

Supplementary Table 1: Proportion of the 1600 patients positive for each of the serological markers.

Number of positive patients (n%)	
Anti-CCP2	
Positive	1020 (64%)
Low (<75 E/ml)	101 (6%)
High (=75 to 1500 E/ml)	691 (43%)
Extreme (≥1500 E/ml)	228 (14%)
ACPA-subspecificities	
Fil307-324 (CCP-1)	688 (43%)
Vim60-75	702 (44%)
Vim2-17	518 (32%)
Fibβ36-52	794 (50%)
Fibα563-583	682 (43%)
Fibα580-600	322 (20%)
Fibα621-635	636 (40%)
Fibα36-50	269 (17%)
Fibβ60-74	912 (57%)
Eno5-21 (CEP-1)	801 (50%)
Pept Z1	846 (53%)
Pept Z2	739 (46%)
Pept-1	575 (36%)
Pept-5	840 (53%)
Bla26	506 (32%)
CII359-369 (citC1)	157 (10%)
ACPA-peptides	
0 peptides	357 (22%)
1-3 peptides	291 (19%)
4-9 peptides	400 (25%)
10-16 peptides	552 (34%)
RF isotype	
IgA RF	692 (43%)
IgG RF	529 (33%)
IgM RF	916 (57%)
Any RF	1023 (64%)

Supplementary Table 2: Overlap in the occurrence of anti-CCP2 (stratified by levels), reactivity with the ACPA microarray (stratified for number of individual ACPA reactivities) and RF overall and by isotype (n=number of patients).

	IgA RF, n(%)		IgG RF, n(%)		IgM RF, n(%)		Any RF, n(%)	All RF, n(%)	Total, n(%)
	positive	negative	positive	negative	positive	negative	positive	negative	
Anti-CCP2 positive	628(91)	392(43)	478(90)	542(51)	825(90)	195(29)	900(88)	120(21)	1020(64)
Anti-CCP2 negative	64(9)	516(57)	51(10)	529(49)	91(10)	489(71)	123(12)	457(79)	580(36)
<i>Anti-CCP2 levels:</i>									
Low (<75 E/ml)	45(7)	56(6)	34(6)	67(6)	68(7)	33(5)	74(7)	27(5)	101(6)
High (=75 to <1500 E/ml)	425(61)	266(29)	311(59)	380(35)	566(62)	125(18)	615(60)	76(13)	691(43)
Extreme (≥1500 E/ml)	158(23)	70(8)	133(25)	95(9)	191(21)	37(5)	211(21)	17(3)	228(14)
ACPA positive (≥1)	668(54)	575(46)	508(41)	735(59)	880(71)	363(29)	969(78)	274(22)	1243(78)
<i>ACPA number of reactivities:</i>									
ACPA 0 (neg)	24(7)	333(93)	21(6)	336(94)	36(10)	321(90)	54(15)	303(85)	357(22)
ACPA 1-3	63(22)	228(78)	45(15)	246(85)	96(33)	195(67)	114(39)	177(61)	291(19)
ACPA 4-9	227(57)	173(43)	173(43)	227(57)	323(81)	77(19)	356(89)	44(11)	400(25)
ACPA 10-16	378(68)	174(32)	290(53)	262(47)	461(84)	91(16)	499(90)	53(10)	552(34)
Total	692(100)	908(100)	529(100)	1071(100)	916(100)	684(100)	1023(100)	577(100)	1600(100)

Supplementary Table 3: Regression with occurrence of IgA, IgG, IgM RF and anti-CCP2 evaluated together as independent variables, and signs and symptoms evaluated individually as dependent variables. The models are adjusted for age, sex, inclusion year and symptom duration; β = standardized beta coefficient. Statistically significant associations are depicted in bold.

	IgA RF		IgG RF		IgM RF		Anti-CCP2	
	<i>p</i> -value	β	<i>p</i> -value	β	<i>p</i> -value	β	<i>p</i> -value	β
CRP	0.925	-0.003	0.652	0.013	0.111	0.057	0.382	0.029
ESR	0.012	0.082	0.068	0.053	0.025	0.079	0.389	0.028
SJC	0.424	-0.026	0.091	0.049	0.735	0.012	<0.0001	-0.135
TJC	0.871	-0.005	0.781	0.008	0.966	-0.002	0.001	-0.107
DAS28	0.909	0.004	0.193	0.039	0.112	0.058	0.080	-0.059
DAS28CRP	0.091	-0.057	0.301	0.031	0.117	0.057	0.023	-0.077
Patient global VAS	0.090	-0.057	0.836	-0.006	0.085	0.063	0.731	-0.012
HAQ	0.521	0.022	0.482	0.021	0.918	-0.004	0.995	0.0002

Supplementary Table 4: RA-patients (n=1600) stratified for occurrence of RF isotypes (columns) or ACPA positivity determined either as any peptide reactivity or anti-CCP2 status (rows) and ESR (upper part) and SJC (lower part). Results are shown for univariate analysis (t-test). Analyses are unadjusted to allow numerical data to be compared both for RF and ACPA statistics. Statistically significant associations are depicted in bold.

		IgA RF				IgG RF				IgM RF			
		All patients (n=1600)	neg (n=908)	pos (n=692)	P-value	neg (n=1071)	pos (n=529)	P-value	neg (n=684)	pos (n=916)	P-value		
Mean ESR													
any ACPA peptide reactivity	neg (n=357)	28.8	28.8	29.4	0.9	28.8	30.0	0.795	29.2	26.0	0.408		
	pos (n=1243)	33.9	29.7	37.4	<0.0001	31.6	37.2	<0.0001	28.2	36.2	<0.0001		
	P-value	0.0003	0.568	0.124		0.059	0.2		0.551	0.012			
Anti-CCP2	neg (n=580)	29.2	28.6	34.2	0.061	29.0	31.0	0.249	28.5	32.8	0.088		
	pos (n=1020)	34.7	30.3	37.5	<0.0001	32.3	37.5	0.001	28.9	36.1	0.0002		
	P-value	<0.0001	0.251	0.307		0.019	0.08		0.845	0.215			
All patients (n=1600)			29.4	37.2	<0.0001	30.7	36.9	<0.0001	28.6	35.8	<0.0001		

Mean
SJC

any ACPA peptide reactivity	neg (n=357)	11.1	11.2	10.8	0.745	11.2	10.8	0.797	11.3	9.4	0.079
	pos (n=1243)	9.4	9.5	9.3	0.639	9.3	9.6	0.251	9.5	9.4	0.785
	P-value	<0.0001	<0.0001	0.214		<0.0001	0.325		<0.0001	0.964	
Anti-CCP2	neg (n=580)	10.8	11.0	9.8	0.145	10.9	9.8	0.213	11.0	10.0	0.259
	pos (n=1020)	9.2	9.0	9.3	0.254	8.8	9.6	0.011	8.7	9.3	0.145
	P-value	<0.0001	<0.0001	0.52		<0.0001	0.806		<0.0001	0.259	
All patients (n=1600)			10.1	9.4	0.012	9.9	9.7	0.503	10.3	9.4	0.001

Supplementary Table 5: Association between ACPA positivity and signs and symptoms in the 577 IgA, IgG and IgM RF-negative patients among 1600 RA patients from the EIRA cohort. ACPA positivity was determined as a positive anti-CCP2 test to the left, and as any ACPA peptide reactivity to the right. Each sign or symptom was evaluated individually. Data presented are least square mean levels from regression analyses, with all comparisons adjusted for age, sex, inclusion year and symptom duration. Statistically significant differences are depicted in bold.

Patients negative for all RF isotypes (n=577)						
	Anti-CCP2			any ACPA peptide		
	positive (n=120)	negative (n=457)	p-value	positive (n=274)	negative (n=303)	p-value
CRP (mg/l)	31.04	27.01	0.215	28.40	27.24	0.663
ESR (ml/h)	26.32	24.65	0.456	25.30	24.65	0.719
SJC	9.02	10.82	0.003	9.80	11.13	0.006
TJC	7.80	9.34	0.022	8.37	9.73	0.013
DAS28	4.88	5.06	0.163	4.94	5.12	0.108
DAS28CRP	4.90	5.14	0.054	5.00	5.19	0.046
Patient global VAS	46.76	48.75	0.456	46.67	50.11	0.108
HAQ	1.04	1.04	0.965	1.02	1.05	0.529

Supplementary Table 6: The association between any ACPA peptide reactivity and low number of swollen joints show more significances among ever smokers (right part) than among never smokers (left part). Data presented are least square mean levels from regression analyses, with all models adjusted for age, sex, inclusion year and symptom duration. Statistically significant differences are depicted in bold.

	IgM RF negative patients					
	Never smokers			Ever smokers		
	(n=256)		<i>p</i> -value	(n=428)		<i>p</i> -value
	any ACPA peptide pos (n=139)	any ACPA peptide neg (n=117)		any ACPA peptide pos (n=224)	any ACPA peptide neg (n=204)	
CRP (mg/l)	23.33	28.91	0.116	29.68	25.96	0.267
ESR (mm/h)	22.09	25.93	0.163	25.63	22.56	0.136
SJC	8.88	10.80	0.011	9.74	11.07	0.015
TJC	7.75	8.68	0.264	7.85	9.90	0.001
DAS28	4.82	5.07	0.163	4.89	5.08	0.091
DAS28CRP	4.88	5.09	0.178	4.95	5.22	0.011
Patient global VAS	46.65	51.22	0.193	45.84	49.06	0.183
HAQ	0.89	0.98	0.273	1.04	1.07	0.613

Supplementary Table 7: Effect of RF positivity on signs and symptoms in the 580 anti-CCP2 negative patients among 1600 RA patients from the EIRA cohort. Each RF isotype was investigated individually. Data presented are least square mean levels from regression analyses, with all models adjusted for age, sex, inclusion year and symptom duration. Statistically significant associations are depicted in bold.

	Anti-CCP2 negative patients (n=580)								
	IgA RF			IgG RF			IgM RF		
	positives (n=64)	negatives (n=516)	p-value	positives (n=51)	negatives (n=529)	p-value	positives (n=91)	negatives (n=489)	p-value
CRP	26.24	26.98	0.857	28.03	26.78	0.782	30.64	26.23	0.208
ESR	31.42	24.28	0.014	28.28	24.65	0.249	29.44	24.19	0.033
SJC	9.71	10.45	0.341	9.82	10.42	0.482	9.73	10.48	0.259
TJC	8.45	9.15	0.429	8.48	9.14	0.497	8.51	9.18	0.377
DAS28	5.10	5.00	0.559	5.02	5.01	0.945	5.10	4.99	0.446
DAS28CRP	4.93	5.07	0.383	4.94	5.06	0.475	5.05	5.05	0.962
Patient global VAS	47.26	46.26	0.771	40.43	47.03	0.084	48.95	45.88	0.301
HAQ	0.99	1.00	0.944	0.92	1.01	0.328	0.99	1.00	0.928

Supplementary Table 8: Mean(median) RF values corresponding to different bars in figure 2A-C. The 98th percentile was used as cut-off for each of the RF isotypes.

	IgA RF(IU/ml)		IgG RF(µg/ml)		IgM RF(IU/ml)	
	positive	negative	positive	negative	positive	negative
0 peptides	72.1(45.9)	4.9(4.2)	92.4(50.3)	5.1(2.9)	77.9(39.1)	1.5(0.9)
1-3 peptides	49.5(32.7)	5.9(4.7)	81.2(60.5)	8.3(5.1)	67.9(40.9)	2.1(1.1)
4-9 peptides	86.4(49.2)	9.2(8.4)	106(61.9)	17.7(17.4)	95.5(54.4)	5.2(5.3)
10-16 peptides	93.7(51.7)	9.9(9.9)	108.6(63.8)	19.1(19.1)	112.9(74.7)	5.2(5.5)

Supplementary Figure 1: Dot plots representing the distribution of swollen and tender joint counts for a-b) anti-CCP2 positive vs. negative, and for c-d) ACPA peptide positive (≥ 1 peptides) vs. negative (0 peptides) patients. Red lines represent mean levels.



