The diagnostic value of anti-cyclic citrullinated peptide antibodies and rheumatoid factor in patients with rheumatoid arthritis

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Objective: To identify the diagnostic value of anti-cyclic citrullinated peptide (anti-CCP) antibodies and rheumatoid factor (RF) in patients with rheumatoid arthritis (RA).

Methods: Serum levels of anti-CCP antibodies were determined by enzyme-linked immunosorbent assay, and levels of RF were determined by nephelometry in 145 patients with RA and 75 patients with non-RA rheumatic diseases.

Results: Among the 145 patients with RA, 119 patients (82.1%) tested positive for anti-CCP antibodies, and 116 patients (80.0%) tested positive for RF. The sensitivity, specificity, positive predictive value, and negative predictive value of anti-CCP antibodies for diagnosing RA were 82.1%, 88.0%, 93.0%, and 71.7% respectively. Those for RF were 80.0%, 62.7%, 81.1%, and 61.0% respectively. The presence of either anti-CCP antibodies or RF increased sensitivity to 88.3%, and when they both were present, the specificity increased to 94.7%. The positive rates for anti-CCP antibodies in the RF-positive RA, RF-negative RA, and non-RA patients were 93.1%, 37.9%, and 12.0% respectively.

Conclusion: With its high sensitivity and specificity, the anti-CCP antibodies assay is a useful test for diagnosing RA. The use of anti-CCP antibodies and RF in combination further increases the diagnostic value for RA.

Key words: Rheumatoid arthritis, anti-CCP antibodies, rheumatoid factor, diagnostic value

Introduction

The diagnosis of rheumatoid arthritis (RA) is primarily based on clinical manifestations and serologic tests [1]. Conventionally, the serology test routinely used in RA is the determination of serum rheumatoid factor (RF). However, it has little predictive value in the general population, since the overall disease prevalence is relatively low. The more specific autoantibodies for the diagnosis of RA, anti-cyclic citrullinated peptide (anti-CCP) antibodies, were discovered in 1964 [2]. Accumulating evidence shows that anti-CCP antibodies are very useful in the diagnosis of RA [3]. They may be present very early in the disease course [4] and are also considered as a prognostic factor for articular destruction [5].

Several studies have shown that anti-CCP antibodies are moderately sensitive but highly specific for the
diagnosis of RA, and their specificity is higher than that of RF [7-10]. Therefore, we conducted a retrospective study to identify the diagnostic value of anti-CCP antibodies and RF in patients with RA.

Materials and Methods

Materials

Two hundred and twenty patients (163 females, 57 males; mean age ± SD, 45.2 ± 16.0 years) randomized selected from 2219 patients with rheumatic diseases who visited the Division of Allergy, Immunology, and Rheumatology, Taichung Veterans General Hospital from 2001 to 2005 were enrolled in this study. Of the 220 patients, 145 (65.9%) patients (106 females, 39 males; mean age ± SD, 48.2 ± 14.9 years) who fulfilled 1987 American College of Rheumatology (ACR) Criteria for RA [1] were included in the RA group. The other 75 patients were classified as non-RA group (57 females, 18 males; mean age ± SD, 39.4 ± 16.3 years). The non-RA group included patients with systemic lupus erythematosus (SLE) (n=19), primary Sjögren's syndrome (SS) (n=33), spondyloarthropathy (n=10), Behcet's disease (n=3), mixed connective tissue disease (n=3), osteoarthritis (n=4), adult onset Still’s disease (n=1), antiphospholipid syndrome (n=1), and gouty arthritis (n=1). The age, gender, clinical characteristics, titers of RF and anti-CCP antibodies of each patient were recorded. All serum samples were obtained and stored at −20°C until assayed. The study was approved by the Ethics committee of Taichung Veterans General Hospital.

Methods

Serum levels of anti-CCP antibodies were determined by the second-generation enzyme-linked immunosorbent assay (INOVA diagnostics, San Diego, CA, USA). The serum samples were evaluated in duplicate, with the upper normal limit of 20 IU/mL being assumed in accordance with the manufacturer's recommendations (cut-off level 20 IU/mL). RF-IgM was determined by nephelometry (Dade Behring, Marburg, Gemh, USA) (cut-off level 15 IU/mL). The inter- and intra-assay variabilities of anti-CCP antibodies and RF were both less than 9%.

Statistical analysis

Continuous variables are expressed as the median ± interquartile range (IQR). The subgroups were compared using the Mann-Whitney U test. Categorical variables, expressed as percentages, were analyzed by Fisher’s exact test or Yate’s correction of contingency. Comparisons of sensitivity and specificity were made using chi-square test. The most appropriate cut-off values for anti-CCP antibodies and RF were determined by receiver operating characteristic (ROC). We also identified the diagnostic sensitivity, specificity, positive predictive values (PPV), negative predictive values (NPV), and area under curve (AUC) in both tests. All statistical tests were 2-sided and were assessed at the 0.05 significance level.

Results

Frequencies of anti-CCP antibodies and RF in patients with rheumatic diseases

The demographic data and clinical features are summarized in Table 1. Significantly higher values of anti-CCP antibodies were found in the RA patients than in non-RA patients (117.4 ± 123.3 IU/mL vs. 7.7 ± 5.9 IU/mL, p<0.001). Similarly, higher levels of the RF in the RA patients than non-RA patients was found (72.0 ± 205.5 IU/mL vs. 10.0 ± 21.9 IU/mL, p<0.001). Anti-CCP antibodies was tested positive in 119 of 145 patients (82.1%) with RA and 9 of 75 patients (12.0%) with non-RA rheumatic diseases, including 8 of 19 patients with SLE (42.1%), and 1 of 33 patients with primary SS (3.0%). Serum RF was detected in 116

<table>
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<th>Table 1. Demographic data and clinical characteristics of patients with RA and patients with non-RA rheumatic diseases</th>
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<td>RA (n=145)</td>
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Abbreviations: RA = rheumatoid arthritis, IQR = interquartile range, anti-CCP = anti-cyclic citrullinated peptide antibody, RF = rheumatoid factor
of 145 patients (80%) with RA and 27 of 75 patients (23.1%) with non-RA rheumatic diseases. These patients with rheumatic diseases other than RA had positive RF, including 16 out of 33 patients (48.5%) with SS, 6 out of 19 patients (31.6%) with SLE, 3 out of 10 patients (30%) with spondyloarthropathy, and 2 out of 3 patients (66.7%) with mixed connective tissue disease (Table 2). Among RF-positive RA patients, 108 of 116 patients (93.1%) were anti-CCP antibodies-positive. Among RF-negative RA patients, 11 of 29 patients (37.9%) were anti-CCP antibodies-positive.

### Diagnostic value of anti-CCP antibodies and RF in patients with RA

Based on the cut-off value suggested by the manufacturer, the anti-CCP antibodies had sensitivity, specificity, PPV, and NPV for a diagnosis of RA of 82.1%, 88.0%, 93.0%, and 71.7% respectively. Those for RF were 80.0%, 62.7%, 81.1%, and 61.0% respectively. For anti-CCP antibodies in combination with RF, they were 74.5%, 94.7%, 96.4%, and 65.7% respectively. If either one was present, they were 88.3%, 57.3%, 80.5%, and 70.5% respectively (Table 3).

As shown in Figure 1, the AUC for anti-CCP antibodies was 0.881 (95% confidence interval (CI) of 0.831 to 0.921), whereas the AUC for RF was 0.784 (95% CI of 0.724 to 0.837). The most appropriate cut-off value of anti-CCP antibodies for diagnosing RA was 34.7 IU/mL and that of RF was 21.3 IU/mL.

### Discussion

In the present study, the sensitivity and specificity of anti-CCP antibodies for diagnosing RA were 82.1% and 88.0% respectively. The high sensitivity and specificity of our study were consistent with the results of previous studies [10-11]. The frequency of anti-CCP antibodies in RF-negative RA patients was 37.9%, similar to that of previous report (40%) [12], suggesting that the determination of anti-CCP antibodies is more beneficial in the diagnosis of RA in RF-negative patients. Furthermore, the AUC for anti-CCP antibodies was 0.881, which was better than that for RF (0.784). The higher value of AUC made anti-CCP antibodies a better diagnostic tool than RF in the diagnosis of RA. However, there was crossover between the ROC curves of anti-CCP antibodies and RF, which means that anti-CCP antibodies were not superior to RF in all circumstances in the study.

In our study, the sensitivity and the specificity of RF for diagnosing RA were 80.0% and 62.7% respectively, which were similar to the results of previous studies [6,13-16]. The PPV of RF for RA (81.1%) was higher.
than that in one report (56%); whereas the NPV (61%) was lower [17]. The reason is that with RF, as with any diagnostic test, the predictive value is affected by the estimated likelihood of disease prior to conducting the test [18]. It has a lower PPV if the test is conducted among patients with non-RA rheumatic diseases (SLE, primary SS, and cryoglobulinemia) or few clinical features of systemic rheumatic disease. The sensitivity, specificity, and PPV in our study were all relatively higher than those in other reports [17]. Our patients who had existing arthritic or rheumatic problems may have contributed to this discrepancy. Detailed history taking and physical examination made anti-CCP antibodies and RF have better diagnostic value for RA.

The presence of either anti-CCP antibodies or RF increased the sensitivity of anti-CCP antibodies for diagnosing RA from 82.1% to 88.3%; however when they both were presented, the specificity increased from 88.0% to 94.7%. In view of practical use, it would be helpful to perform both the RF and anti-CCP antibodies assays in patients with suspected RA.

In addition, we observed anti-CCP antibodies in patients with other rheumatic diseases including 8 of 19 patients with SLE (42.1%), and 1 of 33 patients with primary SS (3.0%). Three patients in the non-RA group who had high anti-CCP antibodies titer (>60 IU/mL) also had SLE. When we used the appropriate cut-off level value (anti-CCP antibodies 34.7 IU/mL) for diagnosing RA, only 4 (21%) SLE patients tested positive for this antibody. Our data were consistent with those of a recent study in which anti-CCP antibodies were detected in 15% of patients with SLE, and 14% in those with SS [19]. Besides, 6 of 19 (31.6%) SLE patients and 16 of 33 (48.5%) SS patients had RF. In another study, 104 patients with SLE were evaluated for arthritis. Of the patients with erosive arthritis (n=12), 8 patients tested positive for anti-CCP antibodies (66.7%) and 6 patients also met criteria for RA [20]. However, the 8 SLE patients with positive anti-CCP antibodies in our study either didn’t receive further radiographic exams of peripheral joints or lost follow up, and thus, the rate of erosive arthritis was not known. As for primary SS, it has been reported that small number of patients tested positive for anti-CCP antibodies. In these patients, SS was closely associated with synovitis and therefore was a predictor of progression to RA or a more inflammatory process of synovial tissue [21]. The only SS patient with anti-CCP antibodies in our study didn’t have erosive arthritis. Further follow-up and evaluation may be needed to determine the disease progression in such cases.

In conclusion, anti-CCP antibodies have better diagnostic value than RF in diagnosing RA in Taiwanese patients. Whether we can use anti-CCP antibodies as a substitute for RF in 1987 ACR Criteria for RA Classification is currently under discussion [22]. Detail history taking and physical examination make the use of RF and anti-CCP antibodies more reliable tools. Anti-CCP antibodies and RF in combination further increases the diagnostic value for RA.

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References

抗環瓜氨酸肽抗體及類風濕因子在類風濕關節炎的診斷價值

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目的：研究抗環瓜氨酸肽抗體及類風濕因子在類風濕關節炎的診斷價值。 方法與材料：在145個類風濕關節炎病人及75個非類風濕關節炎病人身上，以酵素免疫分析法測定抗環瓜氨酸肽抗體數值，另外以濁度測定法測量類風濕因子之高低。結果：在145個類風濕關節炎病人中，119 (82.1%)個病人有抗環瓜氨酸肽抗體，116 (80%)個有類風濕因子。抗環瓜氨酸肽抗體的敏感度、專一度、陽性預測率、陰性預測率各別是82.1%、88.0%、93.0%、71.7%；而類風濕因子則各別為80%、62.7%、81.1%、61.0%。若抗環瓜氨酸肽抗體或類風濕因子兩者其一為陽性，則敏感度有88.3%；若兩者皆為陽性，則專一度高達94.7%。在類風濕因子陽性、類風濕因子陰性、及非類風濕關節炎這三組中，抗環瓜氨酸肽抗體的陽性率各為93.1%、37.9%、12.0%。結論：抗環瓜氨酸肽抗體因為有高度的敏感性及專一性，因此是一個診斷類風濕關節炎很好的工具，若抗環瓜氨酸肽抗體及類風濕因子合併使用，可更進一步增加診斷類風濕關節炎的能力。

關鍵詞：類風濕關節炎、抗環瓜氨酸肽抗體、類風濕因子、診斷價值