ORIGINAL ARTICLE

Antibodies against citrullinated peptides are associated with clinical and radiological outcomes in patients with early rheumatoid arthritis: a prospective longitudinal inception cohort study

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ABSTRACT

Introduction Anticitrullinated peptide antibody (ACPA) responses for 22 citrullinated peptides in patients with early rheumatoid arthritis (RA) were analysed and related to radiological and clinical outcome during the first 2 years in a prospective inception cohort.

Methods The ACPA reactivities were assessed in 1022 patients with early RA (symptoms <12 months) using the custom-made microarray chip (Thermo Fisher Scientific, Uppsala, Sweden) in a prospective longitudinal study of observational assessments of Disease Activity Score (DAS28 and its components) and radiology during the first 24 months, accounting for the treatment.

Results Frequency of ACPA reactivities varied between 13.3% and 63.1%. Of the anticyclic citrullinated peptide-2 (anti-CCP2) antibody-negative patients, ACPA reactivities were positive in 32.6%. Smoking, human leucocyte antigen-shared epitope (HLA-SE), anti-CCP2/ rheumatoid factor, protein tyrosine phosphatase non-receptor type 22 (1858C/T) and DAS28 were significantly associated with number of ACPA reactivities. The ACPA reactivities modified differently the development of DAS28 over 24 months (identified using trajectories). Anti-Filaggrin307-324, anti-hnRNP (Peptide)-Z1 and anti-F4-CIT-R antibodies anticipated lower DAS28 values (p<0.01–0.05), while positivity for anti-Fibrinogen(Fib) β62-78(74), and anti-Fibα563-583 predicted higher DAS28 (p<0.01 both). Interaction between anti-Fibß36-52, anti-Pept-5 and anti-Bla-26 antibodies, respectively, and DAS28 during 24 months decreased significantly the DAS28 values (p<0.01–0.05). Corticosteroids and biologicals were related to DAS28-area under the curve and Larsen score 24 months. Anti-vimentin2-17 antibodies remained significantly associated with Larsen score at baseline and 24 months, respectively, and radiological progression, besides biologicals at 24 months adjusted for sex and age.

Conclusions Several ACPA reactivities modified significantly the DAS28 development during the first 24 months and were significantly associated with Larsen score at baseline, 24 months and radiological progression.

Key messages

What is already known about this subject?
► Previous studies on a smaller number of antibodies have been unable to find associations with disease characteristics, although one small study based on three antibodies found an association with baseline data in early rheumatoid arthritis.

What does this study add?
► We can conclude from our study, comprising a larger cohort of patients, that there are differences in clinical characteristics related to the different antibodies.
► Reactivities of different anticitrullinated peptide antibody are related to different clinical development and radiological destruction.
► Higher number of different detectable antibodies indicated a more severe disease course.

How might this impact on clinical practice?
► In clinical practice, there is a need for new antibodies with abilities to identify different disease phenotypes, and the results of this study is a start.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterised by inflammation of the joints leading to the destruction of cartilage and bone. The aethiopathology behind the disease is not fully known but is believed to result from a combination of genetic and environmental factors, for example, HLA-DRB1 shared epitope (SE) genes and smoking.[1] The identification of associated autoantibodies targeting peptides including modified self-epitopes, such as anticitrullinated peptide/protein antibodies (ACPAs), has improved the diagnostic precision at an early stage of the disease.

Citrullination of proteins has been demonstrated to occur during the various inflammatory stages and in the tissues involved in inflammatory processes.[2–4] ACPAs have been detected with high specificity in serum and synovial fluid.[5 6]

ACPA-positive patients are suggested to have a more severe disease progression in terms of radiographic joint damage,[7–9] extra-articular manifestations, comorbidity[10–12] and disease activity.[13 14] The anticyclic citrullinated peptide-2 (anti-CCP2) assay includes a mixture of peptides with high sensitivity and specificity that has made it possible to identify different phenotypes of patients with RA.[1] However, information as to which citrullinated peptides the anti-CCP2 antibody test is targeting is not revealed.

During recent years, there has been a substantial investigation of the citrullinated autoantigens important for the initiation and progression of ACPA responses in RA. Presently, the citrullinated peptides/proteins identified in inflamed tissues, mostly joints, are, for example, α-enolase,[15] collagen type II,[16–18] fibrin/fibrinogen,[19–21] filaggrin,[5 22–24] vimentin[25–27] and hnRNP.[28] ACPA recognising these peptides are specific for the citrulline side chain but could be more or less specific for the peptide backbone and do in many cases recognise linear epitopes present in many different citrullinated proteins and peptides.[29 30] Between HLA-SE alleles and subsets of positive ACPA in patients with RA there are different associations suggesting diverse mechanisms in the development of different ACPA.[25 31–33] We have previously identified an ACPA-positive subgroup among the anti-CCP2-negative patients by using a multiplex array.[34] The subgroup presented the same HLA-SE and/or smoking associations that has previously been described for anti-CCP2-positive patients.[34] Further investigations of specific antibodies and their relationship to disease progression could be useful both in clinical practice and for understanding disease induction in RA. A better knowledge of the association of specific autoantibodies with the disease course and severity at an early stage of the disease could be helpful for optimising the treatment.

In this prospective longitudinal cohort study, using a multiplex array, we have investigated the presence of 22 different ACPA reactivities at baseline in patients with early RA and their associations to different clinical and radiological phenotypes and outcomes during the first 2 years after disease onset. We have also examined the significance of the number of individual ACPA specificities for different phenotypes.

PATIENTS AND METHODS
Subjects
A total of 1022 patients (692 women/330 men) with early RA (ie, symptom duration of ≤12 months) fulfilling the 1987 American College of Rheumatology classification criteria for RA[35] were consecutively included in the study. The prospective inclusion of patients proceeded between the years of 1996 and 2012. Data of Disease Activity Score (DAS28),[36] including swollen joint count (SJC) and tender joint count (TJC), erythrocyte sedimentation rate (ESR; mm/hour) and Patients Global Assessment (PGA; visual analogue scale) and C reactive protein (CRP; mg/L), Health Assessment Questionnaire (HAQ) and pain (visual analogue scale) were assessed at inclusion and at 6, 12, 18 and 24 months during the follow-up.

Posterior-anterior radiographs of hands, wrists and feet were read in chronological order by two trained readers (EB and SR-D) who were blinded for the antibody data (n=458) (<2 months after diagnosis) and after 24 months (n=429) and graded according to the Larsen score method.[37] Radiological progression was defined as the increase in the Larsen score between baseline and 24 months, and the smallest detectable change was calculated for the two readers and was determined as four (calculated according to reference ref 38). Sensitivity analysis between patients who performed X-rays and those without did not differ significantly concerning disease activity (DAS28, DAS-AUC, swollen and tender joints and laboratory data) and treatment. The patients were classified either as non-smokers, ever or current smokers.

Data regarding protein tyrosine phosphatase non-receptor type 22 (*PTPN22*) 1858 C/T was extracted from Immunochip analysis (SNP&SEQ Technology Platform, Uppsala, Sweden). Genotyping of HLA-DRB1 was performed and defined as HLA-DRB1*0401/0404/0405/0408/0101 as previously described.[39] With respect to the clinical situation identified by the physicians, the patients were treated with the aim of achieving low disease activity/remission. The treatment was introduced at inclusion, that is, when the RA diagnoses was settled or later when needed and constituted of conventional disease-modifying antirheumatic drugs (cDMARDs; methotrexate, sulfasalazine, chloroquine, myocrisine, azathioprine, cyclosporine and leflunomide), corticosteroids and in some cases biological disease-modifying antirheumatic drugs (bDMARDs; adalimumab, etanercept and infliximab) (table 1). Response to treatment was evaluated according to EULAR response criteria.[40]

There was no patient or public involvements in planning or performing this study besides discussions at the early arthritis clinic.

ACPA microarray
Plasma samples were collected at inclusion at the early arthritis clinic before treatment initiation and were stored frozen at −80°C until assayed. IgG-specific ACPA were analysed in plasma using a custom-made microarray based on the custom-made microarray chip (Thermo Fisher Scientific, ImmunoDiagnostics, Uppsala, Sweden) and the autoantibody status against peptides: α-enolase peptide5-21 (CEP-1), collagen type II (CII359-369, F4-R-CIT, F4-CIT-CIT and F4-CIT-R), fibrinogen (Fib) α36-50 (Fibα36-50), Fibα563-583, Fibα580-600, Fibα621-635, Fibβ36-52, Fibβ60-74, Fibβ62-78(72), Fibβ62-78(74),

Table 1 Descriptive data of 1011 patients with early RA at baseline and after 24 months of disease

Variables	Baseline	24 months
Age, median (IQR), years	59.0 (18)	–
Symptom duration until inclusion and diagnose, median (IQR), months	6 (5)	–
Smoker ever, n (%)	637 (64.7)	–
Current smoker, n (%)	205 (20.3)	–
RF+, n (%)	735 (74.2)	–
Anti-CCP2+, n (%)	680 (67.5)	–
HLA-SE, n/n total, (%)	624/1001 (62.3)	–
PTPN22 T-carrier, n (%)	637 (64.7)	–
BMI, median (IQR)	25.8 (5.4)	–
DAS28, median (IQR)	4.7 (1.9)	3.0 (1.9)
DAS28-AUC*, median (IQR)	–	82.7 (28.1)
TJC, median (IQR)	5 (8)	1.0 (4.0)
TJC AUC*, median (IQR)	–	63.0 (64.1)
SJC, median (IQR)	6 (7)	1.0 (4.0)
SJC AUC*, median (IQR)	–	69.0 (58.1)
HAQ, median (IQR)	0.9 (0.9)	0.5 (0.75)
HAQ AUC*, median (IQR)	–	14.0 (10.0)
CRP, median (IQR), mg/L	10 (21)	5.0 (7.0)
CRP AUC*, median (IQR)	–	222.0 (206.0)
ESR, median (IQR), mm/h	22 (28)	12.0 (15.0)
ESR AUC*, median (IQR)	–	406.5 (262.5)
Pain, median (IQR), mm	45 (42)	24.0 (38.0)
Pain AUC*, median (IQR)	–	738.0 (355.1)
PGA, median (IQR), mm	47 (40)	27.0 (39.0)
PGA AUC*, median (IQR)	–	810.0 (373.2)
Larsen score, median (IQR)	5 (8)	9.0 (8.8)
cDMARDs†, n (%)	879 (89.4)	952 (96.2)
cDMARDs, median (IQR), months	–	24.0 (5.0)
Corticosteroids‡, n (%)	494 (50.6)	159 (22.9)
Corticosteroids, median (IQR), months	–	8.0 (22.0)

Continued

Table 1 Continued

Variables	Baseline	24 months
bDMARDs§, n (%)	0 (0)	93 (9.4)
bDMARDs, median (IQR), months	–	12 (10)¶

*AUC calculated for 24 months.
†Conventional DMARDs prescribed at baseline: methotrexate, sulfasalazine, chloroquine, azathioprine and ciclosporin and also during the 24 months: myocrisine and leflunomide.
‡Corticosteroids ≤7.5 mg daily of prednisolone prescribed at baseline or during the 24 months.
§Biological DMARDs: adalimumab, etanercept and infliximab after baseline.
¶Median time for start of therapy after onset 12 months.
anti-CCP2, anticyclic citrullinated peptide-2; AUC, area under curve; BMI, body mass index; CRP, C reactive protein; DAS, Disease Activity Score; DMARDs, disease-modifying antirheumatic drugs; ESR, erythrocyte sedimentation rate; HAQ, Health Assessment Questionnaire; PGA, Patients Global Assessment; RA, rheumatoid arthritis ; SJC, swollen joint count;TJC, tender joint count.

Filaggrin (Fil307-324), vimentin (Vim)2-17, Vim60-75 and hnRNP-A3 (Pept) (Bla-26, Pept-1, Pept-5, PeptZ1 and PeptZ2) was determined at baseline (see ref[34] and online supplementary table 1). Twenty-one different citrullinated peptides and their arginine-containing counterpart were analysed. This technology with validation of the chip-based technique in comparison with ELISA-based technology and diagnostic performance has previously been published.[41] For the statistical calculations, we determined the difference in fluorescence of the peptides between the citrullinated peptide and its arginine-containing counterpart and used the delta value continuously. We used the uncorrected form of the C1 peptide (CII359-369) since it is an autoantigen on its own, and the conformational epitopes are modified by citrullination.[34] The cut-off value was set at the 98th percentile of 477 healthy controls for all of the antibodies to be able to compare them to each other.

Anti-CCP2 antibody test and rheumatoid factor (RF)
Anti-CCP2 antibodies were analysed using ELISA protocols according to the manufacturer's instructions (Euro-Diagnostica AB, Malmö, Sweden) with a cut-off for positivity determined at 25 arbitrary units/mL with 98% specificity. RF was analysed according to routine clinical protocol (Waaler-Rose haemagglutination test) with a 95% specificity.

Statistical and analytical methods
Statistical analyses were performed using SPSS for Windows, V.24. Cumulated values over the 24-month period were calculated according to the trapezoid model[42] referred to as area under the curve (AUC). There was a lack of all clinical data for 11 patients who were excluded from further analyses.

Table 2 Frequency of citrullinated/mutated antibodies in 1011 patients at baseline, with OR and 95% CIs calculated on controls (n=477) analysed at the laboratory and stratified for antibodies against cyclic citrullinated peptide (anti-CCP2)

Antibody against citrullinated/mutated peptides	Frequency n (%)	OR (95% CI)	Anti-CCP2 positive (n=680) n (%)	Anti-CCP2 negative (n=327) n (%)
αEno5-21 (CEP-1)	551 (53.9)	60.8 (31.1 to 119.0)	523 (76.9)	18 (5.5)
CII359-369	306 (29.9)	22.2 (11.3 to 43.6)	290 (42.6)	12 (3.7)
F4-R-CIT	492 (48.1)	17.6 (11.5 to 27.0)	463 (68.1)	22 (6.7)
F4-CIT-CIT	411 (40.2)	15.4 (9.7 to 24.5)	387 (56.9)	18 (5.5)
F4-CIT-R	264 (25.8)	5.8 (3.9 to 8.8)	250 (36.8)	9 (2.8)
Fibα36–50	263 (15.7)	18.0 (9.2 to 35.4)	243 (35.7)	18 (5.5)
Fibα563–583	387 (37.9)	35.7 (17.6 to 72.7)	376 (55.3)	7 (2.1)
Fibα580–600	205 (20.1)	13.0 (6.6 to 25.7)	195 (28.7)	9 (2.8)
Fibα621–635	398 (38.9)	33.2 (16.9 to 65.0)	375 (55.1)	18 (5.5)
Fibβ36–52	562 (55.9)	63.5 (32.5 to 124.3)	531 (78.1)	24 (7.3)
Fibβ62–78 (72)	136 (13.3)	8.0 (4.0 to 15.8)	120 (17.6)	12 (3.7)
Fibβ62–78 (74)	262 (25.6)	18.0 (9.1 to 35.2)	248 (36.5)	9 (2.8)
Fibβ60–74	645 (63.1)	89.0 (45.4 to 174.2)	608 (89.4)	26 (8.0)
Fil307-324 (CCP1)	561 (54.9)	63.3 (32.3 to 123.8)	536 (78.8)	17 (5.2)
Vim2-17	222 (21.7)	14.4 (7.3 to 28.4)	208 (30.6)	11 (3.4)
Vim60-75	578 (56.6)	76.2 (37.5 to 154.9)	551 (81.0)	19 (5.8)
Bla-26	338 (33.1)	23.1 (12.2 to 43.8)	315 (46.3)	19 (5.8)
Pept-1	250 (24.5)	21.7 (10.2 to 46.5)	238 (35.0)	10 (3.1)
Pept-5	530 (51.9)	56.0 (28.6 to 109.6)	500 (73.5)	24 (7.3)
PeptZ1	480 (47.0)	46.1 (23.5 to 90.1)	458 (67.4)	14 (4.3)
PeptZ2	506 (49.5)	51.0 (26.1 to 99.7)	479 (70.4)	20 (6.1)

α-Enolase peptide 5-21 (CEP-1), collagen type II (CII359-369, F4-R-CIT, F4-CIT-CIT and F4-CIT-R), fibrinogen (Fib) α36-50, Fibα5673–583, Fibα580-600, Fibα621–635, Fibβ36–52, Fibβ62–78 (72), Fibβ62–78 (74), Fibβ60–74, Filaggrin (Fil307-324), vimentin (Vim) 2-17, Vim60-75 and mutated proteins (Bla26, Pept-1, Pept-5, PeptZ1 and PeptZ2).

Associations between antibodies and RA were calculated and presented as OR with 95% CIs. Spearman rank correlation was used for calculation between the different ACPA specificities. The associations between autoantibody status, as well as disease activity (DAS28-AUC) and radiological findings (Larsen score at baseline and 24 months and radiological progression), respectively, as outcomes were investigated separately using simple and multivariable linear regression. In the multivariable analyses all variables significant in simple variable analysis were included. Baseline characteristics and DAS28-AUC were also analysed similarly for associations with the number of ACPA reactivities positive as dependent variable. Latent class mixed models were used, using version 1.7.8 of the lcmm package in R (R version 3.4.3), to identify groups having different DAS28 trajectories based on the DAS28 values over the 24 months and to investigate interactions between antibodies and the shape of a trajectory. Imputation was performed for missing values of DAS28 or its components.[43] The percentage of imputations performed for each time point and measures were: 7.3% at baseline, 21.0% at 6 months, 22.4% at 12 months, 37.4% at 18

months and 30.2% at 24 months. Sensitivity analyses of the data before and after imputation yielded 1%–2% different values. P values ≤0.05 were considered statistically significant. The p values were corrected for the number of performed test within the same analysis. All calculations performed on radiological data and DAS28 values and its components, respectively, were adjusted for sex and age when not presented otherwise.

RESULTS

Descriptive data at baseline and at 24 months concerning the included patients is presented in table 1.

Of those who were treated with cDMARDs, 56.6% were good responders, 12.9% were moderate and 30.5% were non-responders at 24 months. Corticosteroid treatment yielded 58.7% good responders, 11.8% moderate responders and 29.6% non-responders. Of the bDMARDs, 63.4% were good responders, 16.1% moderate responders and 20.4% non-responders at 24 months. Of the patients treated with corticosteroids, only 2.2% were without cDMARDs.

ACPA reactivities

Individual ACPAs were present in 13.3%–63.1% of the patients with the highest frequencies for anti-Fibß60-75 (63.1%), followed by anti-Vim60-75 (56.6%), anti-Fibß36-52 (55.9%), anti-Fil309-324 (54.9%), anti-CEP-1 (53.9%) and anti-Pept-5 (51.9%) antibodies (table 2). Of all the patients, 67.5% were positive for anti-CCP2 antibodies. The OR for having RA was highest for the anti-Vim60-75 and anti-Fibβ60-74 antibodies (OR=76.2 (95% CI 37.5 to 154.9) and OR=89.0 (95% CI 45.4 to 174.2), respectively) and with frequencies of 56.6% and 63.1% (table 2). In the anti-CCP2-positive patients, anti-Fil307-324, anti-Fibβ36-52, anti-Vim60-75 and anti-Fibβ60-74 were the most prevalent positive antibodies. Also, in the anti-CCP2-negative group, anti-Fibβ36-52 and anti-Fibβ60-74 together with anti-Pept-5 were most prevalent (table 2). Among the anti-CCP2-negative patients, 32.6% were positive for any of the other ACPA reactivities. There were very high degrees of correlation between the different antibodies (online supplementary figure). Of the patients, 22.6% were negative for all ACPA reactivities and anti-CCP2 antibody test. The strongest correlations occurred between anti-Fibß36-52 and anti-Fil307-324 (r_s=0.692), or anti-Fibß60-74 (r_s=0.695), or anti-PeptZ2 (r_s=0.672), between anti-Vim60-75 and anti-Fibß60-74 (r_s=0.705), between anti-PeptZ1 and anti-PeptZ2 (r_s=0.798), between anti-F4-R-CIT and anti-PeptZ1 (r_s=0.700) or anti-Fil307-324 (r_s=0.694) or anti-F4-CIT-CIT (r_s=0.782), respectively, calculated on number of positivity. The antibody with the lowest degrees of correlation was anti-CII359-369.

The frequencies of all the antibodies increased when selected for the presence of HLA-SE and smoking (online supplementary table 2). Being HLA-SE positive and ever smoker showed the highest frequency of ACPA positivity with the exception of anti-F4-CIT-R, anti-Fibβ62-78(72) and anti-Vim2-17, compared with being positive for one of the factors or double negative (online supplementary table 2).

ACPA reactivities in relation to DAS28 and its components

In simple linear regression anti-Fil307-324, anti-Vim2-17 and anti-F4-R-CIT antibodies were significantly associated with continuous data of DAS28-AUC after 24 months, whereas anti-Vim60-75 and anti-Pept-5 showed borderline significance. Anti-Vim2-17 was the only antibody remaining significant in multivariable linear regression analysis (table 3). Treatment in months with cDMARDs did not affect the results. Treatment with corticosteroids and bDMARDs was related to DAS28-AUC (table 3), with significantly higher values for DAS28-AUC (96.4 vs 82.0 for bDMARDs and 85.1 vs 79.8 for corticosteroids, respectively, p<0.001 for both). None of the antibodies, when analysed separately, showed significant associations with AUC values of SJC, TJC, PGA, HAQ or ESR, although in multivariable regression analysis, anti-Vim2-17 antibodies were significantly associated with CRP-AUC after 24 months (p<0.05).

Number of ACPA reactivities and disease-related factors

The number of different ACPA reactivities in simple regression analysis was related to being ever smoker, current smoker, HLA-SE, RF and anti-CCP2 positivity, increased DAS28-AUC and carriage of the T-variant of *PTPN22* adjusted for sex and age (table 4). In multiple regression analysis excluding anti-CCP2 and RF, the values were similar to simple regression analysis for the included factors. Exchanging ever smoker to current smoker yielded similar results with a slightly higher significant p value (0.013). Analysis without including

Table 3 Simple and multivariable linear regression with DAS28-AUC at 24 months as dependent variable and ACPA reactivities and treatment as covariates presented with significant results

ACPA reactivities and treatment	Simple			Multivariable		
	β value	P value	95% CI	β value	P value	95% CI
Fil307-324	3.57	0.011	0.80 to 6.34	−1.44	0.502	−5.63 to 2.76
Vim60-75	2.74	0.055	−0.06 to 5.53	0.09	0.965	−3.75 to 3.92
Vim2-17	4.91	0.004*	1.60 to 8.23	3.57	0.047	0.05 to 7.08
Pept-5	2.84	0.043	0.09 to 5.59	−0.32	0.875	−4.34 to 3.70
F4-R-CIT	2.88	0.041	0.12 to 5.64	−0.37	0.852	−4.28 to 3.53
cDMARDs†, months	−0.21	0.810	−0.25 to 0.20	–	–	–
bDMARDs‡, months	0.72	0.000	0.44 to 1.00	0.66	0.000	0.38 to 0.95
Corticosteroids§, months	0.42	0.000	0.29 to 0.56	0.39	0.000	0.25 to 0.53

Filaggrin (Fil307-324), vimentin (Vim) 2-17, Vim60-75, mutated proteins (Pept-5) and collagen type II (F4-R-CIT). Adjusted for sex and age.
*Indicates p value remaining significant after correction for number of tests performed.
† Conventional DMARDs: methotrexate, sulfasalazine, chloroquine, mycrisine, azathioprine, ciclosporin and leflunomide.
‡Biological DMARDs: adalimumab, etanercept and infliximab.
§Corticosteroids ≤7.5 mg daily of prednisolone.
ACPA, anticitrullinated peptide antibody; bDMARDs, biologicals disease-modifying antirheumatic drugs; cDMARDs, conventional disease-modifying antirheumatic drugs.

Table 4 Simple and multivariable linear regression with number of ACPA reactivities positive (anti-CCP2 antibodies excluded) as dependent variable

	Simple			Multivariable*			Multivariable†			Multivariable‡		
	β value	95% CI	P value	β value	95% CI	P value	β value	95% CI	P value	β value	95% CI	P value
Ever Smoker+/−	2.11	1.26 to 3.00	<0.000§	1.18	0.45 to 1.91	0.002	1.75	0.80 to 2.71	<0.001	2.29	1.25 to 3.33	<0.001
HLA-SE+/−	3.55	2.74 to 4.36	<0.000§	0.98	0.26 to 1.69	0.007	2.63	1.72 to 3.55	<0.001	3.46	2.48 to 4.45	<0.001
RF+/−	7.24	6.42 to 8.06	<0.000§	–	–	–	5.77	4.74 to 6.80	<0.001	–	–	–
Anti-CCP2+/−	10.60	9.98 to 11.14	<0.000§	10.11	9.34 to 10.89	<0.001	–	–	–	–	–	–
PTPN22 1858 T-carriage+/−	1.23	0.18 to 2.29	0.022	0.57	0.17 to 1.30	0.039	1.01	0.05 to 1.97	0.039	1.09	0.05 to 2.14	0.041
BMI, m²/kg	0.10	−0.01 to 0.21	0.073	0.10	0.01 to 0.17	0.023	0.08	−0.02 to 0.19	0.129	0.09	−0.03 to 0.21	0.124
DAS28-AUC	0.03	0.01 to 0.04	0.010	0.02	0.00 to 0.03	0.036	0.02	0.00 to 0.04	0.024	0.03	0.01 to 0.05	0.009

Adjusted for sex and age at disease onset.

*Analyses including all factors from simple analysis except for RF status, multivariable.

†Analyses including all factors from simple analysis except for anti-CCP2 antibody status, multivariable.

‡Analyses including all factors from simple analysis except for RF and anti-CCP2 antibody status, multivariable.

§Indicates p value remaining significant after correction for number of performed tests.

ACPA, anticitrullinated peptide/protein antibody; anti-CCP2, anticyclic citrullinated peptide antibodies; BMI, body mass index; DAS28-AUC, area under curve for 28-joint disease activity score during 24 months; HLA-SE, human leucocyte antigen-shared epitope; *PTPN22*, protein tyrosine phosphatase non-receptor type 22; RF, rheumatoid factor.
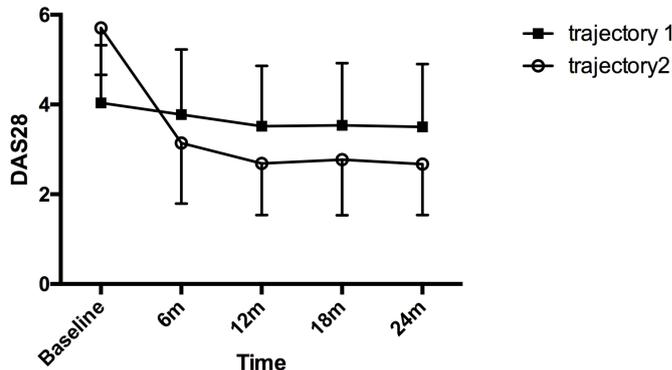

Figure 1 Development of DAS28 during the first 24 months of the RA disease stratified into two trajectories (n=617, n=394, respectively), adjusted for sex and age. DAS, Disease Activity Score; RA, rheumatoid arthritis.

anti-CCP2 antibodies yielded significant results for *PTPN22,* T-carriage (table 4). Adjustments for treatment (DMARDs or corticosteroids) did not affect the results (data not shown).

ACPA reactivities and trajectories of DAS28

Using the latent class mixed model, two groups with different DAS28 trajectories were identified (61.0% and 39.0% of the patients, respectively) based on DAS28 values from the different time points of follow-up during the 24 months. The first trajectory described the DAS28 development during the first 24 months as fairly stable above a value of 3.2 during the time period (figure 1, trajectory 1), while the second trajectory showed a rapid decrease in DAS28 during the initial 6 months and remained at a level below 2.8 for the following 24 months (figure 1, trajectory 2). The presence of some of the antibodies increased the odds for the patients following trajectory 2, that is, anti-Fil307-324 (OR=7.9, p<0.01), anti-PeptZ1 (OR=6.9, p<0.05) and anti-F4-CIT-R (OR=8.5, p<0.01), while being positive for anti-Fibα563-583 (OR=0.2, p<0.01), anti-Fibß74 (OR=0.28, p<0.01), anti-Bla-26 (OR=0.41, p<0.071) and anti-F4-CIT-CIT (OR=0.36, p<0.07) increased the chance for following trajectory 1. Patients belonging to trajectory 1 had 1.7 months longer treatment with corticosteroids (10.9 months vs 9.2 months, respectively). The DMARD treatment was similar in both groups.

Analyses of interactions between the antibodies and the course of DAS28 during the first 24 months, adjusted for age and sex, revealed an interaction of several of the antibodies on the development of DAS28, namely less reduction of the low level of trajectory 2 or decreasing the level to less than 3.2 for trajectory 1. For the individuals with higher predicted DAS28 (trajectory 1), positivity for anti-CEP1 (p<0.065), anti-Fibß36-52 (p<0.01), anti-Pept-5 (p<0.05) and anti-Bla-26 (p<0.01) antibodies decreased the DAS28 values during the 24 months, while antibodies against Vim2-17 (p<0.001) increased the DAS28 values over time for both groups represented by trajectories 1 and 2. None of the antibodies predicted the EULAR

treatment response at 24 months (data not shown). At 24 months, the good response rate was 41.8% and moderate 26.3%.

ACPA reactivities and radiological findings

The median value of the Larsen score was 5.0 at baseline and 9.0 at 24 months with a significant progression (p<0.001). The number of detectable antibodies, with and without including anti-CCP2, was related to Larsen score at baseline, 24 months and the radiological progression analysed using simple analyses of linear regression (table 5). However, in multivariable linear regression including RF and bDMARDs, the number of ACPA reactivities with and without anti-CCP antibodies, respectively (dependent variable) were borderline significant at Larsen score 24 months (p=0.052 and p=0.051, respectively) and non-significant for radiological progression (data not shown). Furthermore, 11 of the separate ACPA reactivities were significantly associated with the radiological findings (Larsen score) at 24 months (table 5). Positivity for anti-CEP-1, anti-Fil307-324 and anti-Vim2-17 antibodies were also associated with the radiological findings at baseline, after 24 months and progression between these time points. In addition, anti-F4-CIT-R and anti-Fibß36-52 were significantly associated with radiological progression and anti-Pept-5 antibodies with Larsen score at baseline in simple linear regression (table 5). The radiological findings and progression were not affected by the treatment with csDMARDs, but biological treatment was related to the Larsen score at 24 months in simple linear regression analyses (table 5). In multivariable linear regression including all separately significantly associated ACPA reactivities as covariates, RF, treatments and adjustments (age and sex), only anti-Vim2-17 antibodies remained significantly associated with the radiological findings at baseline and 24 months, respectively, and radiological progression (p<0.01 for all analyses, data not shown) and biological treatment in multivariable analysis at 24 months besides anti-Vim2-17 antibodies (p<0.001).

DISCUSSION

In this prospective longitudinal cohort study, by using 22 antibodies against different citrullinated peptides, we have been able to define different clinical and radiological development in patients with early RA. The most frequently appearing antibodies, anti-CEP-1, anti-Fibß36-52, anti-Fil307-324, anti-Pept-5 and anti-Vim60-75, were all related to the radiological findings and progression during the first 24 months after diagnosis. These antibodies were also related to DAS28-AUC and/or the modification of the DAS28 progression during the 24 months.

By identifying two trajectories representing different development of DAS28 during the 24 months, we found that certain antibodies had different impact on the slope of the DAS28 curves. Some antibodies, for example,

anti-Fil307-324, anti-PeptZ1 and anti-F4-CIT-R gave a more favourable course, while others, for example, anti-Fibα563-583, anti-Fibβ62-78(74), anti-Bla-26 or anti-F4-CIT-CIT acted the opposite. Still others were unrelated to the DAS28 development over time. During the 24 months DAS28 in trajectory 1, that is, the one with less reduction during the study period, improved for those individuals being positive for anti-CEP-1, anti-Fibß36-52, anti-Pept-5 and anti-Bla-26 antibodies resulting in decreasing DAS28 after 6 months. Positivity for the anti-Vim2-17 antibody was associated with a worse DAS28 progression in both trajectories after 12 months. In a study by Willemze et al, no association was found with any of the reactivities in relation to the baseline characteristics that they tested, that is, age at inclusion, SJC, morning stiffness, ESR, CRP and radiological joint destruction.[44] They analysed eight different ACPA reactivities using ELISAs (against citrullinated αEno5-20, Vim1-16, Vim59-74, Fibα27-43, Fibß36-52, myelin basic protein and mutated citrullinated vimentin) of which four were also included in our analyses. In another study of 374 patients with early RA, 20 different citrullinated peptides were analysed using surface plasmon resonance imaging technique (ie, without focusing solely on IgG ACPA) without showing any association with the clinical characteristics.[45] As a consequence of the results from our study, comprising a larger cohort of patients, we can conclude that there are differences in clinical characteristics related to the different antibodies.

A number of antibodies, although several appeared at fairly low frequencies, for example, anti-Fibß62-78(72), anti-Fibß62-78(74), anti-Pept-1, anti-Vim2-17 and anti-F4-CIT-R antibodies, were also related to the radiological findings at 24 months. The findings of relationships between certain antibodies and radiological findings and progression contradict the results from other studies.[44–46] However, when all antibodies were included in the same analysis, only anti-Vim2-17 antibodies remained significantly related to radiological findings both at baseline, at 24 months and to radiological progression. As previously shown, we can confirm that patients who were positive for the anti-Vim2-17 antibody had a more severe radiographic prognosis than patients negative for the autoantibody,[47] although not related to the more frequently appearing anti-Vim60-75 antibody. The number of antibodies with the citrullinated reactivities, with and without including anti-CCP2 antibodies, was strongly related to the radiological findings both at baseline, at 24 months and with radiological progression. Thus, the number of positive antibodies revealed a more severe disease as is suggested from the relationships with the radiological findings and DAS28-AUC. Another study has shown that the number of autoantibodies, including RF, anti-CCP2 and anti-carbamylated protein antibodies, was associated with the initial clinical presentation of RA in terms of age at onset, BMI, ever smoking and having a family history of disease, duration of symptoms, ESR level and SJC.[48] However, they only examined positivity for anti-CCP2

Table 5 Simple variable linear regression with Larsen score at baseline, at 24 months and radiological progression as dependent variables, respectively, adjusted for sex and age

A	B	C	D	E	F	G	H	I	J
Antibody number/against	Larsen score at Baseline			Larsen score at 24 months			Radiological progression		
	β-value	95% CI	p-value	β-value	95% CI	p-value	β-value	95% CI	p-value
No. positive ACPA	0.12	0.04, 0.21	0.006*	0.22	0.09, 0.35	0.001*	0.11	0.02, 0.19	0.015
No. positive ACPA including anti-CCP2 positivity	0.12	0.04, 0.20	0.005	0.21	0.09, 0.33	0.001*	0.10	0.02, 0.18	0.014
αEno5-21 (CEP-1)	1.21	0.05, 2.36	0.041	2.62	0.95, 4.30	0.002*	1.19	0.09, 2.29	0.034
F4-CIT-R			ns	2.28	0.05, 4.11	0.014	1.38	0.19, 2.57	0.023
Fibβ36-52			ns	1.95	0.23, 3.67	0.027	1.16	0.03, 0.05	0.043
Fibβ62–78 (72)			ns	2.87	0.42, 5.33	0.022			ns
Fibβ62–78 (74)			ns	2.19	0.34, 4.04	0.021			ns
Fil307-324 (CCP1)	1.55	0.43, 2.67	0.007	2.31	4.00, 6.19	0.008	1.19	0.08, 2.29	0.036
Vim2-17	2.55	1.18, 3.92	0.001*	4.36	2.40, 6.32	0.001*	2.10	0.82, 3.39	0.001*
Bla-26			ns	1.96	0.23, 3.68	0.026			ns
Pept-1			ns	2.05	0.13, 3.96	0.037			ns
Pept-5	1.18	0.02, 2.34	0.046	1.63	0.06, 3.32	0.059			ns
PeptZ1			ns	2.10	0.45, 3.75	0.013			ns
RF			ns	3.05	1.05, 5.05	0.003*	2.01	0.70, 3.32	0.003*
Conventional DMARDs, months	−					ns			ns
Biological DMARDs, months	−			0.58	0.30, 0.87	<0.001*			ns
Corticosteroids, months	−					ns			ns

Presented with significant results for number of positive ACPA with and without including anti-CCP2 antibodies, separate ACPA reactivities and treatment.
α-Enolase peptide 5-21 (CEP-1), collagen type II (F4-CIT-R), fibrinogen (Fib) β36-52, Fibβ62-78 (72), Fibβ62-78 (74), filaggrin (Fil307-324), vimentin (Vim) 2-17 and mutated proteins (Bla-26, Pept-1, Pept-5 and PeptZ1), RF, conventional DMARDs: methotrexate, sulfasalazine, cochloroquine, myocrisine, azathioprine, ciclosporin and leflunomide. Biological DMARDs: adalimumab, etanercept and infliximab.
*Indicates p value remaining significant after correction for number of tests performed.
ACPA, anticitrullinated peptide/protein antibody; DMARDs, disease-modifying antirheumatic drugs ; ns, non-significant; RF, rheumatoid factor.

antibodies, RF and anticarbamylated antibodies. We also found that being a smoker or having positivity for HLA-SE, RF, anti-CCP2 antibodies, higher DAS28-AUC and higher BMI were related to the number of antibodies positive.

Despite high correlations between some of the antibodies, they did not appear in defined pairs in relation to the different outcomes, for example, radiological findings or measures of inflammation (DAS28, ESR/CRP, SJC and TJC). However, the impact of each antibody is difficult to dissect, as most of them are present concurrently and some of them are cross-reactive. About 50% of the patients had at least reactivity against eight of the ACPA that we analysed.

We have included the treatment in all analyses, but since most of the patients were given cDMARDs at inclusion, it was not possible to evaluate the outcomes in relation to cDMARD. Biologicals were installed on mean at the 12 months visit and that was related to higher DAS28-AUC and Larsen score at 24 months. Patients treated with corticosteroids, calculated as months of treatment, were also used in patients with significantly higher DAS28-AUC but not with any difference on the radiological findings and progress. This study did not confirm a more favourable radiological progress as has been suggested in patients treated early with corticosteroids.[49] However, this was a clinical observational study of the impact of ACPA reactivities on disease outcomes and not a randomised treatment trial. The number and type of ACPA reactivities at baseline was unknown for the clinician except for anti-CCP2 results when choosing treatment.

One limitation in the study is the lack of randomisation or standardisation of the patients and treatment. The patients were included from the general practice of the early arthritis clinic, and the treatment was according to the physician, individualised to the patient's clinical characteristics. The study comprised a large cohort of patients, consecutively included representing the genetically homogenous population of northern Sweden. Data were missing at random to some extent, and multiple imputations were performed although sensitivity analyses showed limited differences. Some of the measures of disease activity, particularly TJC, HAQ and PGA, are subjective and affected by interpersonal variations, which could affect the analyses and consequently the relationships to antibodies. Therefore, more objective measurements of disease activity such as cytokines representing inflammation could probably give more reliable information on the relationships between antibodies and inflammation.

From this study on patients with early RA, we can conclude that our sample suggests that there may be differences in clinical characteristics, for example, development of disease activity and radiological destruction related to the different antibodies. However, the occurrence of a higher number of different detectable antibodies indicated a more severe disease course.

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