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EXTENDED REPORT

Autoantibodies to citrullinated fibrinogen compared with anti-MCV and anti-CCP2 antibodies in diagnosing rheumatoid arthritis at an early stage: data from the French ESPOIR cohort

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ABSTRACT

Objectives To compare the performance of anticitrullinated peptides/protein antibodies (ACPA) detected by three immunoassays in the French ESPOIR cohort of patients with early rheumatoid arthritis (RA) and undifferentiated arthritis (UA) and to study the relationship between ACPA and disease activity.

Methods A diagnosis of RA (1987 American College of Rheumatology (ACR) criteria) was established at baseline in 497 patients and after a 2-year follow-up in 592 patients. At baseline, antibodies to citrullinated fibrinogen (AhFibA), antimutated citrullinated vimentin (anti-MCV) and anticyclic citrullinated peptide (anti-CCP2) were assayed and the individual and combined diagnostic sensitivities and predictive values of the tests were determined. Relationships between ACPA positivity and the 28-joint disease activity score and Health Assessment Questionnaire scores were analysed.

Results At a diagnostic specificity of at least 98%, the three tests exhibited similar diagnostic sensitivities (47–48.5%). When considering as positive patients with at least one positive test, the sensitivity increased to 53.5% with a probable loss of specificity. Among the patients classified as having UA at baseline, 30% were positive for one ACPA, the positive predictive values for RA of the three tests ranging from 73% to 80% but increasing when two tests were associated. Whatever the test used, the addition of ACPA positivity to the 1987 criteria enhanced their sensitivity by 6%, close to that of the 2010 ACR/European League Against Rheumatism (EULAR) criteria.

Conclusions In early arthritis, AhFibA, anti-MCV and anti-CCP2 showed similar diagnostic sensitivity with a high diagnostic specificity and a similar high positive predictive value for RA. Adding ACPA to the 1987 ACR criteria significantly increased the number of patients classified as having RA, confirming the validity of the recent inclusion of the serological criterion in the ACR/EULAR criteria.

INTRODUCTION

Rheumatoid arthritis (RA), the most common chronic inflammatory joint disease, is characterised by synovial joint inflammation, progressive joint destruction and disability.[1] Early diagnosis and treatment can improve patient outcome. Rheumatoid factor (RF) was the first biological criterion to be included in the American College of Rheumatology (ACR) criteria for RA classification.[2] The main disadvantages of RF are its low diagnostic specificity and its possible absence during the first year of the disease.[3] In contrast, antibodies to citrullinated protein (anticitrullinated peptides/protein antibodies (ACPA)) are highly specific for RA. In addition, ACPA are present in serum before the onset of RA symptoms[4] and are predictive of progression to RA in patients with undifferentiated arthritis (UA).[5] Thus, ACPA are valuable for RA-specific treatment decisions early in the disease course and were recently added to the ACR/European League Against Rheumatism (EULAR) criteria for RA.[6] Different subfamilies of ACPA have been described, depending on the peptide/protein target used for their detection. All ACPA target antigens share citrullyl residues, resulting from post-translational modification of arginyl residues by peptidylarginine deiminase and identified for the first time in epitopes targeted by antifilaggrin antibodies.[7 8] Currently, the ACPA assay most widely used for RA diagnosis is the second-generation anticyclic citrullinated peptide (anti-CCP2) immunoassay. A recent meta-analysis suggested that the diagnostic specificity of anti-CCP2 antibodies for RA is about 96%, while their sensitivity ranges from 67% to 78% in patients with established RA (more than 2 years' duration) and is about 57% in early RA.[9–11]

Despite the very good diagnostic performance of anti-CCP2, the exact nature of the antigen used in the test is unknown, meaning that this test provides no useful pathophysiological information. In contrast, two citrullinated protein targets have been found in RA inflamed joints. As citrullinated fibrin was found to be the main ACPA autoantigen in the synovial tissue of patients with RA,[12 13] an ELISA was developed to detect antibodies to human citrullinated fibrinogen (AhFibA).[14–16] More recently, citrullinated vimentin was also found in the synovium of patients with RA.[17] A 'mutated' citrullinated vimentin (MCV), considered to be a valid autoantigen,[18] was produced as

a recombinant protein and used to develop an anti-MCV ELISA. It is still unclear, however, whether these assays detect different or largely overlapping subpopulations of ACPA. The diagnostic performance of ACPA tests has already been compared,[13 16 18–21] but to date not that of AhFibA and anti-MCV tests.

In addition to their high diagnostic specificity, ACPA identify a more severe phenotype of RA. Moreover, it has been reported that anti-MCV is more closely related to the 28-joint disease activity score (DAS28) than anti-CCP2, raising questions as to the relative prognostic values of the different ACPA subfamilies.[18 20]

The first aim of this study was to compare the diagnostic performance of AhFibA assay with that of anti-MCV and anti-CCP2 assays in the French ESPOIR study population, a large cohort of patients with early arthritis followed for 2 years after inclusion. The 1987 ACR criteria for RA were used as a reference and the added value of ACPA was analysed. Moreover, this combination was compared with the new ACR/EULAR 2010 criteria. We also analysed the association of the three tests with disease activity.

METHODS

Patients

The patients belonged to the French ESPOIR cohort (a French acronym for 'Study and follow-up of undifferentiated early arthritis'), composed of 813 patients with early arthritis (of <6 months' duration) who had not received disease-modifying antirheumatic drugs (DMARDs) or steroids at inclusion.[22] All the patients were at risk of progressing to RA. Blood samples were obtained at inclusion and stored at −80°C until use, and the patients were then seen every 6 months during the first 2 years.[2] The diagnosis was established according to 1987 ACR criteria, both at baseline and after 2 years. At baseline, patients whose clinical features did not fulfil any of the existing classification criteria for rheumatic diseases were considered to have UA. ACR/EULAR 2010 criteria were also collected for comparative analysis.

The protocol of the ESPOIR study was approved in July 2002 by the Montpellier ethics committee and all the patients signed an informed consent form before their inclusion.

Disease activity was evaluated with the Health Assessment Questionnaire (HAQ)[23] and the DAS28.[24]

Table 1 Clinical characteristics of patients

	All patients (n=685)	Patients diagnosed with RA* at baseline (n=497)	Patients diagnosed with RA* after 2 years (n=592)
Number of women (%)	524 (76.5%)	378 (76.1%)	453 (76.5%)
Age (years) mean± SD	48.44±12.21	49.16±11.83	49.06±11.95
Time (mean±SD) between first joint pain and inclusion (days)	223.7±264.7	222.1±271.8	228.1±271.4
Time (mean±SD) between first swelling and inclusion (days)	151.1±186.7	151.3±201.7	152.1±194.8
Time (mean±SD) between first persistent swelling and inclusion (days)	103.5±53.1	100.1±51.5	103.2±53.3

*According to the 1987 ACR criteria.
RA, rheumatoid arthritis.

ACPA assays

Anti-CCP2 and anti-MCV were detected with commercial ELISA methods (Immunoscan, Eurodiagnostica, Arnheim, The Netherlands; and Orgentec SAS, Mainz, Germany, respectively) according to the manufacturer's instructions. However, instead of the recommended positivity thresholds, we used thresholds providing a diagnostic specificity of 98% in a previously described control population with other rheumatic diseases.[25] AhFibA was assayed as previously described[14–16] and the positivity threshold was chosen to give a diagnostic specificity of 98.5%. We also compared the performances of the tests using the manufacturer's thresholds for anti-CCP2 and anti-MCV and the 95% specificity threshold for AhFibA. ACPA levels were evaluated with the three tests on baseline serum samples.

Statistical analysis

Diagnostic sensitivities of AhFibA, anti-CCP2 and anti-MCV for RA were evaluated on baseline serum samples from patients who were diagnosed with RA after 2 years of follow-up using the 1987 ACR criteria. The diagnostic sensitivities were compared using the MacNemar $\chi 2$ test. Correlations of ACPA titres obtained by the three tests were studied with the Spearman rank test.

The ability to predict progression to RA during follow-up was evaluated by calculating in patients not classified as RA at baseline (UA patients) the positive predictive value (PPV: proportion of patients with UA with a positive ACPA test who progressed

Table 2 Diagnostic sensitivity (% of positive RA patients) of ACPA tests alone and in combination at baseline in the group of patients diagnosed as having RA* after 2 years (n=592). Sensitivities are shown for thresholds giving a diagnostic specificity of 0.98 for the three tests (left part) and for the manufacturers' thresholds of anti-CCP2 and anti-MCV, and a threshold giving a specificity of 0.95 for AhFibA (right part)

ACPA test	0.98 specificity threshold	Positive RA patients		Manufacturer's threshold	Positive RA patients	
		n	%(95% CI)		n	%(95% CI)
Anti-CCP2	40 U/ml	278	47 (43 to 51)	25 U/ml	282	47.8 (43.8 to 51.8)
Anti-MCV	35 U/ml	280	47.3 (43.3 to 51.3)	20 U/ml	296	50 (46 to 54)
AhFibA	0.119 (OD)	287	48.5 (44.5 to 52.5)	0.056 (OD)	311	52.5 (48.5 to 56.5)
Anti-CCP2 or anti-MCV	As above	301	50.8† (46.8 to 54.9)	as above	310	52.4 (48.3 to 56.4)
Anti-CCP2 or AhFibA	As above	300	50.7† (46.6 to 54.7)	as above	316	53.5 (49.5 to 57.6)
Anti-MCV or AhFibA	As above	308	52.2† (48.2 to 56.2)	as above	335	56.7† (52.8 to 60.7)
At least one of the three ACPA	As above	317	53.5‡ (49.5 to 57.6)	as above	339	57.3‡ (53.3 to 61.2)

*According to the 1987 ACR criteria after 2 years of follow-up.
†p<0.001 vs a single ACPA test.
‡p<0.001 vs one ACPA alone or two ACPA combined.
anti-CCP2, anticyclic citrullinated peptide antibodies; anti-MCV, antimutated citrullinated vimentin antibodies; AhFibA, anticitrullinated human fibrinogen antibodies; ACPA, anticitrullinated protein antibodies; OD, optical density; RA, rheumatoid arthritis

to RA after 2 years) and the negative predictive value (NPV: proportion of patients with UA with a negative ACPA test who did not progress to RA after 2 years).

We assessed the value of ACPA positivity as a new serological criterion for RA classification by two approaches: replacing RF positivity by ACPA positivity or adding ACPA positivity to that of RF. We compared the sensitivities obtained with those given by both the 1987 criteria and the ACR/EULAR 2010 criteria. Clinical characteristics and the association between ACPA positivity and the DAS28 and HAQ scores were compared using the Student t test. Correlations between ACPA titres obtained with each test and the DAS28 and HAQ scores were studied with the Spearman rank test. p Values <0.05 were considered significant.

Statistical analyses were performed with R software version 2.9.2 (R Development Core Team (2009), R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Clinical characteristics of patients

Among the patients included in the cohort, 685 were re-evaluated after 2 years (161 men and 524 women, mean age 48.44±12.21 years) and constituted the study group. Of these, 497 (72.5%) were classified at baseline as having RA according to the 1987 ACR criteria (table 1). The patients with RA had very recent onset disease as the mean interval between onset of the first swollen joint and inclusion in the cohort was less than 6 months, and the interval between the first persistently swollen joint and inclusion was less than 3 months. The remaining 188 patients (27.5%) did not fulfil the ACR criteria for any defined form of arthritis and were classified as having UA. After 2 years of follow-up, 95 of the 188 patients with UA were diagnosed with RA while the remaining 93 patients were still considered to have UA. Thus, a total of 592 patients (86.4%) were diagnosed with RA during the 2-year study period.

Table 3 Distribution of the 592 RA serum samples according to the presence (+) or absence (−) of anti-CCP2, anti-MCV or AhFibA at a fixed diagnostic specificity of 0.98

Anti-CCP2	Anti-MCV	AhFibA	Number of sera
+	−	−	8
−	+	−	17
−	−	+	16
+	−	+	13
+	+	−	5
−	+	+	6
+	+	+	252
−	−	−	275

ACPA, anticitrullinated protein antibodies; anti-CCP2, anticyclic peptide antibodies; anti-MCV, antimutated citrullinated vimentin antibodies; AhFibA, anticitrullinated human fibrinogen antibodies; RA, rheumatoid arthritis; +, positive; −, negative.

Diagnostic sensitivities of anti-CCP2, anti-MCV and AhFibA in early RA

At baseline, using thresholds providing a diagnostic specificity of at least 98%, the diagnostic sensitivities of AhFibA, anti-MCV and anti-CCP2 in the 592 cases of RA diagnosed during the study period ranged from 47% to 48.5% (not significantly different, table 2). Sensitivity increased significantly when two tests were combined (by 3–4%; p<0.001) and rose to 53.5% when the three tests were combined, a level significantly higher than that of each individual test. Use of the manufacturer's thresholds for anti-CCP2 and anti-MCV and of a threshold giving 95% specificity for AhFibA gave similar results (table 2).

Correlations between AhFibA, anti-MCV and anti-CCP2 titres

The titres of AhFibA, anti-MCV and anti-CCP2 correlated strongly with one another $p<10^{-3}$; see figure S1 in online supplement). Moreover, the correlation coefficients were very similar (r=0.832 for anti-CCP2 with anti-MCV, r=0.758 for anti-MCV with AhFibA and r=0.816 for anti-CCP2 with AhFibA). However, discrepancies were noted for some sera (table 3): 8 sera were positive for anti-CCP2 only, 17 for anti-MCV only and 16 for AhFibA only. Discrepancies were noted in 44 cases between CCP2 and MCV, in 35 cases between CCP2 and AhFibA and in 51 cases between anti-MCV and AhFibA. Finally, 527 of the 592 RA serum samples (89%) gave concordant results in the three tests and less than 7% were positive in only one test. These results showed that the ACPA subfamilies detected by the three tests largely overlapped.

Table 4 Ability of three ACPA tests alone and in combination to predict progression to RA in the 188 patients with UA at inclusion, of whom 95 were diagnosed as having RA after 2 years

	Sensitivity		Specificity		PPV	NPV			
ACPA test	n	% (95% CI)	n	% (95% CI)	% (95% CI)	% (95% CI)	LR+	LR−	DOR
Anti-CCP2	28	29.5 (20.3 to 38.6)	7	92.5 (87.1 to 97.8)	80 (66.7 to 93.3)	56.2 (48.3 to 64.1)	3.93	0.76	5.16
Anti-MCV	29	30.5 (21.3 to 39.8)	11	88.2 (81.6 to 94.7)	72.5 (58.7 to 86.3)	55.4 (47.4 to 63.4)	2.58	0.79	3.28
AhFibA	30	31.6 (22.2 to 40.9)	11	88.2 (81.6 to 94.7)	73.2 (59.6 to 86.7)	55.8 (47.8 to 63.8)	2.68	0.78	3.45
Anti-CCP2 or anti-MCV	30	31.6 (22.2 to 40.9)	12	87.1 (80.3 to 93.9)	71.4 (57.8 to 85.1)	55.5 (47.4 to 63.5)	2.45	0.79	3.12
Anti-CCP2 or AhFibA	32	33.7 (24.2 to 43.2)	13	86 (79 to 93.1)	71.1 (57.9 to 84.4)	55.9 (47.8 to 64.1)	2.41	0.77	3.12
Anti-MCV or AhFibA	32	33.7 (24.2 to 43.2)	17	81.7 (73.9 to 89.6)	65.3 (52 to 78.6)	54.7 (46.4 to 63)	1.84	0.81	2.27
At least one of the three ACPA	33	34.7 (25.2 to 44.3)	18	80.6 (72.6 to 88.7)	64.7* (51.6 to 77.8)	54.7 (46.4 to 63.1)	1.79	0.81	2.21
Anti-CCP2 and anti-MCV	27	28.4 (37.5 to 19.4)	6	93.5 (88.6 to 98.5)	81.8 (68.7 to 95)	56.1 (48.3 to 63.9)	4.37	0.77	5.71
Anti-CCP2 and AhFibA	26	27.4 (18.4 to 36.3)	5	94.6 (99 to 99.2)	83.9 (70.9 to 96.8)	56.1 (48.3 to 63.8)	5.07	0.77	6.61
Anti-MCV and AhFibA	27	28.4 (19.4 to 37.5)	5	94.6 (90 to 99.2)	84.4 (71.8 to 97)	56.4 (48.6 to 64.2)	5.26	0.76	6.95
Anti-CCP2 and anti-MCV and AhFibA	26	27.4 (18.4 to 36.3)	5	94.6 (90 to 99.2)	83.9 (70.9 to 96.8)	56.1 (48.3 to 63.8)	5.07	0.77	6.61

n, Number of positive patients.
*p<0.001 vs a single ACPA test.
anti-CCP2, anticyclic peptide antibodies; anti-MCV, antimutated citrullinated vimentin antibodies; AhFibA, anticitrullinated human fibrinogen antibodies; ACPA, anticitrullinated protein antibodies; DOR, diagnostic OR; LR, likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; RA, rheumatoid arthritis; UA, undifferentiated arthritis. See Methods section for detailed definitions.

Table 5 Sensitivity of the 1987 ACR criteria for RA at baseline, after replacing RF positivity by ACPA positivity (ACPA instead of RF) or by adding ACPA positivity to RF positivity: comparison with 2010 ACR/EULAR criteria

	Number of patients classified as having RA at baseline	Sensitivity (%)
1987 ACR criteria	497	72.5
One ACPA instead of RF	532	77.6*
At least one ACPA added to RF	543	79.3*
2010 ACR/EULAR criteria (with anti-CCP2)	551	80.4*
2010 ACR/EULAR criteria (with anti-MCV)	553	80.7*
2010 ACR/EULAR criteria (with AhFibA)	557	81.3*

*p<0.001 vs 1987 ACR criteria.
ACR, American College of Rheumatology; ACPA, anticitrullinated protein antibodies; EULAR, European League Against Rheumatism; RA, rheumatoid arthritis; RF, rheumatoid factor.

Predictive value of the three ACPA tests for progression to RA

Among the 95 patients with UA at inclusion who were classified as having RA after 2 years of follow-up, about 30% were positive for ACPA at baseline whatever the test (diagnostic specificity >98%). However, about 10% of the patients still classified as UA at 2 years were also positive (table 4). The PPV for progression to RA were 80%, 72.5% and 73.2% for anti-CCP2, anti-MCV and AhFibA, respectively. The NPV of the three tests ranged from 55.4% to 56.2% (p=0.6–1, not significant). We then examined the predictive values of combinations of the three tests (table 4). The proportion of patients who developed RA among those who were positive in at least one test at baseline rose to 34.7%. However, this test combination led to a loss of specificity, PPV and NPV compared with each individual test. In contrast, when positivity was defined by positive results in two or three tests, specificity increased to 94.6% with only a slight loss of sensitivity (from 31.6% with AhFibA alone to 27.4% with the three tests combined). Use of the manufacturer's thresholds for anti-CCP2 and anti-MCV gave similar results for PPV (76.3% and 68.2% for anti-CCP2 and anti-MCV, respectively; not significant), NPV (56% and 54.9%, respectively; not significant) and for the association of both tests (80% and 56.2% for PPV and NPV, respectively) (see table S1 in online supplement). Thus, when two or three tests were positive at baseline, the PPV and NPV for progression to RA increased. These results confirm that ACPA positivity helps to predict the onset of RA in patients with UA. None of the tests performed better than any other, while the use of two tests significantly improved diagnostic performance.

Inclusion of ACPA positivity in the 1987 ACR/EULAR criteria and comparison with the ACR/EULAR 2010 criteria

We assessed the value of adding ACPA to the 1987 RA classification criteria. When we replaced RF positivity by one ACPA positivity in the 1987 criteria, 532 patients (77.6%) instead of 497 (72.5%) were classified as having RA (p<0.001, table 5). When we added ACPA positivity to RF positivity, 543 patients (79.3%) instead of 497 (72.5%) were classified as having RA (p<0.001). Compared with those 543 patients, 551 patients (80.7%) fulfilled the 2010 criteria at baseline (not significant). Using anti-MCV or AhFibA instead of anti-CCP2 in the 2010 criteria gave similar results. After 2 years the percentage of patients who fulfilled the 1987 criteria (86.4%) or the 2010 criteria (87.7%) was not significantly different but remained significantly higher than that obtained at baseline, even with the new serological criterion (p<0.001).

Table 6 Association between positivity in the ACPA tests and disease activity as measured with HAQ and DAS28

		HAQ	DAS28
Anti-CCP2			
	Positive (n=278)	1.0±0.7*	5.3±1.3*
	Negative (n=314)	0.9±0.7	5±1.3
Anti-MCV			
	Positive (n=280)	1±0.7*	5.3±1.3*
	Negative (n=312)	0.9±0.6	4.9±1.3
AhFibA			
	Positive (n=287)	1±0.7	5.3±1.3*
	Negative (n=305)	0.9±0.6	5±1.3

Values are mean±SD.
ACPA, anticitrullinated protein antibodies; anti-CCP, anticyclic citrullinated peptide antibodies; anti-MCV, antimutated citrullinated vimentin antibodies; AhFibA, anticitrullinated human fibrinogen antibodies; DAS28, disease activity score; HAQ, Health Assessment Questionnaire.
*p<0.05 versus negative patients.

Association of ACPA with disease activity

We examined whether baseline ACPA titres were associated with disease activity, as evaluated with the DAS28 and HAQ scores. The ACPA titre correlated only weakly with DAS28 and HAQ (r<0.15). HAQ scores were significantly higher in patients with anti-CCP2 or anti-MCV positivity (p<0.02) and tended to be higher in AhFibA-positive patients (p=0.05). Similarly, ACPA-positive patients had higher DAS28 scores than ACPA-negative patients (p<0.05, table 6). Thus, ACPA positivity was associated with disease activity, whatever the test used.

DISCUSSION

The ESPOIR cohort is composed of 813 patients with early-stage RA or UA (symptom onset <6 months before enrolment), of whom 685 patients could be followed up for 2 years.[22] This cohort is particularly useful for evaluating the diagnostic performance of clinical and biological parameters of RA in the very early stages of the disease when therapeutic decisions need to be taken.

A total of 592 patients (86.7%) were classified as having RA after 2 years according to the 1987 ACR criteria. At inclusion, using thresholds previously established to have a same high diagnostic specificity of at least 98%,[16 25–27] for reasons of comparability, AhFibA, anti-MCV and anti-CCP2 had similar diagnostic sensitivities of about 50%. Previous comparative studies have been heterogeneous with respect to disease duration (early or established RA) and/or the thresholds used to define positivity. In addition, most studies only compared anti-CCP2 and anti-MCV. For early arthritis, our results are in line with those of similar cohorts. In a cohort composed of patients with early RA (<1 year duration), sensitivities of 55.3% and 59.3% were obtained for anti-CCP2 and anti-MCV, respectively.[28] In another cohort of patients with UA, sensitivities of 50% and 57% were obtained for anti-CCP2 and anti-MCV, respectively.[29] Anti-CCP2 and AhFibA have been compared less frequently. These tests had respective diagnostic sensitivities of 64% and 61%, respectively, in patients with arthritis of average duration 3 years and of 65% and 70%, respectively, in patients with established RA.[16] In the present study we obtained lower diagnostic sensitivities for both tests, probably owing to the very short disease duration at inclusion in the ESPOIR cohort (<6 months).

Our study is the first to compare the diagnostic performance of tests for ACPA targeting mutated citrullinated vimentin and citrullinated fibrinogen and shows that they have similar diagnostic sensitivity. Although discrepancies between the three tests were found for a few serum samples only, we examined whether a combination of two or three tests could improve the diagnostic performance for RA. When patients with at least one positive test were considered positive, diagnostic sensitivity improved to 53.5% but specificity was probably lowered. Moreover, we checked that applying the manufacturer's thresholds for anti-CCP2 and anti-MCV and the 95% specificity threshold for AhFibA similarly improved the results obtained by combining the three tests. In our cohort, only 7% of the RA sera were positive in only one test, confirming that it is a rare event. We confirmed a strong correlation between anti-CCP2 and anti-MCV titres[21] and between anti-CCP2 and AhFibA titres.[16] Additionally, we found a strong correlation between AhFibA and anti-MCV titres. The close concordance and strong correlations among ACPA tests suggest that the three tests largely (but not entirely) detect the same antibodies.

Of the 95 patients with UA at inclusion who were classified as having RA after 2 years, 30% were positive in each ACPA test at baseline. Our results confirm that ACPA detection at baseline is predictive of progression to RA—albeit with relatively low specificity—and show that none of the three tests is better than any other. Combining the tests and considering as positive those patients with at least one positive test did not enhance the prediction of progression to RA. However, the number of patients with UA was small, and some of the 93 patients who did not develop RA after 2 years might have progressed later as these patients were not classified as having another disease at the end of follow-up. Nevertheless, our results are in keeping with those of a larger cohort of patients with UA in which considering as positive those patients with at least one positive test did not increase the predictive accuracy compared with a single positive test.[29] Interestingly, considering as positive those patients with more than one positive test significantly increased the diagnostic specificity up to 95%, but also improved the PPV for progression to RA whatever the thresholds used. Thus, when a patient with UA is positive in one ACPA test, the risk of developing RA is far higher if another test is also positive.

When analysing the benefit of adding ACPA to RF in the ACR 1987 criteria, we showed that adding ACPA positivity slightly but significantly improved the sensitivity by 6%, in keeping with the findings published by Liao *et al*,[30] and reached the performances of the ACR/EULAR 2010 criteria. This confirms the impact of the new serological criterion in the classification of RA.[31 32]

It has been suggested that defining specific ACPA patterns could be useful for predicting disease outcome.[33] Mathsson *et al* found that anti-MCV was better than anti-CCP2 for predicting a poor radiological outcome,[20] but this remains controversial.[34 35] These discrepancies may be explained by differences in treatment status across the studies. In our cohort in which ACPA tests were done before any treatment with DMARD or steroids, ACPA positivity correlated with the DAS28 and HAQ scores. However, the strong overlap between AhFibA, anti-MCV and anti-CCP2 argues against any association between disease prognosis and specific patterns of ACPA. We rather think that specific ACPA patterns may be described among ACPA-positive sera by testing them against various citrullinated peptide epitopes. Such analyses allow classification of patients into subgroups of antigen specificities with possibly different disease outcomes. For example, it has been suggested that the risk alleles HLA-DRB1 and PTPN22 and also cigarette smoking do not constitute risk factors in all anti-CCP2-positive patients but only in a subgroup defined by serum reactivity with α-enolase peptide 1.[36 37] It was recently shown that the same risk factors are associated with serum reactivity with vimentin citrullinated peptides and, to a lesser extent, with a peptide derived from fibrinogen.[38] However, this remains controversial[39] and needs further investigation.

In conclusion, we have shown that AhFibA, anti-MCV and anti-CCP2 have similar diagnostic performance for RA in early arthritis and confirm the ability of ACPA detection to predict progression from UA to RA. Moreover, we found that none of the tests performed better but that, when a patient was positive in one ACPA test and did not meet the ACR criteria, the risk of developing RA was higher if another test was positive. Finally, adding ACPA positivity to the 1987 ACR criteria significantly increased the number of patients correctly classified as having early stage RA, validating the decision of the ACR/EULAR committee to add ACPA detection as a new RA criterion.

Contributors PNR, LN, GS and SCM designed the study, analysed the results and wrote the manuscript. CD and NR performed all the statistical analyses. LC, SGM and MC were involved in the acquisition and analysis of the results. PD, MD, AC and OM participated in the critical revision of the manuscript.

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Notes