Diagnostic value of serum and joint fluid anti-CCP (citrulline-containing peptide) antibody for rheumatoid arthritis and its relationship with other inflammatory indicators

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To investigate the diagnostic sensitivity and specificity of serum and joint fluid citrulline-containing peptide (CCP), variant antibody (anti-CCP antibody) for active rheumatoid arthritis (RA) and to explore their relationship with other clinical inflammatory indicators, the levels of serum and joint fluid anti-CCP antibodies, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and rheumatoid factors (RF) were detected in 104 active RA patients including 68 with knee joint effusion. The sensitivity of serum anti-CCP antibody to RA was 92.3% (96/104) and that of joint fluid anti-CCP antibody was 82.4% (56/68) (P>0.05). The specificity of serum and joint fluid anti-CCP antibody was 100%. There was no significant correlation between anti-CCP antibody and ESR, CRP, score of DAS28, and RF in RA patients positive for serum anti-CCP antibody (P>0.05). After treatment for half month with integrated traditional Chinese and Western medicine, all indicators were improved. The levels of ESR, CRP, RF, score of DAS28 and serum anti-CCP antibody were decreased significantly after treatment when compared with those before treatment (P<0.05). In our study, two patients with early RA (course<2 months) were negative for serum anti-CCP antibody but positive for joint fluid anti-CCP antibody, and seroconversion was noted with the prolongation of disease duration. Combination of serum anti-CCP antibody and joint fluid anti-CCP antibody is helpful for the “early diagnosis” of RA and evaluation of the severity of RA, thus providing basis for the early treatment. The negativity and positivity of anti-CCP antibody are interchangeable under certain conditions.

Key words: Anti-CCP antibody, rheumatoid arthritis, early diagnosis.

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic inflammation of the joints, eventually resulting in erosive changes and joint deformities. It is the most common inflammatory arthritis, affecting about 0.14% of the world population (Gabriel, 2001). Early diagnosis of RA and early intervention can effectively control the disease progression by reducing the bone and joint damage and improve prognosis (American College of Rheumatology Subcommittee on Rheumatoid Arthritis Guidelines, 2002). Thus, early diagnosis of RA has been a focus in numerous researches. Previously, the diagnosis of RA mainly relied on clinical manifestations and serum biomakers, in which serum rheumatoid factor (RF) is frequently detected. However, the sensitivity of RF is approximately 60 to 80% in RA, and the specificity is also low since RF is detectable in many other diseases including some connective tissue diseases, chronic liver diseases and infectious diseases, and even in a few healthy individuals (Smolen, 1996; Eggeland and Munthe, 1983;
Thorsteinsson et al., 1975). Therefore, despite the fact that RF is included the criteria for the classification of RA, its diagnostic value is unsatisfactory, especially in the disease at early stage.

About one-third of patients with persistent arthritis do not meet the classification criteria, which renders the diagnose RA at the early stage difficult (Vallbracht and Helmke, 2005). Using the citrulline-containing peptide (CCP) variants, Schellekens et al. (1998) developed an enzyme-linked immunosorbent assay (ELISA) to detect auto-antibody, anti-CCP antibody (Vasishtha, 2002), which is highly specific and predictive for RA, but has poor sensitivity (Schellekens et al., 2000; Bizzaro et al., 2001). Anti-CCP, which stands for anti-cyclic citrullinated peptide antibody, is a new and exciting blood test to help doctors confirm a diagnosis of rheumatoid arthritis. Anti-CCP is a very useful test to order during the diagnostic evaluation of a person who may have rheumatoid arthritis. After the first generation of anti-CCP test (CCP1) (Van et al., 2002; Visser et al., 2002), a second generation of anti-CCP test (CCP2) is introduced.

The sensitivity of anti-CCP2 test in different populations ranges between 64 and 74% whereas the specificity between 90 and 99% (Suzuki et al., 2003; Dubucquoi et al., 2004; Kastbom et al., 2004; Vallbracht et al., 2004; Zendman et al., 2006). A correlation of anti-CCP antibodies with the radiographic joint damage has also been reported (De Rycke et al., 2004; Forslind et al., 2004; Van Gaalen et al., 2004; Ronnelid et al., 2005). In China, anti-CCP antibody has a specificity of 90% in the diagnosis of RA and can also be used to evaluate the disease activity (Zeng et al., 2003; Li et al., 2010). The present study aimed to investigate the sensitivity and specificity of anti-CCP antibody in the serum and joint fluid in the diagnosis of RA and the relationship between anti-CCP antibody and other RA makers.

### RESULTS

#### Characteristics of RA and OA patients

The characteristics of these patients are presented in Table 1. The scores of DAS28 in all RA patients were greater than 3.2. In RA group, the mean age was 50.0±13.6 years (range: 16 to 71 years), and mean disease duration 6.7±6.9 years (range: 2 month to 20 years). 68 of 104 (65.4%) patients with RA had knee swelling and synovia, while all of the patients with OA had knee synovia. In OA group, the mean age was 66.0±14.3 years (range: 15 to 73 years) and mean disease duration 5.4±4.2 years (range: 1 to 12 years). No statistically significant differences were found between RA patients and OA patients in sex, age, and disease duration (Table 1). All RA patients were treated with Traditional Chinese Medicine and a synthetic disease-modifying anti-rheumatic drug (DMARD) (Table 1).

#### Exceptional two cases of early RA patients

Two patients with early RA (disease duration < 2 months) were negative for serum anti-CCP antibody and positive

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>RA (n = 104)</th>
<th>OA (n = 50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female/male</td>
<td>76/28</td>
<td>30/20</td>
</tr>
<tr>
<td>Age (mean±SD)</td>
<td>50.0(13.6)</td>
<td>66.0(14.3)</td>
</tr>
<tr>
<td>Disease duration (mean±SD)</td>
<td>6.7(6.9)</td>
<td>5.4(4.2)</td>
</tr>
</tbody>
</table>
Table 2. Characteristics of two patients with early RA.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Case 1</th>
<th>Case 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Age (years)</td>
<td>45</td>
<td>26</td>
</tr>
<tr>
<td>Disease duration (days)</td>
<td>45</td>
<td>37</td>
</tr>
<tr>
<td>RF (IU/ml)</td>
<td>138.5</td>
<td>120.0</td>
</tr>
<tr>
<td>First serum anti-CCP (RU/ml)</td>
<td>8.0</td>
<td>15.0</td>
</tr>
<tr>
<td>First joint fluid anti-CCP (RU/ml)</td>
<td>210.0</td>
<td>234.5</td>
</tr>
<tr>
<td>RF 3 months later (IU/ml)</td>
<td>473.0</td>
<td>348.0</td>
</tr>
<tr>
<td>Serum anti-CCP 3 months later (RU/ml)</td>
<td>257.4</td>
<td>264.7</td>
</tr>
<tr>
<td>RF after 2 months of treatment (RU/ml)</td>
<td>96.6</td>
<td>83.7</td>
</tr>
<tr>
<td>Serum anti-CCP after 2 months of treatment (RU/ml)</td>
<td>108.6</td>
<td>121.5</td>
</tr>
</tbody>
</table>

Table 3. The serum levels of inflammatory indicators and anti-CCP before and after treatment.

<table>
<thead>
<tr>
<th>Serum indicator</th>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR (mm/h)</td>
<td>75.7±23.97</td>
<td>49.17±28.86</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>54.78±41.32</td>
<td>8.66±6.23</td>
</tr>
<tr>
<td>RF (IU/ml)</td>
<td>418.36±690.12</td>
<td>349.86±593.34</td>
</tr>
<tr>
<td>DAS28</td>
<td>6.66±1.27</td>
<td>3.74±1.01</td>
</tr>
<tr>
<td>Anti-CCP (RU/ml)</td>
<td>282.45±189.23</td>
<td>212.23±139.38</td>
</tr>
</tbody>
</table>

for joint fluid anti-CCP antibody and seroconversion was observed with the prolongation of disease duration (Table 2).  

Sensitivity/specificity of anti-CCP

Of the 104 patients with RA, 96 were positive for serum anti-CCP antibody. Of the 68 RA patients who had knee joint effusion, 56 were positive for joint fluid anti-CCP antibody. All patients with OA were negative for both serum and joint fluid anti-CCP antibody. The sensitivity of serum anti-CCP antibody to RA was 92.3% (96/104) and that of joint fluid anti-CCP antibody to RA was 82.4% (56/68), showing no significant difference between two groups ($X^2 = 0.248$, $P>0.05$). The specificity of serum and joint fluid anti-CCP antibody was 100%, respectively.

Correlation between serum anti-CCP antibody and inflammatory indicators in RA group before and after treatment

For the 90/104 patients (92.3%) positive for serum anti-CCP antibody, the scores of DAS28 were greater than 5.1 (6.66±1.27) showing active disease. However, 84/104 (76.9%) patients were positive for RF, which was 418.36±690.12 RU/ml. The mean content of anti-CCP antibody was 282.45±189.23 RU/ml. There was no significant correlation between anti-CCP antibody and ESR, CRP, DAS28 and RF in RA patients positive for serum anti-CCP ($r = 0.624$, 0.628, 0.687 and 0.865, respectively; $P>0.05$). Significant correlation was found between ESR and DAS28 ($r = 0.485$, $P = 0.016$) in these patients. In the serum anti-CCP antibody positive group, after about 15 days of treatment with integrated traditional Chinese and Western medicine, all indicators were improved. 

The levels of ESR, CRP, RF, DAS28 and serum anti-CCP antibody were decreased significantly after treatment when compared with those before treatment ($P = 0.001$, 0.001, 0.049, 0.001 and 0.001, respectively) (Table 3). There were no significant correlations between serum anti-CCP and ESR, CRP, DAS28 and RF ($r = 0.549$, 0.633, 0.721 and 0.935, respectively, $P>0.05$) after treatment. However, significant correlations were noted between DAS28 and ESR and CRP ($r = 0.835$ and 0.664, respectively, $P = 0.001$ and 0.001, respectively).

Correlation of joint fluid and serum anti-CCP antibody with other laboratory indicators in RA patients with knee joint effusion

In the 56/68 patients (82.4%) positive for serum anti-CCP and having knee joint effusion, the mean score of DAS28 was 6.97±1.42, mean RF level 437.72±650.12, mean serum anti-CCP antibody level 349.33±187.44, and mean joint fluid anti-CCP antibody level 345.62±212.85, which were higher than those in patients without knee joint effusion. There was no significant difference between serum anti-CCP levels and joint fluid anti-CCP levels
(r = 0.387, P = 0.112), and there was no significant correlation between serum anti-CCP levels and joint fluid anti-CCP(r = 0.387, P = 0.112). There were no significant correlations between serum anti-CCP and ESR, CRP, score of DAS28, and RF in the serum anti-CCP antibody positive RA patients (r = 0.123, -0.081, 0.264, and 0.041, respectively, P>0.05), and no significant correlations between joint fluid anti-CCP and ESR, CRP, score of DAS28, and RF in the serum anti-CCP positive RA patients (r = 0.295, -0.236, 0.318, and 0.165, respectively, P>0.05) were found. However, there was significant correlation between ESR and score of DAS28 (r = 0.51, p = 0.03).

DISCUSSION

In recent years, the pathogenic role and the diagnostic value of anti-CCP antibody have been evaluated in RA patients. Most of studies show detection of anti-CCP antibody has high specificity for RA (Schellekens et al., 2000) and the presence of anti-CCP antibody has been noted in early RA (Rantapaa-Dahlqvist et al., 2003). Moreover Nielen et al. (2004) showed the appearance of anti-CCP antibody in the circulation may occur several years before RA onset and anti-CCP antibody represents a marker of future RA. In the present study, our results showed two patients with early RA (course<2 months) were negative for serum anti-CCP antibody and but positive for joint fluid anti-CCP antibody. However, seroconversion was found three months later. This finding shows Yin and Yang of anti-CCP antibody can be interchangeable under certain conditions.

Anti-CCP antibody is the first antibody against citrulline-containing peptide variant and its detection has been applied in clinical practice. Their high specificity in the diagnosis of RA is of great importance, as well as the sensitivity especially in RF negative patients, much more RF positivity shows low diagnostic specificity (Smolen, 1996). Our study revealed that the diagnostic sensitivity (92.3%) of serum anti-CCP antibody in RA patients of Southwestern China is higher than that of Northern China (80.4%) (Li et al., 2010) and Beijing city (47.1%) (Zeng et al., 2010). Caspi et al. (2006) revealed the sensitivity of serum anti-CCP antibody was 58% and the combination of serum and joint fluid anti-CCP antibody increased the sensitivity up to 74%. Our study showed the sensitivity of joint fluid anti-CCP antibody was 82.4%.

The specificity of serum anti-CCP and joint fluid were 100%, which was consistent with in the study of Thai (Monchand et al., 2010; Zhao et al., 2010).

Our study indicated all the markers were improved after treatment for half a month with the integrated Traditional Chinese and Western Medicine when compared with those before treatment. The levels of ESR, CRP, RF, DAS28 and serum anti-CCP antibody were decreased significantly after treatment. It has been reported that anti-CCP antibody is associated with score of DAS28 in Han RA patients of Northern China (Li et al., 2010). But our results did not reveal significant correlation between serum anti-CCP antibody and ESR, CRP, DAS28, and RF, and significant correlation between DAS28 and ESR, and CRP was observed in serum anti-CCP antibody positive patients. There was no significant difference between serum anti-CCP antibody level and joint fluid anti-CCP antibody level.

In addition, significant correlation between serum and joint fluid anti-CCP antibody levels was found in RA patients positive for serum anti-CCP antibody and having knee joint effusion, in whom there was no significant correlation between joint fluid anti-CCP antibody level and ESR, CRP, score of DAS28, and RF. However, significant correlation was noted between ESR and score of DAS28. In conclusion, our results reveal the negativity and positivity of anti-CCP antibody are interchangeable under certain conditions. RA can be diagnosed at early stage and the severity of RA can be assessed by combining serum anti-CCP antibody with joint fluid anti-CCP antibody, which may be beneficial for the subsequent treatment. The inflammation in the joints of RA patients can be improved significantly after treatment with the integrated Traditional Chinese and Western medicine.

REFERENCES

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