diseases served as disease controls. They included 26 patients with systemic lupus erythematosus (SLE), 23 with primary Sjögren's syndrome (pSS), 11 with systemic sclerosis, 11 with polymyositis or dermatomyositis, 28 with ankylosing spondylitis, 5 with mixed connective tissue disease (MCTD), and 8 with Behçet's disease. All patients gave informed consent. Sixty serum samples from blood donors were used as healthy controls. The duration of disease was < 1 year in the 198 patients with RA (150 women, 48 men).

Antibody measurements. Anti-Sa antibody measurements were performed using a commercial ELISA kit (Euroimmun, Lubeck, Germany). The Elecsys® ACPA2 ECLIA from Roche Diagnostics (Mannheim, Germany) was determined by a fully automated ECLIA processing system, Ecobas e601. RF [immunoglobulin M (IgM)] in serum was measured by the rate of nephelometry (Immage; Beckman Coulter, Fullerton, CA, USA).

Clinical and laboratory measures. The following clinical and laboratory data were collected: age, sex, disease duration, number of swollen joints, number of tender joints, 28-joint Disease Activity Score (DAS28), IgG, IgM, IgA, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), complement (C3, C4), antinuclear antibody (ANA), SSA, and SSB.

Statistical methods. Analyses were performed using SPSS for Windows, version 13.0. Data were expressed as the mean ± SD, and differences between groups were analyzed with the t-test. The chi-square test was used to compare qualitative variables between groups. Correlations were tested by Pearson's correlation test. Statistical significance was set at $p < 0.05$.

RESULTS

Comparison between RA and disease control. There was no significant difference in age, sex, ESR, and CRP between patients with RA and patients without RA (Table 1). In patients with RA, the mean serum anti-Sa, ACPA2, and RF levels were significantly higher compared to patients with other rheumatic diseases ($p < 0.05$).

Sensitivity and specificity of antibodies. RF had the highest sensitivity in these antibodies. It was positive in 89.39% (177/196) of the cases of early RA and in 29.65% (51/172) of controls. Anti-CCP2 was positive in 80.8% (160/196) of cases of early RA and in 5.81% (10/172) of controls, including 3 patients with SLE, 3 with pSS, 2 with myositis, 2 with MCTD, and none of the healthy individuals. The sensitivity of ACPA was 80.8% and the specificity 94.18%. Anti-Sa antibody showed the highest specificity (98.02%) and only 1 control with SLE showed anti-Sa positive, but it had a lower sensitivity (50%; Table 2).

Combination of anti-Sa, ACPA2, and RF (IgM). In our study, the combination of anti-Sa and ACPA2 positivity had the highest specificity (99.42%) and positive predictive value (99%), but it had a rather low sensitivity (50.0%). The combination of anti-RF and ACPA2 showed the highest sensitivity (80.30%), with a specificity of 95.93% (Table 3).

Associations between anti-Sa and clinical features in RA. Among the 198 patients with early RA, there were no significant differences between the 99 who were anti-Sa-negative and the 99 who were anti-Sa-positive with respect to age, sex, ANA, SSA, SSB, ESR, CRP, IgA, IgG, IgM, C3, and C4. The mean titer of ACPA2 and RF was significantly higher in the anti-Sa-positive group compared to the negative group (ACPA2, 342.39 ± 154.39 U/ml vs 188.79 ± 217.64 U/ml, $p < 0.001$; and RF, 696.82 ± 1178.67 U/ml vs 188.79 ± 217.64 U/ml, $p = 0.007$). The DAS28 scores of the anti-Sa-positive patients were significantly higher than the negative group (4.96 ± 1.22 vs 4.70 ± 0.97; $p = 0.01$).

Both anti-Sa and ACPA2 belong to the family of antibodies against citrullinated antigens. A significant correlation was found between ACPA2 and anti-Sa levels in RA ($R = 0.766$; $p < 0.001$).

Table 1. Clinical and laboratory characteristics of the patients and controls. All data are mean ± SD unless otherwise indicated.

Characteristics	Patients with RA	Disease Controls	p
Age, yrs	47.37 ± 13.63	45.71 ± 14.93	0.485
Women, n	150	77	0.185
ESR, mm/h	46.17 ± 21.37	42.91 ± 18.35	0.531
CRP, mg/dl	19.52 ± 15.19	17.52 ± 12.33	0.421
RF, U/ml	451.59 ± 99.84	50.27 ± 61.04	0.005*
ACPA2, U/ml	277.16 ± 192.67	6.85 ± 0.86	< 0.001*
Anti-Sa, U/ml	54.01 ± 71.69	6.42 ± 8.07	< 0.001*

* P values < 0.05 were considered statistically significant. RA: rheumatoid arthritis; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; RF: rheumatoid factor; ACPA2: anticitrullinated protein antibodies, second generation.

Table 2. Sensitivity, specificity, and predictive values of antibodies for the diagnosis of rheumatoid arthritis. All data are percentages.

	RF	ACPA2	Anti-Sa
Sensitivity	89.39	80.80	50
Specificity	70.34	94.18	98.84
PPV	77.63	94.12	98.02
NPV	85.21	85.38	63.19

ACPA2: anticitrullinated peptide antibodies, second generation; RF: rheumatoid factor; PPV/NPV: positive/negative predictive value.

Table 3. Diagnostic sensitivity and specificity of anti-Sa, ACPA2, and RF combination assays using optimal cutoff values. All data are percentages.

	ACPA2 + Sa	Sa + RF	RF + CCP2
Sensitivity	50	42.93	80.30
Specificity	99.42	98.84	95.93
PPV	99	97.70	97.55
NPV	63.33	60.07	81.16

ACPA2: anticitrullinated protein antibodies, second generation; RF: rheumatoid factor; PPV/NPV: positive/negative predictive value.

DISCUSSION

A number of reports have demonstrated the high diagnostic value of ACPA in the diagnosis of RA.

We determined the titer of ACPA2, anti-Sa antibodies, and RF in patients with RA compared with a control group. We found that patients with RA had higher titers of ACPA2, anti-Sa antibodies, and RF than did the disease controls ($p < 0.001$).

RF had the highest sensitivity compared to the other antibody, whereas anti-Sa antibodies had the highest specificity for early RA, in agreement with other studies. It has been reported that anticitrullinated vimentin antibodies have been detected by immunoblotting in 23%–43% of patients with RA[8,9]. Specificity of this assay varied between 94% and 99%. We measured with ELISA the serum anti-Sa levels in patients with RA and found that the sensitivity of anti-Sa (50%) was higher in patients with RA than that reported[8,9]. Detecting anti-Sa by ELISA is thus more sensitive than by immunoblotting[10].

We measured ACPA by the automated chemiluminescence enzyme immunoassay (CLEIA) processing system. It has been reported that no difference was observed in the sensitivity and specificity between CLEIA and ELISA for ACPA[11]. In our study, the sensitivity of ACPA was 80.8%, with specificity of 91.09%. The statistical values obtained in our study are similar to those previously described[12,13,14]. Out of all evaluated combinations, when anti-Sa antibodies were present in conjunction with ACPA2, it yielded higher diagnostic power in specificity (99.42%) than other combinations.

Our study suggests that there is a certain correlation between anti-Sa and ACPA2 in patients with early RA. This indicates that the target antigens of anti-Sa and ACPA2 may have some shared epitopes, or that one of the physiological targets of ACPA2 may be citrullinated vimentin. Further, we evaluated the associations between anti-Sa and clinical or laboratory variables of RA. Anti-Sa showed no significant statistical correlation with mostly clinical and laboratory factors such as ESR, CRP, ANA, SSA, SSB, IgA, IgG, IgM, C3, and C4. Anti-Sa was correlated with disease activity measures such as DAS28, serum ACPA2 levels, and RF levels.

Our results come from a newly developed ECLIA for detection of ACPA2 and anti-Sa antibody-based ELISA system. The anti-Sa and ACPA2 assay are very valuable tools for the diagnosis of early RA. The combined application of ACPA2 and anti-Sa tests can improve the laboratory diagnosis of early RA, thus promoting timely treatment.

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