EXTENDED REPORT

The association between anti-carbamylated protein (anti-CarP) antibodies and radiographic progression in early rheumatoid arthritis: a study exploring replication and the added value to ACPA and rheumatoid factor

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Handling editor Tore K Kvien

▶ Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2015-208870).

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Received 6 November 2015
Revised 31 March 2016
Accepted 9 April 2016
Published Online First 27 April 2016

To cite: Ajeganova S, van Steenbergen HW, Verheul MK, *et al*. *Ann Rheum Dis* 2017;**76**:112–118.

ABSTRACT

Objective Anti-carbamylated protein (anti-CarP) antibodies are reported to associate with more radiographic progression within the total rheumatoid arthritis (RA) population and anti-citrullinated peptide antibody (ACPA)-negative subgroup. We explored the association of anti-CarP with radiographic progression in RA and aimed to replicate the association and evaluate the added value of anti-CarP antibodies in relation to ACPA and rheumatoid factor (RF).

Methods 576 Swedish and 628 Dutch patients with RA (2394 and 3247 sets of radiographs, respectively) were longitudinally studied. Replication was restricted to the Swedish patients. In both cohorts, the association of anti-CarP with radiographic progression was determined in strata of patients with similar ACPA and RF status; results of both cohorts were combined in fixed-effect meta-analyses. The net percentage of patients for whom the radiographic progression in 5 years was additionally correctly classified when adding anti-CarP to a model including ACPA and RF was evaluated.

Results Anti-CarP associated with radiographic progression in the total Swedish RA population (beta=1.11 per year, $p=8.75\times10^{-13}$) and in the ACPA-negative subgroup (beta=1.14 per year, p=0.034). Anti-CarP associated with more radiographic progression in the strata of ACPA-positive/RF-negative, ACPA-negative/RF-positive and ACPA-positive/RF-positive patients with RA (respective p values 0.014, 0.019 and 0.0056). A model including ACPA and RF correctly classified 54% and 57% of the patients; adding anti-CarP to this model did not increase these percentages (54% and 56% were correctly classified).

Conclusions Anti-CarP antibodies associated with more severe radiographic progression in the total and ACPA-negative RA population. Anti-CarP-positivity had a statistically significant additive value to ACPA and RF, but did not improve correct classification of patients.

INTRODUCTION

Rheumatoid arthritis (RA) is considered a prototype autoimmune disease with anti-citrullinated peptide antibodies (ACPAs) and rheumatoid factors (RFs) being its hallmark autoantibodies.[1] However, approximately 30–40% of patients with early RA do not have these autoantibodies.[2–4] Although it is not exactly known how RA-related autoantibodies exert their effect,[5] autoantibody-positive and autoantibody-negative RA, mainly ACPA-positive and ACPA-negative, are considered as two different disease entities with different genetic backgrounds[6 7] and different disease outcomes. In general, patients with autoantibodies have more severe disease course with more joint damage progression.[8 9] However, a substantial part of seronegative patients also develop severe joint destruction and there is a need to identify biomarkers predictive for future radiographic progression in patients who lack ACPA and RF.[10]

In this respect, the identification of novel autoantibody systems within RA is relevant.[11–13] One of these newly identified autoantibodies are anti-carbamylated protein (anti-CarP) antibodies, which were detected in 36–45% of the sera of all patients with RA and in 3–16% of the ACPA-negative patients.[11 14–16] Indeed, the presence of anti-CarP was observed to associate with the severity of joint destruction within ACPA-negative patients with RA.[11] These findings contribute to our understanding of autoimmune processes in RA and might be valuable to identify patients with a worse prognosis.

However, in order to determine the usefulness of anti-CarP antibodies in patients with early RA for identification of patients at risk for radiographic progression, several items need to be addressed. First, the association of anti-CarP with radiographic progression has to be replicated in a large cohort with longitudinal radiographic follow-up. At present, there is some evidence suggestion replication, but these studies were considerably smaller than the identification cohort;[15 17] hence, compelling replication is still lacking. Second, in clinical practice, ACPA and RF are generally determined and both autoantibodies associate with more severe radiographic progression. These questions prompted us to perform the present study. Our first aim was to replicate the association of anti-CarP with radiographic progression in RA. Then, in order to determine the additive value of anti-CarP to ACPA and RF, we aimed to assess this association in subgroups of patients with similar ACPA and RF status. We aimed then to assess the clinical

Table 1 Baseline characteristics

	BARFOT	Leiden EAC
Total number of patients	576	628
Total number of sets of radiographs	2394	3247
Radiographic follow-up (years)	8	7
Year of diagnosis	1993–1999	1993–2006
Female, number (%)	368 (63.9)	427 (68.0)
Age at diagnosis in years, mean (SD)	57.3 (15.0)	56.8 (15.8)
CRP at inclusion (mg/L)	20 (8–49)	12 (4–28)
ESR at inclusion (mm/h)	30 (16–51)	33 (19–54)
ACPA-positive, number (%)	340 (59.0)	337 (53.7)
RF-positive, number (%)	340 (59.0)	370 (58.9)
Anti-CarP-positive, number (%)	209 (36.3)	293 (46.7)

ACPA, anti-citrullinated peptide antibody; BARFOT, Better Anti-Rheumatic FarmacoTherapy; CarP, carbamylated protein; CRP, C reactive protein; EAC, Early Arthritis Clinic; ESR, erythrocyte sedimentation rate; RF, rheumatoid factor.

usefulness of anti-CarP beyond ACPA and RF by the net percentage of patients for whom radiographic progression was additionally correctly classified with information on anti-CarP status. For the first aim, patients from the Better Anti-Rheumatic FarmacoTherapy (BARFOT) cohort (576 patients with 2394 radiographs) were studied. For the second and third aims, patients from both BARFOT and the Leiden Early Arthritis Clinic (EAC) cohort (EAC comprised 628 patients with 3247 radiographs) were evaluated.

PATIENTS AND METHODS

Patients

Two large European RA cohorts that included and followed patients before the widespread introduction of biologics were studied. All patients fulfilled the 1987 American College of Rheumatology RA criteria.[18] Baseline clinical characteristics and autoantibody status are presented in table 1 and figure 1.

BARFOT cohort

This multicentre Swedish cohort consisted of 576 patients with early RA included in 1993–1999.[19] Radiographs of hands and feet were performed at baseline and at 1, 2, 5 and 8 years follow-up. In total, 2394 radiographs were available and chronologically scored by the Sharp–van der Heijde scoring (SHS) method by one of two experienced readers who were blinded to the clinical data. The between-reader intraclass correlation coefficient (ICC) was 0.94 and the within-reader ICC 0.998. Treatment strategies differed between 1993–June 1996 and July 1996–1999, resulting in less severe radiographic progression in the second period. Patients included in 1993–June 1996 were treated with mostly mild disease-modifying antirheumatic drugs, and in July 1996–1999 methotrexate was commenced early. The baseline sera were tested for IgM-RF, ACPA (anti-CCP2, Eurodiagnostica, Malmö, Sweden) and IgG anti-CarP antibodies against carbamylated fetal calf serum (FCS). Anti-CarP was determined by ELISA[11] (see online supplementary text for details); the cut-off was set at 452 aU/mL based on the 82 control samples from the source population.

Leiden EAC cohort

This cohort contained 628 Dutch patients with early RA included in 1993–2006.[20] In total, 3247 radiographs of hands and feet performed at baseline and yearly follow-ups during 7 years were available and scored using the SHS in chronological order by one experienced reader who was blinded to clinical data (ICC 0.91). Patients included in 1993–1995 were initially treated with non-steroidal anti-inflammatory drugs, in 1996–1998 with hydroxychloroquine or sulfasalazine, and patients included in 1999–2006 were promptly treated with methotrexate. The baseline sera were tested for IgM-RF, ACPA (anti-CCP2, Eurodiagnostica, Arnhem, the Netherlands) and IgG anti-CarP FCS antibodies. The cut-off for anti-CarP positivity was set at 246 aU/mL based on 305 Dutch controls. As the association between anti-CarP and radiographic progression has been previously identified in the EAC, this cohort was used only for evaluation of the additive value of anti-CarP to ACPA and RF.

Statistical methods

Associations between autoantibody status and radiographic joint damage were analysed using multivariate normal regression for longitudinal data (see online supplementary text and reference[21] for details) with radiographic score as outcome and autoantibody status as independent variable. Associations between anti-CarP antibodies and radiographic progression were assessed in the total RA population, in ACPA-positive and ACPA-negative

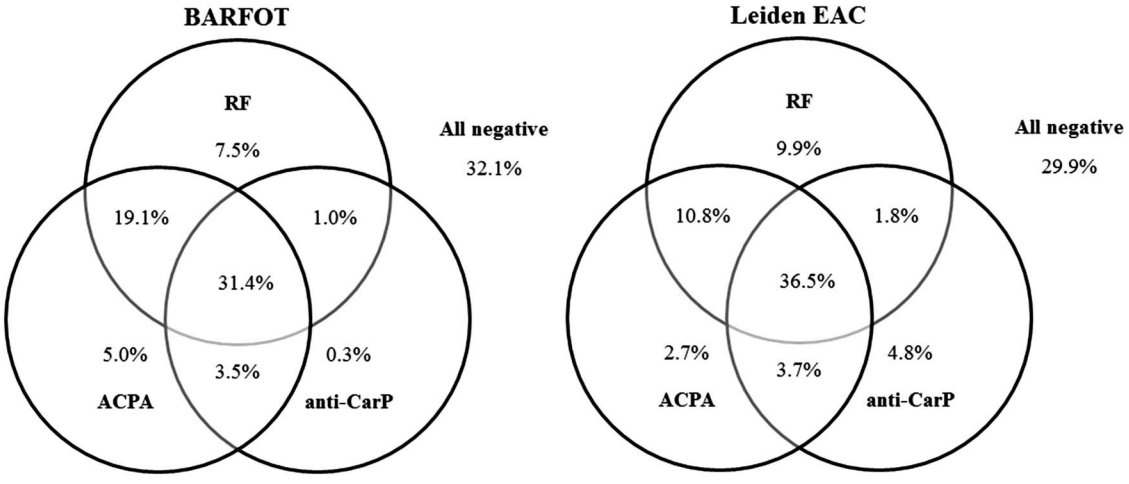

Figure 1 Autoantibody status of patients with rheumatoid arthritis within the Better Anti-Rheumatic FarmacoTherapy (BARFOT) and Leiden Early Arthritis Clinic (EAC) cohorts. ACPA, anti-citrullinated peptide antibody; CarP, carbamylated protein; RF, rheumatoid factor.

patients, in the subgroups of patients: ACPA-negative/RF-negative, ACPA-positive/RF-negative, ACPA-negative/RF-positive and ACPA-positive/RF-positive, and in the groups by number of presented autoantibodies. We anticipated that analyses within subgroups have insufficient power to detect true associations in the individual cohorts. Therefore, the effect sizes and SEs of the analyses in the cohorts were combined in inverse-weighted variance meta-analyses. This meta-analysis weights the results with a low SE stronger than the results with a high SE, preventing an over-representation of less precise data. As we assumed that the cohorts share the true effect, fixed-effect models were applied.

To evaluate the usefulness of anti-CarP antibodies, in addition to ACPA and RF status, in identifying patients with no, moderate or severe progression, we assessed the net percentage of correctly reclassified patients.[22] The progression in SHS during 5 years ($\Delta SHS_{0-5\ years}$) was categorised in three severity groups: $\Delta SHS_{0-5\ years} \leq 5$, 6–25 and >25 units, indicating no/little, moderate and severe radiographic progression. The cut-offs were chosen according to those previously suggested: minor radiological progression if a change ≤1 SHS unit per year and rapid progression if an increase >5 SHS units per year.[23–25] As described in the online supplementary text, the improvement in predictive performance gained by adding information on anti-CarP-status to ACPA and RF was determined (the net percentage correct reclassifications minus incorrect reclassifications of radiographic progression).

All multivariate normal regression and linear regression analyses were adjusted for age, gender and inclusion period (as proxy for different treatment strategies).

RESULTS

Replication of association of anti-CarP antibodies with progression of joint damage

First, the association between anti-CarP antibodies and radiographic progression was studied within the BARFOT cohort. Patients carrying anti-CarP antibodies had a 1.11-fold rate of joint destruction per year compared with patients without anti-CarP, $p=8.75\times10^{-13}$ (figure 2A). This equals a 230% (1.11^8) higher rate of joint destruction over 8 years. When adjusting the analysis for ACPA and RF, anti-CarP remained significantly associated with more radiographic progression, beta=1.06 per year, $p=6.90\times10^{-4}$.

Subsequently, analyses were stratified for ACPA status; this was done as in the identification cohort anti-CarP was strongly associated with radiographic progression in ACPA-negative patients with RA.[11] Also within the 236 ACPA-negative Swedish patients with RA, presence of anti-CarP was significantly associated with radiographic progression, beta=1.15 per year, p=0.034 (figure 2B). In addition, within the ACPA-positive patients, patients with anti-CarP had a significantly higher rate of joint destruction, beta=1.05 per year, p=0.0021 (figure 2C).

Thus, within 576 Swedish patients with RA with longitudinal radiographic follow-up, the association of anti-CarP with joint damage progression was replicated.

Association of anti-CarP antibodies with radiographic progression in strata of patients with similar ACPA and RF status

We then evaluated whether anti-CarP antibodies were associated with radiographic progression within subgroups of patients having similar combinations of ACPA and RF, that is, in the following strata: ACPA-negative/RF-negative, ACPA-positive/RF-negative, ACPA-negative/RF-positive and ACPA-positive/RF-positive. These subgroup analyses explored the additive value of anti-CarP status to known ACPA and RF status.

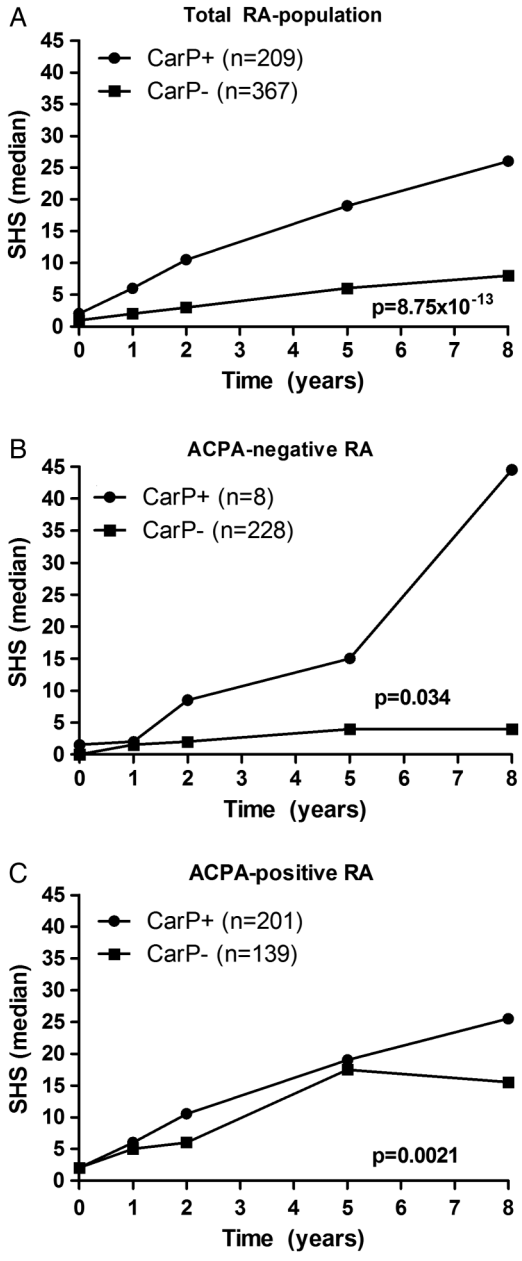

Figure 2 Presence of anti-carbamylated protein (CarP) antibodies in relation to radiographic progression in all patients with rheumatoid arthritis (RA) (A), anti-citrullinated peptide antibody (ACPA)-negative patients with RA (B) and ACPA-positive patients with RA (C) in the Better Anti-Rheumatic FarmacoTherapy cohort. SHS, Sharp–van der Heijde scoring.

Within ACPA-negative/RF-negative patients, the presence of anti-CarP antibodies did not significantly associate with radiographic progression, ACPA−/RF−/CarP+ versus ACPA−/RF−/CarP−, fixed-effects meta-analysis p=0.074 (figure 3A). Baseline C reactive protein (CRP) differed in the cohorts (table 1). Meta-analysis with additional adjustment for baseline CRP showed similar result, p=0.11.

Within the ACPA-positive/RF-negative patients, anti-CarP positivity associated with more severe joint damage progression, ACPA+/RF−/CarP+ versus ACPA+/RF−/CarP−, meta-analysis p=0.014 (figure 3B), CRP-adjusted p=0.028. Similarly, within

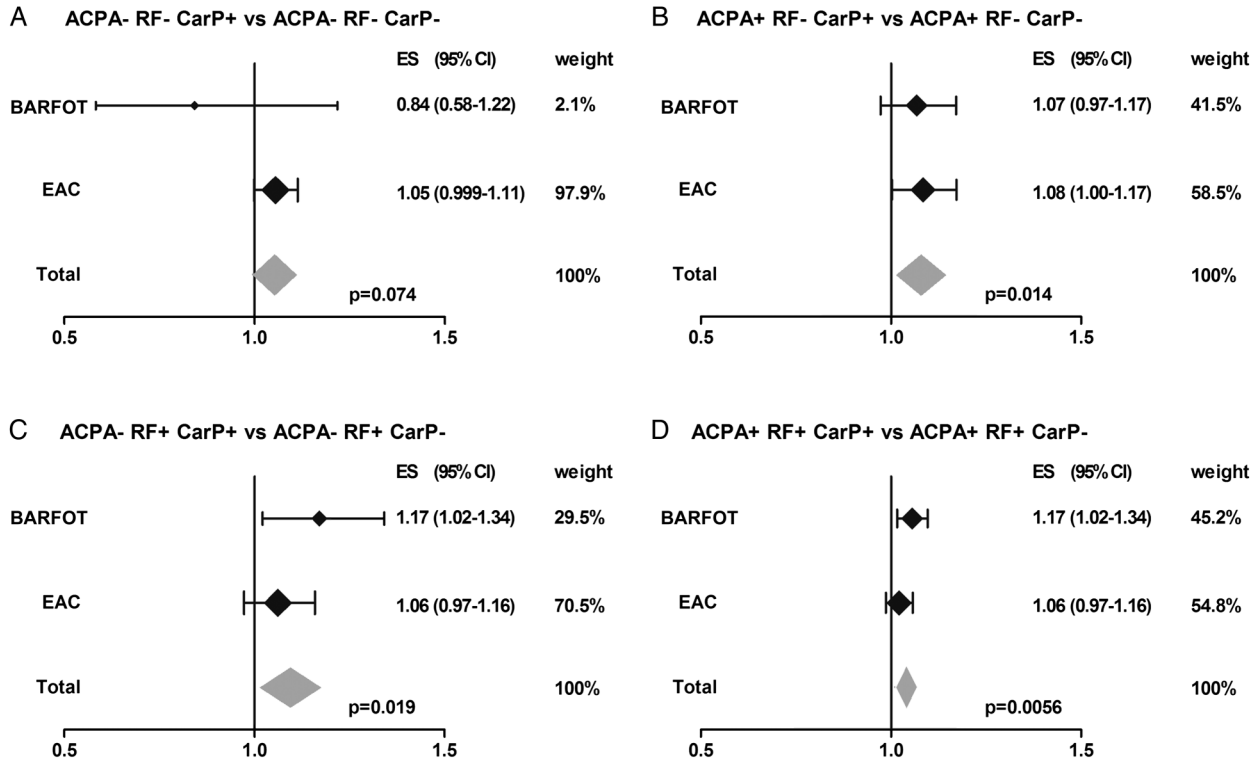

Figure 3 Presence of anti-carbamylated protein (CarP) antibodies in relation to radiographic progression in subgroups of patients with similar anti-citrullinated peptide antibody (ACPA) and rheumatoid factor (RF) status. BARFOT, Better Anti-Rheumatic FarmacoTherapy; EAC, Early Arthritis Clinic.

ACPA-negative/RF-positive patients, patients carrying anti-CarP had more radiographic progression, ACPA−/RF+/CarP+ versus ACPA−/RF+/CarP−, meta-analysis p=0.019 (figure 3C), CRP-adjusted p=0.039. Finally, within ACPA-positive/RF-positive patients, anti-CarP positivity significantly associated with more radiographic progression, ACPA+/RF+/CarP+ versus ACPA +/RF+/CarP−, meta-analysis p=0.0056 (figure 3D), CRP-adjusted p=0.011.

Thus, a statistically significant association of anti-CarP positivity with more severe radiographic progression was observed in all subgroups of patients with RA except for the stratum of patients negative for both ACPA and RF. The effect sizes of associations of anti-CarP antibodies with radiographic progression in the subgroups of the individual cohorts ranged 1.05-fold to 1.17-fold rate of joint destruction per year compared with patients without anti-CarP.

Number of additionally correctly classified patients with radiographic progression after evaluation of anti-CarP status in addition to ACPA and RF

In order to estimate the usefulness of anti-CarP testing in clinical practice, we evaluated the net number of correctly reclassified patients with radiographic progression when adding information on the anti-CarP-status to known ACPA and RF status. We analysed whether the net number of patients with severe progression (defined as $\Delta SHS_{0-5\ years}$ >25), moderate progression ($\Delta SHS_{0-5\ years}$ 6–25) or no/little progression ($\Delta SHS_{0-5\ years}$ ≤5) that was correctly classified by adding anti-CarP status was increased. First, the predicted $\Delta SHS_{0-5\ years}$ was analysed by a model including ACPA and RF, and subsequently, by a model that also included anti-CarP status. For each model, predicted $\Delta SHS_{0-5\ years}$ was plotted against observed $\Delta SHS_{0-5\ years}$ categorised in three severity groups. The number of patients who were correctly classified by the models was determined.

Within the BARFOT cohort, 433 patients had radiographic data at baseline and after 5 years; in 233 of them (53.8%), radiographic progression was correctly classified by a model including ACPA and RF status (see online supplementary figure S1A). When anti-CarP-status was added, the number of correctly classified patients was 232 (53.6%), that is, no increase in the net percentage of patients whose radiographic progression was correctly predicted (table 2A and online supplementary figure S1B).

Likewise, within 311 patients of the Leiden EAC with available radiographic data at baseline and after 5-year follow-up, 177 patients (56.9%) were correctly classified for radiographic outcome by the model including ACPA and RF status, and 170 patients (56.0%) by the model also including anti-CarP status. Thus, there was no increase in correct classifications when adding information on anti-CarP status to ACPA and RF status in the EAC cohort either (table 2B and online supplementary figure S1C,D).

These data suggest that information on anti-CarP antibodies does not have an additive value to ACPA and RF for predicting joint damage severity in clinical situation. When analysing progression in SHS over different time periods, similar results were obtained (data not shown).

Association of number of autoantibodies with radiographic progression

A higher number of presented autoantibodies was associated with more radiographic progression in both cohorts (see online supplementary figure S2).

DISCUSSION

This study aimed to explore the association of anti-CarP antibodies with radiographic progression in RA. Here, 1204 patients

Table 2 Numbers of patients with observed and predicted ΔSHS over 5 years (in total and by categories of severity of radiographic progression) by models without and with anti-CarP status in the patients of the BARFOT and the Leiden EAC cohorts

	(A) BARFOT cohort			
	Observed progression over 5 years			
	No (ΔSHS ≤5)	Moderate (ΔSHS 6–25)	Severe (ΔSHS >25)	Total
Predicted progression over 5 years by model without anti-CarP status				
No (ΔSHS ≤5)	**119**	38	12	169
Moderate (ΔSHS 6–25)	68	**114**	82	264
Severe (ΔSHS >25)	0	0	**0**	0
Predicted progression over 5 years by model with anti-CarP status				
No (ΔSHS ≤5)	**119**	40	12	171
Moderate (ΔSHS 6–25)	68	**112**	81	261
Severe (ΔSHS >25)	0	0	**1**	1
Total	187	152	94	433
	(B) Leiden EAC cohort			
	Observed progression over 5 years			
	No (ΔSHS ≤5)	Moderate (ΔSHS 6–25)	Severe (ΔSHS >25)	Total
Predicted progression over 5 years by model without anti-CarP status				
No (ΔSHS ≤5)	**47**	23	4	74
Moderate (ΔSHS 6–25)	37	**96**	56	189
Severe (ΔSHS >25)	4	10	**34**	48
Predicted progression over 5 years by model with anti-CarP status				
No (ΔSHS ≤5)	**46**	23	3	72
Moderate (ΔSHS 6–25)	39	**97**	64	200
Severe (ΔSHS >25)	3	9	**27**	39
Total	88	129	94	311

Correct classifications of a model including only ACPA and RF status and a model including ACPA, RF and anti-CarP status were compared. Both models included also age, gender and treatment strategy. Within the BARFOT cohort, the models without and with anti-CarP status correctly classified respectively 233 (53.8%) and 232 (53.6%), out of 433 patients, yielding no improvement in correct reclassifications. Within the Leiden EAC, the model without and with anti-CarP status correctly classified respectively 177 (56.9%) and 170 (56.0%) out of 311 patients; also here yielding no improvement in correct reclassifications.
ACPA, anti-citrullinated peptide antibody; BARFOT, Better Anti-Rheumatic FarmacoTherapy; CarP, carbamylated protein; EAC, Early Arthritis Clinic; RF, rheumatoid factor; SHS, Sharp–van der Heijde scoring.

with RA with 5641 longitudinal sets of radiographs were studied from the Swedish BARFOT and the Dutch Leiden EAC cohorts. In the Swedish patients, the association of anti-CarP antibodies with radiographic progression in the total and in the ACPA-negative patients was replicated. Furthermore, anti-CarP had a statistically significant additive value to ACPA and RF in predicting radiographic progression in the subgroups of patients who were ACPA-positive/RF-negative, ACPA-negative/RF-positive and ACPA-positive/RF-positive. With regard to the number of correctly classified patients, information on ACPA and RF yielded a correct classification of only a part of the patients and information on anti-CarP antibodies did not improve this.

The discovery of association between anti-CarP antibodies and more severe radiographic progression in both the total RA population and the ACPA-negative subgroup was made in patients with RA of the Leiden EAC.[11] Thus far, this association was not replicated in a large cohort with multiple radiographs over time. The present study is the first providing independent replication of the association between anti-CarP antibodies and radiographic progression within the ACPA-negative population in a large cohort of patients with RA with longitudinal radiographs over 8 years. Anti-CarP antibodies are predominantly present in RA with the reported sensitivity and specificity for RA of 44% and 89%, respectively.[26] Intriguingly, already in the presymptomatic individuals the presence of anti-CarP antibodies is associated with RA development in ACPA-negative individuals and also with worse radiological progression independent of ACPA.[15] In a small cohort of patients with RA, the presence of anti-CarP antibodies and anti-CarP combined with ACPA has been reported to correlate with joint erosion score.[17]

Autoantibodies are frequently present together. In our populations, 55% and 53% of the patients with RA were positive for at least two out of three assessed autoantibodies. The large majority of anti-CarP-positive patients were also positive for ACPA and/or RF. To unravel the effects of the individual autoantibodies, analyses can either be adjusted or stratified for the presence of other autoantibodies. The advantage of the latter approach is that it most clearly depicts the effect in a subgroup of patients, but with the limitation of a decrease in power due to smaller subgroups. In our previous analysis, stratified by ACPA and RF serostatus, RF did not show an additive effect on bone erosions in ACPA-positive patients, but RF was associated with more severe erosive disease in ACPA-negative patients.[27] In the present study, we were interested in the effect of anti-CarP antibodies in addition to both ACPA and RF, generally assessed in clinical practice. Therefore, we performed analyses in four subgroups of patients with similar ACPA and RF status. As the subgroups in the cohorts were small, the results of the two cohorts were combined in meta-analyses. We observed that anti-CarP antibodies had a statistically significant association with more severe radiographic progression in the subgroups of ACPA-positive/RF-negative, ACPA-negative/RF-positive and ACPA-positive/RF-positive patients. Whether this finding is attributed to the effect of anti-CarP per se or whether it might

be boosted and/or associated with higher levels of ACPA and RF in the subgroup of anti-CarP-positive compared with anti-CarP-negative patients should be addressed in further studies.

No association between anti-CarP antibodies and radiographic outcome was found in the ACPA-negative/RF-negative group in this study. The used anti-CarP assay is not commercial. Healthy controls are needed to be taken along to determine the cut-off for a positive test. The variation in the anti-CarP reactivity within the Swedish controls was higher than that within the Dutch controls, which resulted in a higher cut-off for the BARFOT cohort. ACPA-negative/RF-negative/anti-CarP-positive patients generally have low levels of anti-CarP antibodies. Further, in the BARFOT the number of ACPA-negative/RF-negative/anti-CarP-positive patients was very small. We cannot exclude that if a lower threshold for positivity was used, the number of patients in this subgroup was larger. Indeed, when the Dutch cut-off was applied for the BARFOT data set, the meta-analysis within the ACPA-negative/RF-negative strata was significant (data not shown). However, we think that use of population-specific controls is an advantage. Further validation studies on other independent data sets are certainly needed. At present, the replication of the findings in the independent cohorts seems rationale to overcome possible disparities in the participants and shortcomings of non-standardised test. In our opinion, the similarity of the findings of the primary analyses in the cohorts and the approach of combining the large data sets support the validity of our results.

For patients who are both ACPA and RF-negative, there is a lack of biomarkers to identify a risk for radiographic progression. Within this subgroup of the EAC, presence of anti-CarP antibodies (4.8% single anti-CarP-positive patients) significantly associated with more radiographic progression. A small number of anti-CarP-positive patients in the ACPA-negative/RF-negative subgroup of the BARFOT cohort (0.3% of the patients) did not permit to definitely conclude on the association between anti-CarP antibodies and radiographic progression in ACPA-negative/RF-negative patients (type 2 error). Larger studies and standardised anti-CarP assay are needed to enable validation of multi-antibody testing for prediction of radiographic progression in patients with RA.

The mechanism underlying the association of anti-CarP antibodies with radiographic progression is unknown, as is the case for the association of RF with joint destruction. Recent studies suggest that ACPA might activate osteoclasts directly.[28 29] The extent of functional consequences of the presence of antibodies to carbamylated proteins is unexplored.

To assess the clinical relevance of anti-CarP antibodies, we quantified the net percentage of patients for whom the radiographic progression in 5 years was additionally correctly identified when adding anti-CarP status to a model including ACPA and RF. The progression in SHS during the first five years after inclusion, categorised as no/mild/severe progression, was used as outcome. This analysis (based on data at only two timepoints) is less powerful than repeated measurement methods (based on data at all follow-ups), but a clear effect would be observed if it were present. With information on ACPA and RF, only 54% and 57% of patients were correctly classified. In both cohorts, adding information on anti-CarP did not improve correct classification. These data indicate that although anti-CarP positivity had a statistically significant ACPA and RF-independent association with radiographic progression, in clinical practice, when the common autoantibodies ACPA and RF are assessed, anti-CarP status is not useful to differentiate patients who will develop no, mild or severe radiographic progression. Whether levels of anti-CarP antibodies could enable a better differentiation of a poorer radiographic prognosis should be addressed with an optimised anti-CarP antibody test.

In conclusion, the present study provided replication for the association of anti-CarP antibodies with more radiographic progression in the total RA population and in the ACPA-negative subgroup. Analyses within strata of patients with similar ACPA and RF status showed that the presence of anti-CarP antibodies was statistically significantly associated with more severe radiographic progression. These findings might contribute to our understanding of the role of autoimmunity in regulating disease severity. The clinical usefulness of determination of anti-CarP status in addition to ACPA and RF for prognosis of radiographic progression was, however, not observed here.

Contributors All authors were involved in drafting the manuscript or revising it critically for important intellectual content, and all authors approved the final version of the manuscript to be published. SA and HWvS performed data analyses. SA, HWvS, TWJH and AHMvdH-vM contributed to the concept of the study and data interpretation. SA, HWvS, MKV, KF, IH, REMT, BS and LAT contributed to data acquisition.

Funding The authors acknowledge the financial support from the Dutch Arthritis Foundation, The Netherlands Organization for Scientific Research, the IMI JU funded project BeTheCure, contract no 115142-2, the Swedish Rheumatism Association and King Gustav V 80 year's Foundation. Both AHMH and LAT are each supported by a ZON-MW Vidi grant.

Competing interests REM T, TWJH and LAT are on a patent application for the use of anti-carbamylated protein antibodies in diagnostics.

Patient consent Obtained.

Ethics approval The medical ethical committees of the participating centres approved the study.

Provenance and peer review Not commissioned; externally peer reviewed.

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Ann Rheum Dis 2017 76: 112-118 originally published online April 26, 2016
doi: 10.1136/annrheumdis-2015-208870