

Diagnostic Accuracy of Anticarbamylated Protein Antibodies in Established Rheumatoid Arthritis: A Monocentric Cross-Sectional Study

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Objective. To evaluate the diagnostic accuracy of anticarbamylated protein antibodies (CarP), alone and in combination with traditional biomarkers (rheumatoid factor [RF] and anticitrullinated peptide antibodies [ACPA]), in established rheumatoid arthritis (RA).

Methods. A commercially available enzyme-linked immunosorbent assay (ELISA) kit was used to assess CarP concentrations in serum samples of 200 established RA and 206 controls (115 healthy donors and 55 patients with other rheumatic diseases). Main outcome measures were sensitivity, specificity, and area under the curve (AUC; 95% confidence interval [CI]). Difference in accuracy was evaluated by comparison of the respective AUCs.

Results. A serum CarP cut-off of 1.47 ng/ml or more differentiated patients with RA from controls with 30% sensitivity, 97.1% specificity, and good accuracy (AUC[95%CI] = 0.83[0.79-0.86], $P < 0.0001$). However, it showed moderate diagnostic accuracy in seronegative RA patients: sensitivity 17.9%, specificity 96.9%, and AUC (95% CI) = 0.69 (0.63-0.75). The diagnostic accuracy of CarP_ACPA and CarP_RF combinations was significantly superior to that of ACPA and RF alone ($P < 0.0001$ and $P = 0.015$, respectively), but not to that of ACPA_RF combination ($P = 0.089$). In addition, the CarP_ACPA_RF combination did not improve the diagnostic accuracy of the ACPA_RF combination (AUC mean difference [95% CI] = 0.006 [−0.001 to 0.015], $P = 0.10$). The number of positive autoantibodies (0, 1, 2, or 3) was not significantly associated with moderate-severe disease (Disease Activity Score-28 [DAS-28] > 3.2) in adjusted multiple regression analysis.

Conclusion. CarP has good diagnostic accuracy in established RA but not in seronegative RA. The addition of CarP to ACPA and RF alone or in combination does not significantly enhance the diagnostic accuracy of ACPA_RF combination.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease that affects synovial joints and leads to bone damage, disability, and excess of mortality (1,2). Although the pathogenesis of RA is largely unknown, chronic inflammation is thought to be the result of immune-mediated mechanisms in subjects harbouring a genetically favourable substrate (1).

Despite continuing efforts to identify new diagnostic biomarkers, early diagnosis of RA remains a challenging and highly individualized process. The 2010 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) classification criteria for RA included autoantibodies (rheumatoid

factor [RF] and anti-cyclic citrullinated peptide antibodies [ACPA]) as biomarkers of the disease (3). However, a sizeable subgroup of RA patients is negative for both ACPA and RF (the so-called seronegative RA) (4). Therefore, there is an urgent need to develop simple and affordable biomarkers for the accurate diagnosis of RA, especially in the early phase of disease and in seronegative patients.

Among candidate markers of RA, antibodies against carbamylated proteins (CarP) have been extensively studied in recent years. CarP are described in the preclinical (5) and early phases of RA (6) and are associated with severe disease (7), bone erosions (8), and all-cause mortality (9). Of note, CarP were shown to be positive in seronegative RA patients (10).

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A good accuracy of CarP has been demonstrated in different cohorts of RA patients (10–12), but its usefulness in the diagnosis of RA in routine clinical practice is uncertain (13). In particular, there is a paucity of data about the additive value of testing CarP over and above ACPA and RF to classify RA patients as well as the diagnostic accuracy of CarP in patients lacking these traditional antibodies. Regueiro et al, reported only a limited value of testing CarP in addition to traditional biomarkers for the classification of early arthritis (14). Accordingly, in a recent meta-analysis, the combination of CarP, ACPA, and RF with respect to ACPA and RF alone showed a significant, although modest, increase in specificity (at the cost of a loss of sensitivity) in the prediction of RA in individuals at risk, but no significant improvement in the classification of patients with established RA (15).

Based on this background, we sought to further explore the contribution of CarP testing, alone and in addition to ACPA and RF, for the classification of RA in a large monocentric cohort of patients with established RA compared with healthy controls and patients with other rheumatic diseases (RDs).

PATIENTS AND METHODS

Patients and controls. Established RA patients satisfying the 2010 ACR/EULAR classification criteria (3) consecutively enrolled in the BIOmarkers of Subclinical Atherosclerosis in RA—The Bio-RA study between October 2015 and November 2018 were included. We also enrolled an age- and gender-matched control population that included healthy donors (HDs), referred to the blood donors bank of the Azienda Ospedaliero-Universitaria of Sassari (Italy), and consecutive patients with RDs referred to the rheumatology outpatient's clinic of the Azienda Ospedaliero-Universitaria of Sassari (Italy).

In RA patients, the following disease-specific scores, disease descriptors, and treatment data collected on the day of the inclusion in the Bio-RA study were available for analysis: C-reactive protein (CRP) concentrations, erythrocyte sedimentation rate (ESR) values, Disease Activity Score-28 (DAS-28), Health Assessment Questionnaire (HAQ) score, current steroid use, daily steroid dose in prednisone equivalent mg/day, current treatment with synthetic disease-modifying antirheumatic drugs (DMARDs), and current use of tumor necrosis factor- α -inhibitors or other biological DMARDs.

The Bio-RA study was approved by the Ethics Committee of the Azienda ASL 1 of Sassari (Italy) (2219/CE-2015) and was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from each study participant.

CarP test, ACPA, and RF. CarP were detected using a quantitative, commercially available enzyme-linked immunosorbent assay (ELISA) kit (Novatein Biosciences) according to manufacturer's instructions. ACPA were detected using a second-generation ELISA (anti-cyclic citrullinated peptide) kit (Delta

Biologicals) while immunoglobulin M RF was determined as part of routine analysis by immunonephelometry (Behring) according to the manufacturer's instructions. The cut-off for each antibody was set as the mean + 2 Standard Deviations (SD) in the control group.

Statistical analysis. Results are expressed as mean values (mean \pm SD) or absolute number and percentages (n [%]). Statistical differences between groups were assessed using unpaired Student's *t*-tests or the Mann-Whitney rank sum test, as appropriate. Differences between categorical variables were evaluated by the chi-squared test or Fisher exact test as appropriate. Correlations between variables were assessed by Pearson's correlation or Spearman's correlation as appropriate.

The ability of the different tests to discriminate between RA and controls as well as between RA, RDs, and HDs was assessed using receiver operating characteristic (ROC) curve analysis. Selection of the optimal cut-off values for sensitivity and specificity of the combination of different tests was made according to the Youden Index. Positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (+LR), and negative LR (–LR) were also calculated. AUCs of different tests, alone and in combination, were compared with the nonparametric method by DeLong et al (16).

Multiple regression analysis (ENTER method) was also performed to evaluate the association between the number of positive antibodies and the severity of disease.

Analyses were performed using SPSS 20 (version 20.0, 2011; IBM Corp.) and MedCalc for Windows (Version 15.0, MedCalc Software). Graphs were created using GraphPad Prism 7 (GraphPad Software 7825).

A $P \leq 0.05$ was considered statistically significant.

RESULTS

Patients and controls. A total of 200 patients with established RA and 206 controls (151 HD and 55 patients with RDs) were studied. The subgroup of RDs included 14 patients with systemic sclerosis, 14 with systemic lupus erythematosus, 12 with Sjogren's syndrome, 4 with ankylosing spondylitis, 6 with psoriatic arthritis, and 5 with osteoarthritis.

As expected, according to the RA epidemiology, the female gender was the prevalent one. Age and gender distribution were similar between patients and control groups by matching as per protocol (Table 1). RA patients had a relatively long disease duration (mean 9.48 years), moderate mean disease activity (DAS-28 = 3.87 ± 1.1), and were mostly under immunosuppressive and anti-inflammatory treatment at the time of assessment (Table 3).

Accuracy of CarP for the diagnosis of established RA. Serum cut-offs for CarP, ACPAs, and RF were 1.47 ng/ml or

Table 1. Concentrations and positivity of CarP, ACPA, and RF across all group

| Variable | RA n = 200 | All Controls n = 206 | HD n = 151 | RDs n = 55 | RA vs CTRLs, <i>P</i> | RA vs HD, <i>P</i> | RA vs RDs, <i>P</i> |
|-------------------|---------------|----------------------------|---------------|---------------|-----------------------|--------------------|---------------------|
| Age, yr | 60.9 ± 8.8 | 58.8 ± 14 | 61.3 ± 12 | 59.8 ± 16 | 0.07 | 0.71 | 0.56 |
| Female sex, n (%) | 156 (78) | 155 (75.2) | 113 (74.8) | 42 (76.4) | 0.51 | 0.48 | 0.79 |
| Anti-CarP, ng/ml | 2.75 ± 4.63 | 0.32 ± 0.57 | 0.26 ± 0.26 | 0.49 ± 1.01 | <0.001 | <0.001 | <0.001 |
| ACPA, U/ml | 24.4 ± 39.3 | 2.0 ± 1.2 | 1.9 ± 1.0 | 2.3 ± 1.5 | <0.001 | <0.001 | <0.001 |
| RF, IU/ml | 109.7 ± 116 | 17.5 ± 32.3 | 13.9 ± 22.5 | 27.5 ± 49.3 | <0.001 | <0.001 | <0.001 |
| CarP+, n (%) | 60 (30) | 6 (2.9) | 3 (2) | 3 (5.5) | <0.001 | <0.001 | <0.001 |
| ACPA+, n (%) | 129 (64.5) | 2 (1) | 1 (0.7) | 1 (1.8) | <0.001 | <0.001 | <0.001 |
| RF+, n (%) | 115 (57.5) | 6 (2.9) | 2 (1.3) | 4 (7.3) | <0.001 | <0.001 | <0.001 |

Abbreviation: CarP, anticarbamylated protein antibodies; HD, healthy donors; ACPA, anticitrullinated peptide antibodies; RA, rheumatoid arthritis; RDs, rheumatic diseases; RF, rheumatoid factor. RDs represents patients with rheumatic diseases other than RA (14 systemic sclerosis; 14 systemic lupus erythematosus; 12 Sjogren’s syndrome; 4 ankylosing spondylitis; 6 psoriatic arthritis; 5 osteoarthritis).

greater, 4.57 UI/ml or greater, and 73.7 UI/ml or greater, respectively. CarP serum concentrations were significantly higher in RA patients than in the whole group of controls (2.75 ± 4.63 vs 0.32 ± 0.57 ng/ml, *P* < 0.0001) (Table 1). CarP serum concentrations were also significantly higher in RA when compared with controls subgroups taken singularly: (CarP in RA = 2.75 ± 4.63 vs CarP

in HD 0.49 ± 1.01 ng/ml and vs CarP in RDs 0.26 ± 0.26 ng/ml, *P* < 0.0001 for all comparisons) (Table 1).

CarPs were positive in 60 (30%) subjects from the RA group vs only 6 (2.9%) of controls (*P* < 0.0001) (Figure 1A and Table 1) giving a sensitivity and a specificity of CarP for established RA, respectively, of 30 and 97.1% (Table 2). The CarP test resulted

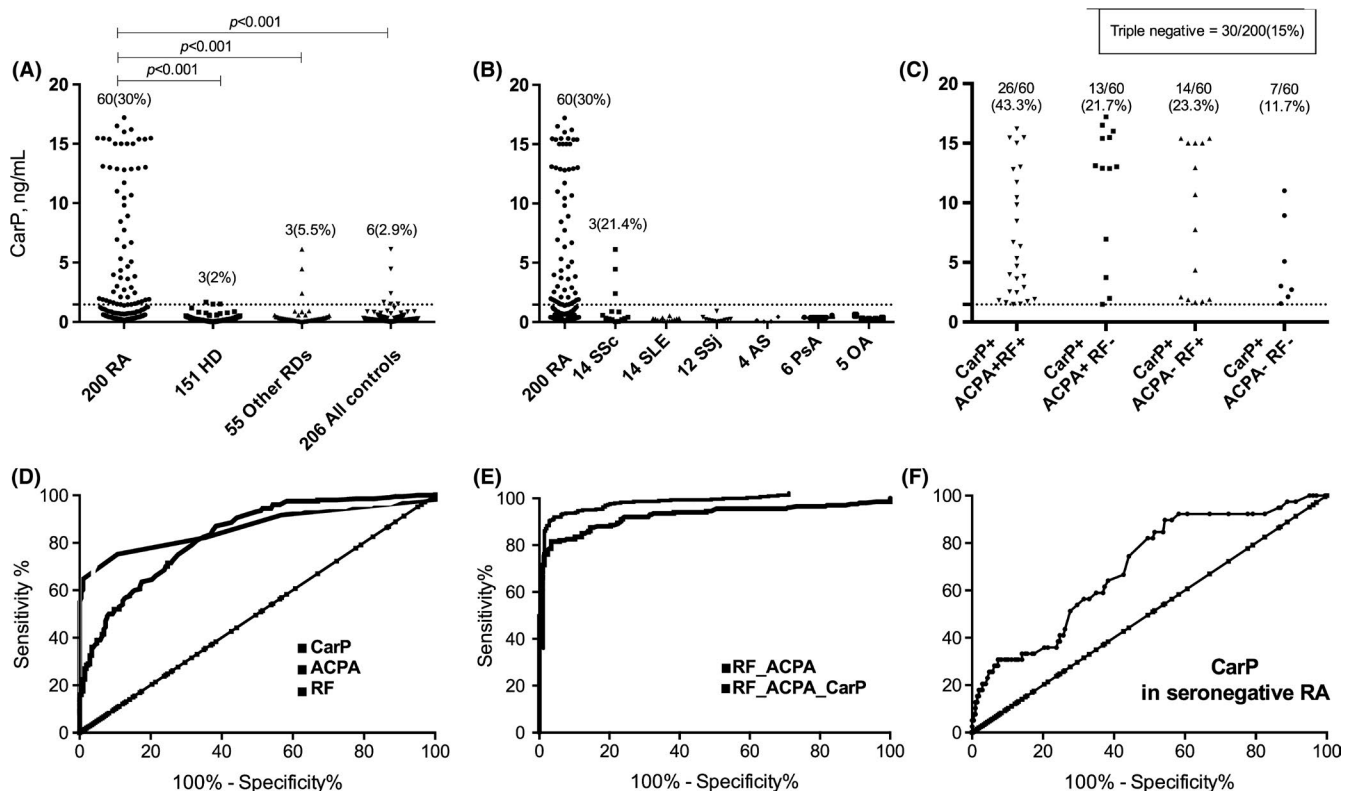


Figure 1. CarP positivity across groups and receiver operating characteristics (ROC) curves of CarP, ACPA, and RF. **A**, Distribution of CarP across all groups. **B**, Distribution of CarP positivity in other rheumatic diseases. **C**, Frequency of CarP positivity in RA groups stratified according to ACPA and RF positivity. **D**, ROC curves of CarP, ACPA, and RF. **E**, ROC curves of combinations of CarP_RF_ACPA. **F**, ROC curve of CarP in seronegative RA. ACPA, anticitrullinated protein antibodies; AS, ankylosing spondylitis; CarP, anti-carbamylated protein antibodies; HD, healthy donors; OA, osteoarthritis; PsA, psoriatic arthritis; RA, rheumatoid arthritis; RDS, other rheumatic diseases; RF, rheumatoid factor; SLE, systemic lupus erythematosus; SSc, systemic sclerosis; SSJ, Sjogren’s syndrome.

positive in only three healthy subjects and in three patients from the RDs group affected by the systemic sclerosis (SSc) (Figure 1B).

Accuracy of CarP for the diagnosis of established RA was good with AUC (95%CI) = 0.830 (0.790-0.866), $P < 0.0001$ (Figure 1D and Table 2). PPV, NPV, +LR, and -LR of CarP testing for the diagnosis of established RA were 90.9, 58.8, 10.3, and 0.72, respectively (Table 2).

In our series of patients, the accuracy of CarP for the diagnosis of established RA was similar to that of ACPA (CarP AUC [95% CI] = 0.830 [0.790-0.866] vs ACPA AUC [95%CI] = 0.862 [0.825-0.894], mean difference [95% CI] = 0.0319 [-0.0184 to 0.0821], $P = 0.21$), but was inferior to that of RF (RF AUC [95%CI] = 0.889 [0.850-0.914] vs CarP AUC [95%CI] = 0.830[0.790-0.866], mean difference [95%CI] = 0.0581 [0.0108-0.105], $P = 0.016$) (Table 2).

The CarP test was positive in 7 of 39 (17.9%) seronegative RA patients vs only 6 (2.9%) controls $P < 0.0001$, giving a sensitivity, specificity, and accuracy of 17.9%, 96.9%, and 0.699 (0.637-0.757), respectively (Figure 1F and Table 2).

Accuracy of CarP in combination with ACPA and RF.

The accuracy of the CarP_RF combination for the diagnosis of established RA was significantly higher than the accuracy of RF alone (CarP_RF AUC [95% CI] = 0.907 [0.874-0.933] vs RF AUC [95% CI] = 0.889 [0.850-0.914], mean difference [95% CI] = 0.0185 [0.0034-0.0335], $P = 0.015$) (Table 2). Similarly, the accuracy of the CarP_ACPA combination was significantly higher than that of ACPA alone (CarP_ACPA AUC [95% CI] = 0.909 [0.876-0.935] vs ACPA AUC [95% CI] = 0.862 [0.825-0.894], mean difference [95% CI] = 0.0031 [-0.0299-0.0362], $P = 0.85$) (Table 2).

However, the accuracy of the CarP-RF and CarP-ACPA combinations was not significantly different from that of the ACPA-RF combination (CarP_RF AUC [95% CI] = 0.907 [0.874-0.933] vs ACPA_RF AUC [95%CI]=0.926 [0.896-0.949], mean difference [95% CI] = 0.0186 [-0.0033-0.0406], $P = 0.096$; CarP_ACPA AUC [95% CI] = 0.909 [0.876-0.935] vs ACPA_RF AUC [95% CI] = 0.926 [0.896-0.949], mean difference [95% CI] = 0.0170 [-0.0026-0.0365], $P = 0.089$) (Table 2).

Table 2. Test's characteristics and comparisons of AUCs of CarP alone and in combination with RF and ACPA

| Test | AUC (95% CI) | <i>P</i> | Sensitivity (95%CI) | Specificity (95%CI) | PPV (95% CI) | NPV (95% CI) | +LR (95% CI) | -LR (95% CI) |
|---|------------------------|----------|---------------------|---------------------|---------------------|---------------------|----------------------|-------------------|
| CarP | 0.830 (0.790-0.866) | <0.0001 | 30.0 (23.7-36.9) | 97.1 (93.8-98.9) | 90.9 (81.6-95.8) | 58.8 (56.5-61.1) | 10.3 (4.6-23.3) | 0.72 (0.7-0.8) |
| ACPA | 0.862 (0.825-0.894) | <0.0001 | 64.5 (57.4-71.1) | 99.0 (96.5-99.9) | 98.5 (94.2-99.6) | 74.2 (79.4-77.6) | 66.4 (16.7-264.9) | 0.36 (0.3-0.4) |
| RF | 0.889 (0.850-0.914) | <0.0001 | 57.5 (50.3-64.4) | 96.6 (93.1-98.6) | 94.3 (88.7-97.2) | 70.1 (66.5-73.4) | 16.9 (8.1-35.4) | 0.44 (0.4-0.5) |
| ACPA_RF combination | 0.926 (0.896-0.949) | <0.0001 | 81.5 (75.4-86.6) | 96.6 (93.1-98.6) | 95.9 (91.8-98.0) | 84.3 (80.1-87.8) | 23.9 (11.5-49.8) | 0.19 (0.1-0.3) |
| CarP_ACPA combination | 0.909 (0.876-0.935) | <0.0001 | 77.5 (71.1-83.1) | 96.6 (93.1-98.6) | 95.7 (91.4-97.9) | 81.6 (77.3-85.1) | 22.8 (11.0-47.4) | 0.23 (0.2-0.3) |
| CarP_RF combination | 0.907 (0.874-0.933) | <0.0001 | 81.5 (75.4-86.6) | 93.6 (89.5-96.6) | 92.6 (88.1-95.5) | 83.9 (79.6-87.5) | 12.9 (7.6-21.9) | 0.20 (0.1-0.3) |
| CarP_ACPA_RF combination | 0.932 (0.904-0.955) | <0.0001 | 84.5 (78.7-89.2) | 95.6 (91.9-98.0) | 94.9 (90.8-97.3) | 86.4 (82.1-89.8) | 19.3 (10.2-36.7) | 0.16 (0.1-0.2) |
| CarP in seronegative RA | 0.699 (0.637-0.757) | <0.0001 | 17.9 (7.5-33.5) | 96.9 (93.5-98.9) | 53.8 (29.3-76.7) | 85.7 (83.8-87.4) | 5.9 (2.1-16.7) | 0.85 (0.7-1.0) |
| Test | AUC Mean Difference | | SE | 95%CI | | Z Statistic | <i>P</i> | |
| RF vs CarP | 0.0581 | | 0.0241 | (0.0108 to 0.105) | | 2.410 | 0.016 | |
| ACPA vs CarP | 0.0319 | | 0.0256 | (-0.0184 to 0.0821) | | 1.243 | 0.21 | |
| CarP_RF combination vs RF alone | 0.0185 | | 0.0076 | (0.0034 to 0.0335) | | 2.411 | 0.015 | |
| CarP_RF combination vs ACPA_RF combination | 0.0186 | | 0.0112 | (-0.0033 to 0.0406) | | 1.663 | 0.096 | |
| CarP_ACPA combination vs ACPA alone | 0.0464 | | 0.0111 | (0.0246 to 0.0682) | | 4.169 | <0.0001 | |
| CarP_ACPA combination vs ACPA_RF combination | 0.0170 | | 0.0100 | (-0.0026 to 0.0365) | | 1.696 | 0.089 | |
| CarP_ACPA_RF combination vs ACPA_RF combination | 0.0068 | | 0.0041 | (-0.0013 to 0.0150) | | 1.635 | 0.10 | |

Abbreviation: ACPA, anticitrullinated peptide antibodies; AUC, area under curve; CarP, anticarbamylated protein antibodies; CI, confidence interval; LR, likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; RF, rheumatoid factor.

Table 3. RA clinical and laboratory features according to CarP, ACPA, and RF autoantibodies positivity

| Variable | RA n = 200 | CarbP+ n = 60 | CarbP- n = 140 | ACPA+ n = 129 | ACPA- n = 71 | RF+ n = 115 | RF- n = 85 |
|--------------------|------------|---------------|----------------|---------------|-------------------------|-------------|------------|
| Age, yr | 60.9 ± 8 | 62.3 ± 8 | 60.3 ± 9 | 60.7 ± 9 | 61.2 ± 8 | 61.0 ± 8 | 60.7 ± 9 |
| Female gender, % | 78.0 | 71.7 | 80.7 | 82.2 | 70.4 | 79.1 | 76.5 |
| Current smokers, % | 24.0 | 26.7 | 22.9 | 22.5 | 24.8 | 22.6 | 25.9 |
| ESR, mm/h | 31 ± 22 | 33 ± 23 | 30 ± 21 | 33 ± 23 | 28 ± 19 | 31 ± 20 | 30 ± 24 |
| CRP, mg/dl | 0.67 ± 0.9 | 0.85 ± 1.3 | 0.59 ± 0.6 | 0.75 ± 1 | 0.55 ± 0.6 | 0.70 ± 1.0 | 0.63 ± 0.7 |
| DAS-28 | 3.87 ± 1.1 | 3.96 ± 1.2 | 3.82 ± 1.1 | 4.01 ± 1.2 | 3.62 ± 0.9 ^a | 3.88 ± 3.8 | 3.85 ± 1.3 |
| DAS-28 > 3.2, % | 70.5 | 71.7 | 70.0 | 71.3 | 69.0 | 73.9 | 65.9 |
| HAQ | 0.69 ± 0.6 | 0.78 ± 0.6 | 0.65 ± 0.6 | 0.73 ± 0.6 | 0.63 ± 0.5 | 0.65 ± 0.5 | 0.75 ± 0.6 |
| Steroid use, % | 42.4 | 45.6 | 40.8 | 45.2 | 37.8 | 42.1 | 42.9 |
| Steroid dose, mg/d | 3.77 ± 3.4 | 3.85 ± 3.3 | 3.72 ± 3.5 | 3.71 ± 3.5 | 3.92 ± 3.4 | 3.35 ± 2.2 | 4.31 ± 4.5 |
| DMARDs use, % | 70.2 | 69.1 | 70.8 | 67.7 | 74.3 | 68.4 | 72.6 |
| TNFi use, % | 21.7 | 19.1 | 23.1 | 20.2 | 24.3 | 21.9 | 21.4 |
| Other bDMARDs, % | 11.7 | 14.3 | 10.5 | 15.3 | 5.4 | 10.9 | 12.9 |

Abbreviation: ACPA, anticitrullinated peptide antibodies; bDMARDs, biological DMARDs; CarP, carbamylated protein antibodies; CRP, C-reactive protein; DAS-28, Disease Activity Score—28 joints calculated with ESR; DMARDs, synthetic disease-modifying anti-rheumatic drugs; ESR, erythrocyte sedimentation rate; HAQ, Health Assessment Questionnaire; RA, rheumatoid arthritis; RF, rheumatoid factor; SENS, Simple Erosion Narrowing Score; TNFi, tumor necrosis factor α -inhibitors. Values are expressed as means (SD); ^a $P = 0.032$.

Moreover, the accuracy of the CarP_ACPA_RF combination was not significantly different from that of the ACPA_RF one (CarP_ACPA_RF AUC [95% CI] = 0.932 [0.904-0.955] vs ACPA_RF AUC [95% CI] = 0.926 [0.896-0.949], mean difference [95% CI] = 0.0068 [-0.0013-0.0150], $P = 0.10$) (Figure 1E and Table 2).

Correlation analysis between CarP positivity and RA features. We found no significant differences in serum CarP concentrations according to demographic and clinical characteristics of RA patients (Table 3). Mean DAS-28 values were significantly higher in ACPA+ versus ACPA- RA patients. However, in bivariate correlation, we found no association between autoantibody positivity and values of DAS-28 greater than 3.2, which indicates moderate-severe disease. Moreover, in multiple logistic analysis adjusted for demographic factors and immunosuppressive therapy, the number of positive autoantibodies (0, 1, 2, or 3) was not significantly associated with the presence of moderate-severe disease (Table 4).

DISCUSSION

Although the diagnosis of RA is still based on clinical grounds, the demonstration in sera of specific autoantibodies is of significant diagnostic value and may also have prognostic implications. A plethora of biomarkers have been studied for the diagnosis of RA (17,18), but apart from ACPA and RF, no commercial test is currently available in clinical practice.

In this study we expanded the current evidence about the performance of the CarP test for the diagnosis of estab-

lished RA. We demonstrated that a commercially available CarP test has a good accuracy (AUC > 0.8) for the diagnosis of established RA. However, although the test specificity was good (97.1%), its sensitivity (30%) was not satisfactory: this suggests that this commercially available CarP test does not perform well in ruling out RA, as confirmed by the low NPV and -LR values. Our results are in line with those of a recent meta-analysis reporting pooled sensitivity and specificity of dif-

Table 4. Factors associated with DAS-28 greater than 3.2 in the RA sample

| Factor | Multiple Logistic Analysis | | |
|-----------------------------------|----------------------------|-------------------|--------|
| | B | OR (95%CI) | P |
| Age, years | 0.07 | 1.07 (1.02-1.12) | <0.01 |
| Female sex, % | 1.66 | 5.28 (2.35-11.85) | <0.001 |
| Smoke | -0.61 | 0.54 (0.22-1.29) | 0.16 |
| Number of positive autoantibodies | | | 0.58 |
| 0 (Ref.) | | | |
| 1 | -0.82 | 0.43 (0.10-1.83) | 0.25 |
| 2 | -0.89 | 0.40 (0.10-1.56) | 0.19 |
| 3 | -0.83 | 0.43 (0.12-1.53) | 0.19 |
| Steroid use | -1.73 | 0.17 (0.07-0.40) | <0.001 |
| DMARDs use | -0.47 | 0.62 (0.26-1.45) | 0.27 |
| TNF inhibitors use | -0.67 | 0.51 (0.19-1.33) | 0.16 |

Abbreviation: B, unstandardised regression coefficient; CI, confidence interval; DAS-28, Disease Activity Score—28 joints; DMARD, drug-modifying antirheumatic drug; OR, odds ratio; RA, rheumatoid arthritis; TNF, tumor necrosis factor. OR is based on the risk of the dependent variable (DAS-28 > 3.2), given the presence of the independent variable.

ferent CarP tests for the diagnosis of RA at 42% and 96%, respectively (19).

In the present study, we were also focused on understanding whether testing CarP over and above ACPA and RF may add some diagnostic benefit. Therefore, we specifically looked at the accuracy of different combinations of CarP with ACPA or RF, or both. The comparison of the accuracy of three different combinations of CarP (CarP_ACPA, CarP_RF, and CarP_RF_ACPA) did not show significant differences with respect to the ACPA_RF combination. This suggests that the incorporation of CarP in routinely ordered tests (ACPA and RF tests) is not useful for the diagnosis of RA.

In agreement with our results, Regueiro et al (14) showed that the incorporation of the anti-CarP antibodies into different combinations with ACPA and RF in the ACR/EULAR classification of RA resulted in only a modest increase in sensitivity (2.2% higher) at the cost of decreased specificity (8.1% lower).

Moreover, no data reporting the cost-benefit ratio of adding CarP to conventional autoantibodies for the diagnosis of RA have been published to date. Therefore, based on our data and the available evidence, the incremental value of testing CarP for the diagnosis of RA is unclear.

In our series, we also demonstrated the presence of CarP positivity in 7 of 39 (17.9%) seronegative RA patients: this figure is similar to that reported in ACPA and RF– (20) (8%) and in ACPA– patients (5,10,11) (8%-30%).

Therefore, we evaluated whether CarP testing may be of some diagnostic benefit in this group of RA patients. In the stratum of ACPA and RF seronegative RA patients, the CarP test demonstrated low sensitivity (17.9%), high specificity (96.9%), and only moderate accuracy (AUC < 0.7), which suggests that CarP is not useful in seronegative patients.

Of note, a low rate of CarP positivity was observed in the control group of RDs: 5.8% of patients with SSc (20) and 28.3% of patients with systemic lupus erythematosus (21). CarP-positive patients from the SSc group (three patients) all had a history of a chronic seronegative RA-like nonerosive arthritis. It is therefore conceivable that CarP positivity may be associated with joint inflammation also in other connective tissue diseases.

Despite some data reporting a significant association between CarP and a severe course of RA (7), we did not observe significant differences in DAS-28 mean values, CRP, ESR, and HAQ between CarP+ and CarP– RA patients. Moreover, in multiple logistic analysis, we found no association between the number of positive autoantibodies and presence of moderate-severe disease (DAS-28 > 3.2).

Some limitations of our study should be described. First, the cross-sectional nature of our study and the absence of radiographic data did not allow us to evaluate the presence of an association between CarP levels and severe, progressive, and erosive course of RA disease. Second, we enrolled patients under

immunosuppressive treatment at the moment of CarP testing: although not documented to date, a negative effect of treatment with immunosuppressants on serum concentrations of CarP cannot be ruled out. Third, we should also consider the bias in the assessment of CarP performance introduced by the inclusion of RF and ACPA in the 2010 EULAR classification criteria. We selected these criteria because of the lack of complete x-ray data. However, it should be also emphasized that the use of the 1987 RA classification criteria might also have biased the results, although to a lesser extent, because of the inclusion of the RF (14). Last, because of the small sample size of the “other RDs” group, no firm conclusions could be drawn regarding the prevalence of CarP in other RDs.

In conclusion, our data confirmed a good performance of CarP for the diagnosis of established RA. However, the additional value of CarP over conventional ACPA and RF biomarkers for the diagnosis of RA appears minimal.

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AUTHOR CONTRIBUTIONS

Dr. Erre drafted the article. All authors revised the article critically for important intellectual content, approved the final version to be published, and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Erre, Carru, Zinellu, Passiu.

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