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Exploring seropositive rheumatoid arthritis: from immunological depths to clinical course

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Part 3

The AMPA profile in relation to clinical phenotype and disease outcomes in RA patients

Rheumatoid arthritis phenotype at presentation differs depending on the number of autoantibodies present

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Abstract

Objectives In rheumatoid arthritis (RA), seropositive and seronegative disease may be two entities with different underlying pathophysiological mechanisms, long-term outcomes and disease presentations. However, the effect of the conjoint presence of multiple autoantibodies, as proxy of a more pronounced humoral autoimmune response, on clinical phenotype remains unclear. Therefore, this study investigates the association between the number of autoantibodies and initial clinical presentation in two independent cohorts of early RA patients.

Methods Autoantibody status (rheumatoid factor, anti-citrullinated protein antibodies and anti-carbamylated protein antibodies) was determined at baseline in the Leiden Early Arthritis Cohort (EAC, n=828) and the Swedish BARFOT study (Better Anti-rheumatic Farmaco-therapy, n=802). The association between the number of autoantibodies and baseline clinical characteristics was investigated using univariable and multivariable ordinal regression.

Results In both cohorts the following independent associations were found in multivariable analysis: patients with a higher number of RA-associated antibodies were younger, more often smokers, had a longer symptom duration and a higher erythrocyte sedimentation rate at presentation compared to patients with few autoantibodies.

Conclusions The number of autoantibodies, reflecting the breadth of the humoral autoimmune response, is associated with clinical presentation of RA. Pre-disease pathophysiology is thus reflected by the initial clinical phenotype.

Introduction

Approximately 60% of early rheumatoid arthritis (RA) patients are positive for RA-associated autoantibodies like rheumatoid factor (RF), anti-citrullinated protein antibodies (ACPA) and anti-carbamylated protein antibodies (anti-CarP). Anti-CarP is a novel autoantibody system in RA and is associated with the development of RA in arthralgia patients and radiographic damage (1, 2). Autoantibodies may develop years before disease onset and are commonly seen as markers for underlying autoimmune pathophysiology. Thus, clinical phenotype at presentation may be a reflection of pathophysiological mechanisms taking place before disease onset.

Seropositive and seronegative RA differ in terms of risk factors and disease course. Seropositivity, especially ACPA-positivity is associated with risk factors like smoking and HLA shared epitope (SE) alleles (3). Seropositive patients also have a worse disease outcome with more radiological damage over time (4). In contrast, seronegative patients have recently been described to have more joint inflammation at initial presentation (5). Even though novel autoantibodies may be discovered in the future, seropositive and seronegative RA as defined at the moment are likely to differ in underlying pathophysiology and phenotype (5, 6).

However, within the seropositive subset a varying number of autoantibodies can be found, with the presence of several autoantibodies indicating a break of tolerance to more autoantigens. Yet the specific clinical implications of a broader humoral autoimmune response remain unclear, although this could be very important since broadening of the autoantibody response could be amenable to therapeutic intervention. Therefore this study investigates the association between the number of autoantibodies, as proxy of a broader humoral autoimmune response, and initial clinical presentation in RA patients.

Patients and Methods

Patients

Two independent early RA cohorts from the Netherlands and Sweden were analysed: the Leiden Early Arthritis Clinic (EAC) and the Better Anti-Rheumatic Farmaco Therapy project (BARFOT) (details described elsewhere) (7, 8). All patients had a short symptom duration (<24 months EAC, <12 months BARFOT), were DMARD-naive and had known status for all three autoantibodies (RF, ACPA, anti-CarP). All patients fulfilled the 1987 ACR criteria for RA (in EAC within 1 year of follow-up and in BARFOT at inclusion).

Informed consent was obtained and the studies were approved by the local medical ethics committees. At baseline, information about demographics, smoking, family history and disease characteristics was recorded. The location of initial symptoms was documented only in EAC.

Autoantibody measurements

All antibody measurements were performed in baseline sera. IgM-RF was measured by commercial enzyme-linked immunosorbent assay (ELISA) in EAC and agglutination test (SERODIA-RA) in BARFOT. In both cohorts, ACPA were determined by anti-CCP2 ELISA (Euro-Diagnostica) with manufacturer's cut-offs. Antibodies against carbamylated fetal calf serum (anti-CarP-FCS) were measured using in-house ELISAs in Leiden for both cohorts as described previously (2). The cut-off was set at the mean plus two times the standard deviation of the specific anti-CarP antibody reactivity of healthy controls matched for country of origin (Netherlands and Sweden). The presence of ACPA isotypes, ACPA fine specificities and SE alleles was determined in EAC for a subset of patients as described previously (9, 10).

Statistical analysis

Multiple imputations were used in both cohorts to deal with random missing data. Markov Chain Monte Carlo imputations (m=20) using linear and logistic regression for continuous and categorical variables respectively were done in SPSS version 23. Ordinal regression was performed to compare baseline characteristics between patients harbouring 0-3 autoantibodies and results of each imputed dataset were pooled to yield odds ratios (ORs) and 95% confidence intervals (95% CI), which signify the increase in association with every additional autoantibody. The Holm-Bonferroni method was applied to correct for multiple testing (11). Variables with a univariable p-value <0.10 were included in a multivariable model, excluding highly correlated variables. Associations between SE positivity and baseline characteristics were analysed using Mann-Whitney U tests or Chi-square tests.

Results

In both cohorts, the distribution of the number of autoantibodies was similar. The majority of patients was either seronegative (31% EAC, 33% BARFOT) or triple-positive for RF, anti-CCP2 and anti-CarP (35% EAC, 29% BARFOT). Baseline characteristics differed between the two cohorts at several points (see online supplementary table S1), reflecting differences in inclusion criteria and referral systems.

Several phenotypic characteristics were significantly associated with the number of autoantibodies in both EAC and BARFOT. In univariable and multivariable analysis, the following independent associations were found in both cohorts (Table 1, 2): patients with additional RA-associated antibodies were younger, more often smokers, had longer symptom duration and higher ESR at presentation (Figure 1). Furthermore some associations were found in one cohort but could not be replicated, like BMI, SJC28 and TJC28.

To investigate whether underlying genetic risk factors could partly explain our findings, we analysed the association between shared epitope alleles (presence versus absence) and initial clinical presentation in EAC. SE-positive patients indeed had a significant longer symptom duration and a trend towards being younger, more often smokers and having a higher ESR, but associations were less strong than with the number of autoantibodies.

Thereafter it was investigated whether the increasing prevalence of one autoantibody in particular among patients with an increasing number of autoantibodies, might be responsible for the observed observations. Stratifications for all three autoantibodies were performed, comparing single-positive patients with patients harbouring 1 or 2 additional autoantibodies (data for anti-CCP2 shown in online supplementary tables S2, S3). This resulted in small groups with limited power, but for most variables there was still a trend visible for increasing numbers of autoantibodies. Overall, the effect of the higher number of autoantibodies seemed stronger than the effect of individual autoantibodies.

Besides the number of autoantibodies, other features of a broad autoantibody response are increased levels, ACPA fine specificity and isotype usage. In EAC, there was an association between clinical phenotype and these features (see online supplementary table S4-11), although not as significant and consistent as with the number of autoantibodies. This could be due to inclusion of only ACPA-positive individuals in these sub-analyses.

Table 1: EAC univariable and multivariable ordinal regression analysis for increasing number of autoantibodies present.

n=828	0 (n=261)	1 (n=138)	2 (n=139)	3 (n=290)	Ordinal OR (95% CI)	p-value	Multivariable Ordinal OR (95% CI)	p-value
Age, years, M ± SD	60 ± 16	59 ± 17	53± 16	55± 15	Per 10 years: 0.85 (0.79-0.92)	**<0.001***	0.87 (0.79-0.95)	**0.001**
Female, n (%)	171 (66)	99 (72)	95 (68)	190 (66)	0.98 (0.75-1.27)	0.868		
BMI, kg/m^2, M ± SD	26.5 ± 4.0	26.0 ± 3.8	25.1 ± 3.1	25.3 ± 3.8	0.93 (0.90-0.97)	**<0.001***	0.95 (0.92-0.99)	**0.022**
Smoking (ever), n (%)	115 (45)	70 (52)	71 (53)	167 (59)	1.51 (1.17-1.94)	**0.001***	1.52 (1.17-1.97)	**0.001**
Family history of RA, n (%)	50 (20)	35 (26)	37 (28)	85 (30)	1.47 (1.11-1.96)	**0.008**	1.45 (1.08-1.95)	**0.015**
(Sub-)Acute onset symptoms, n (%)	157 (66)	82 (59)	66 (48)	145 (51)	0.70 (0.54-0.90)	**0.005***	0.80 (0.61-1.05)	0.104
Symptom duration, months, M ± SD	4.6 ± 4.9	4.9 ± 4.9	6.0 ± 5.3	6.1 ± 5.3	Per 3 months: 1.14 (1.05-1.23)	**0.001***	1.09 (1.00-1.18)	**0.044**
Location start of symptoms, n (%)								
· Small joints	150 (60)	75 (54)	78 (56)	166 (58)	1 (ref)			
· Large joints	36 (14)	22 (16)	20 (14)	53 (18)	1.22 (0.85-1.73)	0.279		
· Both	64 (26)	41 (30)	41 (30)	68 (24)	0.96 (0.72-1.29)	0.806		
Symmetric start of symptoms, n (%)	179 (77)	101 (79)	93 (72)	177 (68)	0.70 (0.52-0.94)	**0.017**		
Start symptoms in, n (%)								
· Upper extremity	120 (54)	71 (60)	46 (37)	92 (36)	1 (ref)			
· Lower extremity	24 (11)	14 (12)	17 (14)	46 (18)	2.18 (1.46-3.24)‡	**<0.001**		
· Both	78 (35)	34 (29)	61 (49)	115 (46)	1.71 (1.29-2.27)‡	**<0.001***		

Table 1: Continued

n=828	0 (n=261)	1 (n=138)	2 (n=139)	3 (n=290)	Ordinal OR (95% CI)	p-value	Multivariable Ordinal OR (95% CI)	p-value
ESR, mm/hour, M ± SD	36 ± 25	37 ± 26	38 ± 27	42 ± 29	Per 10 mm/hr: 1.06 (1.02-1.11)	**0.009**	1.14 (1.08-1.21)	**<0.001**
CRP, mg/liter, M ± SD	32 ± 36	27 ± 31	30 ± 42	30 ± 33	1.00 (1.00-1.00)	0.743		
SJC in 28 joints, M ± SD	9 ± 6	8 ± 6	7 ± 5	7 ± 5	0.95 (0.93-0.97)	**<0.001***	0.95 (0.93-0.97)	**<0.001**
TJC in 28 joints, M ± SD	11 ± 8	9 ± 6	8 ± 7	9 ± 6	0.97 (0.95-0.99)	**0.002***		
HAQ score, M ± SD	1.2 ± 0.7	1.2 ± 0.7	1.0 ± 0.7	1.0 ± 0.7	0.81 (0.67-0.97)	**0.023**	0.85 (0.69-1.05)	0.126
SHS score, M ± SD	8 ± 9	9 ± 10	9 ± 14	9 ± 11	1.01 (0.99-1.02)	0.435		
VAS general health, M ± SD	41 ± 26	44 ± 24	41 ± 25	41 ± 25	1.00 (0.99-1.01)	0.760		
DAS28-ESR, M ± SD	5.2 ± 1.4	5.1 ± 1.1	4.8 ± 1.4	5.1 ± 1.1	0.93 (0.84-1.03)	0.143		

ORs of imputed datasets are pooled. *Significant after correction for multiple testing (only performed in univariable analysis). ǂTest of parallel lines significant. Joint symptoms refer to any signs or symptoms of synovitis (e.g. pain, swelling, tenderness). Onset of symptoms was (sub-)acute when symptoms started within one week. Patient data were partly missing for a number of baseline variables, in particular for BMI, TJC28, HAQ, VAS general health and DAS28-ESR. Multivariable analysis was performed after exclusion of variables not available in BARFOT and of TJC28 due to high correlation.

Table 2: BARFOT univariable and multivariable ordinal regression analysis for increasing number of autoantibodies present.

n=802	0 (n=265)	1 (n=111)	2 (n=195)	3 (n=231)	Ordinal OR (95% CI)	p-value	Multivariable Ordinal OR (95% CI)ǂ	p-value
Age, years, M ± SD	61 ± 17	56 ± 17	55 ± 15	58 ± 14	Per 10 years: 0.92 (0.85-0.99)ǂ	**0.034**	0.85 (0.78-0.93)	**<0.001**
Female, n (%)	169 (64)	78 (70)	136 (70)	143 (62)	0.95 (0.73-1.24)	0.707		
BMI, kg/m^2, M ± SD	25.7 ± 4.2	25.1 ± 4.2	25.0 ± 4.4	25.5 ± 4.6	0.99 (0.96-1.03)	0.629		
Smoking (ever), n (%)	137 (52)	58 (53)	121 (63)	153 (67)	1.59 (1.23-2.06)	**<0.001***	1.62 (1.25-2.10)	**<0.001**
Family history of RA, n (%)	87 (34)	34 (31)	71 (37)	78 (35)	1.07 (0.82-1.40)	0.628		
(Sub-)Acute onset symptoms, n (%)	141 (55)	51 (47)	93 (49)	119 (52)	0.91 (0.71-1.17)	0.452		
Symptom duration, months, M ± SD	5.6 ± 3.1	5.9 ± 3.1	6.4 ± 3.0	6.3 ± 3.0	Per 3 months: 1.19 (1.05-1.34)	**0.006**	1.22 (1.07-1.39)	**0.002**
ESR, mm/hour, M ± SD	31 ± 25	31 ± 23	37 ± 24	46 ± 27	Per 10 mm/hr: 1.19 (1.13-1.25)	**<0.001***	1.24 (1.17-1.30)	**<0.001**
CRP, mg/liter, M ± SD	29 ± 34	26 ± 31	31 ± 30	44 ± 44	1.01 (1.01-1.01)ǂ	**<0.001***		
SJC in 28 joints, M ± SD	11 ± 6	10 ± 6	10 ± 5	11 ± 5	1.00 (0.98-1.03)	0.786		
TJC in 28 joints, M ± SD	9 ± 7	8 ± 7	6 ± 6	8 ± 6	0.98 (0.96-1.00)ǂ	**0.024**	0.98 (0.95-1.00)	**0.018**
HAQ score, M ± SD	1.0 ± 0.7	0.9 ± 0.6	1.0 ± 0.6	1.1 ± 0.6	1.05 (0.86-1.28)ǂ	0.638		
SHS score, M ± SD	3 ± 8	3 ± 5	5 ± 8	4 ± 7	1.01 (0.99-1.03)	0.351		
VAS general health, M ± SD	45 ± 25	44 ± 25	45 ± 26	46 ± 25	1.00 (1.00-1.01)	0.758		
DAS28-ESR, M ± SD	5.1 ± 1.3	5.0 ± 1.4	5.1 ± 1.2	5.5 ± 1.1	1.19 (1.07-1.32)ǂ	**0.001***		

ORs of imputed datasets are pooled. *Significant after correction for multiple testing (only performed in univariable analysis) ǂTest of parallel lines significant. Joint symptoms refer to any signs or symptoms of synovitis (e.g. pain, swelling, tenderness). Onset of symptoms was (sub-)acute when symptoms started within one week. Patient data were partly missing for a number of baseline variables, in particular for BMI. Multivariable analysis was performed after exclusion of DAS28-ESR and CRP due to high correlation.

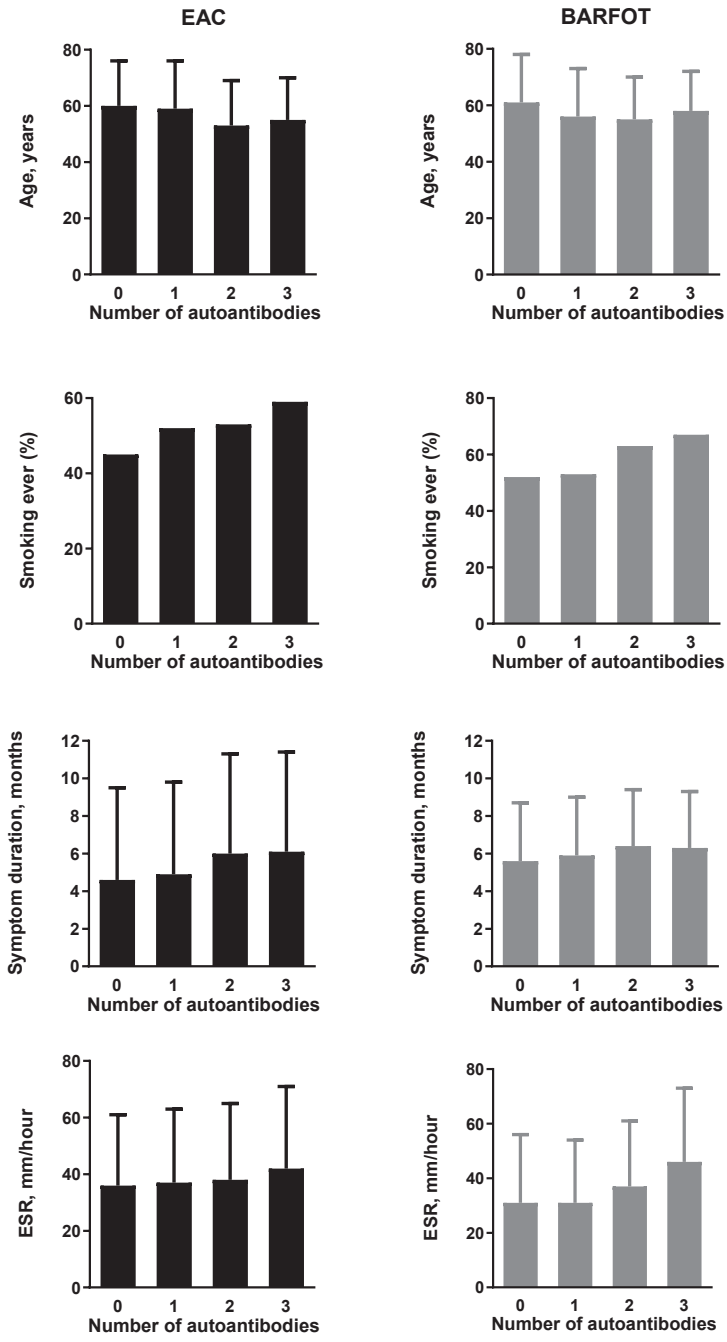

Figure 1: Number of autoantibodies and clinical characteristics. Means and standard deviations or percentages of clinical characteristics, which were significant in multivariable analysis in both cohorts, for increasing number of autoantibodies.

Discussion

This study describes for the first time the association between initial clinical presentation and the number of autoantibodies. We found that younger age, smoking, longer symptom duration and higher ESR were independently associated with having additional autoantibodies. We replicated these results in a second independent cohort.

Patients with a higher number of autoantibodies also have higher autoantibody levels, more fine specificities and broadened isotype usage. These features also appeared to be associated with clinical phenotype. Since all reflect the breadth of underlying autoimmune pathophysiology, this suggests an association between initial clinical presentation and the extent of humoral autoimmunity.

The underlying genetic predisposition associated with autoantibody positivity could possibly explain part of the observations, e.g. why patients with multiple autoantibodies develop RA at a younger age (12). This idea is supported by the association between clinical presentation and HLA SE alleles, representing part of the genetic predisposition. The association between the presence of additional autoantibodies and longer symptom duration is in line with a previous publication (13). It was recently reported that seronegative individuals have higher joint counts at presentation (5). We found in addition that increasing numbers of autoantibodies are associated with lower tender joint counts (and swollen joint counts in EAC). This may possibly reflect the higher number of joints required for seronegative individuals to fulfil classification criteria as well as intrinsic pathophysiologic differences. Overall, the findings suggest that the genetic predisposition underlying autoantibody-positive RA may result in an early, smouldering disease onset which is difficult to recognize promptly by patients and their general practitioners.

Our study has several limitations. Some variables had a large amount of missing data. However, since these data were generally missing at random and multiple imputations yielded very similar results, it seems unlikely that missing data biased our conclusions. Although this study focuses on the simultaneous presence of autoantibodies, stratifications for the different autoantibodies were performed to determine whether the observed effects are due to the presence of a single autoantibody. This analysis lacks power to draw solid conclusions, but there were still trends visible. Although we cannot exclude certain effects of specific autoantibodies, overall the effect of a higher number of autoantibodies seemed stronger than the effect of individual autoantibodies. Another limitation is the dissimilarity in baseline characteristics between both cohorts, which might be due to difference in inclusion criteria or referral systems between the Netherlands and Sweden (7, 8).

Nevertheless, the use of two large, independent cohorts enabled us to distinguish between patients with 1 versus 2 versus 3 autoantibodies and thereby discover new dose-dependent associations consistent across different populations. To further enhance our understanding of the link between humoral autoimmunity and clinical phenotype, more studies are warranted investigating disease evolution, possibly by studying immunological processes in arthralgia patients progressing towards RA. Our study also has implications for the early recognition and treatment of RA. The fact that patients with additional autoantibodies had a longer symptom duration, while they are particularly predisposed to a more severe disease course, might indicate the need for better targeted early recognition strategies to reach these patients.

In conclusion, the number of autoantibodies present is associated with clinical phenotype at presentation, indicating that the breadth of the humoral autoimmune response affects the initial clinical presentation of RA.

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Supplementary information

Supplementary table S1: Baseline characteristics of EAC and BARFOT.

	EAC (n=828)	BARFOT (n=802)	p-value
Age, years M ± SD	57 ± 16	58 ± 16	0.384
Female, no. (%)	555 (67)	526 (66)	0.561
BMI, kg/m², M ± SD	25.8 ± 3.9	25.3 ± 4.4	0.080
Smoking (ever), no. (%)	423 (52)	469 (59)	**0.006**
Family history of RA, no. (%)	207 (26)	270 (34)	**<0.001**
(Sub-)Acute onset symptoms, no. (%)	450 (54)	403 (52)	0.096
Symptom duration, months, M ± SD	5.4 ± 5.2	6.0 ± 3.0	**<0.001***
ESR, mm/hour, M ± SD	39 ± 27	37 ± 26	0.125*
CRP, mg/liter, M ± SD	30 ± 35	34 ± 37	**0.002***
SJC in 28 joints, M ± SD	7 ± 6	10 ± 6	**<0.001***
TJC in 28 joints, M ± SD	9 ± 7	8 ± 6	**<0.001***
HAQ score, M ± SD	1.1 ± 0.7	1.0 ± 0.7	0.241
SHS score, M ± SD	9 ± 11	4 ± 7	**<0.001***
VAS general health, M ± SD	42 ± 25	45 ± 25	**0.038***
DAS28-ESR, M ± SD	5.1 ± 1.3	5.2 ± 1.3	0.144

The cohorts are compared using T-tests or Mann-Whitney U tests (the latter indicated by *) for continuous data and Chi-square tests for categorical data. Symptoms refer to any signs of synovitis.

Supplementary table S2: In EAC stratification for anti-CCP2.

n=433	Anti-CCP2 only (n=20)	Anti-CCP2 +1 (n=123)	Anti-CCP2 +2 (n=290)	Ordinal OR (95% CI)	p-value
Age, years M ± SD	53 ± 15	53 ± 16	55 ± 15	1.09 (0.96-1.25)[#]	0.188
Female, no. (%)	17 (85)	87 (71)	190 (66)	0.69 (0.45-1.07)	0.098
BMI, kg/m², M ± SD	25.5 ± 3.3	25.1 ± 3.2	25.3 ± 3.8	1.00 (0.94-1.06)	0.996
Smoking (ever), no. (%)	8 (42)	62 (52)	167 (59)	1.44 (0.96-2.17)	0.077
Family history of RA, no. (%)	5 (25)	34 (28)	85 (30)	1.12 (0.72-1.75)	0.608
(Sub-)Acute onset symptoms, no. (%)	13 (65)	55 (46)	145 (51)	1.08 (0.73-1.62)	0.691
Symptom duration, months, M ± SD	4.6 ± 3.8	6.2 ± 5.4	6.1 ± 5.3	1.01 (0.90-1.13)[#]	0.919
Location start of symptoms, no. (%)					
Small joints	9 (45)	73 (59)	166 (58)	reference	
Large joints	5 (25)	17 (14)	53 (19)	1.14 (0.66-1.99)	0.641
Both	6 (30)	33 (27)	68 (24)	0.84 (0.53-1.34)	0.469
Symmetric start of symptoms, no. (%)	14 (74)	82 (72)	177 (68)	0.84 (0.53-1.33)	0.450
Start of symptoms in, no. (%)					
Upper extremity	9 (47)	42 (38)	92 (36)	reference	
Lower extremity	4 (21)	16 (15)	46 (18)	1.25 (0.66-2.35)	0.496
Both	6 (32)	52 (47)	115 (46)	1.13 (0.71-1.78)	0.610
ESR, mm/hour, M ± SD	30 ± 27	39 ± 28	42 ± 29	1.07 (0.99-1.15)[#]	0.085
CRP, mg/liter, M ± SD	20 ± 27	29 ± 41	30 ± 33	1.00 (1.00-1.01)	0.616
SJC in 28 joints, M ± SD	5 ± 3	7 ± 6	7 ± 5	1.01 (0.97-1.05)	0.537
TJC in 28 joints, M ± SD	5 ± 4	8 ± 6	9 ± 6	1.01 (0.98-1.05)	0.538
HAQ score, M ± SD	0.9 ± 0.6	1.0 ± 0.7	1.0 ± 0.7	1.06 (0.77-1.45)	0.738
SHS score, M ± SD	7 ± 6	9 ± 14	9 ± 11	1.00 (0.98-1.02)	0.699
VAS general health, M ± SD	54 ± 14	40 ± 25	41 ± 25	1.00 (0.99-1.01)	0.524
DAS28-ESR, M ± SD	4.4 ± 1.4	4.7 ± 1.4	4.7 ± 1.4	1.13 (0.95-1.34)	0.160

Single positive patients are compared to patients harbouring additional autoantibodies using ordinal regression analysis. ORs of imputed datasets are pooled. [#]OR for age is per 10 years, OR for symptom duration is per 3 months, OR for ESR is per 10 mm/hr.

Supplementary table S3: In BARFOT stratification for anti-CCP2.

n=461	Anti-CCP2 only (n=46)	Anti-CCP2 +1 (n=184)	Anti-CCP2 +2 (n=231)	Ordinal OR (95% CI)	p-value
Age, years, M ± SD	51 ± 15	55 ± 15	58 ± 14	1.23 (1.08-1.39)#	**0.001***
Female, n (%)	38 (83)	130 (71)	143 (62)	0.57 (0.39-0.84)	**0.004***
BMI, kg/m², M ± SD	24.2 ± 3.8	25.0 ± 4.4	25.5 ± 4.6	1.03 (0.98-1.08)	0.270
Smoking (ever), n (%)	21 (47)	113 (62)	153 (67)	1.48 (1.02-2.13)	**0.037**
Family history of RA, n (%)	15 (33)	67 (37)	78 (35)	0.97 (0.67-1.41)	0.887
(Sub-)Acute onset symptoms, n (%)	24 (52)	86 (48)	118 (52)	1.10 (0.77-1.56)	0.606
Symptom duration, months, M ± SD	6.7 ± 3.0	6.5 ± 2.9	6.3 ± 3.0	0.92 (0.77-1.10)#	0.368
ESR, mm/hour, M ± SD	32 ± 25	36 ± 24	46 ± 27	1.18 (1.10-1.27)#	**<0.001***
CRP, mg/liter, M ± SD	26 ± 35	30 ± 30	44 ± 44	1.01 (1.01-1.02)	**<0.001***
SJC in 28 joints, M ± SD	8 ± 5	10 ± 5	11 ± 5	1.05 (1.01-1.09)	**0.018**
TJC in 28 joints, M ± SD	7 ± 6	7 ± 6	8 ± 6	1.02 (0.98-1.05)	0.371
HAQ score, M ± SD	1.0 ± 0.6	1.0 ± 0.6	1.1± 0.6	1.29 (0.96-1.73)	0.094
SHS score, M ± SD	4 ± 6	4 ± 8	4 ± 7	0.99 (0.96-1.02)	0.647
VAS general health, M ± SD	45 ± 23	46 ± 25	46 ± 25	1.00 (0.99-1.01)	0.866
DAS28-ESR, M ± SD	4.8 ± 1.2	5.1 ± 1.1	5.5 ± 1.1	1.34 (1.14-1.58)	**<0.001***

Single positive patients are compared to patients harbouring additional autoantibodies using ordinal regression analysis. ORs of imputed datasets are pooled. #OR for age is per 10 years, OR for symptom duration is per 3 months, OR for ESR is per 10 mm/hr. *Significant after correction for multiple testing by the Holm-Bonferroni method.

Supplementary table S4: Amount of ACPA isotypes according to number of autoantibodies present.

	Number of autoantibodies present (RF, anti-CCP2 and anti-CarP)		
n=231	**1 (n=14) (only ACPA positive), n (%)**	**2 (n=60) n (%)**	**3 (n=157) n (%)**
1 ACPA isotype	7 (50)	24 (40)	33 (21)
2 ACPA isotypes	6 (43)	15 (25)	21 (13)
3 ACPA isotypes	1 (7)	21 (35)	103 (66)

The number of ACPA isotypes (IgG, IgA and IgM) present in patients grouped according to the number of autoantibodies present (RF, anti-CCP2 and anti-CarP) in EAC. ACPA isotype measurements were restricted to anti-CCP2 positive patients.

Supplementary table S5: The number of ACPA isotypes present and clinical characteristics at presentation in EAC.

	Number of ACPA isotypes present (ACPA IgG, IgA and IgM)				
n=231	**1 (n=64)**	**2 (n=42)**	**3 (n=125)**	**Ordinal OR (95% CI)**	**p-value**
Age, years, M ± SD	54 ± 17	53 ± 17	58 ± 13	1.18 (1.00-1.40)[#]	**0.048**
Smoking (ever), n (%)	23 (38)	20 (48)	76 (62)	2. 16 (1.30-3.60)	**0.003**
Symptom duration, months, M ± SD	6.0 ± 5.3	5.2 ± 3.7	6.7 ± 5.3	1.10 (0.94-1.28)[#]	0.259
ESR, mm/hour, M ± SD	42 ± 28	41 ± 23	49 ± 30	1.09 (1.00-1.20)[#]	0.052

Clinical characteristics are compared between patients with an increasing number of ACPA isotypes (IgG, IgA and IgM) using ordinal regression analysis. ACPA isotype measurements were restricted to anti-CCP2 positive patients. ORs of imputed datasets are pooled. [#]OR for age is per 10 years, OR for symptom duration is per 3 months, OR for ESR is per 10 mm/hr.

Supplementary table S6: Amount of ACPA fine specificities according to number of autoantibodies present.

	Number of autoantibodies present		
n=273	**1 (n=15) (only ACPA positive), n (%)**	**2 (n=77) n (%)**	**3 (n=181) n (%)**
0 fine specificities	4 (27)	10 (13)	14 (8)
1 fine specificity	3 (20)	22 (29)	27 (15)
2 fine specificities	4 (27)	18 (23)	33 (18)
3 fine specificities	2 (13)	15 (19)	40 (22)
4 fine specificities	2 (13)	7 (9)	41 (23)
5 fine specificities	0	4 (5)	21 (12)
6 fine specificities	0	1 (1)	5 (3)

The number of ACPA fine specificities present in patients grouped according to the number of autoantibodies present (RF, anti-CCP2 and anti-CarP) in EAC. ACPA fine specificities: vimentin 1-16: STCitSVSSSSYCitCitMFGG, vimentin 59-74: VYATCitSSAVCitLCitSSVP, fibrinogen α 27-43: FLAEGGGVCitGPRVVERH, fibrinogen β 36-52: NEEGFFSACitGHRPLDKK, α-enolase 5-20: KIHACitEIFDSCitGNPTV and myelin basic protein (MBP). ACPA fine specificity measurements were restricted to anti-CCP2 positive patients.

Supplementary table S7: The number of ACPA fine specificities present and clinical characteristics at presentation in EAC.

	Number of ACPA fine specificities present				
n=273	**0 or 1 (n=80)**	**2 or 3 (n=112)**	**4, 5 or 6 (n=81)**	**Ordinal OR (95% CI)**	**p-value**
Age, years, M ± SD	52 ± 16	57 ± 15	54 ± 15	1.06 (0.92-1.22)[#]	0.443
Smoking (ever), n (%)	38 (51)	59 (54)	46 (58)	1.25 (0.80-1.95)	0.329
Symptom duration, months M ± SD	6.5 ± 5.0	5.7 ± 5.1	6.5 ± 5.5	0.99 (0.87-1.13)[#]	0.914
ESR, mm/hour, M ± SD	37 ± 24	42 ± 28	45 ± 28	1.08 (1.00-1.18)[#]	0.056

ACPA fine specificity measurements were restricted to anti-CCP2 positive patients. Clinical characteristics are compared between patients with 0 or 1, 2 or 3 and 4, 5 or 6 ACPA fine specificities using ordinal regression analysis. ORs of imputed datasets are pooled. [#]OR for age is per 10 years, OR for symptom duration is per 3 months, OR for ESR is per 10 mm/hr.

Supplementary table S8: The autoantibody levels for patients grouped according to the number of autoantibodies present (RF, anti-CCP2, anti-CarP) in EAC.

	Number of autoantibodies present		
	1	**2**	**3**
Levels RF, M ± SD	(n=77) 22 ± 28	(n=102) 53 ± 51	(n=275) 62 ± 59
Levels anti-CCP2, M ± SD	(n=17) 490 ± 678	(n=99) 702 ± 867	(n=262) 1329 ± 2223
Levels anti-CarP, M ± SD	(n=39) 345 ± 99	(n=46) 607 ± 402	(n=290) 699 ± 455

All levels are in aU/ml. Only the levels of patients positive for that specific autoantibody are taken into account.

Supplementary table S9: RF levels and clinical characteristics at presentation in EAC.

	RF negative (n=318)	**Low RF positive (n=230)**	**High RF positive (n=224)**	**Ordinal OR (95% CI)**	**p-value**
Age, years M ± SD	59 ± 16	56 ± 16	56 ± 15	0.91 (0.83-0.98)[#]	**0.018**
Smoking (ever), n (%)	138 (44)	118 (52)	134 (63)	1.72 (1.31-2.25)	**<0.001**
Symptom duration, months, M ± SD	4.8 ± 4.9	5.7 ± 4.9	5.9 ± 5.4	1.11 (1.02-1.20)[#]	**0.014**
ESR, mm/hour, M ± SD	36 ± 24	40 ± 27	42 ± 27	1.06 (1.01-1.12)[#]	**0.019**

Clinical characteristics are compared between RF negative patients, patients that are low positive for RF (levels below median) and patients that are high positive for RF (levels above median) using ordinal regression analysis. ORs of imputed datasets are pooled. [#]OR for age is per 10 years, OR for symptom duration is per 3 months, OR for ESR is per 10 mm/hr.

Supplementary table S10: Anti-CCP2 levels and clinical characteristics at presentation in EAC.

	Anti-CCP2 negative (n=348)	Low anti-CCP2 positive (n=189)	High anti-CCP2 positive (n=189)	Ordinal OR (95% CI)	p-value
Age, years M ± SD	60 ± 16	54 ± 16	56 ± 14	0.86 (0.79-0.94)‡#	**0.001**
Smoking (ever), n (%)	161 (47)	94 (52)	107 (59)	1.40 (1.06-1.84)	**0.018**
Symptom duration, months, M ± SD	4.6 ± 4.9	5.5 ± 4.9	6.6 ± 5.4	1.19 (1.10-1.30)#	**<0.001**
ESR, mm/hour, M ± SD	37 ± 25	37 ± 26	44 ± 28	1.07 (1.02-1.13)#	**0.010**

Clinical characteristics are compared between anti-CCP2 negative patients, patients that are low positive for anti-CCP2 (levels below median) and patients that are high positive for anti-CCP2 (levels above median) using ordinal regression analysis. ORs of imputed datasets are pooled. #OR for age is per 10 years, OR for symptom duration is per 3 months, OR for ESR is per 10 mm/hr. ‡Test of parallel lines significant.

Supplementary table S11: Anti-CarP levels and clinical characteristics at presentation in EAC.

	Anti-CarP negative (n=453)	Low anti-CarP positive (n=188)	High anti-CarP positive (n=187)	Ordinal OR (95% CI)	p-value
Age, years M ± SD	58 ± 16	55 ± 16	55 ± 15	0.89 (0.82-0.97)#	**0.006**
Smoking (ever), n (%)	216 (49)	102 (55)	105 (58)	1.34 (1.03-1.75)	**0.029**
Symptom duration, months, M ± SD	4.9 ± 5.0	5.7 ± 5.5	6.2 ± 5.0	1.11 (1.03-1.20)#	**0.010**
ESR, mm/hour, M ± SD	37 ± 26	39 ± 27	43 ± 29	1.06 (1.01-1.11)#	**0.020**

Clinical characteristics are compared between anti-CarP negative patients, patients that are low positive for anti-CarP (levels below median) and patients that are high positive for anti-CarP (levels above median) using ordinal regression analysis. ORs of imputed datasets are pooled. #OR for age is per 10 years, OR for symptom duration is per 3 months, OR for ESR is per 10 mm/hr.