Anticitrullinated Protein Antibodies and Rheumatoid Factor Fluctuate in Early Inflammatory Arthritis and Do Not Predict Clinical Outcomes

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ABSTRACT. ***Objective.*** In inflammatory arthritis, rheumatoid factor (RF) and anticitrullinated protein antibodies (ACPA) are believed to be associated with more severe clinical outcomes. Our objective was to determine whether ACPA and RF remain stable in early inflammatory arthritis and whether their trajectories over time or baseline levels predicted clinical outcomes.

Methods. The study population consisted of patients enrolled in the Canadian Early Arthritis Cohort Study with baseline and at least 12-month followup values of RF and ACPA. Primary outcomes were Disease Activity Score (DAS) remission and the presence of erosions at 12 and 24 months. Other objectives included swollen joint count, Health Assessment Questionnaire score, and DAS.

Results. At baseline, 225/342 (66%) patients were ACPA-positive and 334/520 (64%) were RF-positive. At 24 months, 15/181 (8%) ACPA-positive patients became negative. A larger number of patients changed from ACPA-negative to positive: 13/123 (11%). For RF, fluctuations were more common: 67/240 (28%) reverted from positive to negative and 21/136 (18%) converted from negative to positive. RF and ACPA fluctuations did not predict disease outcomes. Patients who remained ACPA-positive throughout followup were more likely to have erosive disease (OR 3.86, 95% CI 1.68, 8.92).

Conclusion. RF and ACPA have the potential to revert and convert during the early course of disease. Fluctuations in RF and ACPA were not associated with clinical outcomes. (First Release Feb 1 2013; J Rheumatol 2013;40:1259–67; doi:10.3899/jrheum.120736)

Key Indexing Terms:
ANTICITRULLINATED PROTEIN ANTIBODIES RHEUMATOID FACTOR
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Rheumatoid arthritis (RA) is the most common inflammatory arthritis, affecting about 1% of the population. Left untreated, it can lead to irreversible joint deformities, disability, and increased mortality. Early identification and treatment of RA with disease-modifying antirheumatic drugs (DMARD) improve outcomes significantly[1]. Recently, new diagnostic criteria for RA [2010 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) criteria] have been developed to identify patients earlier in the disease course[2]. The criteria include the presence and levels of anticitrullinated protein antibodies (ACPA) in addition to the previously used rheumatoid factor (RF)[2].

ACPA are as sensitive as RF for the diagnosis of RA; however, they are more specific. The most common clinically used ACPA, anticyclic citrullinated peptide-2 (anti-CCP2), has a specificity of > 95% in early RA compared to RF, with a specificity of 70%–80%[3]. These

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antibodies are present in some individuals > 10 years prior to disease onset, but appear to remain stable after RA diagnosis[4,5,6]. It is uncertain whether antibody levels fluctuate with treatment response or predict outcomes[7,8,9,10,11,12,13,14,15,16]. Therefore, the role of repeat antibody testing in the management of RA is unknown. A recent study has shown that changes in anti-CCP2 and RF status were not predictive of erosive disease[16]. That study identified another ACPA (anti-Sa) as a prognostic marker for erosions. However, there are few studies investigating the usefulness of ACPA and RF fluctuations in early inflammatory arthritis for predicting other disease outcomes, such as disease activity and physical function[15].

Our objective was to determine whether ACPA or RF positivity change over time and whether these changes predict erosive disease and remission in a large prospective cohort of patients with early inflammatory arthritis (EIA), the Canadian Early Arthritis Cohort Study (CATCH). We also analyzed whether antibody levels at baseline predict disease outcomes.

MATERIALS AND METHODS

Patients. Data were collected on patients enrolled in the CATCH study, a multicenter observational prospective inception cohort of patients with EIA, from July 2007 to July 2011. Inclusion criteria for CATCH are age > 16 years, between 6 weeks and 12 months of persistent synovitis, and ≥ 2 swollen joints or 1 swollen metacarpophalangeal or proximal interphalangeal joint with ≥ 1 of the following: positive RF, positive ACPA, morning stiffness > 45 min, response to nonsteroidal antiinflammatory drugs (NSAID), or painful metatarsophalangeal squeeze test. Patients were followed every 3 months using a standard protocol. Treatment with DMARD, NSAID, corticosteroids (oral, intramuscular, and/or intra-articular), and biologic agents was based on physician's discretion with the aim of obtaining zero swollen joints.

Our study was approved by the research ethics boards of all the centers involved and consent was obtained according to the Declaration of Helsinki.

RF and ACPA. Patients were included if they had baseline and at least 12-month followup values for RF or ACPA. Data on antibodies were not available from 7 of 18 centers involved in CATCH. All other centers had baseline antibody values, but followup data were available in roughly 50% of enrolled patients. All RF assays measured IgM RF, but methods were not standardized across centers. Depending on the site, 2 different anti-CCP2 IgG kits were used (Euroimmune™ and Inova™). Cutoff values for positivity used by the different assays were recorded in the database. For each individual patient, the same kit was always used for baseline and followup anti-CCP2 tests. There were no differences in study outcomes for the 2 different kits. For RF, moderate/high titers were arbitrarily set at ≥ 160 units/ml. For ACPA, moderate/high titers were deemed to be ≥ 3 times the upper limit of normal following the 2010 ACR/EULAR RA diagnostic criteria. The status of antibodies from inclusion to last available followup data was stratified into 4 categories: always negative, conversion (negative to positive), always positive, and reversion (positive to negative).

Disease variables and outcomes. Outcomes were assessed by a rheumatologist. Time of RA onset was defined as patient-reported commencement of symptoms that were persistent. The demographic information included age, sex, and smoking status (current smoker, ex-smoker, or never smoked). Clinical outcomes were regularly recorded at all centers every 3 months. Disease activity was determined using the Disease Activity Score-28 joint count (DAS28) and remission was defined as DAS28 < 2.6. Patient function was evaluated using the Health Assessment Questionnaire (HAQ). Swollen joint count (SJC) was a 28-joint count. Presence or absence of erosions was determined using plain radiographs of the hands and feet performed at baseline and at 6 and every 12 months, as reported by the local radiologist and/or as reviewed by the treating rheumatologist. Radiographic data were available in roughly 90% of enrolled patients at baseline and 70% at followup. Presence or absence of erosions and new erosions was known but Sharp scores were not available. Followup antibody measurements were conducted at the same time as disease outcome measures.

Statistical analysis. Student t test or the Mann-Whitney U test was used to compare continuous variables; chi-square or Fisher's exact tests were used to compare categorical variables. ANOVA was used to determine risk of continuous outcomes for the different trajectories of antibodies. Multiple logistic regression was performed to evaluate whether antibody trajectory or level was an independent predictor of outcomes. Variables significantly associated with the outcomes of interest by univariate analyses were included in the logistic regression model if $p < 0.1$. The following variables were tested: age, sex, duration of disease, smoking history, baseline SJC, baseline HAQ score, presence of baseline erosions, baseline DAS28 score, and baseline treatment with antirheumatic agents. Bonferroni correction was performed for multiple comparisons. For analyses of the antibody trajectories, 6 comparisons were performed and significance was set at a p value of $0.05/6 = 0.0083$. For analyses of the antibody levels, 3 comparisons were performed and significance was set at a p value of $0.05/3 = 0.017$. Missing outcomes in followup data were not included in the analyses. Statistical analyses were performed using SAS software, version 9.3 (SAS Institute).

RESULTS

Patient characteristics. As of July 2012, 1431 patients were enrolled in CATCH; 342 had baseline and 12-month followup ACPA data available and 520 had RF data available. At 24 months, 277 and 376 patients had ACPA and RF data available, respectively. The baseline demographics, presence of erosions, DAS28, and HAQ score were not significantly different in the included versus excluded patients. Baseline characteristics are shown in Table 1. There were also no significant differences between patients with baseline and followup ACPA values and those with RF data available. Mean age was 52 years and disease duration at enrollment was 6.5 months. The population was 77.5% female. A history of smoking was reported in 58.8%. The majority of patients met 1987 ACR RA criteria or 2010 ACR/EULAR criteria (67.2% and 74.6%, respectively).

ACPA-positive patients were younger, had longer disease duration, and were more likely to meet criteria for RA ($p < 0.0001$) than ACPA-negative patients. Similarly, RF-positive patients had longer disease duration and were more likely to meet the RA criteria ($p < 0.05$). There was no significant difference in smoking history between ACPA-positive and negative patients, but a higher proportion of RF-positive patients had a history of smoking compared to those who were RF-negative ($p = 0.0473$). Most of the anti-CCP2-positive patients were also RF-positive (78.1%), whereas 57.4% of ACPA-negative patients expressed RF ($p < 0.0001$). RF-positive patients were also more likely to be ACPA-positive (66.2% vs 36.1%; $p < 0.0001$).

Table 1. Patient characteristics at baseline.

Characteristic	All	n	ACPA–	n	ACPA+	n	p	All	n	RF–	n	RF+	n	p
			ACPA							RF				
Age, mean yrs (SD)	51.56 (13.23)	342	56.46 (11.86)	123	48.8 (13.18)	219	< 0.0001	53.54 (13.54)	520	54.62 (14.94)	186	52.94 (12.67)	334	NS
Female	265 (77.5)	342	90 (732)	123	175 (79.9)	219	NS	392 (75.4)	520	134 (72)	186	258 (77.3)	334	NS
Symptom duration, mean mo (SD)	6.53 (3.21)	342	6.06 (.35)	123	6.81 (3.1)	219	0.0373	6.09 (3.17)	520	5.72 (2.99)	186	6.29 (3.25)	334	0.0473
Ever-smoker	201 (58.8)	342	65 (52.9)	123	136 (62.1)	219	NS	304 (58.5)	520	97 (52.2)	186	207 (62)	334	0.0293
SJC28, median (range)	6 (0–28)	336	8 (0–28)	117	5 (0–26)	219	0.0231	6 (0–28)	514	8 (0–28)	183	5 (0–26)	331	< 0.0001
TJC28, median (range)	6 (0–28)	336	7 (0–28)	117	6 (0–27)	219	NS	6 (0–28)	514	8 (0–28)	183	5 (0–26)	331	< 0.0001
ESR, mean (SD)	26.08 (22.7)	330	25.43 (21.86)	120	26.44 (23.21)	210	NS	26.28 (23)	510	25.1 (23.21)	184	26.94 (22.88)	326	NS
CRP, mean (SD), mg/l	13.52 (19.74)	330	13.05 (17.84)	118	13.78 (20.76)	212	NS	14 (18.22)	502	13.91 (18.94)	180	14.1 (17.83)	322	NS
Erosions	62 (20.1)	308	19 (17.4)	109	43 (21.6)	199	NS	102 (22.5)	454	37 (23.1)	160	65 (22.1)	294	NS
DAS28, mean (SD)	4.83 (1.62)	321	4.91 (1.6)	113	4.78 (1.63)	208	NS	4.78 (1.53)	496	5 (1.57)	175	4.66 (1.49)	321	0.0174
HAQ, median (range)	0.88 (0–3)	340	1 (0–2.75)	122	0.88 (0–3)	218	NS	0.88 (0–3)	513	1 (0–3)	181	0.88 (0–3)	332	NS
RF-positive	216 (70.4)	307	66 (57.4)	115	150 (78.1)	192	0.0001	334 (64.2)	520	—	—	—	—	—
Anti-CCP2-positive	219 (64)	342	—	—	—	—	—	224 (56.1)	399	48 (36.1)	133	176 (66.2)	266	< 0.0001
1987 ACR RA criteria	225 (67.2)	335	67 (56.8)	118	158 (72.8)	217	0.0028	334 (65.4)	511	93 (51.1)	182	241 (73.3)	329	< 0.0001
2010 ACR/EULAR RA criteria	255 (74.6)	342	74 (60.2)	123	181 (82.6)	219	< 0.0001	373 (71.7)	520	100 (53.8)	186	273 (81.7)	334	< 0.0001
DMARD	309 (91.2)	339	110 (91.7)	120	199 (90.9)	219	NS	466 (90.7)	514	159 (87.9)	181	307 (92.2)	333	NS
Biologics	7 (2.1)	339	1 (0.8)	120	6 (2.7)	219	NS	9 (1.8)	514	2 (1.1)	181	7 (2.1)	333	NS
Corticosteroids	150 (44.3)	339	56 (46.7)	120	94 (42.9)	219	NS	254 (49.4)	514	83 (45.9)	181	171 (51.4)	333	NS

ACPA: anticitrullinated protein antibodies; RF: rheumatoid factor; SJC28: swollen joint count-28; TJC28: tender joint count-28; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; DAS28: Disease Activity Score-28; ACR: American College of Rheumatology; EULAR: European League Against Rheumatism; DMARD: disease-modifying antirheumatic drugs; NS: nonsignificant; HAQ: Health Assessment Questionnaire; RA: rheumatoid arthritis.

At inclusion, patients had moderate to severe disease, with median SJC of 6 (range 0–28)/28, TJC of 6 (range 0–28)/28, HAQ score = 0.88 (range 0–3), and mean DAS28 = 4.83 (SD 1.62). A large percentage (20.1%) had erosive disease at baseline. More than 90% of patients were treated with a DMARD and > 40% were receiving corticosteroids (doses < 20 mg daily). Treatment rates with antirheumatic drugs were not different in antibody-negative versus antibody-positive patients. SJC was significantly higher in ACPA-negative patients compared to ACPA-positive patients ($p = 0.0231$), but other measures of disease activity were not different. RF-negative patients also had higher SJC ($p < 0.0001$) and DAS28 score ($p = 0.0174$) than RF-positive patients.

Tables 2 and 3 summarize clinical characteristics of patients at 12 and 24 months' followup. Clinical outcomes were significantly improved at 12 months' followup, with median SJC of 1, median HAQ score = 0.25, and mean DAS28 score = 2.84 ($p < 0.05$). DAS remission was achieved in 52.6% of patients. These improvements continued at 24 months' followup. There was no significant change in the proportion of ACPA-positive and RF-positive patients at baseline and followup.

At 12 months' followup, a larger proportion of ACPA-positive patients had erosive disease compared to ACPA-negative patients (33.1% vs 27.3%, respectively; $p = 0.0058$). This difference was not seen at 24 months. At 24 months, ACPA-positive patients had a lower SJC than ACPA-negative patients ($p = 0.004$). Other measures of disease activity at followup were not different between

Table 2. Patient characteristics at followup by ACPA status.

Characteristic	12 Months All	n	ACPA–	n	ACPA+	n	p	24 Months All	n	ACPA–	n	ACPA+	n	p
SJC28, median (range)	1 (1–18)	336	1 (0–13)	111	1 (0–18)	225	NS	0 (0–17)	269	1 (0–14)	91	0 (0–17)	178	0.004
TJC28, median (range)	1 (1–18)	336	1 (0–17)	111	1 (0–18)	225	NS	0 (0–20)	269	1 (0–16)	91	0 (0–20)	178	NS
ESR, mean (SD)	14.64 (15.46)	338	14.49 (16.19)	117	14.72 (15.1)	221	NS	14.27 (13.39)	270	13.24 (13.4)	95	14.83 (13.39)	175	NS
CRP, mean (SD), mg/l	5.3 (7.92)	334	4.96 (7.67)	115	5.48 (8.06)	219	NS	4.91 (7.26)	272	4.22 (5.65)	96	5.29 (7.99)	176	NS
Erosions	68 (27.3)	249	15 (16.9)	89	53 (33.1)	160	0.0058	55 (27.8)	198	15 (25)	60	40 (29)	138	NS
DAS28, mean (SD)	2.84 (1.34)	325	2.89 (1.29)	110	2.81 (1.36)	215	NS	2.69 (1.36)	259	2.78 (1.33)	87	2.64 (1.38)	172	NS
DAS28 < 2.6	180 (52.6)	342	59 (50.4)	117	121 (35.4)	225	NS	165 (59.6)	277	56 (57.1)	98	109 (60.9)	179	NS
HAQ, median (range)	0.25 (0–3)	334	0.25 (0–2.25)	113	0.25 (0–3)	221	NS	0.13	261	0.13 (0–2.5)	87	0.19 (0–2.63)	174	NS
RF-positive	169 (54.3)	311	36 (32.1)	112	133 (66.83)	199	< 0.0001	137 (55)	249	33 (36.7)	90	104 (65.4)	159	< 0.0001
Anti-CCP2-positive	225 (65.8)	342	—	—	—	—	—	179 (64.6)	277	—	—	—	—	—
1987 ACR RA criteria	225 (67.2)	335	62 (18.5)	112	163 (48.7)	223	0.0011	191 (69.5)	275	61 (62.9)	97	130 (73)	178	NS
2010 ACR/EULAR RA criteria	255 (74.6)	342	72 (61.5)	117	183 (81.3)	225	< 0.0001	219 (79.1)	277	66 (67.4)	98	153 (85.5)	179	0.0004
DMARD	323 (95.3)	339	109 (95.6)	114	214 (95.1)	225	NS	259 (93.8)	276	90 (92.8)	97	169 (94.4)	179	NS
Biologics	35 (10.3)	339	9 (7.9)	114	26 (11.6)	225	NS	51 (18.5)	276	21 (21.6)	97	30 (16.8)	179	NS
Corticosteroids	79 (23.3)	339	28 (24.6)	114	51 (22.7)	225	NS	70 (25.4)	276	33 (34)	97	37 (20.7)	179	0.0149

ACPA: anticitrullinated protein antibodies; RF: rheumatoid factor; SJC28: swollen joint count-28; TJC28: tender joint count-28; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; DAS28: Disease Activity Score-28; ACR: American College of Rheumatology; EULAR: European League Against Rheumatism; DMARD: disease-modifying antirheumatic drugs; NS: nonsignificant; HAQ: Health Assessment Questionnaire; RA: rheumatoid arthritis.

ACPA-positive and ACPA-negative patients and treatment with antirheumatic agents was similar. At 24 months ACPA-negative patients were more likely to be treated with corticosteroids (34% vs 20.7%; p = 0.0149). RF-positive patients appeared to have more severe disease at followup: they had higher DAS28 score, ESR, and CRP and were less likely to achieve remission (p < 0.05). There was no difference in the rate of erosive disease in RF-positive versus RF-negative patients. RF-positive patients were less likely to be treated with a biologic agent at 24 months' followup (9.3% vs 20.6%; p = 0.0022).

Trajectory of ACPA and RF over time. Fluctuations in both ACPA and RF antibody status occurred at the 12- and 24-month followup periods (Table 4). With ACPA, 10.6% of patients who were initially negative converted to positive at 12 months and 13.5% converted by 24 months. Similar findings were seen with RF (11.3% conversion at 12 mo and 18.3% at 24 mo). Reversion was less frequent with ACPA (3.2% positive at baseline and became negative at 12 mo and 8.3% at 24 mo). RF frequently reverted over time: 25.4% positive at baseline became negative at 12 months and 27.9% at 24 months' followup. Reversion was more common in patients with low RF levels: 52/85 patients with low levels versus 33/85 with high levels (p = 0.0006) at 12 months; and 40/67 patients with low levels versus 27/67 with high levels (p < 0.0001) at 24 months. ACPA levels were not significantly associated with the rate of reversion.

Effect of antibody trajectory on disease outcomes. Patients who were ACPA-positive at baseline and 12 months' followup were independently more likely to have erosive disease than patients who were always ACPA-negative (OR 3.86, 95% CI 1.68, 8.92; Table 5). About RF, there was a lower likelihood of achieving remission (OR 0.59, 95% CI 0.39, 0.87) on univariate analysis; however, this association was not seen when multiple logistic regression was performed (Table 4). Also, we did not detect an association between ACPA or RF conversion and reversion and erosive disease or remission. With respect to SJC, HAQ, and DAS28 score, there were no statistically significant differences between the antibody trajectory groups at 12 or 24

Table 3. Patient characteristics at followup by RF status.

Characteristic	12 Months							24 Months						
	All	n	RF–	n	RF+	n	p	All	n	RF–	n	RF+	n	p
SJC28, median (range)	1 (0–24)	514	0 (0–24)	244	1 (0–12)	270	NS	0 (0–17)	361	0 (0–17)	174	0 (0–16)	187	NS
TJC28, median (range)	1 (0–25)	514	1 (0–25)	244	1 (0–19)	270	NS	0 (0–23)	361	0 (0–23)	174	0 (0–23)	187	NS
ESR, mean (SD)	14.49 (14.88)	510	11.72 (11.35)	244	17.03 (17.14)	266	< 0.0001	14.98 (14)	367	12.89 (12.69)	178	16.94 (14.9)	189	0.0055
CRP, mean (SD), mg/l	5.24 (7.91)	508	4.21 (6.81)	245	6.19 (8.72)	263	0.0045	5.47 (8.56)	367	4.62 (6.8)	177	6.27 (9.88)	190	NS
Erosions	87 (27)	322	39 (25.7)	152	48 (28.2)	170	NS	66 (26.7)	247	28 (24.1)	116	38 (29)	131	NS
DAS28, mean (SD)	2.79 (1.29)	493	2.63 (1.22)	230	2.94 (1.34)	263	0.0093	2.71 (1.35)	347	2.58 (1.31)	166	2.82 (1.38)	181	NS
DAS28 < 2.6	272 (52.3)	520	145 (58)	250	127 (47)	270	0.0124	221 (58.8)	376	113 (62.1)	182	108 (55.7)	194	NS
HAQ, median (range)	0.25 (0–3)	502	0.25 (0–2.38)	241	0.25 (0–3)	261	NS	0.25 (0–2.5)	355	0.25 (0–2.5)	168	0.25 (0–2.38)	187	NS
RF-positive	270 (51.9)	520	—	—	—	—	—	194 (51.6)	376	—	—	—	—	—
Anti-CCP2-positive	203 (62.3)	326	69 (45.4)	152	134 (77)	174	< 0.0001	150 (60.5)	248	59 (47.6)	124	91 (73.7)	124	< 0.0001
1987 ACR RA criteria	334 (65.4)	511	142 (58.4)	243	192 (71.6)	268	0.0017	242 (64.9)	373	111 (61.3)	181	131 (68.2)	192	NS
2010 ACR/EULAR RA criteria	373 (71.7)	520	160 (64)	250	213 (78.9)	270	0.0002	278 (73.9)	376	123 (67.6)	182	155 (79.9)	194	0.0066
DMARD	481 (93.6)	514	231 (94.7)	244	250 (92.6)	270	NS	347 (93)	373	171 (95)	180	176 (91.2)	193	NS
Biologics	50 (9.7)	514	29 (11.9)	244	21 (7.8)	270	NS	55 (14.8)	373	37 (20.6)	180	18 (9.3)	193	0.0022
Corticosteroids	122 (23.7)	514	56 (23)	244	66 (24.4)	270	NS	94 (25.2)	373	38 (21.1)	180	56 (29)	193	NS

Anti-CCP: anticyclic citrullinated peptide; RF: rheumatoid factor; SJC28: swollen joint count-28; TJC28: tender joint count-28; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; DAS28: Disease Activity Score-28; ACR: American College of Rheumatology; EULAR: European League Against Rheumatism; DMARD: disease-modifying antirheumatic drugs; NS: nonsignificant; HAQ: Health Assessment Questionnaire; RA: rheumatoid arthritis.

Table 4. ACPA and RF trajectory over followup period.

Status	12 Months Followup		24 Months Followup	
	ACPA, n (%)	RF, n (%)	ACPA, n (%)	RF, n (%)
Negative at baseline	123	186	96	136
Negative at followup	110 (89.4)	165 (88.7)	83 (86.4)	115 (84.6)
Conversion at followup	13 (10.6)	21 (11.3)	13 (13.5)	21 (18.3)
Positive at baseline	219	334	181	240
Positive at followup	212 (96.8)	249 (74.6)	166 (91.7)	173 (72.1)
Reversion at followup	7 (3.2)	85 (25.4)	15 (8.3)	67 (27.9)

ACPA: anticitrullinated protein antibodies; RF: rheumatoid factor.

months' followup (data not shown). Baseline antibody status or trajectory over time was not significantly associated with treatment with antirheumatic drugs at baseline or followup in multivariable analysis (data not shown).

Effect of ACPA and RF levels on clinical outcomes. Forty percent of patients had moderate or high levels of RF and 56% had moderate or high levels of ACPA. SJC, HAQ score, and DAS28 score at followup was not significantly different based on antibody level at baseline (data not shown). There was also no significant association between baseline levels of RF and erosions or DAS remission (Table 5). The ACPA moderate/high-positive group had an independent and statistically significant higher risk of erosive disease at 12-month followup (OR 3.4, 95% CI 1.61,

Table 5. Effect of antibody trajectory and level on erosions and DAS remission.

	Univariate Analyses		Multiple Logistic Regression	
	Erosions OR (95% CI)	DAS Remission OR (95% CI)	Erosions[a] OR (95% CI)	DAS Remission[b] OR (95% CI)
Followup 12 months				
Antibody trajectory				
ACPA				
Always negative (n = 110)	1	1	1	1
Conversion (n = 13)	1.37 (0.26, 7.17)	1.21 (0.38, 3.83)	1.83 (0.28, 11.85)	1.22 (0.33, 4.56)
Always positive (n = 212)	2.81 (1.42, 5.56)*	1.21 (0.76, 1.91)	3.86 (1.68, 8.92)*	1.11 (0.65, 1.91)
Reversion (n = 7)	3.64 (0.55, 23.97)	2.59 (0.48, 13.92)	3.24 (0.4, 26.2)	1.53 (0.26, 8.94)
RF				
Always negative (n = 165)	1	1	1	1
Conversion (n = 21)	1.8 (0.4, 8.13)	0.49 (0.19, 1.22)	1.22 (0.16, 9.23)	0.55 (0.2, 1.48)
Always positive (249)	1.15 (0.64, 2.07)	0.59 (0.39, 0.87)*	0.58 (0.26, 1.3)	0.59 (0.37, 0.92)
Reversion (n = 85)	1.09 (0.52, 2.29)	0.73 (0.43, 1.24)	0.74 (0.28, 2.01)	0.77 (0.43, 1.28)
Antibody level				
ACPA				
Negative (n = 123)	1	1	1	1
Low positive (n = 26)	2.03 (0.63, 6.53)	2.29 (0.93, 5.65)	3.14 (0.86, 11.5)	2.06 (0.76, 5.58)
Moderate/high positive (n = 193)	2.84 (1.48, 5.46)†	1.12 (0.71, 1.75)	3.4 (1.61, 7.2)†	1.02 (0.6, 1.73)
RF				
Negative (n = 186)	1	1	1	1
Low positive (n = 122)	0.75 (0.39, 1.46)	0.58 (0.37, 0.92)	0.78 (0.37, 1.66)	0.56 (0.33, 0.95)
Moderate/high positive (n = 212)	1.37 (0.77, 2.46)	0.73 (0.49, 1.09)	1.25 (0.64, 2.44)	0.75 (0.48, 1.17)
	Erosions OR (95% CI)	DAS Remission OR (95% CI)	Erosions[c] OR (95% CI)	DAS Remission[d] OR (95% CI)
Followup 24 months				
Antibody trajectory				
ACPA				
Always negative (n = 83)	1	1	1	1
Conversion (n = 13)	1.76 (0.43, 6.98)	0.66 (0.2, 2.12)	3.39 (0.72, 16.05)	0.47 (0.14, 1.59)
Always positive (n = 166)	1.22 (0.58, 2.54)	1.25 (0.73, 2.14)	1.24 (0.53, 2.92)	1.17 (0.66, 2.09)
Reversion (n = 15)	1.23 (0.21, 7.12)	1.15 (0.38, 3.52)	2.24 (0.34, 14.88)	0.98 (0.3, 3.24)
RF				
Always negative (n = 115)	1	1	1	1
Conversion (n = 21)	1.82 (0.54, 6.2)	0.89 (0.35, 2.28)	3.25 (0.86, 12.22)	0.77 (0.29, 2.08)
Always positive (n = 173)	1.45 (0.71, 2.96)	1.28 (0.68, 2.39)	1.87 (0.85, 4.14)	0.74 (0.44, 1.23)
Reversion (n = 67)	1.38 (0.29, 3.24)	0.83 (0.52, 1.34)	1.78 (0.71, 4.44)	1.18 (0.61, 2.28)
Antibody level				
ACPA				
Negative (n = 123)	1	1	1	1
Low positive (n = 26)	1.15 (0.28, 4.81)	1.51 (0.49, 4.68)	1.43 (0.32, 6.38)	1.12 (0.33, 3.8)
Moderate/high positive (n = 193)	1.64 (0.79, 3.43)	0.91 (0.53, 1.59)	1.98 (0.88, 4.44)	0.76 (0.41, 1.41)
RF				
Negative (n = 186)	1	1	1	1
Low positive (n = 122)	0.88 (0.38, 2.06)	0.64 (0.36, 1.39)	1.14 (0.46, 2.81)	0.59 (0.32, 1.1)
Moderate/high positive (n = 212)	1.83 (0.89, 3.77)	0.81 (0.49, 1.32)	2.37 (1.08, 5.22)	0.71 (0.42, 1.2)

Multiple logistic regression model included the following variables associated with the outcome in univariate analyses with $p < 0.1$: [a] baseline erosions, CRP (baseline ACPA positivity for analyses of RF trajectory and levels); [b] age, sex, smoking, baseline DAS28, HAQ, SJC; [c] baseline erosions, DAS28; [d] age, baseline DAS28, HAQ. ACPA: anticitrullinated protein antibodies; RF: rheumatoid factor; CRP: C-reactive protein; DAS28: Disease Activity Score-28; HAQ: Health Assessment Questionnaire 28 score; SJC: swollen joint count. Bonferroni correction for multiple comparisons was formed. * $p < 0.0083$; † $p < 0.017$.

7.2). This increased risk was not detected at 24 months. ACPA levels at baseline did not appear to predict DAS remission (Table 5).

DISCUSSION

RF and ACPA are the most common clinically used RA-associated antibodies. It is unclear whether repeated

measurements of these antibodies throughout the course of disease are useful in the management of patients with early inflammatory arthritis. Therefore, we investigated fluctuations of RF and ACPA in a prospective inception cohort of EIA and determined whether changes in antibody status predicted disease outcomes.

Our data revealed significant fluctuations in antibody status over a median followup period of 21 months: 13.5% of patients who were initially ACPA-negative became positive by 24 months' followup. Conversion to positive status occurred despite significant improvements in disease activity and function. Reversion rate was lower at 8.3%. The rate of RF fluctuations was very high (18% conversion and 28% reversion at 24 mo). Other studies of EIA or early RA have shown lower rates of ACPA conversion of 2% to 6%[16,17,18,19,20]. ACPA and RF status in established RA have also been reported to be stable[6]. However, with respect to RF, our findings are consistent with recent studies that also show high rates of fluctuations for RF (13%–35%)[18,19,20]. We found that reversion to negative antibody status appeared to occur more frequently with low RF levels, but not ACPA. Also, half of the ACPA and RF converters had moderate to high levels. Since ACPA has a very high specificity for RA diagnosis, the significant conversion rate in our study suggests a potential diagnostic role for repeating antibody testing in patients with undifferentiated arthritis.

Many studies have investigated RF and ACPA as predictors of disease severity in RA. The majority of studies have shown that baseline ACPA is an independent predictor of erosive disease in both early and established RA at 1 to 10 years' followup[15,17,21,22,23,24]. These support our finding that persistent ACPA positivity is independently associated with erosive disease. RF has also been shown to predict erosions[25,26], but this may not be an independent effect[21,22,23] except in patients with high RF titer[27]. We did not find that RF positivity even at higher levels was independently associated with erosive disease.

Studies investigating the prognostic utility of RF and ACPA for other disease outcomes have yielded conflicting results. Van der Linden, *et al* found that RF and ACPA-positive patients were less likely to achieve remission at 7 years' followup[25]. Farragher, *et al* found that the presence of ACPA, but not RF, was associated with higher SJC, DAS score, and HAQ score[22]. However, other studies found that the association was only with high-titer ACPA, was lost at longer followup times, or was present only in RF-negative patients[17,24,28]. Wagner, *et al* showed that ACPA did not predict unfavorable outcomes, but that RF did[29]. Ursum, *et al* and van den Broek, *et al* found similar DAS28 scores, rates of DAS remission, and physical function in ACPA-positive versus ACPA-negative patients[19,30].

We also showed that RF and ACPA positivity or conversion from negative to positive did not predict worse DAS28 and HAQ scores or remission rates. Our results are consistent with the findings from the Norfolk Arthritis Register[18]. In another Canadian study, Guzian, *et al* also found that fluctuations in ACPA and RF were not predictive of erosive disease[16]. However, another anti-citrullinated peptide antibody (anti-Sa) was associated with higher rates of erosive disease. Other studies have also suggested that anti-modified citrullinated vimentin (anti-MCV) is better than ACPA at prognostication in RA[23]. Unfortunately, anti-Sa and anti-MCV were not measured in the CATCH cohort. Although the study by Guzian, *et al* investigated a Canadian early arthritis cohort, none of the patients included in that study were enrolled in CATCH.

The lack of association between RF positivity and worse disease outcomes may be partly explained by the higher DAS scores of RF-negative patients at baseline. These patients were more likely to be treated with biologic agents at followup, but outcomes in RF-positive and RF-negative patients remained similar after accounting for this difference. Conversely, ACPA patients did not have worse disease activity at baseline. In addition, ACPA-positive patients were not treated more aggressively with disease-modifying agents even though physicians in this study were not blinded to antibody status. ACPA-negative patients were more likely to require corticosteroids at followup compared to ACPA-positive patients. It is possible that antibody-negative patients are less responsive to methotrexate and biologic therapies[31,32,33], which may contribute to our findings.

The disparate findings from our study compared to other studies of early inflammatory arthritis may be secondary to differences in population and study design. For example, the followup time in our study was shorter than others and RA-associated antibodies may be predictive of worse outcomes only later in the disease course[28]. Other baseline features, such as disease duration at inclusion, sex, smoking status, and disease activity, were similar across all studies[16,18,23]. However, different proportions of patients with undifferentiated arthritis and slight differences in treatment strategies in these studies may also account for differing results. The strengths of our study include the use of a large, prospective cohort of patients with EIA, including those with undifferentiated arthritis. This study used a "real-world" cohort reflecting actual practice, in which patients with inflammatory arthritis were investigated and treated based on physician preferences from both tertiary care and community settings. It is the first North American multicenter study determining whether fluctuations in RA and ACPA predict various disease outcomes. Our study has several limitations: ACPA testing is not uniformly covered by the public healthcare system in Canada; therefore, it was not regularly tested in all centers. However, there were no significant differences in baseline demographics and disease activity between the included and excluded populations

(data not shown). The sample size in some of the trajectory groups was relatively small. Radiographs were read locally and data on modified Sharp scores were not collected. Although the laboratory tests were not standardized centrally, the same tests were used to measure baseline and followup RF and ACPA in each patient. Also, there was no difference in results between the 2 anti-CCP2 kits used (data not shown). Nonetheless these findings need to be corroborated in other cohort studies.

We found that fluctuations in RF and ACPA up to 24 months' followup are common. Since ACPA is highly specific for RA, repeat testing at followup in patients with undifferentiated arthritis may be beneficial. Patients who remained positive for ACPA at 12 months' followup were more likely to have erosive disease. However, there is no consensus on the prognostic utility of ACPA and RF in early RA for other outcome measures. In our study, these antibodies did not predict DAS28 scores, HAQ scores, or DAS remission. Further studies are needed to identify other prognostic markers in EIA.

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