

Redefining seropositive rheumatoid arthritis: Clinical implications of anti-carbamylated protein on remission, radiographic damage and quality of life

Dear Editor,

Rheumatoid arthritis (RA) is a chronic disabling disease where continued disease activity translates to irreversible articular damage. Seropositivity for rheumatoid factor (RF) and anticitrullinated protein antibody (ACPA) are markers of poorer prognosis; with increased disease activity, radiographic progression and even mortality.¹ However, other than ACPA, no other biomarker has emerged for clinical use in the last 10 years.

Anti-carbamylated protein antibody (anti-CarP) has been recently proposed as a candidate biomarker to classify and prognosticate RA. Similar to ACPA, RA patients who are positive for anti-CarP appear to have higher disease activity, increased radiographic damage and higher disability.²⁻⁵ Studies from Asia, however, do not suggest a correlation between anti-CarP and increased disease activity.⁶⁻⁸ Therefore, we aimed to study the prevalence of anti-CarP in our multi-ethnic population of Asian RA patients and its association with Disease Activity Score in 28 Joints (DAS28)-remission at 12 months. Secondary outcomes included functional disability and radiographic progression.

This study was approved by the Singapore National Healthcare Group Domain Specific Review Board E (reference: 2016/00152) and was funded by the Clinician Scientist Unit Pitch for Funds Grant from the Yong Loo Lin School of Medicine, National University of Singapore and National University Health System. We recruited patients with early RA from the Singapore Early Arthritis Cohort, a single-centre cohort of patients with rheumatologist-diagnosed RA and symptom duration of ≤ 1 year. Patients provided informed consent for data and sample collection. We recorded baseline demographic variables including age, sex, ethnicity, body mass index (BMI) and smoking status. The treating rheumatologist assessed disease activity at baseline and 3 monthly intervals using DAS28 scoring system. Disease activity was categorised as: (1) remission-DAS28 < 2.6 ; (2) low disease activity-DAS28 2.6–3.2; (3) moderate disease activity-DAS28 > 3.2 –5.1; and (4) high disease activity-DAS28 > 5.1 .⁹

We measured anti-CarP antibody titre cross-sectionally using a commercially-purchased enzyme-linked immunosorbent assay kit (Sincere Biotech, Beijing, China). Patients' sera were aspirated into wells coated with human

carbamylated protein antigens before biotinylated detection antibodies were added. After the excess detection antibodies were washed away, avidin-biotin-peroxidase complex was then added to the wells. The intensity of the colour change was proportional to the amount of human anti-CarP bound and quantified as optical density (OD) of absorbance at 450 nm in the microplate reader.

We repeated the assay in 40 healthy individuals to establish the receiver operating characteristic curve. The optimal sensitivity and specificity of anti-CarP was established for the diagnosis of RA. We found the optimal cut-off to be at 1.60 OD, allowing a sensitivity of 60% and a specificity of 95% for RA diagnosis. The treating physicians were blinded to the results of the anti-CarP antibody assay and made all treatment decisions independently. We measured disability using the modified Health Assessment Questionnaire (mHAQ) at baseline and 12 months after cohort entry.¹⁰

Two independent accessors (SA and JC) quantified radiographic damage of the hands using the van der Heijde-modified total Sharp score (mTSS) at baseline and 1–3 years after follow-up.¹¹ Radiographs were accessed chronologically such that the more recent radiograph was compared against the baseline. Lastly, we performed a multivariable logistic regression for the dependent outcome of DAS28-remission at 12 months.

We recruited 100 patients for this study. The mean (standard deviation) age was 49.8 (12.5) years. Seventy-two (72%) were female and 62 (62%) were Chinese. The median (interquartile range [IQR]) disease duration was 10.2 (6.9–15.1) weeks. The median (IQR) baseline DAS28 was 4.5 (2.9–5.9). At baseline, 78 (78%) patients had disability (mHAQ > 0). Of the 93 patients who had baseline hand radiographs, 21 (22.6%) had baseline radiographic damage. In 6 patients, ACPA status was unknown. Sixty (60%) were anti-CarP positive and 35 (37.2%) were positive for ACPA, RF and anti-CarP. Out of the 31 patients who had negative RF and negative or unknown ACPA, 14 (14.9% of cohort and 45.1% of seronegative patients) were positive for anti-CarP alone.

Only anti-CarP positivity and baseline high disease activity were associated with DAS28-remission at 12 months in the multivariable

Table 1. Multivariable logistic regression for DAS28-remission at 12 months.

Variable	n (%)	Univariable logistic regression		Multivariable logistic regression		
		OR (CI)	P	OR (CI)	SE	P
Anti-CarP	60 (60)	3.0 (1.31–6.88)	0.01	3.41 (1.08–10.7)	1.99	0.04
Serologies						
RF and anti-CCP negative	31 (33.0)			Reference		
Either RF or anti-CCP positive	11 (11.7)	0.99 (0.25–3.93)	0.99	1.10 (0.17–7.04)	1.04	0.92
RF and anti-CCP positive	52 (55.3)	1.12 (0.45–2.75)	0.80	0.89 (0.28–2.81)	0.52	0.84
Baseline disease activity						
Remission	17 (17.4)			Reference		
Low disease activity	10 (10.2)	0.50 (0.05–4.67)	0.54	0.13 (0.01–1.67)	0.17	0.12
Moderate disease activity	32 (32.7)	0.29 (0.05–1.65)	0.16	0.10 (0.02–0.68)	0.10	0.02
High disease activity	39 (39.8)	0.18 (0.04–0.90)	0.04	0.06 (0.01–0.41)	0.06	<0.01
Treatment						
csDMARD monotherapy	26 (26)	Reference				
Combination csDMARDs or bDMARD	74 (74)	1.13 (0.46–2.76)	0.80	1.97 (0.58–6.67)	1.23	0.28
Baseline radiographic damage	11 (20)	1.79 (0.65–4.95)	0.26	1.27 (0.33–4.95)	0.88	0.73
Tertiary education	23 (38.3)	0.77 (0.34–1.77)	0.54	0.42 (0.12–1.45)	0.27	0.17
Ethnicity						
Chinese	42 (70)			Reference		
Malay	39 (68.4)	0.61 (0.22–1.71)	0.35	0.56 (0.14–2.25)	0.40	0.41
Indian	8 (13.3)	0.60 (0.20–1.77)	0.35	0.79 (0.20–3.13)	0.56	0.74
Females	46 (76.7)	0.43 (0.17–1.11)	0.08	0.48 (0.13–1.85)	0.33	0.29

anti-CarP: anti-carbamylated protein antibody; anti-CCP: anti-citrullinated protein antibody; bDMARD: biologic disease-modifying anti-rheumatic drugs; CI: confidence interval; csDMARD: conventional synthetic disease-modifying anti-rheumatic drugs; OR: odds ratio; RF: rheumatoid factor; SE: standard error

regression model (odds ratio [OR] confidence interval [CI] 3.41 [1.08–10.7], $P=0.04$ and OR [CI] 0.06 [0.01–0.41], $P<0.01$, respectively, Table 1). Positivity for both RF and ACPA was not associated with DAS28-remission at 12 months in the multivariable model (OR [CI] 0.89 [0.28–2.81], $P=0.84$, Table 1).

Sixty-six patients had follow-up radiographs of the hands, of which 10 (15.2% of 66 patients) demonstrated radiographic progression. Spearman's correlation coefficients to assess inter-rater agreement of the mTSS scores were 0.79 and 0.71 for baseline radiographs and follow-up radiographs, respectively. Rank correlation independence test was statistically significant

($P<0.001$). On multivariable logistic regression, neither anti-CarP nor positivity for both RF/ACPA showed an association with radiographic progression; (OR [CI] 0.64 [0.14–2.81], $P=0.55$ and OR [CI] 1.98 [0.34–11.61], $P=0.45$, respectively). Anti-CarP was not associated with disability at 12 months in the multivariable model.

While the prevalence of anti-CarP varies between RA cohorts depending on the assay used and definition of RA, it appears to be more prevalent in non-Western cohorts. For instance, anti-CarP was found in up to 77% of Egyptian RA patients, in contrast to ~20–40% of Caucasian cohorts.^{3-5,12,13} Also, while anti-CarP has been invariably associated with higher disease activity and

radiographic progression in Western cohorts, similar findings have not been convincingly replicated in Asians.

Our study has several limitations, including a small sample size, cross-sectional measurement of anti-CarP and follow-up hand X-rays at variable times in the disease course. Last, we have examined the outcome of DAS28-remission only at 1 timepoint of 12 months, instead of employing a prospective design with repeated measures. Nonetheless, we have examined a multitude of clinically important RA outcomes including DAS28-remission, mHAQ and radiographic progression. We have also, for the first time, described the prevalence and clinical relevance of anti-CarP in a multi-ethnic Southeast Asian cohort.

In summary, anti-CarP was found in 60% of all early RA patients and 45% of RF/ACPA negative patients in a multi-ethnic Southeast Asian cohort. Anti-CarP was associated with 3.4 times higher odds of DAS28-remission at 12 months and may serve as a favourable prognostic marker in a subset of Southeast Asian patients with early RA.

Disclosure

All authors declare no conflict of interest.

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Jiacai Cho^{1,2} MBBS, Anselm Mak^{1,2} MBBS,
Sachin Agrawal^{2,3,4} MBBS,
Preeti Dhanasekaran² BSc,
Peter P Cheung^{1,2} MBBS,
Manjari Lahiri^{1,2} MBBS

¹ Division of Rheumatology, Department of Medicine, National University Hospital of Singapore, Singapore

² Yong Loo Lin School of Medicine, National University of Singapore, Singapore

³ Department of Radiology, National University Hospital of Singapore, Singapore

⁴ Department of Radiology, Alexandra Hospital, Singapore

Correspondence: Dr Jiacai Cho, Division of Rheumatology, Department of Medicine, National University Hospital, 5 Lower Kent Ridge Road, Singapore 119074.
Email: Jiacai_cho@nuhs.edu.sg