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

Shuichiro Nakabo, Hajime Yoshifuji, Motomu Hashimoto, Yoshitaka Imura, Ran Nakashima, Kosaku Murakami, Nobuo Kuramoto, Shinji Ito, Junko Satoh, Masao Tanaka, Takao Fujii, Tsuneyo Mimori, and Koichiro Ohmura

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
CONNECTIVE TISSUE DISEASES
RHEUMATOID ARTHRITIS
ANTICYCLIC CITRULLINATED ANTIBODIES
Cross-reaction between anti-CCP and anti-CarP antibodies in non-RA patients with CTD and 12 out of 80 HC because the pre-test probability deeply affects the sensitivity and specificity of anti-CarP antibodies in our data (Figure 1). 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 our data, 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).

Then we obtained the results of commercially available disease-specific autoantibody tests in each patient by retrospective chart review, and assessed the correlation between each antibody and anti-CarP antibodies. The positivity of anti-SS-A/Ro antibodies was associated with that of anti-CarP antibodies (Supplementary Table 1, available with the online version of this article). The levels of anti-CarP antibodies were significantly 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).

Finally, we reviewed the charts of patients with SLE and studied the correlation between arthritis and anti-CarP positivity. Arthritis was present in 171 patients (71.0%) at least once during the course of the disease. Anti-CarP antibodies were positive in 36 patients with arthritis (21.1%) and 17 patients without arthritis (24.3%). There was no significant correlation between the positivity of anti-CarP and arthritis (p = 0.582).

**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.

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–)/–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).
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
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 uremia and inflammation in atherosclerotic lesions, it was reported that anti-CarP antibodies are seen only in CTD. 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 glands. 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 antibodies and anti-CCP antibodies in non-RA CTD groups. Although the cross-reaction between anti-CCP and anti-CarP antibodies has been a concern, 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


13. Shi J, van Steenbergen HW, van Nies JA, Levarht EW, Huizinga...

