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ORIGINAL ARTICLE

Anti-carbamylated protein antibodies positivity and disease activity in Hispanic patients with established rheumatoid arthritis: An observational study

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ABSTRACT

Objectives: We aimed to determine the prevalence of anti-carbamylated protein (anti-CarP) antibodies in Mexican Hispanics with established rheumatoid arthritis (RA) and to assess their relationship with disease activity.

Methods: A cohort study was conducted in 278 patients with established RA during an 18-month follow-up. We measured IgG/IgM/IgA rheumatoid factor (RF), IgG anticitrullinated protein antibodies (ACPA) and IgG/IgM/IgA anti-CarP antibodies using enzyme-linked immunosorbent assay (ELISA). For disease activity, we performed the 28-joint disease activity score with erythrocyte sedimentation rate (DAS28-ESR). Repeated measures one-way ANOVA was used to test the association between anti-CarP IgG antibody status and longitudinal DAS28-ESR scores. Patients were evaluated at baseline and at 6, 12, and 18 months during follow-up.

Results: Anti-CarP IgG antibodies were positive in 47.8% of patients and, accounting for all isotypes, in 9.5% of patients with negative RF and ACPA. Triple antibody positivity was present in 42.6% of patients in our sample. Anti-CarP IgG antibody positivity did not show statistically significant differences in mean DAS28-ESR when compared to anti-CarP IgG antibody negative patients at baseline, 6, 12 or 18 months.

Conclusion: Anti-CarP IgG antibodies are not associated to a higher disease activity in Hispanic patients with established RA. Our findings suggest that the clinical value of measuring anti-CarP antibodies in RA diminishes over time.

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Introduction

Anti-carbamylated protein (anti-CarP) antibodies, a new autoantibody system described in rheumatoid arthritis (RA), is characterized by antibodies against proteins that contain homocitrulline residues. These post-translational protein modifications are produced by a non-enzymatic chemical reaction, involving cyanate in the conversion of lysine into homocitrulline. In susceptible individuals, extensive carbamylation may trigger the development of an autoimmune response [1,2]. Carbamylated and citrullinated peptides complement each other in the generation of the autoimmune response. The immune-activating effects of carbamylation may enhance the arthritogenic properties of citrullinated peptides, providing new information regarding the mechanism underlying the pathogenesis of autoimmune arthritis [3–7].

Clinically relevant anti-CarP antibodies are detected in up to 45% of RA patients (45% IgG and 43% IgA isotypes). Anti-CarP antibodies have been associated with higher disease activity, disability, bone erosions and mortality [8–12]. Premenopausal RA women have shown higher serum levels of anti-CarP antibodies, which have been correlated with articular erosive changes and a reduction in systemic trabecular and local bone mineral density [11]. The association of anti-CarP antibody positivity with increased mortality has been found to be independent of the presence of rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA). This increase was mostly specific of respiratory system causes of death, although this association is unprecedented and should be regarded with caution until replicated [12].

The current trend of anti-CarP antibodies in RA is that they seem to confer a higher disease activity status [8,10,11],

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although Kaneko et al. found no correlation between serum CarP levels and the 28-joint disease activity score with C-reactive protein (DAS28-CRP) [5]. Both of these findings are not mutually exclusive, since the presence of carbamylated proteins (the target of anti-CarP antibodies) alone does not seem to be sufficient to break tolerance and induce autoimmunity [4]. The description of a new system of autoantibodies associated with poorer clinical outcomes in RA is particularly relevant in patients negative to ACPA and RF, given their substantial value in the 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria for definite RA [13].

RA can be divided into early and established forms. While it is accepted that early RA represents the first years after symptom-onset, a consensus on the time frame for the precise beginning of established RA is currently lacking. Following the definition of early RA, established RA should refer to all patients with disease duration greater than one or two years, irrespective of clinical or radiological joint damage [14]. This distinction is relevant because the earliest clinically apparent phases of RA represent important therapeutic windows within which treatment is associated with improved long-term outcomes [15]. Because most of the studies regarding anti-CarP antibodies have focused on patients in early phases of RA [16], information regarding anti-CarP antibodies in patients with established RA is scarce. Furthermore, few studies have analyzed anti-CarP antibodies in Hispanic patients with RA [17]. We aimed to longitudinally evaluate the relationship between anti-CarP antibody positivity and disease activity in an established RA Hispanic cohort. We hypothesize that Hispanic patients with established RA positive to anti-CarP antibodies have a higher disease activity as measured by DAS28 with erythrocyte sedimentation rate (DAS28-ESR) during an 18-month follow up.

Materials and methods

Participants

We performed an observational, descriptive, longitudinal cohort study. Patients over 18 years of age were invited to participate if they fulfilled the 2010 ACR/EULAR classification criteria for RA [13] and were evaluated in the Rheumatology Department of the University Hospital "Dr. José E. González" in Monterrey, México from January 2017 to August 2018. We included patients with established RA, with at least one year of disease duration, as suggested previously [14]. Patients with overlap syndromes or pregnancy were excluded. Overlap syndrome was defined as the fulfillment of the classification criteria of two or more connective tissue diseases in the same patient [18], or the diagnosis by a rheumatologist of another systemic autoimune rheumatic disease. The study was carried out in 4 visits during an 18-month follow-up (baseline, 6, 12 and 18 months). Subjects that did not fulfill the four follow-up visits were eliminated from the final analysis.

Demographic and clinical characteristics, such as age, sex, body mass index, time to RA diagnosis and smoking status were collected. Disease severity was evaluated by means of DAS28-ESR with conventional cutoffs [19]. Physical examination of swollen and tender joint counts were measured by a board-certified rheumatologist at each visit. Additional data, including comorbidities and medications (prednisone, methotrexate, leflunomide, sulfasalazine, anti-malarics and biological disease-modifying antirheumatic drugs [bDMARDs]) were retrieved from the clinical records of the patients.

Laboratory tests

At baseline, ESR, RF, ACPA and anti-CarP antibody laboratories were performed. ESR was measured by the Westergreen method with a cutoff of >15 mm/Hr for men and >20 mm/Hr for women. ACPA IgG antibodies were measured with a 2nd generation enzyme-linked immunosorbent assay (ELISA) kit (Euroimmun, Lübeck, Germany) with a positive cutoff of ≥5 RU/mL as provided by the manufacturer. RF isotypes (IgA, IgM, IgG) were performed with an ELISA kit (Euroimmun, Lübeck, Germany) with positive cutoffs of ≥20 RU/mL as provided by the manufacturer.

The IgG anti-CarP antibodies were measured by ELISA, as previously described by Shi et al. [20]. Positive cutoff points were established for a positive reaction as mean plus two standard deviations (SD) for anti-CarP, obtained from 60 healthy blood donor controls compared to 126 RA patients. We obtained an area under the ROC curve of 0.779 (CI 95% 0.720 to 0.845). The cutoff of 90 AU/mL had a sensitivity of 38.7% and a specificity of 94.9%. We also measured anti-CarP IgM and IgA antibodies with the same methodology.

Statistical methods

The statistical analysis was performed using SPSS statistical software v.25.0 (IBM, Armonk, NY). Qualitative variables are expressed as absolute frequency and percentages. The Kolmogorov-Smirnov test was used to evaluate normality in continuous variables. Variables with a normal distribution are presented as mean and standard deviation (SD), whereas variables with a non-normal distribution are expressed as median and 25th and 75th percentiles (p25 – p75). Repeated measures one-way ANOVA test was performed to evaluate the relationship between disease activity and antibody positivity. A p-value $< .05$ was considered as statistically significant.

Ethical considerations

The protocol was approved by the institutional research and ethics committee (RE16-00007). Written informed consent was obtained from all participants before inclusion, and all procedures performed in this study were in accordance with the 1964 Helsinki declaration and its later amendments.

Results

Demographics

We included 278 RA patients in the analysis, with no patients lost to follow-up. A total of 254 (91.4%) were women. Mean age in years was 51.09 (SD 11.13). The median time of RA evolution was 5 years (3–10). Current or past smoking was referred by 114 (41%) subjects. The most frequent comorbidities were type 2 diabetes mellitus (T2DM), present in 38 (13.7%) patients followed by hypertension in 53 (19.1%), and hypothyroidism in 20 (7.2%). Demographic and clinical characteristics of patients divided by anti-CarP IgG antibody status are detailed in Table 1.

Antibody status

Anti-CarP antibodies were tested in 278 patients, of whom 263 had available RF and ACPA serology (Table 2). Anti-CarP IgG was positive in 133 (47.8%) patients. The mean anti-CarP IgG AU/mL in the cohort was 227.52 (SD 402.5). Anti-CarP IgM and IgA analysis yielded 89 (32%) and 74 (26.6%) positive patients, respectively.

Among the 263 patients with all three antibodies tested, positivity to anti-CarP antibodies accounting for any isotype (IgA, IgM and IgG) was present in 178 (67.7%) patients (Figure 1). Patients negative to RF (any isotype) and IgG ACPAs were positive to anti-CarP in 25 (9.5%) individuals of the sample. Triple positivity accounting for all isotypes to anti-CarP, RF and ACPAs was found in 112 (42.6%) of patients. Triple antibody negativity was present in 21 (8%) patients.

Follow-up

The pooled basal DAS28-ESR mean was 4.13 (SD 1.48), with a median of 4.09 (2.9–5.3). At the 6-month visit mean was 3.7 (SD 1.3) with a median of 3.5 (2.6–4.6). At the 12-month visit mean was 3.6 (SD 1.3) with a median of 3.3 (2.6–4.4), and at the 18-month visit mean was 3.7 (SD 1.3) with a median of 3.6 (2.7–4.5).

To explore the clinical differences in activity indexes (DAS28-ESR) at baseline and at 6, 12, and 18 months in follow-up according to the anti-CarP IgG status, we performed a repeated measures ANOVA test. The mean DAS28-ESR per visit divided by anti-CarP IgG status is depicted in Figure 2. With the non-sphericity assumed by Greenhouse-Geisser analysis, we obtained a non-significant statistical difference among groups in the repeated measures one-way ANOVA ($p = .829$). The difference was also non-significant when the model was adjusted to ACPA positivity and

Table 1. Demographic and clinical characteristics of patients divided by anti-CarP IgG antibody status.

Total cohort	$n = 278$	Anti-CarP IgG positive ($n = 133$)	Anti-CarP IgG negative ($n = 145$)	p
Demographic features				
Age in years, mean (SD)	51 (11.9)	52.8 (10.6)	49.5 (11.3)	.01
Female, *n* (%)	254 (91.4)	119 (89.5)	135 (93.1)	.2
Clinical features				
BMI in kg/m^2, mean (SD)	28.1 (5.3)	27.9 (5.4)	28.3 (5.3)	.5
Time to RA diagnosis in years, median (p25 – p75)	5 (3–10)	5 (3–10)	5 (3–10)	.2
Smoking status, *n* (%)	114 (41)	59 (44.4)	55 (37.9)	.2
T2DM, *n* (%)	38 (13.7)	21 (15.8)	17 (11.7)	.3
Cardiovascular disease, *n* (%)	9 (3.2)	3 (2.3)	6 (4.1)	.3
Hypertension, *n* (%)	53 (19.1)	26 (19.5)	27 (18.6)	.8
Hypothyroidism, *n* (%)	20 (7.2)	9 (6.8)	11 (7.6)	.7
DAS28-ESR Basal visit, mean (SD)	4.1 (1.4)	4.15 (1.4)	4.1 (1.5)	.7
VAS patient in mm, mean (SD)	37.2 (29.7)	37.4 (29.8)	37.0 (29.8)	.8
TJC, mean (SD)	6.3 (7.9)	6.1 (7.8)	6.5 (8.1)	.6
SJC, mean (SD)	2 (3.3)	2.0 (3.1)	2.0 (3.5)	.9
ESR in mm/Hr, mean (SD)	30.5 (13.3)	32.0 (13.4)	29.3 (13.2)	.08
Treatment				
Prednisone, *n* (%)	207 (74.5)	99 (74.4)	108 (74.5)	.9
Prednisone dose in mg/day, mean (SD)	5 (4.9)	5.2 (5.1)	4.9 (4.7)	.5
Methotrexate, *n* (%)	207 (74.5)	91 (68.4)	116 (80%)	.02
Methotrexate dose in mg/week, mean (SD)	13.2 (9.1)	11.8 (9.1)	14.5 (8.9)	.01
Leflunomide, *n* (%)	125 (45)	63 (47.4)	62 (42.8)	.4
Sulfasalazine, *n* (%)	53 (19.1)	20 (15)	33 (22.8)	.1
Anti-malaric, *n* (%)	29 (10.4)	13 (9.8)	16 (11)	.7
bDMARD, *n* (%)	39 (14)	21 (15.8)	18 (12.4)	.1
Patients with all antibodies tested	**$n = 263$**	**Anti-CarP IgG positive ($n = 127$)**	**Anti-CarP IgG negative ($n = 136$)**	**p**
RF IgA positive, *n* (%)	155 (58.9)	82 (64.6)	73 (53.7)	.07
RF IgM positive, *n* (%)	188 (71.5)	98 (77.2)	90 (66.2)	.04
RF IgG positive, *n* (%)	44 (16.7)	27 (21.3)	17 (12.5)	.05
ACPA IgG positive, *n* (%)	144 (54.8)	84 (66.1)	60 (44.1)	.001
Anti-CarP IgA positive, *n* (%)	70 (26.6)	40 (31.5)	30 (22.1)	.08
Anti-CarP IgM positive, *n* (%)	86 (32.7)	58 (45.7)	28 (20.6)	.001

SD: standard deviation; BMI: body mass index; RA: rheumatoid arthritis; IQR: interquartile range; T2DM: type 2 diabetes mellitus; DAS28-ESR: Disease Activity Score in 28 joints using the erythrocyte sedimentation rate; VAS: visual analog scale; TJC: tender joint count; SJC: swollen joint count; bDMARD: biological disease-modifying anti-rheumatic drug; RF: rheumatoid factor; Ig: immunoglobulin; ACPA: anti-citrullinated peptide antibody; Anti-CarP: anti-carbamylated protein antibodies.

Table 2. Antibody status in the pooled cohort of 278 patients.

	Mean (SD)	Antibody positivity, *n* (%)	95% CI
RF IgA[a]	266.9 (460.5)	155 (58.9)	53.0–64.9
RF IgM[a]	406.8 (611.9)	188 (71.5)	66.0–77.0
RF IgG[a]	36.1 (249.6)	44 (16.7)	12.2–21.3
ACPA IgG[a]	191.01 (411.1)	144 (54.8)	48.7–60.8
Anti-CarP IgA[b]	212.9 (464.2)	74 (26.6)	21.4–31.8
Anti-CarP IgM[b]	381.6 (762)	89 (32)	26.5–37.5
Anti-CarP IgG[b]	227.5 (402.5)	133 (47.8)	41.9–53.8

[a]Data were available for 263 patients. Units are RU/mL.
[b]Data were available for 278 patients. Units are AU/mL. RF: rheumatoid factor; ACPA: anticitrullinated protein antibodies; Anti-CarP: anti-carbamylated protein; Ig: immunoglobulin; SD: standard deviation; 95% CI: 95% confidence intervals.

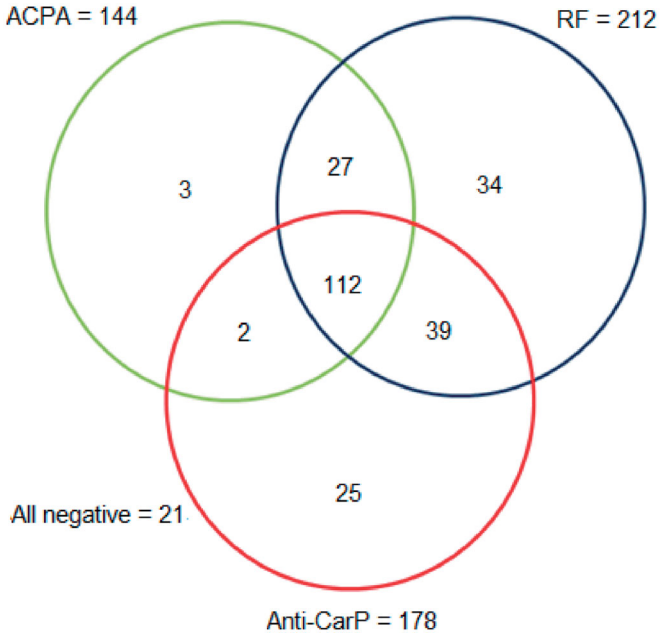

Figure 1. Antibody status from patients with all antibodies tested ($n = 263$) fulfilling 2010 ACR/EULAR classification criteria for RA. Assessed antibodies included ACPA (IgG), RF (IgA, IgM and IgG) and anti-CarP (IgA, IgM and IgG). ACPA: anticitrullinated protein antibodies; Anti-CarP: anti-carbamylated protein antibodies; Ig: immunoglobulin; RF: rheumatoid factor.

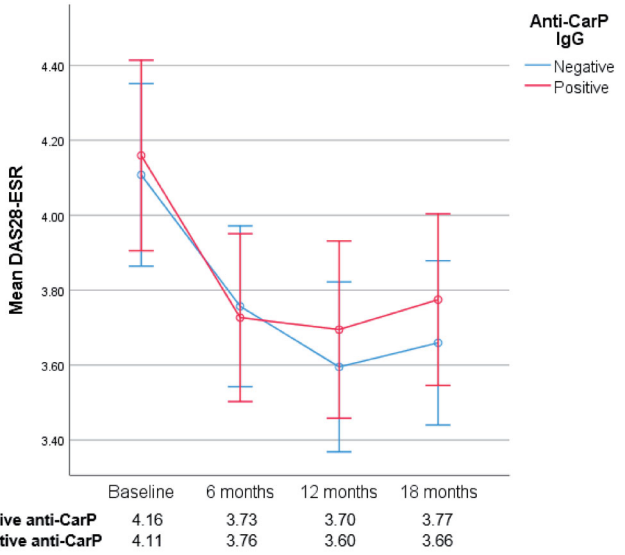

Figure 2. Mean DAS28-ESR scores over an 18-month follow-up by anti-CarP antibody status with error bars corresponding to 95% CI. With the non-sphericity assumed by Greenhouse-Geisser analysis, a non-significant statistical difference was found among groups in the repeated measures one-way ANOVA ($p = .829$). DAS28-ESR: 28-joint disease activity score with erythrocyte sedimentation rate; Anti-CarP: anti-carbamylated protein antibodies; Ig: immunoglobulin.

Table 3. Patients with remission and LDA as measured by DAS28-ESR according the anti-CarP IgG antibody status during 4 visits in the 18-month follow-up.

	Anti-CarP IgG positive ($n = 133$)	Anti-CarP IgG negative ($n = 145$)	*p*
Remission			
Baseline	18 (13.5%)	31 (21.4%)	.086
6 months	33 (24.8%)	26 (17.9%)	.161
12 months	27 (20.3%)	40 (27.6%)	.156
18 months	28 (21.1%)	33 (22.8%)	.731
LDA			
Baseline	40 (30.1%)	50 (64.5%)	.433
6 months	52 (39.1%)	60 (41.4%)	.698
12 months	52 (39.1%)	72 (49.7%)	.077
18 months	47 (35.3%)	52 (35.9%)	.927

LDA: low disease activity; DAS28-ESR: Disease Activity Score in 28 joints using the erythrocyte sedimentation rate; Anti-CarP: anti-carbamylated protein.

methotrexate use. We have therefore not been able to reject the null hypothesis that anti-CarP antibody positivity is not associated to a higher disease activity in Mexican Hispanics as measured by DAS28-ESR during an 18-month follow up.

No statistically significant difference between anti-CarP IgG positive patients vs. anti-CarP IgG negative patients was found regarding remission rate classification (Table 3) at baseline, or at 6, 12, or 18 months during follow-up. Furthermore, no significant difference was found between both groups regarding low disease activity (LDA) (which also included patients in remission, given DAS28-ESR disease activity cutoffs) classified by DAS28-ESR during the 4 aforementioned visits. An equally nonsignificant difference was found when assessing RF and ACPA serology across all isotypes at baseline and at 6, 12 and 18 months (data not shown).

Discussion

We investigated the prevalence of anti-CarP antibody positivity and its association with disease activity in Mexican Hispanic patients with RA. The prevalence of RA is estimated to be around 0.4% for Latin America as a whole, while epidemiological studies reckon a prevalence of 1% for most European populations. In our study, 91.4% of the patients were women, well above the female-to-male ratio of 3:1 in USA and Europe. However, Latin American surveys show that women are more frequently affected than men, with a ratio of 7-8:1, similar to our findings. A similar ratio of 6.9:1 has also been reported in South African Blacks [21,22].

In our cohort of Mexican Hispanic patients with established RA, anti-CarP IgG antibody positive patients did not show statistically significant different disease activity measured by DAS28-ESR over 18 months than patients with negative anti-CarP IgG antibodies. Our rate of positive anti-CarP IgG antibodies patients was 47.8%, similar to the Swedish Better Anti-Rheumatic PharmacOTherapy

(BARFOT) and Dutch Leiden Early Arthritis Clinic (EAC) cohorts, where the anti-CarP IgG positivity was 36% and 47%, respectively [23].

Anti-CarP IgG antibody positivity patterns vary by population. The biggest cohort assessing the overall presence of anti-CarP IgG antibodies is the Norfolk Arthritis Register (NOAR) [24], with a sample of 1995 patients with recent-onset inflammatory polyarthritis from the UK. Overall positivity to anti-CarP antibodies was 23%, although this figure increased to 30% if only the 61% of patients who fulfilled the 2010 ACR/EULAR classification criteria for RA were considered. Other series from France [8] and Sweden [25] found an anti-CarP IgG positivity rate in close to one third of their patients.

The presence of positive anti-CarP antibodies in patients with negative RF and ACPA was 9.5% in our sample, similar to the 11.8% found in the French population [8] and superior to the 5% found in the Dutch Leiden EAC [23] and the UK NOAR cohorts [24]. Anti-CarP antibody measurement may be useful for diagnosis in a subset of patients with negative RF and ACPA seronegative RA, early-onset arthritis, or prediction of progression to RA [26–28].

Triple antibody positivity was present in 42.6% of our sample, similar to the 36.5% observed in the Leiden EAC and the 31.4% from Swedish BARFOT cohorts [23]. Triple positivity (RF, ACPA and anti-CarP) has been found to be associated with progression from early RA to established RA but prospective studies in healthy populations are needed to elucidate the usefulness of these measurements in family members or asymptomatic high-risk individuals [29].

The prevalence of triple antibody negativity varied widely in the published cohorts (30–56%) which reflect the different inclusion criteria between the studies (early, probable, or definite RA, inflammatory arthritis) [8,23,24]. Our cohort with established RA, had a lower triple antibody negativity than the other cohorts (8%) probably due to the strict inclusion criteria (2010 ACR/EULAR classification criteria for RA and at least one year of disease duration).

Anti-CarP antibodies have been associated to a poor prognosis in RA, including radiological damage, long-term disability and increased disease activity [8,10,23–25]. The NOAR, that included 1995 patients with recent-onset inflammatory polyarthritis of which 61% fulfilled the 2010 ACR/EULAR classification criteria for RA, found that anti-CarP positivity was associated with higher disease activity at baseline and over 15 years. However, their pool of patients had a median disease duration of 34 weeks at inclusion, and only one third of their patients were under treatment with a DMARD at baseline. In comparison, up to 80% of our patients were users of prednisone, methotrexate or other DMARDs, and our cohort had been treated for nearly 5 years before the serological anti-CarP analysis. Similarly, data from the French Etude et Suivi des Polyarthrites Indifférenciéees Récentes (ESPOIR) [8] and the Leiden EAC [30] cohorts found an association between higher disease activity and/or radiographic progression and anti-CarP positivity but the median/mean evolution time at baseline was 21 and 23.3 weeks, respectively [30]. The difference between our findings may rely on the distinct evolution time, inclusion of patients already under treatment, sample size, and population.

A recent analysis from the Swedish EIRA cohort [26] in patients with newly diagnosed RA and negative RF and ACPA antibodies found that the presence of anti-CarP antibodies was associated with lower disease activity, contrary to previous reports where anti-CarP antibodies associated with higher disease activity and worse clinical outcomes [8,20,31]. Previous studies have analyzed disease activity or radiological progression over time but have not included fine-specificities antibodies. This cohort measured ACPA with fine-specificities instead of conventional anti-cyclic citrullinated peptide (anti-CCP) assays and found that close to 40% of anti-CarP-positive patients within the seronegative RA population were actually ACPA positive. More studies are needed to determine if radiological progression may be primarily associated with ACPA (determined with multiplex assays), instead of anti-CarP antibodies.

Early RA or pre-RA offers the best opportunity to intervene and modify the disease course. Previous studies have explored the additional value of anti-CCP3 testing to further risk stratify anti-CCP2 positive at-risk individuals, improving the prediction to inflammatory arthritis (IA) [32]. Adding anti-CarP serology in early RA may also help identify patients at higher risk of progression to RA and predict poorer clinical outcomes [28,33]. The benefit of measurement in patients with a diagnosis of RA and long-standing disease seems limited to predict higher disease activity as supported by our findings. RF and ACPA positivity is associated with a worse long-term prognosis and a diminished likelihood to achieve drug-free remission (DFR) [34], whether anti-CarP antibodies also follow this trend is less clear. Recently, a study by de Moel et al. [35] investigated whether the breadth of the autoantibody profile is associated with treatment outcomes, namely, initial DAS44 response (not specified if ESR or CRP), initial DFR and long-term sustained DFR. They found that patients with a broad autoantibody profile at baseline had a significantly better early treatment response, but this association was not found regarding long-term sustained DFR. They also studied whether changes in autoantibody levels were associated with disease activity/treatment outcomes and whether they were modified by treatment intensity in early RA. They found that autoantibody levels decrease upon initiation and escalation of immunosuppressive treatment, but found no association between autoantibody levels and DAS44 over time or EULAR response. This suggests that anti-CarP antibody levels are modifiable by current therapies, but this itself is of limited clinical relevance [36]. Because we only measured autoantibody levels at baseline in patients with established RA, we cannot be sure whether this finding would also be present in our cohort and is an area of opportunity for future studies longitudinally assessing the clinical importance of anti-CarP antibodies. No other studies have investigated the effect of composition of the baseline autoantibody profile on early response to conventional DMARD therapy or long-term DFR [35]. This is a matter of debate because

aforementioned cohorts [8,23,24] have observed persistent worse clinical outcomes, while recent studies [35] and our findings suggest that the relevance of the baseline autoantibody profile (that includes anti-CarP measurement) diminishes over time. A recent study assessing the autoantibody-response maturation of ACPA and anti-CarP antibodies compared patients with clinically suspect arthralgia that did and did not progress to IA. They found that the presence and levels of the autoantibody isotypes did not significantly increase over time in neither group [37]. The findings of this study suggest that autoantibody-response maturation occurs in the asymptomatic phase of the disease, and broadening of the autoantibody response is not specific for progression from arthralgia to clinical arthritis. Anti-CarP utility regarding disease activity may be limited to early or treatment-naïve RA. More population-based studies are needed to support this observation.

Some strengths of this study should be highlighted. This is the first study to analyze anti-CarP antibodies in Mexican Hispanics. Additionally, it is one of the first to analyze disease activity and anti-CarP antibodies in an RA cohort with long-standing disease. Furthermore, the study has a large sample and an 18-month follow-up in Hispanics, a population commonly underrepresented in clinical trials.

Some limitations are worth noting. Our study does not correspond to an inception cohort, so that patients with established RA lack a uniform zero time in the natural history of the disease (early RA, RA diagnosis or beginning of treatment) at the time of their recruitment. A partial follow-up introduces bias and limits the precision of the findings for the purpose of prognostic cohorts [38]. Although there was a statistically significant difference in the decline of disease activity over time in the pooled analysis (as if patients had started treatment at the time of enrolment), this reduction was not different when divided by anti-CarP seropositivity. We think our findings are still relevant since studies focusing on established RA are lacking, and patients with this entity probably make up for the majority of the RA population [14]. Thus, sample size would significantly decrease if these patients were excluded. There is clearly a discrepancy among inception cohorts showing persistent worse disease activity even after their early RA patients turn into established RA patients [8,24], and the studies that support our findings, reporting no significant association between anti-CarP antibody positivity and DAS28 [39,40]. Additionally, the results in mortality from established RA patients have been similar to those reported from inception cohorts [12,41]. The generalizability of our results may also be limited because all of our patients were recruited from a single center. The majority of our patients lack major medical health insurance, which, incidentally, might be a contributing factor in the low usage of bDMARDs despite moderate or high disease activity. Finally, we did not assess radiographic progression, and DAS28 does not predict radiographic outcomes as well as other Boolean-based definitions of remission [42]. More studies assessing the prognostic value of anti-CarP antibodies measured by other composite indices of RA activity and a longer time of follow-up are needed.

In conclusion, in our sample of Hispanic patients with long-standing RA, anti-CarP IgG positivity was 47.8%. Patients negative to RF and ACPAs were positive to anti-CarP (any isotype) antibodies in 9.5%. Anti-CarP IgG positivity was not associated to a higher disease activity measured by DAS28-ESR at baseline, or at 6, 12, or 18 months during follow-up. These findings suggest that the clinical relevance of measuring anti-CarP antibodies diminishes over time. More studies are needed to elucidate the role of anti-CarP antibodies in patients with long disease duration.

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Conflicts of interest

None.

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