Anti-carbamylated Protein Antibodies Are Detectable in Various Connective Tissue Diseases

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ABSTRACT. Objective. Anti-carbamylated protein (anti-CarP) antibodies are possible diagnostic biomarkers of anticitrullinated protein antibody (ACPA)-negative rheumatoid arthritis (RA). We aimed to elucidate the prevalence of anti-CarP antibodies in non-RA connective tissue diseases (CTD) because CTD are important in the differential diagnosis of ACPA-negative RA.

Methods. The sera from 266 patients with RA and 616 patients with CTD and 80 healthy controls were examined using an in-house anti-CarP ELISA.

Results. The prevalence and the level of anti-CarP antibodies in several CTD were comparable to those in ACPA-negative RA.

Conclusion. Anti-CarP antibodies are not useful for differentiating ACPA-negative RA from CTD. (First Release May 15 2017; J Rheumatol 2017;44:1384–8; doi:10.3899/jrheum.161432)

Key Indexing Terms:
AUTOANTIBODIES RHEUMATOID ARTHRITIS
CONNECTIVE TISSUE DISEASES ANTICYCLIC CITRULLINATED ANTIBODIES
Anticyclic citrullinated peptide antibodies. Anticyclic citrullinated peptide antibodies (anti-CCP) were measured by MESACUP-2 test CCP (Medical and Biological Laboratories) following the manufacturer’s instruction.

Statistical analysis. All statistical analyses were conducted using R version 3.1.16. The Mann-Whitney U test was used for comparison of antibody levels. Pearson’s chi-squared test or Fisher’s exact test was used for categorical data. We drew the receiver-operator characteristic (ROC) curves and calculated their area under the curve (AUC) using the ROCR package7 in R.

RESULTS
Anti-CarP antibodies were positive in 53.6% (112/209) of ACPA-positive patients with RA and 21.4% (12/56) of ACPA-negative patients with RA, consistent with previous reports1,2,3.

On the other hand, anti-CarP antibodies were also frequently seen in other CTD (Table 1). When non-RA patients with CTD were used as controls, the sensitivity and specificity of anti-CarP antibodies for diagnosing RA were 46.8% and 76.3%, respectively.

We then assessed whether the specificity could be improved by raising the cutoff value and permitting a lower sensitivity. However, a high anti-CarP level was mainly seen in ACPA-positive RA, and the level in ACPA-negative RA was not higher than that in non-RA CTD (Figure 1). AUC of ROC curves for non-RA CTD versus total RA and ACPA-negative RA were 0.663 and 0.461, respectively (Figure 2). Consequently, a high level of anti-CarP antibodies is specific to ACPA-positive RA, and anti-CarP antibodies are not informative for differentiating ACPA-negative RA from non-RA CTD.

Next, we tested anti-CCP antibody in 493 out of 617 non-RA patients with CTD and 12 out of 80 HC because the cross-reaction between anti-CCP and anti-CarP antibodies has been a concern8,9. Anti-CCP antibody was positive in 29 (5.9%) non-RA patients with CTD. The prevalence in each disease is shown in Table 1. In non-RA patients with CTD, the numbers of patients were as follows: anti-CarP-positive (CarP+/anti-CCP positive (CCP+)), 9; CarP+/anti-CCP–negative (CCP–), 105; anti-CarP–negative (CarP–)/anti-CCP+, 20; and CarP+/CCP–, 359. There was no significant correlation between the positivity of anti-CarP and anti-CCP antibodies in non-RA patients with CTD (p = 0.298, Pearson’s chi-squared test).

DISCUSSION
It was recently reported that anti-CarP antibodies were seen in 27%-31.1% of primary Sjögren syndrome (pSS) cases10,11, in 8.3%-16.8% of SLE cases11,12, and in 5.8% of systemic sclerosis (SSc) cases11. Our data expanded these findings and revealed that anti-CarP antibodies are detectable in various non-RA CTD.

Because our data lack information about the joint involvement of non-RA CTD except for SLE, the exact diagnostic value in differentiating ACPA-negative RA from other inflammatory arthritis in daily clinical practice is unknown. Shi, et al reported the good efficacy of anti-CarP antibodies in diagnosing ACPA-negative RA in their early arthritis cohort13. However, their cohort did not often contain CTD with autoantibodies such as SLE, SSc, mixed CTD, and pSS, which showed a higher prevalence of anti-CarP antibodies in our data (Figure 1). The difference in the efficacy of anti-CarP antibodies can be explained by the difference in the components of each cohort. Consequently, although the pre-test probability deeply affects the sensitivity and specificity of anti-CarP antibodies in daily clinical practice, our data suggest that anti-CarP antibodies cannot be used for differentiating ACPA-negative RA from non-RA CTD.
Our data showed that the prevalence of anti-CarP antibodies tended to be higher in CTD that are usually autoantibody-positive than in those that are usually autoantibody-negative (Supplementary Figure 1, available with the online version of this article). And the positivity of anti-CarP antibodies were significantly correlated with that...

Table 1. Prevalence of anti-CarP antibodies and anti-CCP antibody in rheumatoid arthritis (RA) and other connective tissue diseases (CTD).

<table>
<thead>
<tr>
<th>Disease</th>
<th>n</th>
<th>Age, mean ± SD</th>
<th>Sex, F/M</th>
<th>Anti-CarP+, n (%)</th>
<th>Anti-CCP+, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA (ACPA+)</td>
<td>209</td>
<td>57.1 ± 13.7</td>
<td>166/43</td>
<td>112 (53.6)</td>
<td>209/209 (100)</td>
</tr>
<tr>
<td>RA (ACPA–)</td>
<td>56</td>
<td>63.0 ± 12.8</td>
<td>46/10</td>
<td>12 (21.4)</td>
<td>0/56 (0)</td>
</tr>
<tr>
<td>SLE</td>
<td>241</td>
<td>41.3 ± 13.4</td>
<td>224/17</td>
<td>53 (22)</td>
<td>9/189 (4.8)</td>
</tr>
<tr>
<td>PM/DM</td>
<td>41</td>
<td>54.1 ± 12.6</td>
<td>34/7</td>
<td>3 (7.3)</td>
<td>5/41 (12.2)</td>
</tr>
<tr>
<td>SSC</td>
<td>100*</td>
<td>57.7 ± 13.2</td>
<td>94/6</td>
<td>23 (23)</td>
<td>3/61 (4.9)</td>
</tr>
<tr>
<td>MCTD and overlap syndrome</td>
<td>47</td>
<td>49.8 ± 13.2</td>
<td>43/4</td>
<td>18 (38.3)</td>
<td>4/55 (11.4)</td>
</tr>
<tr>
<td>Primary SS</td>
<td>73</td>
<td>57.9 ± 14.4</td>
<td>72/1</td>
<td>26 (35.6)</td>
<td>1/62 (1.6)</td>
</tr>
<tr>
<td>Vasculitis</td>
<td>37*</td>
<td>52.4 ± 17.5</td>
<td>29/8</td>
<td>8 (21.6)</td>
<td>2/37 (5.4)</td>
</tr>
<tr>
<td>BD</td>
<td>36*</td>
<td>47.2 ± 14.4</td>
<td>24/12</td>
<td>3 (8.3)</td>
<td>3/35 (8.6)</td>
</tr>
<tr>
<td>AOSD</td>
<td>13*</td>
<td>50.7 ± 17.4</td>
<td>9/4</td>
<td>2 (15.4)</td>
<td>1/11 (9.1)</td>
</tr>
<tr>
<td>PMR</td>
<td>13</td>
<td>70.1 ± 10.0</td>
<td>9/4</td>
<td>1 (7.7)</td>
<td>1/13 (7.7)</td>
</tr>
<tr>
<td>SpA</td>
<td>8*</td>
<td>46.5 ± 15.2</td>
<td>3/5</td>
<td>4 (50)</td>
<td>0/8 (0)</td>
</tr>
<tr>
<td>Others**</td>
<td>11*</td>
<td>52.8 ± 17.9</td>
<td>9/2</td>
<td>5 (45.5)</td>
<td>0/3 (0)</td>
</tr>
<tr>
<td>HC</td>
<td>80</td>
<td>ND</td>
<td>ND</td>
<td>3 (3.8)</td>
<td>0/12 (0)</td>
</tr>
</tbody>
</table>

*Including 3 overlapping patients (a patient with AOSD and SpA, a patient with BD and relapsing polychondritis in the group of Others, and a patient with SSc and vasculitis). They were counted in both disease groups. Anti-CCP was measured in 493 out of 617 non-RA CTD patients and 12 of HC. ** Includes relapsing polychondritis, sarcoidosis, primary antiphospholipid syndrome, and osteoarthritis. Anti-CarP: anti-carbamylated protein; ND: not determined; ACPA: anti-citrullinated protein antibody; anti-CCP: anticyclic citrullinated peptide antibodies; SLE: systemic lupus erythematosus; PM/DM: polymyositis/dermatomyositis; SSc: systemic sclerosis; MCTD: mixed CTD; SS: Sjögren syndrome; BD: Behçet disease; AOSD: adult-onset Still disease; PMR: polymyalgia rheumatica; SpA: spondyloarthritis; HC: healthy controls.

Figure 1. Anti-CarP levels in RA and other CTD. The horizontal line represents the cutoff value (mean + 2 SD of anti-CarP antibody levels in healthy controls). Anti-CarP: anti-carbamylated protein; RA: rheumatoid arthritis; CTD: connective tissue disease; ACPA: anti-citrullinated protein antibodies; SLE: systemic lupus erythematosus; PM/DM: polymyositis/dermatomyositis; SSc: systemic sclerosis; MCTD: mixed CTD; SS: Sjögren syndrome; BD: Behçet disease; AOSD: adult-onset Still disease; PMR: polymyalgia rheumatica; SpA: spondyloarthritis; HC: healthy controls.
of anti-SS-A/Ro antibodies. This suggests the existence of a common pathway to produce anti-CarP antibodies and several autoantibodies.

On the other hand, although protein carbamylation occurs in various situations, such as uremia14 and inflammation in atherosclerotic lesions15, it was reported that anti-CarP antibodies are seen only in CTD16. Although we could not show the association between the presence of anti-CarP antibodies and joint symptoms in patients with SLE, Bergum, et al reported that anti-CarP antibodies in pSS are associated with more severe symptoms and stronger inflammation in minor salivary glands10. These findings suggest that induction of anti-CarP antibodies requires not only protein carbamylation, but also some other immune response.

Finally, our data showed that there is no significant association between the presence of anti-CarP and anti-CCP antibodies in non-RA CTD groups. Although the cross-reaction between anti-CCP and anti-CarP antibodies has been a concern8,9, our data indicate that the detection of anti-CarP antibodies is not the result of cross-reaction of anti-CCP antibody.

The mechanism to develop anti-CarP antibodies and its pathological roles are of interest, but our study revealed that anti-CarP antibodies are not useful for differentiating ACPA-negative RA from non-RA CTD.

ONLINE SUPPLEMENT
Supplementary material accompanies the online version of this article.

REFERENCES

