RMD Open
Rheumatic & Musculoskeletal Diseases

ORIGINAL RESEARCH

Utility of testing for third-generation anticyclic citrullinated peptide (anti-CCP3) antibodies in individuals who present with new musculoskeletal symptoms but have a negative second-generation anticyclic citrullinated peptide (anti-CCP2) antibody test

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To cite: Di Matteo A, Mankia K, Garcia-Montoya L, *et al*. Utility of testing for third-generation anticyclic citrullinated peptide (anti-CCP3) antibodies in individuals who present with new musculoskeletal symptoms but have a negative second-generation anticyclic citrullinated peptide (anti-CCP2) antibody test. *RMD Open* 2024;**10**:e003927. doi:10.1136/rmdopen-2023-003927

► Additional supplemental material is published online only. To view, please visit the journal online (https://doi.org/10.1136/rmdopen-2023-003927).

Received 20 November 2023
Accepted 22 February 2024

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ABSTRACT

Objectives To investigate the role of third-generation anticyclic citrullinated peptide (anti-CCP3) antibodies in predicting progression to inflammatory arthritis (IA) in individuals with new musculoskeletal (MSK) symptoms and a negative second-generation anti-CCP antibody test (anti-CCP2–).

Methods 469 anti-CCP2– individuals underwent baseline anti-CCP3 testing (QUANTA Lite CCP3; Inova Diagnostics) and received a post enrolment 12-month questionnaire. A rheumatologist confirmed or excluded diagnosis of IA. Univariable/multivariable analyses were performed to assess the value of anti-CCP3 in predicting IA development in these anti-CCP2– individuals.

Results Only 16/469 (3.4%) anti-CCP2– individuals had a positive anti-CCP3 test. Of these 16 individuals, 4 developed IA. In addition, 61/469 (13.0%) anti-CCP2– individuals self-reported, to have developed, IA. Progression was confirmed in 43/61 of them (70.5%); of whom 30/43 (69.8%) and 13/43 (30.2%) were given a diagnosis of IA and rheumatoid arthritis (RA), respectively. In qualitative univariable analysis, anti-CCP3 positivity was associated with self-reported progression ($p<0.01$) and IA ($p=0.03$), but not with RA. Anti-CCP3 levels differed significantly between progressors and non-progressors ($p<0.01$) for all three categories. At the manufacturer's cut-off, OR for progression ranged from 2.4 (95% CI 0.5 to 18.6; RA) to 7.5 (95% CI 2.3 to 24.0; self-reported progression). Interestingly, when cut-offs for anti-CCP3 were optimised, lower values (≥5 units) significantly increased the OR for progression in all three categories. In multivariable analysis, anti-CCP3 positivity at the manufacturer's cut-off did not remain associated with IA progression, while this lower cut-off value (≥5 units) was associated with diagnosis of RA ($p=0.02$).

Conclusions Anti-CCP3 testing could improve the prediction of IA development in anti-CCP2– individuals with new MSK symptoms.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Previous studies have demonstrated the potential role of third generation anticyclic citrullinated peptide (CCP3) antibodies in improving prediction of inflammatory arthritis (IA) in CCP2+ individuals with musculoskeletal (MSK) symptoms.
⇒ However, the role of anti-CCP3 antibodies in individuals with MSK symptoms and a negative anti-CCP2 test has not been studied before.

WHAT THIS STUDY ADDS

⇒ The rate of progression to IA (either IA or rheumatoid arthritis (RA)) in anti-CCP2– individuals with MSK symptoms seen in primary care setting was low within a 12-month follow-up period.
⇒ Anti-CCP3 antibodies might improve prediction of IA/RA progression in anti-CCP2– at-risk individuals.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Future studies are warranted to validate the cut-off values for anti-CCP3 antibodies with best prediction accuracy in this population.
⇒ If validated, it is possible that testing anti-CCP3 antibodies in CCP2– individuals could be a logical approach for identifying those likely to progress to IA.

INTRODUCTION

In recent years, several studies have been carried out to identify biomarkers that can delineate the profile of an individual deemed to be at 'high risk' of developing rheumatoid arthritis (RA).[1–6] Among these biomarkers, rheumatoid factor (RF) and anticitrullinated

protein antibodies (ACPAs) are the most frequently studied, but other biomarkers may also provide valuable insights.[7] The presence of certain combinations of serological biomarkers can further improve the potential predictive value of disease progression as is seen in the presence of RF, ACPA and anticarbamylated protein (CarP) antibodies.[3]

Additionally, genetic predisposition, subclinical inflammation on MRI and ultrasound (US), as well as certain cellular markers (eg, circulating B-cell and T-cell biomarkers), have been described as specific risk factors for RA development in 'at-risk' populations.[8–11] Most recently, Fab-linked glycans of ACPA have also been shown to be associated with the development of RA.[12–14]

In 'at-risk' cohorts around the globe, individuals are identified and invited for participation in trials based on the presence of musculoskeletal (MSK) symptoms and a positive ACPA test.[15 16] Positive ACPAs have been found in the serum of individuals with RA several years before the onset of disease.[17 18] In addition, ACPAs have been shown to greatly increase the risk of developing RA in 'at-risk' individuals with MSK symptoms.[19]

ACPAs are most commonly tested using anticyclic citrullinated peptide (CCP) antibody assays which are based on different generations of CCP peptides.[20] In Europe, the second-generation of anti-CCP (anti-CCP2) antibody is most commonly used, while third-generation anti-CCP (anti-CCP3) antibody represents the predominant assay used in the USA.

In a recent study, our group demonstrated the additional value of anti-CCP3 antibodies for the prediction of inflammatory arthritis (IA) development in anti-CCP2+ 'at-risk' individuals with new MSK symptoms. A positive anti-CCP3 antibody test increased the risk of developing IA from 38.9% to 48.4% in high-titre anti-CCP2+individuals, while a negative anti-CCP3 test decreased such risk from 38.9% to 9.8% in the same population.[21]

A subsequent study from our research group showed that in CCP2+ 'at-risk' individuals with MSK symptoms, anti-CCP3 antibodies were associated with the development of US subclinical synovitis. This study raised the hypothesis that anti-CCP2+/CCP3+ individuals without subclinical joint disease might be at a critical transition point in the evolution of RA (ie, the transition from autoimmunity to joint inflammation) and could potentially represent the optimal 'window of opportunity' for the introduction of preventative treatments (ie, before the so-called 'second hit' in RA pathogenesis occurs).[22]

On the other hand, the role of anti-CCP3 antibodies in anti-CCP2 negative (anti-CCP2−) individuals who present to primary care with new MSK symptoms has not been explored. Therefore, it remains unclear whether testing for anti-CCP3 antibodies in such a cohort of individuals would improve the prediction of IA development and whether or not these antibodies have a role in primary care screening.

Thus, the aims of the current study were to explore the prevalence of anti-CCP3 positivity in anti-CCP2− individuals who have presented to primary care with new non-specific MSK symptoms. In addition, to determine the additional value of testing for and identifying anti-CCP3 antibody positivity in predicting progression to IA in anti-CCP2− individuals who present with non-specific MSK symptoms.

METHODS

Study population

Individuals included in the current study are part of the 'The CCP Study: Coordinated Programme to Prevent Arthritis—Can We Identify Arthritis at a Pre-clinical Stage?'. This is an observational study where individuals with a new non-specific MSK symptom (eg, shoulder pain, back pain or hand pain) are tested for anti-CCP2 antibodies at their local general practitioner (GP) surgery.[23 24] In this study, individuals with a positive anti-CCP2 antibody test are invited to Chapel Allerton Hospital in Leeds for further assessments in a dedicated research clinic as part of an observational longitudinal study. Individuals are followed until the development of IA. Those with a negative anti-CCP2 antibody test are sent a postal questionnaire 12 months after enrolment, in order to assess for potential disease progression.

Study design

For the current study, a subpopulation of the whole cohort of anti-CCP2− individuals with MSK symptoms (437 of 6587 CCP2− individuals) was selected.[25] These 437 CCP2− individuals were identified as an age-matched and sex-matched control group for CCP2+ individuals in a previous study.[21] Data on this control group were not presented before. Due to the known low prevalence of progression to IA in CCP2− individuals, we also collated data from another subgroup of anti-CCP2− individuals who were known progressors to IA.[25] As previously described, the total number anti-CCP2− individuals who in our cohort progressed to IA was 53.[25] Of these, 43 had an available stored serum sample for CCP3 testing and were included in this study.[25] This latter group of 43 individuals also included 11 patients who were also in the initial randomly selected 437 anti-CCP2− subpopulation and were, therefore, removed to avoid duplication. Thus, a total of 469 anti-CCP2− individuals who presented to their GP with new MSK symptoms were included in this study. Figure 1 provides a schematic description of the study design.

If any of the 469 anti-CCP2− individuals self-reported progression to IA, which was defined as the development of swelling in at least one joint, their GPs were contacted by a member of our study team to confirm or refute the individual's rheumatological disease status. Individuals whose rheumatological diagnosis was confirmed by a rheumatologist were defined as either RA progressors or IA progressors (which included RA progressors, spondyloarthritis, IA in systemic sclerosis, IA in systemic lupus erythematosus, IA in polymyalgia rheumatica), according

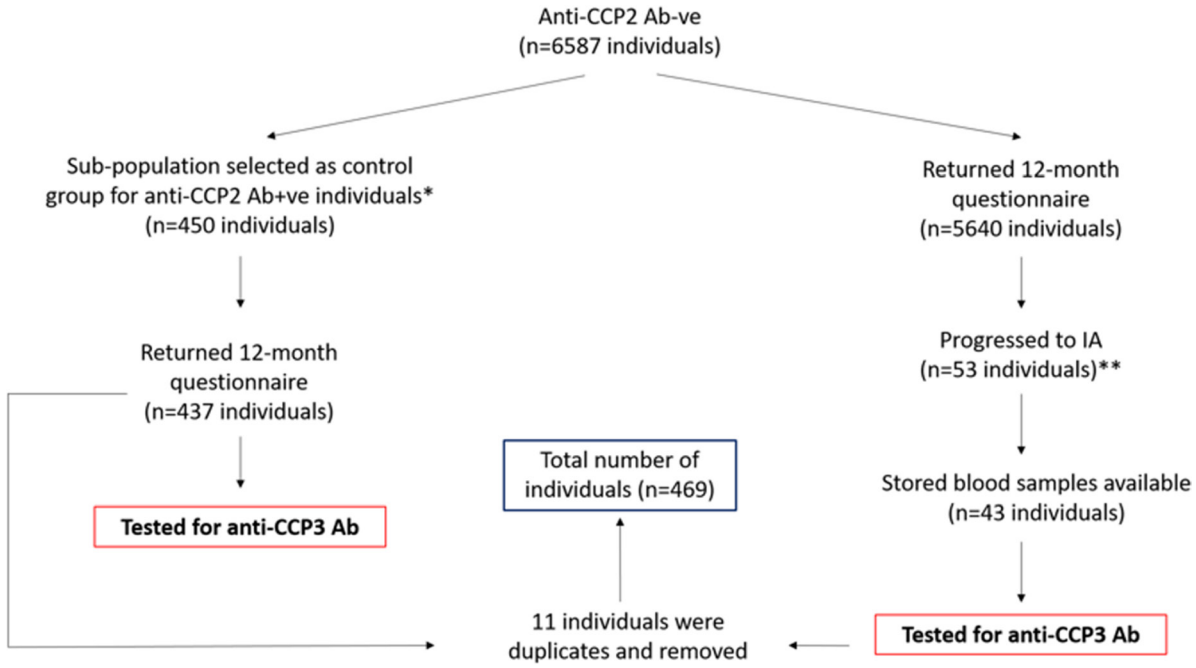

Figure 1 Schematic representation of the study design. *Refers to reference n=21; **refers to reference n=25. +ve, positive; –ve, negative; Ab, antibodies; CCP, cyclic citrullinated peptide; IA, inflammatory arthritis.

to corresponding international diagnostic/classification criteria.

Serological assay

For the 469 anti-CCP2– individuals, stored serum samples obtained from 2008 to 2018 were tested for CCP3 IgG (QUANTA Lite CCP3; Inova Diagnostics). CCP2 and CCP3 testing was performed on the same stored serum samples. The threshold for a positive CCP3 antibody test was >20 units, according to the manufacturer's cut-off. In addition, alternative cut-off values were evaluated derived from optimised F-1 score analysis.

Statistical analysis

Descriptive statistics were used for the main characteristics of the study population and reported as absolute frequencies with the corresponding percentage for categorical variables and mean with SD for continuous variables. Receiver operating characteristic (ROC) analyses with area under the curve assessment (AUC) were performed to investigate the discriminatory power of anti-CCP3 antibody levels for disease progression. Scatter plots with Mann-Whitney statistics were deployed to assess the difference between progressors and non-progressors. P values <0.05 were considered statistically significant. A Sankey plot was generated to visualise the disease progression. Lastly, pre-test/post-test probability plots were created to visualise the impact of anti-CCP3 antibody results on probability of disease progression. To optimise cut-off values of the anti-CCP3 test, F1 scores at different threshold were calculated from the precision and recall of the test. The F1 score combines precision and recall using their harmonic mean, and maximising the F1 score implies simultaneously maximising both precision and recall. Cut-off values with the highest F1 score were used to establish other diagnostic criteria for prediction of progression.

Analysis and visualisation were conducted using the following libraries and software tools: Python, V.3.8; Numpy, V1.24.2; Pandas, V.1.5.3; Scikit-learn, V.1.2.2; SciPy, V.1.10.1; Plotly, V.5.13.1. A total of three multivariable analyses models were generated to assess the association of CCP3 with disease progression. In these models, age and CCP3 were included as continues scale values and gender as a binary category and variables were tested for self-reported progression, IA and RA progression (latter two confirmed by rheumatologist).

RESULTS

The characteristics of the 469 anti-CCP2– individuals included in the study are reported in table 1. Only 16/469 (3.4%) anti-CCP2– individuals had a positive anti-CCP3 test. Of the anti-CCP2– individuals identified as 'progressors' in a previous study, 4/43 (9.3%) were positive for anti-CCP3 antibodies.

Discrimination between progressors and non-progressors

Among the 469 anti-CCP2– individuals, 61 (13.0%) self-reported or were subsequently confirmed to have disease progression. Of these 61, 43 (70.5%) were confirmed to have progressed to IA by a rheumatologist; of whom 30/43 (69.8%) and 13/43 (30.2%) were given a diagnosis of IA and RA, respectively.

A summary of the disease progression is presented in online supplemental figure 1 and online supplemental table 1.

Table 1 Main characteristics of the included population of progressors (self-reported progressors, IA/RA progressors) versus non-progressors

	Entire subpopulation (n=469)	Self-reported progression* (n=61)	IA/RA progressors (n=43)	RA progressors (n=13)	IA/RA non-progressors (n=426)
Age, years (mean±SD)	53.1±15.2	60.2±13.9	60±13.4	60.1±14.0	52.4±15.3
Female sex	342 (72.9%)	33 (54.1%)	24 (55.8%)	8 (61.5%)	318 (74.6%)
Family history of RA	137 (29.2%)	15 (24.6%)	13 (30.2%)	5 (38.5%)	124 (29.1%)
CCP3 positive	16 (3.4%)	8 (13.1%)	4 (9.3%)	1 (7.7%)	8 (1.9%)

*Includes 18 patients who have self-reported progression to IA/RA but where this was not confirmed by a rheumatologist.
Ab, antibodies; CCP3, third-generation cyclic citrullinated peptide antibodies; IA, inflammatory arthritis; RA, rheumatoid arthritis.

Anti-CCP3 antibody levels differed significantly between IA progressors and non-progressors (p<0.001; see figure 2). The ROC analyses showed strong discrimination for anti-CCP3 antibodies between progressors and non-progressors with AUC values of 0.832 (95% CI 0.798 to 0.866) for self-reported progression, 0.887 (95% CI 0.858 to 0.915) for IA and 0.789 (95% CI 0.753 to 0.826) for RA. At the manufacturer's cut-off (≥20 units), the sensitivity for progression to overt disease ranged from 8% for RA to 9% for IA, with high specificity of 97% (see table 2). ORs were 2.4 (95% CI 0.5 to 18.6) for RA and 3.5 (95% CI 1.2 to 11.0) for IA. Interestingly, when cut-offs were optimised for F-1 score, lower cut-off values (≥5 units) significantly increased the OR for progression in all three categories (ie, self-reported progression, IA and RA) (see table 2 and figure 3).

To further evaluate the role of anti-CCP3 antibodies in anti-CCP2− individuals, we assessed the association of age, gender and family history of RA with IA progression. As shown in table 3, older age was statistically associated with self-reported progression (p<0.0001) and RA progression (p=0.0006). Male gender was statistically associated with self-reported progression (p=0.0010) and IA diagnosis by rheumatologist (p=0.0113). Interestingly, there was no association between reported family history for RA with any progression group.

Based on these findings, multivariable analyses were conducted to understand the individual value of anti-CCP3 antibodies in disease progression. A total of three multivariable analyses models were generated (see table 4). Anti-CCP3 antibodies remained statistically significant for self-reported progression (p=3.2E−06) as well as for RA diagnosis by a rheumatologist (p=0.019), but not for IA diagnosis by a rheumatologist (p=0.154).

DISCUSSION

To the best of our knowledge, this is the first study to investigate the role of anti-CCP3 antibodies in predicting evolution to IA in a population of anti-CCP2− individuals presenting to primary care with new non-specific MSK symptoms.

Our results indicate a low prevalence of anti-CCP3 antibodies in anti-CCP2− individuals; only 16/469 (3.4%) of anti-CCP2− individuals showed a positive anti-CCP3 antibody test. This suggests that the agreement between the anti-CCP2 and anti-CCP3 antibody tests is higher in a population with negative anti-CCP2 antibodies than in a population with positive anti-CCP2 antibodies, where the rate of disagreement between the two tests reached up to almost 40%, especially in those with low titre anti-CCP2 positivity.[21] The prevalence of anti-CCP3 antibody positivity in the subgroup of 43 CCP2− individuals which were known progressors was slightly higher, but still low (<10%).

Only a small proportion of anti-CCP2− anti-CCP3 +individuals progressed to IA after 12 months of follow-up. The infrequent progression rate is consistent with the findings from a recent study in which only 0.93% of individuals with anti-CCP2− and MSK symptoms progressed to IA.[25]

Previous studies from our group suggested that testing for anti-CCP3 antibodies in anti-CCP2+ 'at-risk' individuals with MSK symptoms might improve risk stratification for RA development and improve management of these individuals, as well as facilitating the detection of those individuals at imminent risk of any (ie, either clinical or subclinical) joint involvement.[21 22] In addition, in a recent study, it was demonstrated that the combination of both anti-CCP2 and anti-CCP3 antibodies improves the accurate detection of patients with RA.[26] More specifically, using sera from 127 patients analysed using anti-CCP2 (EliA on Phadia 250 instrument, Thermo Fisher Scientific) and anti-CCP3 (QUANTA Flash on BIO-FLASH, Inova Diagnostics) antibody tests, comparable performance was found between the two CCP assays. However, binary logistic regressions indicated that the likelihood of having RA is significantly higher when testing positive for both anti-CCP2 and anti-CCP3 antibody assays compared with anti-CCP2 or anti-CCP3 antibody assays alone. Consequently, it was concluded that in patients with joint complaints suspected of having RA and with a weakly positive anti-CCP2 antibody (≥7 and ≤16 U/mL), anti-CCP3 antibody testing could be of additive value for diagnosing RA.[26]

The results of the current study suggest a potential role for anti-CCP3 antibody testing in CCP2− individuals

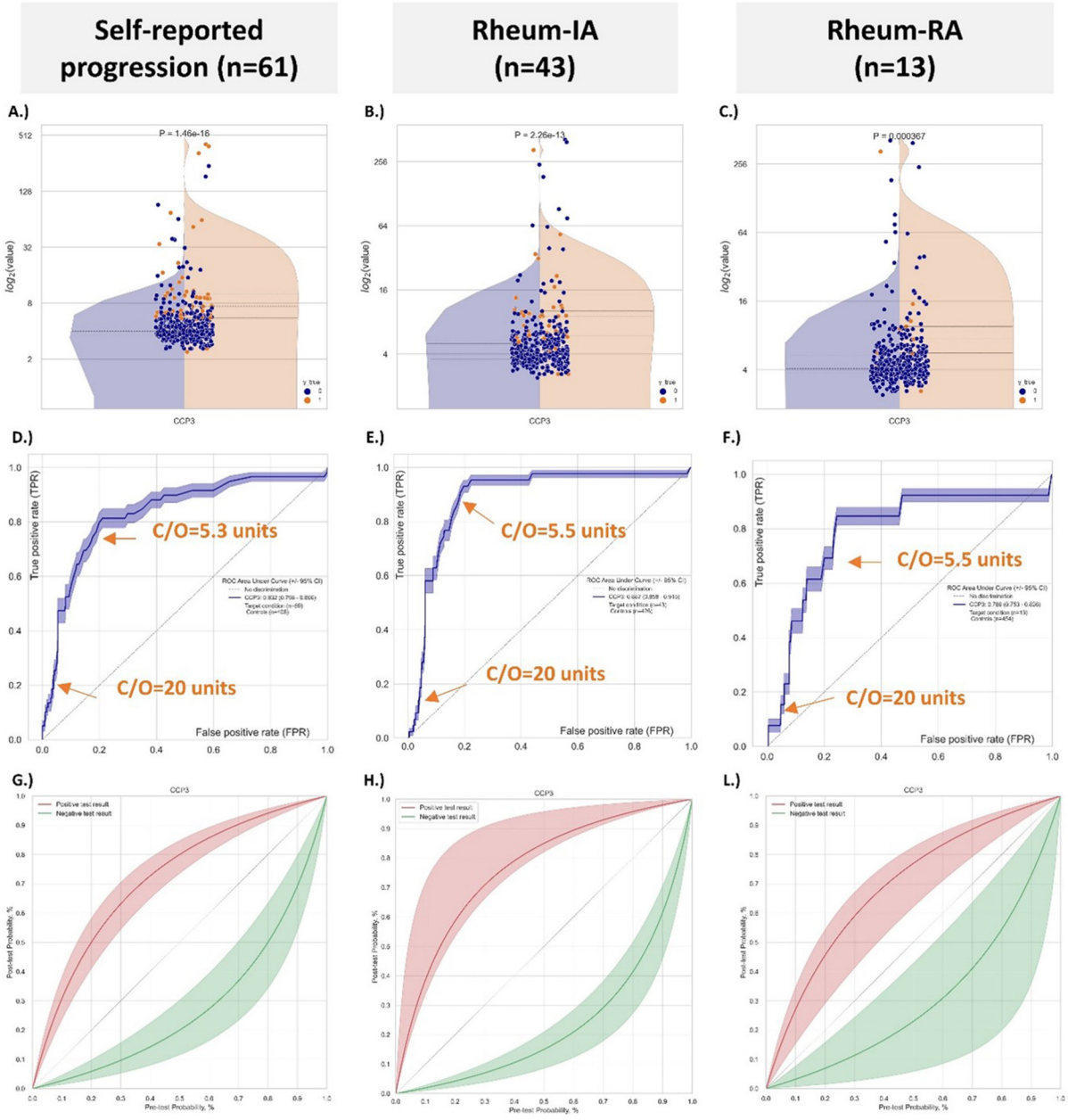

Figure 2 Impact of anti-CCP3 antibody levels in predicting progression to inflammatory arthritis (IA). Anti-CCP3 antibody levels were significantly higher in individuals who progressed. (A–C) The titre differences in individuals who progressed (1; orange dots) to the non-progressors (0; blue dots). The blue and orange shaded areas indicate the distribution of anti-CCP3 results in progressors (orange) and non-progressors (blue). (A) Self-reported progression (B) IA/rheumatoid arthritis (RA) confirmed by a rheumatologist and (C) RA confirmed by a rheumatologist. (D–F) The ROC curves of anti-CCP3 antibodies in the three categories (self-reported progression, rheumatologist-confirmed diagnosis of IA or rheumatologist-confirmed diagnosis of RA). Lastly, (G–L) the corresponding pre-test and post-test probability curves at the optimised cut-off. IA includes patients with RA. CCP3, third-generation anticyclic citrullinated peptide antibodies; C/O, cut-off; ROC, receiver operating characteristic.

who attended their GP with MSK symptoms. Interestingly, low levels (well below the manufacturer's cut-off) of anti-CCP3 antibodies were strongly associated with disease progression. At cut-off values of ≥5 units, the OR for self-reported progression, IA progression and RA progression were 16.5, 42.8 and 10.9, respectively. As indicated in table 2, employing an alternative cut-off for the CCP3 assay resulted in an augmented sensitivity, although at the expense of reduced specificity when compared with the standard cut-off. Furthermore, lower cut-off values were associated with an elevated risk of IA development across all three categories. This raises the question of whether cut-off values for anti-CCP3 antibodies (when used in patients with new MSK symptoms) should be lowered, and to what level. This is of particular importance since the majority of studies designed to establish cut-off values for diagnostic assays are based on patients with established disease, and therefore, fulfilling

Table 2 Performance characteristics of anti-CCP3 antibodies as predictors of disease progression

Cohort	Cut-off	Specificity % (95% CI)	Sensitivity % (95% CI)	Precision % (95% CI)	LR+ (95% CI)	LR– (95% CI)	OR (95% CI)
Self-reported progression (n=61)	20 units	98 (96 to 99)	14 (06 to 25)	50 (25 to 75)	6.7 (2.7 to 16.5)	0.9 (0.8 to 1.0)	7.5 (2.3 to 24.0)
	5.3 units	80 (76 to 84)	80 (68 to 89)	38 (29 to 47)	0.2 (0.2 to 0.2)	4.0 (4.0 to 4.1)	16.5 (8.4 to 32.4)
Rheum-IA* (n=43)	20 units	97 (95 to 98)	9 (4 to 22)	31 (11 to 59)	3.3 (1.6 to 9.1)	0.9 (0.8 to 1.0)	3.5 (1.2 to 11.0)
	5.5 units	81 (77 to 85)	91 (78 to 97)	33 (25 to 42)	0.1 (0.1 to 0.1)	4.9 (4.9 to 4.9)	42.8 (14.9 to 123.3)
Rheum-RA (n=13)	20 units	97 (95 to 98)	8 (1 to 33)	6 (0 to 30)	2.3 (0.4 to 11.2)	1.0 (0.7 to 1.0)	2.4 (0.5 to 18.6)
	5.5 units	77 (72 to 80)	77 (46 to 95)	09 (04 to 15)	0.3 (0.3 to 0.3)	3.3 (3.3 to 3.3)	10.9 (3.0 to 40.5)

*IA also includes patients with RA.
CCP3, third-generation cyclic citrullinated peptide; IA, inflammatory arthritis; LR+, likelihood ratio positive; LR–, likelihood ratio negative; RA, rheumatoid arthritis.

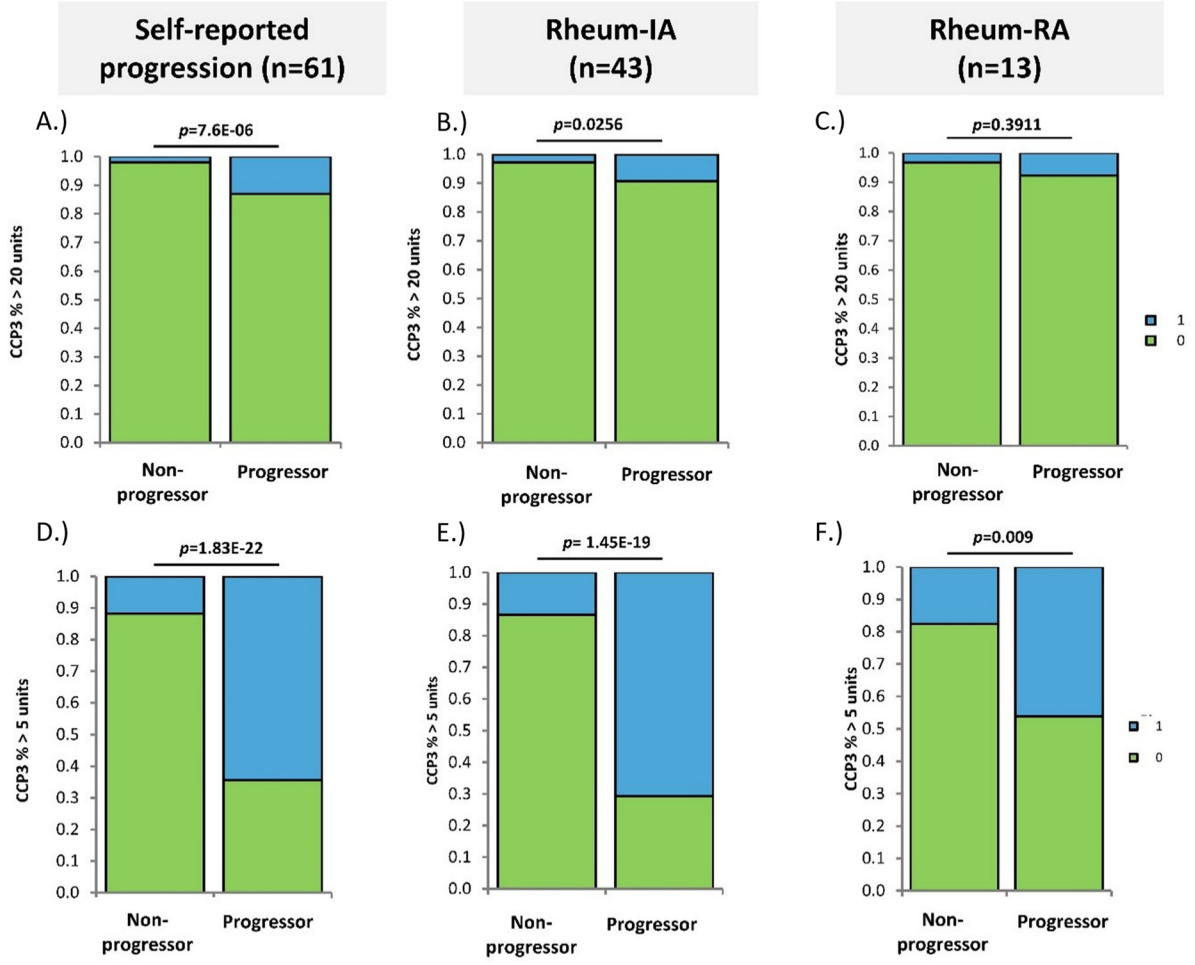

Figure 3 Ability of anti-CCP3 antibody status (qualitative) in predicting progression to inflammatory arthritis (IA). (A–C) Using the manufacturer's cut-off values, anti-CCP3 antibody positivity was significantly higher in individuals who progressed by self-reported progression and IA diagnosis confirmed by a rheumatologist. (D–F) At optimised cut-off values (≥5 units), the difference in anti-CCP3 antibody positivity became more pronounced and also reached significance for RA diagnosis confirmed by a rheumatologist. IA includes patients with RA. CCP3, third-generation anticyclic citrullinated peptide antibodies; RA, rheumatoid arthritis; 1, positive; 0, negative.

Table 3 Demographic and serological features and their associations with disease progression

Diagnosis	Age	Gender (male)	Family history	CCP3 (≥20 units)
Self-reported progression (n=61)	**P<0.0001**	2.6 (1.5–4.8) **p=0.0010**	0.8 (0.4–1.5) p=0.45	7.5 (2.3–24.0) **p=0.0003**
Rheum-IA* n=43	P=0.0683	2.3 (1.2–4.6) **p=0.0113**	1.1 (0.5–2.2) p=0.86	3.5 (1.2–11.0) **p=0.03**
Rheum-RA (n=13)	**P=0.0006**	1.7 (0.4–6.2) p=0.3471	1.5 (0.4–5.4) p=0.54	2.4 (0.1–18.6) p=0.37

Significant p-values are highlighted in bold.
*IA includes also patients with RA.
CCP3, third-generation anti-CCP antibodies; IA, inflammatory arthritis; RA, rheumatoid arthritis.

classification criteria.[6] We acknowledge the low prevalence of anti-CCP3 antibodies among anti-CCP2− individuals, as per the manufacturer's cut-off. This must be considered when considering the practical value of using the anti-CCP3 test in predicting the development of IA in this anti-CCP2-negative population. Further research into this area is needed to better understand the implications of this aspect.

Although ACPA is known as a specific diagnostic marker for RA, our current results indicate that low levels of anti-CCP3 antibodies, also predict progression to other autoimmune conditions that may exhibit inflammatory synovitis. Whether this finding reflects a low level of B-cell activation requires further studies. Lastly, the future development of overlap syndromes of RA and other conditions (eg, systemic lupus erythematosus, systemic sclerosis) cannot be ruled out.

As shown in table 3, we have demonstrated a lack of association between progression to IA and family history for RA. One of the reasons for this finding could be attributed to the limited statistical power of our current study. Additionally, we acknowledge that self-reporting of family history by patients is a limitation, as some individuals may misinterpret 'true' RA with other non-inflammatory joint conditions, such as osteoarthritis.

Our study has some limitations. First, we merged a selected population of known anti-CCP2− progressors to IA with a randomly selected subpopulation of anti-CCP2− individuals (both having non-specific MSK symptoms), therefore, the overall prevalence of progression was very much higher than expected or observed in real life. This meant that rate of progression in this merged population would have created potentially biased results, though analysing rate of progression in the randomly selected anti-CCP2− cohort could be representative of the general population (and our figures support this). Second, our cohort included low numbers of progressors within 12 months of follow-up. Overall, 61 participants reported

Table 4 Demographic and serological features and their association with disease progression

	Coefficients	SE	T stat	P value	Lower 95%	Upper 95%
Self-reported progression						
Intercept	0.0159	0.061	0.245	0.806	−0.111	0.143
Age	0.0030	0.001	3.052	**0.002**	0.001	0.005
Gender	−0.0922	0.034	−2.692	**0.007**	−0.159	−0.025
CCP3	0.0021	0.001	4.715	**3.2E−06**	0.001	0.003
Rheum-IA*						
Intercept	0.0070	0.057	0.122	0.903	−0.106	0.120
Age	0.0023	0.001	2.625	**0.009**	0.001	0.004
Gender	−0.0600	0.030	−1.981	**0.048**	−0.120	−0.001
CCP3	0.0006	0.000	1.428	0.154	−0.001	0.001
Rheum-RA						
Intercept	−0.0060	0.033	−0.181	0.856	−0.071	0.059
Age	0.0007	0.001	1.314	0.190	−0.000	0.002
Gender	−0.0091	0.018	−0.525	0.600	−0.044	0.025
CCP3	0.0005	0.000	2.364	**0.019**	9.04E-05	0.001

Significant p-values are highlighted in bold.
*IA includes also patients with RA
CCP3, third-generation cyclic citrullinated peptide; IA, inflammatory arthritis; RA, rheumatoid arthritis.

disease progression, but only 43 were diagnosed as IA or RA by a rheumatologist resulting in limited statistical power to perform extensive statistical analyses. However, based on the high predictive value of ACPA, this represents an inherent limitation of all studies on ACPA-negative RA. Despite the low number of progressors, we observed statistical difference among patients depending on the cut-off used for anti-CCP3 antibodies. Third, the sensitivity of the patients' reported questionnaire for the diagnosis of IA is unknown, and the period of observation was relatively short (12 months). In other words, it remains unknown how many of the individuals who did not report progression eventually developed or will develop IA. In addition, some important information on the study population was not available, such as body mass index, shared epitope status, current treatment, as these were not included in the patients' questionnaires. The main objective of the current study was to assess the additional value of a second CCP assay for clinically feasible predictive testing. Indeed, it may not be feasible in all centres to have access to multiple antimodified protein antibodies. In the current study, the included population of anti-CCP2− individuals was identified as a control group for anti-CCP2+ patients in a previous study of our group.[21] In these CCP2− individuals, stored serum samples were used to test anti-CCP3 antibodies but not anti-CarP antibodies or RF testing.

Finally, no quantitative results were available for anti-CCP2 antibody, which limits the ability to analyse the value of anti-CCP2 antibody levels on disease progression. Therefore, a definitive answer regarding the best screening strategy in individuals 'at risk' of RA (ie, anti-CCP2 or anti-CCP3 antibody testing) will require a large head-to-head study, with previous attempts at such studies proving inconclusive in patients with RA.[27] This evaluation would enable a direct comparison of the diagnostic efficacy of both tests in this specific 'at-risk' population, facilitating a comprehensive understanding of their respective performances. Furthermore, conducting such studies would allow for the assessment and determination of the optimal screening strategy for identifying at-risk individuals.[28]

CONCLUSIONS

The current preliminary results showed a low prevalence of anti-CCP3 antibodies in individuals with a new MSK symptom and a negative anti-CCP2 antibody test.

Despite the low number of anti-CCP3+ antibody individuals combined with the low disease progression rate in the randomly selected anti-CCP2− subpopulation, our data indicate that anti-CCP3 antibody levels could improve prediction of disease progression to IA (either IA or RA) in this population. Further studies are warranted to validate the findings, especially the observation that lower cut-off values might provide higher accuracy for predicting disease progression.

If validated, it is possible that testing anti-CCP3 antibodies in CCP2− individuals could be a logical approach for increasing detection of those likely to progress to IA.

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Acknowledgements PE is National Institute for Health Research (NIHR) Biomedical Research Centre (BRC) past director and current workstream lead and BRC funds supported this work.

Contributors ADM was one of the clinicians of the study, contributed to design the study, collected and analysed data, and wrote the first draft of the manuscript. ADM is the guarantor of the current study. KM was one of the clinicians of the study, contributed to design the study and was involved in the analysis of data and writing the manuscript. LG-M and JN were clinicians of the study and contributed to the collection and analysis of data. SS and LD contributed to the interpretation of data and writing the manuscript. MM performed the statistical analysis and contributed to the analysis and interpretation of data, and writing the manuscript. PE established the cohort, designed the study and contributed to writing the manuscript. All coauthors contributed to revising the manuscript critically and approved the final version to be published.

Funding The study was supported by the NIHR Leeds BRC (grant IS-BRC-1215-20015).

Competing interests ADM has received speaking fees from Janssen. KM reports personal fees from Abbvie, Lilly, Galapagos, UCB and Serac Healthcare outside the submitted work and research grants from Gilead, Serac Healthcare and Lilly. MM is employed at Werfen, a diagnostic company that commercialises the CCP3 assay. He does not have stocks or shares of the company or other incentives for the product. Testing was done at the University of Leeds and MM was not involved. PE reports providing expert advice to Abbvie, Astra-Zeneca, BMS, Boehringer Ingelheim, Galapagos, Gilead, Lilly, Novartis, Pfizer, Roche, Samsung outside the submitted work. He also reports research grants from AbbVie, BMS, Lilly and Samsung. The remaining authors have declared no conflicts of interest.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and this study was approved by the NHS Health Research Authority National Research Ethics Service Committee Yorkshire & the Humber—Leeds West. All patients signed an informed consent prior to participation in the study.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request.

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REFERENCES

1 Kolfenbach JR, Deane KD, Derber LA, *et al*. Autoimmunity to Peptidyl arginine Deiminase type 4 precedes clinical onset of rheumatoid arthritis. *Arthritis Rheum* 2010;62:2633–9.
2 Sokolove J, Bromberg R, Deane KD, *et al*. Autoantibody EPITOPE spreading in the pre-clinical phase predicts progression to rheumatoid arthritis. *PLoS One* 2012;7:e35296.
3 Verheul MK, Böhringer S, van Delft MAM, *et al*. Triple positivity for anti-Citrullinated protein Autoantibodies, rheumatoid factor, and anti-Carbamylated protein antibodies conferring high specificity for rheumatoid arthritis: implications for very early identification of at-risk individuals. *Arthritis Rheumatol* 2018;70:1721–31.
4 Kelmenson LB, Wagner BD, McNair BK, *et al*. Timing of elevations of autoantibody isotypes prior to diagnosis of rheumatoid arthritis. *Arthritis Rheumatol* 2020;72:251–61.
5 van Steenbergen HW, Aletaha D, Beaart-van de Voorde LJJ, *et al*. EULAR definition of arthralgia suspicious for progression to rheumatoid arthritis. *Ann Rheum Dis* 2017;76:491–6.
6 Aletaha D, Neogi T, Silman AJ, *et al*. Rheumatoid arthritis classification criteria: an American college of rheumatology/European League against rheumatism collaborative initiative. *Arthritis Rheum* 2010;62:2569–81.
7 Mahler M, Martinez-Prat L, Sparks JA, *et al*. Precision medicine in the care of rheumatoid arthritis: focus on prediction and prevention of future clinically-apparent disease. *Autoimmun Rev* 2020;19:102506.
8 Bemis EA, Demoruelle MK, Seifert JA, *et al*. Factors associated with progression to inflammatory arthritis in first-degree relatives of individuals with RA following autoantibody positive screening in a non-clinical setting. *Ann Rheum Dis* 2021;80:154–61.
9 Di Matteo A, Corradini D, Mankia K. “What is the value of ultrasound in individuals 'at-risk' of rheumatoid arthritis who do not have clinical Synovitis”. *Healthcare (Basel)* 2021;9:752.
10 Boeren AMP, Oei EHG, van der Helm-van Mil AHM. The value of MRI for detecting Subclinical joint inflammation in clinically suspect arthralgia. *RMD Open* 2022;8:e002128.
11 Hunt L, Hensor EM, Nam J, *et al*. T cell Subsets: an immunological biomarker to predict progression to clinical arthritis in ACPA-positive individuals. *Ann Rheum Dis* 2016;75:1884–9.
12 Pfeifle R, Rothe T, Ipseiz N, *et al*. Regulation of autoantibody activity by the IL-23-Th17 axis determines the onset of autoimmune disease. *Nat Immunol* 2017;18:104–13.
13 Kissel T, van Schie KA, Hafkenscheid L, *et al*. On the presence of HLA-SE Alleles and ACPA-IgG variable domain Glycosylation in the phase preceding the development of rheumatoid arthritis. *Ann Rheum Dis* 2019;78:1616–20.
14 Hafkenscheid L, de Moel E, Smolik I, *et al*. N-linked Glycans in the variable domain of IgG anti-Citrullinated protein antibodies predict the development of rheumatoid arthritis. *Arthritis Rheumatol* 2019;71:1626–33.
15 Mankia K, Siddle H, Di Matteo A, *et al*. A core set of risk factors in individuals at risk of rheumatoid arthritis: a systematic literature review informing the EULAR points to consider for conducting clinical trials and observational studies in individuals at risk of rheumatoid arthritis. *RMD Open* 2021;7:e001768.
16 Mankia K, Siddle HJ, Kerschbaumer A, *et al*. EULAR points to consider for conducting clinical trials and observational studies in individuals at risk of rheumatoid arthritis. *Ann Rheum Dis* 2021;80:1286–98.
17 Nielen MMJ, van Schaardenburg D, Reesink HW, *et al*. Specific Autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. *Arthritis Rheum* 2004;50:380–6.
18 Rantapää-Dahlqvist S, de Jong BAW, Berglin E, *et al*. Antibodies against cyclic Citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum* 2003;48:2741–9.
19 Bos WH, Wolbink GJ, Boers M, *et al*. Arthritis development in patients with arthralgia is strongly associated with anti-Citrullinated protein antibody status: a prospective cohort study. *Ann Rheum Dis* 2010;69:490–4.
20 Trouw LA, Mahler M. Closing the serological gap: promising novel biomarkers for the early diagnosis of rheumatoid arthritis. *Autoimmun Rev* 2012;12:318–22.
21 Di Matteo A, Mankia K, Duquenne L, *et al*. Third-generation anti-cyclic Citrullinated peptide antibodies improve prediction of clinical arthritis in individuals at risk of rheumatoid arthritis. *Arthritis Rheumatol* 2020;72:1820–8.
22 Di Matteo A, Duquenne L, Cipolletta E, *et al*. Ultrasound Subclinical Synovitis in anti-CCP-positive at-risk individuals with musculoskeletal symptoms: an important and predictable stage in the rheumatoid arthritis continuum. *Rheumatology (Oxford)* 2022;61:3192–200.
23 Rakieh C, Nam JL, Hunt L, *et al*. Predicting the development of clinical arthritis in anti-CCP positive individuals with non-specific musculoskeletal symptoms: a prospective observational cohort study. *Ann Rheum Dis* 2015;74:1659–66.
24 Duquenne L, Hensor EM, Wilson M, *et al*. Predicting inflammatory arthritis in at-risk persons: development of scores for risk stratification. *Ann Intern Med* 2023;176:1027–36.
25 Garcia-Montoya L, Nam JL, Duquenne L, *et al*. Prioritising referrals of individuals at-risk of RA: guidance based on results of a 10-year national primary care observational study. *Arthritis Res Ther* 2022;24:26.
26 Vos I, Van Mol C, Trouw LA, *et al*. Anti-Citrullinated protein antibodies in the diagnosis of rheumatoid arthritis (RA): diagnostic performance of automated anti-CCP-2 and anti-CCP-3 antibodies assays. *Clin Rheumatol* 2017;36:1487–92.
27 Demoruelle MK, Parish MC, Derber LA, *et al*. Performance of anti-cyclic Citrullinated peptide assays differs in subjects at increased risk of rheumatoid arthritis and subjects with established disease. *Arthritis Rheum* 2013;65:2243–52.
28 Di Matteo A, Bathon JM, Emery P. Rheumatoid arthritis. *Lancet* 2023;402:2019–33.