Is It Necessary to Combine Detection of Anticitrullinated Protein Antibodies in the Diagnosis of Rheumatoid Arthritis?

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ABSTRACT. ***Objective.*** Antibodies against citrulline-containing epitopes, such as antiperinuclear factor (APF), antikeratin antibodies (AKA), antifilaggrin antibodies, and anticyclic citrullinated peptide (anti-CCP) antibodies, are specific in rheumatoid arthritis (RA). Detection of APF, AKA, and anti-CCP has been widely used in clinical practice. However, studies on combined detection of these anticitrullinated protein antibodies (ACPA) in the significance of diagnosing RA have been limited. We aimed to detect APF, AKA, and anti-CCP antibodies and to evaluate the significance of combined detection of these ACPA in RA.

Methods. A total of 551 patients with arthritic disorders, 304 with RA and 247 with other rheumatic diseases, were selected at the Department of Rheumatology and Immunology during the past 2 years. AKA and APF were tested by indirect immunofluorescence assay. Anti-CCP was detected using the second-generation ELISA kit.

Results. The sensitivities of anti-CCP, AKA, and APF tests for RA were 76.2%, 43.4%, and 34.5%, respectively, while the specificities were 96.0%, 98.4%, and 99.6%. The combination of anti-CCP, AKA, and APF positivity had the highest specificity (100%), but it yielded a low sensitivity (28.3%). When 2 of the 3 ACPA were positive, the sensitivity and specificity were 48.4% and 99.2%, respectively. When either anti-CCP or AKA or APF was positive, sensitivity increased to 77.3%, but specificity decreased to 94.7%.

Conclusion. Anti-CCP was the most valuable marker in the diagnosis of RA, among the 3 ACPA. Combined detection of anti-CCP, AKA, and APF did not increase the diagnostic capability for RA. (First Release Oct 15 2010; J Rheumatol 2010;37:2462–5; doi:10.3899/jrheum.100399)

Key Indexing Terms:
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Rheumatoid arthritis (RA) is a systemic chronic autoimmune disease characterized by synovial inflammation and joint destruction that often leads to joint deformity and disability. Early diagnosis and treatment is therefore key to improving both the prognosis and the patient's quality of life.

Autoantibody formation commonly occurs in RA. The value of these autoantibodies as diagnostic and/or prognostic tools continues to be debated. The best known antibody is rheumatoid factor (RF). The presence of RF is included in the 1987 American College of Rheumatology (ACR) classification criteria for RA[1], although it has a poor specificity in the diagnosis of RA. Antiperinuclear factor (APF)[2], antikeratin antibody (AKA)[3], anticyclic citrullinated peptide antibodies (anti-CCP)[4], and antimodified citrullinated vimentin[5] are other autoantibodies that have been shown to exist in the sera of patients with RA. These antibodies are all directed against epitopes of citrulline, so they are known as anticitrullinated protein antibodies (ACPA). Some ACPA such as APF, AKA, and CCP have been widely used in diagnosing RA clinically, since they have high specificity for RA. To date, studies on the combined detection of these antibodies in the diagnosis of RA have been limited. We compared the diagnostic value of isolated detection of APF, AKA, and CCP and combined detection of the 3 ACPA, and further evaluated the significance of combined detection of these ACPA in RA.

MATERIALS AND METHODS

Patients and serum samples. Serum samples were obtained from 551 patients who had arthritic complaints admitted to the Department of

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Rheumatology and Immunology, Peking University People's Hospital and Peking University Third Hospital, in the past 2 years. These included 304 patients with RA (237 women, 67 men, mean age 57.2 ± 13.6 yrs) who fulfilled the 1987 ACR criteria for RA. The mean disease duration of RA was 8.9 ± 9.8 years. Other connective tissue diseases were diagnosed in 247 patients (146 women, 101 men, mean age 45.6 ± 17.3 yrs), which included 54 with osteoarthritis, 30 with ankylosing spondylitis, 30 with psoriatic arthritis, 37 with reactive arthritis, 21 with systemic lupus erythematosus, 16 with primary Sjögren's syndrome, and 59 with other connective tissue diseases. Medical records of the patients were collected. Clinical characteristics were reviewed, including morning stiffness and rheumatoid nodules. Hand radiographs were obtained from each patient and they were read by the Radiology Department of Peking University People's Hospital. The radiographs were evaluated according to Steinbrocker's classification[6]: Stage I, osteoporosis, no erosion; Stage II, osteoporosis, slight narrowing of the joint space or subchondral bone destruction; Stage III, osteoporosis, destruction of articular cartilage and extensive bone destruction; and Stage IV, osseous ankylosis in existing osteoporosis and severe bone destruction.

The study protocol was approved by the Medical Ethics Committee of Peking University People's Hospital.

Detection of serum antibodies and other measurements. Serum antibodies directed to anti-CCP were assessed with ELISA using the second-generation ELISA kit (Euroimmun, Lübeck, Germany), and values > 25 RU/ml were considered positive. AKA was examined by indirect immunofluorescence assay (Euroimmun). APF was determined on buccal mucosa cells by indirect immunofluorescence assay. RF was examined by the immunonephelometry method, and the cutoff value for positivity was 20 IU/ml. Erythrocyte sedimentation rate (ESR) was measured by the Westergren method: values ≤ 15 mm/h for men and ≤ 20 mm/h for women were considered normal. C-reactive protein (CRP) was also examined by the immunonephelometry method. Values > 7.9 mg/l were considered positive.

Statistical analysis. Data analysis was performed using the SPSS for Windows software, version 13.0. The sensitivities, specificities, positive predictive value (PPV), and negative predictive value (NPV) of the antibodies were calculated. For normally distributed data, the results were expressed as mean ± SD; differences between groups were analyzed with the t-test. The chi-squared test was used to compare categorical data and percentages between groups. P values < 0.05 were considered statistically significant.

RESULTS

Sensitivity and specificity of the antibodies. The sensitivities of anti-CCP, AKA, and APF were 76.2%, 43.4%, and 34.5%, respectively, which were all lower than that for RF (82.5%). Their specificities were 96.0%, 98.4%, and 99.6%, all higher than that of RF (88.7%). The sensitivities of AKA and APF were lower than that for anti-CCP, although they had high specificity (Table 1).

The association of APF, AKA, and anti-CCP. In those patients with positive anti-CCP, the positivities of AKA and APF were 54.1% and 42.2%, respectively. In those patients with negative anti-CCP, positivities of APF and AKA were only 1.0% and 1.6%. The diagnostic values of these 2 ACPA were not as good as those for anti-CCP. The results indicated that AKA and APF were closely related to anti-CCP in terms of diagnostic value. The value of adding AKA and APF in diagnosing RA in patients lacking anti-CCP is therefore limited.

Table 1. Sensitivities and specificities of 3 anticitrullinated protein antibodies and RF. All numbers are percentages.

Measurements	Sensitivity	Specificity	PPV	NPV
Anti-CCP	76.2	96.0	95.9	76.5
AKA	43.4	98.4	97.1	58.6
APF	34.5	99.6	99.1	55.3
RF	82.5	88.7	89.9	80.5

Anti-CCP: anticyclic citrullinated peptide antibodies; AKA: antikeratin antibody; APF: antiperinuclear factor; RF: rheumatoid factor; PPV: positive predictive value; NPV: negative predictive value.

The diagnostic value of combined detection of the 3 ACPA. When either anti-CCP or AKA or APF was positive, the sensitivity and specificity were 77.3% and 94.7%, respectively. The PPV and NPV were 94.8% and 77.2% (Table 2). This kind of combined detection increased sensitivity but decreased specificity compared to individual ACPA. When either 2 of the 3 ACPA were positive, the sensitivity and specificity were 48.4% and 99.2%, respectively. The combination of anti-CCP, AKA, and APF positivity had the highest specificity (100%), but it had a low sensitivity (28.3%). The PPV and NPV were 100% and 53.1%.

Associations between ACPA and clinical and laboratory features in RA. The associations between ACPA and clinical/laboratory features in patients with RA were evaluated (Tables 3, 4, and 5). Compared with patients with RA who did not have ACPA, there were no significant differences in ACPA-positive patients with respect to morning stiffness and ESR and CRP levels. Patients with ACPA showed rheumatoid nodules more often than patients without ACPA. The positivity of RF in ACPA-positive patients was higher than in ACPA-negative patients. With regard to radiographic damage as shown by the hand images, we did a transversal radiographic assessment according to Steinbrocker's classification. We observed that patients with RA who had anti-CCP antibodies showed significantly more severe radiographic changes (41.1% ≥ Stage III) compared to those without anti-CCP antibodies (24.6% ≥ Stage III; $p < 0.05$). No statistical difference was observed in the radiographic damage between the groups with positive and those with negative AKA or APF.

DISCUSSION

RF is present in about 65%–80% of patients with RA, but it is also frequently observed in patients with other inflammatory diseases and occasionally presents in healthy aged persons. Studies suggest that this marker seems to have prognostic value in RA, but is of limited diagnostic value. In contrast to RF, ACPA are highly specific for RA. Recent studies of ACPA are the most advanced studies in the clinical research on RA. It has been shown that ACPA and the citrullinated protein antigen play important roles in the pathogenesis of RA[7].

In 1964, Nienhuis, *et al* described the antiperinuclear factor (APF)[2]. Fifteen years later, Young, *et al* detected antikeratin antibodies (AKA)[3]. In 1995, those antibodies were shown to belong to the same family of autoantibodies

Table 2. Sensitivities and specificities of combined detection of 3 anticitrullinated protein antibodies. All numbers are percentages.

Combined Detection	Sensitivity	Specificity	PPV	NPV
Anti-CCP or AKA or APF	77.3	94.7	94.8	77.2
(anti-CCP and AKA) or (anti-CCP and APF) or (AKA and APF)	48.4	99.2	98.7	60.9
Anti-CCP and AKA and APF	28.3	100	100	53.1

PPV: positive predictive value; NPV: negative predictive value; anti-CCP: anticyclic citrullinated peptide antibodies; AKA: antikeratin antibodies; APF: antiperinuclear factor.

Table 3. Clinical and laboratory features of patients with RA who have anti-CCP.

Features	Anti-CCP-positive	Anti-CCP-negative	p
Morning stiffness, min	77.5 ± 63.0	68.2 ± 71.7	> 0.05
Nodules, n (%)	30/230 (13.0)	2/73 (2.7)	< 0.05
Radiographic changes (%)			
≤ Stage II	123/209 (58.9)	52/69 (75.4)	< 0.05
≥ Stage III	86/ 209 (41.1)	17/69 (24.6)	< 0.05
RF-positive (%)	211/230 (91.7)	39/73 (53.4)	< 0.05
ESR, mm/h	67.5 ± 32.0	61.3 ± 29.5	> 0.05
CRP, mg/l	40.5 ± 63.6	39.4 ± 3.4	> 0.05

Anti-CCP: anticyclic citrullinated peptide antibodies; RF: rheumatoid factor; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein.

Table 4. Clinical and laboratory features of patients with RA who have antikeratin antibodies (AKA).

Features	AKA-positive	AKA-negative	p
Morning stiffness, min	71.1 ± 55.7	78.3 ± 7.6	> 0.05
Nodules, n (%)	20/132 (15.2)	12/172 (7.0)	< 0.05
Radiographic changes (%)			
≤ Stage II	71/118 (60.2)	104/160 (65)	> 0.05
≥ Stage III	47/118 (39.8)	56/160 (35)	> 0.05
RF-positive (%)	123/132 (93.2)	127/171 (74.3)	< 0.05
ESR, mm/h	66.4 ± 32.4	65.9 ± 30.9	> 0.05
CRP, mg/l	41.1 ± 71.5	39.5 ± 44.6	> 0.05

RF: rheumatoid factor; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein.

Table 5. Clinical and laboratory features of patients with RA who have antiperinuclear factor (APF).

Features	APF-positive	APF-negative	p
Morning stiffness, min	78.0 ± 62.3	73.7 ± 66.8	> 0.05
Nodules, n (%)	16/105 (15.2)	16/199 (8.0)	< 0.05
Radiographic changes (%)			
≤ Stage II	54/95 (56.8)	121/183 (66.1)	> 0.05
≥ Stage III	41/95 (43.2)	62/183 (33.9)	> 0.05
RF-positive (%)	98/105 (93.3)	152/198 (76.8)	< 0.05
ESR, mm/h	67.4 ± 33.2	65.4 ± 30.6	> 0.05
CRP, mg/l	44.5 ± 79.7	37.9 ± 41.6	> 0.05

RF: rheumatoid factor; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein.

against filaggrin and/or its precursor, profilaggrin[8]. In 1998, Schellekens, *et al* found that epitopes recognized by the antifilaggrin antibodies contained citrullinated residues[9]. An ELISA kit was developed by the use of synthetic cyclic peptides derived from the sequence of human filaggrin with a CCP as antigen[4]. Many studies have shown the high specificity of anti-CCP antibodies in RA, and the presence of anti-CCP antibodies was associated with more rapid progress of RA or more severe disease[10,11,12]. Recent studies have shown that some new antibodies against cit-

rulline-containing epitopes, such as anticitrullinated fibrinogen antibodies, antimodified citrullinated vimentin antibodies, and anticitrullinated collagen II antibodies, are also observed in RA[13,14,15].

Among the ACPA, the APF, AKA, and anti-CCP antibodies have been studied extensively and have been used widely in clinical practice. Much research has focused on the diagnostic and prognostic value of each isolated ACPA. The significance of combined detection of these ACPA in diagnosis and prognosis of RA is not well known. In this study, we evaluated and compared the use of anti-CCP, AKA, and APF in the diagnosis of RA as well as the significance of their combined detection. The study showed that anti-CCP had higher sensitivity than AKA and APF, with similar specificity. When either anti-CCP, AKA, or APF were positive, the sensitivity value was slightly increased over anti-CCP alone. However, the specificity decreased. When 2 of the 3 ACPA were positive, the sensitivity and specificity were 48.4% and 99.2%, respectively. The combination of anti-CCP, AKA, and APF positivity had the highest specificity (100%), but it had a rather low sensitivity (28.3%). Based on our study, anti-CCP is the most valuable marker in diagnosing RA, among the 3 ACPA. Further, combined detection of anti-CCP, AKA, and APF does not increase the diagnostic significance in RA.

Using a CCP from human filaggrin as antigen, the antigen epitope containing citrulline recognized by anti-CCP was also the target of AKA and APF. In our study, in the patients with positive anti-CCP antibodies, the positivities of AKA and APF were 54.1% and 42.2%, respectively. In those patients with negative anti-CCP antibodies, the positivities of APF and AKA were only 1.0% and 1.6%. The results indicated that AKA and APF are commonly found with anti-CCP antibodies. The complementary value of AKA and APF in diagnosing patients with RA who did not have anti-CCP antibodies remained limited. Combined detection of the 3 ACPA was not superior to that of anti-CCP alone.

The relationship between ACPA and clinical/laboratory features in patients with RA was also investigated. With regard to radiographic damage, the patients with RA who had anti-CCP showed more severe radiographic progression than patients without anti-CCP in a transversal radiographic assessment. No statistical difference was observed in the radiographic damage between the groups with positive and those with negative AKA or APF. The result indicated that anti-CCP had better prognostic value than AKA and APF. Based on the high sensitivity and specificity of anti-CCP and its association with disease progression, some investigators have proposed introducing anti-CCP antibodies into the classification criteria for RA to improve the sensitivity of the ACR criteria[16,17,18]. Their results showed that the revised criteria improved the sensitivity, especially in patients with early RA. As a good diagnostic and prognostic marker, anti-CCP was supposed to substitute for other ACPA in clinical practice.

Anti-CCP was the most valuable marker in diagnosing RA, among the 3 ACPA that are currently used in clinical practice. The combined detection of anti-CCP, AKA, and APF did not increase the ability to diagnose RA.

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