Prognosis of Seronegative Patients in a Large Prospective Cohort of Patients with Early Inflammatory Arthritis

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ABSTRACT. ***Objective.*** Rheumatoid factor (RF) and anticitrullinated protein antibodies (ACPA) are believed to be associated with more severe rheumatoid arthritis; however, studies in early inflammatory arthritis (EIA) have yielded conflicting results. Our study determined the prognosis of baseline ACPA-negative and RF-negative patients.

Methods. Patients enrolled in the Canadian Early Arthritis Cohort had IgM RF and IgG anticyclic citrullinated peptide antibodies 2 (anti-CCP2) measured at baseline. Remission was defined as a Disease Activity Score of 28 joints (DAS28) < 2.6 using logistic regression accounting for confounders at 12-month and 24-month followup.

Results. Of the 841 patients, 216 (26%) were negative for both RF and anti-CCP2. Compared to seropositive subjects, seronegative subjects were older (57 ± 15 vs 51 ± 14 yrs), more males proportionately (31% vs 23%), and had shorter length of symptoms (166 ± 87 vs 192 ± 98 days), and at baseline had higher mean swollen joint count (SJC; 8.8 ± 6.8 vs 6.5 ± 5.6), DAS28 (5.0 ± 1.6 vs 4.8 ± 1.5), and erosive disease (32% vs 24%, $p < 0.05$). Treatment was similar between the 2 groups. At 24-month followup, seronegative compared to seropositive subjects had greater mean change (Δ ± SD) in disease activity measures: ΔSJC counts (–6.9 ± 7.0 vs –5.1 ± 5.9), ΔDAS28 (–2.4 ± 2.0 vs –1.8 ± 1.8), and ΔC-reactive protein (–11.0 ± 17.9 vs –6.4 ± 17.5, $p < 0.05$). Accounting for confounders, antibody status was not significantly associated with remission. However, at 12-month followup, ACPA-positive subjects were independently more likely to have new erosive disease (OR 2.94, 95% CI 1.45–5.94).

Conclusion. Although seronegative subjects with EIA have higher baseline DAS28 compared to seropositive subjects, they have a good response to treatment and are less likely to develop erosive disease during followup. (First Release Oct 1 2014; J Rheumatol 2014;41:2361–9; doi:10.3899/jrheum.140082)

Key Indexing Terms:
RHEUMATOID ARTHRITIS
RHEUMATOID FACTOR
ANTICITRULLINATED PROTEIN ANTIBODIES
PROGNOSIS

Rheumatoid factor (RF) and anticitrullinated protein antibodies (ACPA) are the most common antibodies associated with rheumatoid arthritis (RA). In some individuals, these antibodies are present many years prior to

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Dr. Barra has received honoraria from Hoffmann-La Roche Ltd., Amgen Canada Inc., AbbVie, and United Chemicals of Belgium (UCB). The CATCH study was designed and implemented by the investigators and financially supported initially by Amgen Canada Inc. and Pfizer Canada Inc. through an unrestricted research grant since inception of CATCH. As of 2011, further support was provided by Hoffmann-La Roche Ltd., UCB Canada Inc., Bristol-Myers Squibb Canada Co., Abbott Laboratories Ltd., and Janssen Biotech Inc. (a wholly owned subsidiary of Johnson & Johnson Inc.).

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Accepted for publication August 27, 2014.

disease onset[1], but in early disease, rates of antibody expression have been reported as low as 50%[2]. Seroconversion from negative to positive is uncommon, occurring in less than 15% at up to 30-month followup[3,4,5,6]. Even when testing for multiple types of ACPA, about 25% of patients with RA remain seronegative[7]. Less is known about the clinical presentation and outcomes of seronegative RA, and studies are challenging given that seronegative RA is harder to classify and may consist of a heterogeneous population. Nevertheless, seronegative RA is thought to represent a separate entity with different pathogenesis and less severe disease.

Evidence supports disparate disease mechanisms in seropositive versus seronegative RA: (1) seropositive RA has higher hereditability than seronegative RA[8]; (2) seropositive, but not seronegative RA, is strongly associated with HLA-DRB1 shared epitope alleles[9]; (3) genome-wide association studies have shown disparate genetic associations for ACPA-positive versus ACPA-negative RA[10,11]; and (4) the strongest environmental risk factor for RA, cigarette smoking, is significantly associated only with seropositive RA[12].

Clinically, numerous studies of early inflammatory arthritis (EIA) have shown that seropositive patients, particularly ACPA-positive patients, are more likely to have progressive erosive disease[13,14,15,16]. Associations between the presence of antibodies and other disease outcomes, such as disease activity scores and physical function, are less clear[13,14,15,16,17,18,19,20]. In our study, we investigated a population of EIA, including early RA and unclassified inflammatory arthritis, to determine the prognosis of seronegative patients.

MATERIALS AND METHODS

Study population. Subjects were from the Canadian Early Arthritis Cohort (CATCH) study, a multicenter, observational, prospective cohort of patients with EIA with ongoing recruitment and followup. Subjects included in this article were recruited from July 2007 to July 2012. Inclusion criteria 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 anticyclic citrullinated peptide 2 (anti-CCP2), morning stiffness > 45 min, response to nonsteroidal antiinflammatory drugs, or painful metatarsophalangeal squeeze test. Subjects with undifferentiated inflammatory arthritis (UIA) and early RA (ERA) were included in the study. Patients are followed every 3 months by a rheumatologist using a standard protocol. Treatment is based on physician discretion. Patients are withdrawn from the study if they are diagnosed by the treating rheumatologist with another rheumatologic condition other than UIA or RA, including osteoarthritis, psoriatic arthritis (PsA), ankylosing spondylitis, systemic lupus erythematosus and other connective tissue diseases, crystal arthropathies, or infectious arthritis. The study was approved by the research ethics boards of all the centers involved, and consent was obtained according to the Declaration of Helsinki.

RA-associated antibody assays. IgM RF was measured, but methods used were not standardized across centers. In 9 of 21 CATCH sites, ACPA was consistently measured (i.e., ACPA was not used selectively based on patient characteristics). At these sites, greater than 90% of enrolled subjects had ACPA measured at baseline. In the remaining sites, less than 25% of enrolled subjects had ACPA measured at baseline. In total, 59% of the cohort had ACPA values measured at baseline (Figure 1). However, 85% of the subjects included in the analysis were from sites that consistently measured ACPA. Depending on the site, 2 different anti-CCP2 IgG (CCP2) assays were used (Euroimmune and Inova). There were no differences in the results based on type of anti-CCP2 assay used (data not shown).

Study variables and outcomes. The following demographic information was included: age, sex, and smoking status (ever smoker defined as current or previous smoker vs never smoker). Time of RA onset was defined as patient-reported initiation of symptoms that were persistent (> 6 weeks). Disease activity was determined using the Disease Activity Score of 28 joints (DAS28) and remission was defined as a DAS28 < 2.6. Patient function was evaluated using the Health Assessment Questionnaire (HAQ). Swollen (SJC) and tender joint count (TJC) was for 28 joints. Inflammatory markers included erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). Seronegative subjects were defined as those negative for both ACPA and RF (ACPA–RF–); all other subjects were defined as seropositive. The following subgroups were also analyzed: ACPA-positive and RF-negative (ACPA+RF–), ACPA-negative and RF-positive (ACPA–RF+), and ACPA-positive and RF-positive (ACPA+RF+).

Erosions were determined using plain radiographs of the hands and feet performed at baseline, 6, and 12 months, and then annually as reported by the local radiologist and/or reviewed by the treating rheumatologist. Erosive disease (binary outcome) was defined as the presence of any erosion. New erosive disease (binary outcome) was defined as the presence of any erosion in a subject who did not have erosion(s) at baseline.

Followup for our study was at 12 and 24 months, and subjects who withdrew from the study were not included in analyses (Figure 1). Reasons for withdrawal included loss to followup, non-ERA/UIA diagnosis, withdrawal of consent, poor understanding of French or English, a comorbidity that precluded frequent followup, or death. There were less than 15% missing data for all variables except radiographic data, which were available in > 75% of subjects with available ACPA and RF (Figure 1). Missing data were not included in the analyses. There were no significant differences in subjects with or without radiographic data at baseline or followup (data not shown). Results were not affected when multiple imputations using Markov Chain Monte Carlo method was performed for the missing antibody, radiographic, and DAS28 values.

Statistical analyses. All statistical analyses were performed using SAS software, version 9.3 (SAS Institute). Categorical data were compared using the Fisher's exact test. Continuous variables were reported as means (SD), and seronegative versus seropositive values were compared using the Student t test ($p < 0.05$ significant). For analyses with the 3 seropositive antibody groups, ANOVA with Bonferroni correction for multiple comparisons was performed (p values were considered significant if < 0.0167).

To determine whether antibody category at baseline was significantly associated with remission and new erosive disease at 12-month and 24-month followup, stepwise forward selection logistic regression was performed. The following baseline variables were tested: sex, age, smoking history, presence of erosions, DAS28 score, HAQ score, SJC, ESR, CRP, and treatment with disease-modifying antirheumatic drugs (DMARD) and/or biologic agents. Variables were included in the model if $p < 0.1$. Results were reported as an OR (95% CI).

Stepwise forward selection multiple linear regression was conducted to determine whether baseline antibody category was associated with DAS28 score at the 12-month and 24-month followup. Variables were included in the model if they were significantly associated with followup DAS28 score ($p < 0.1$). Standardized β coefficients of the included variables were reported with p values, as well as the total R^2.

RESULTS

Seropositive patients do not have more severe disease at baseline. There were 216 seronegative patients (26% ACPA–RF–); 105 were ACPA+RF– (12%), 144 were

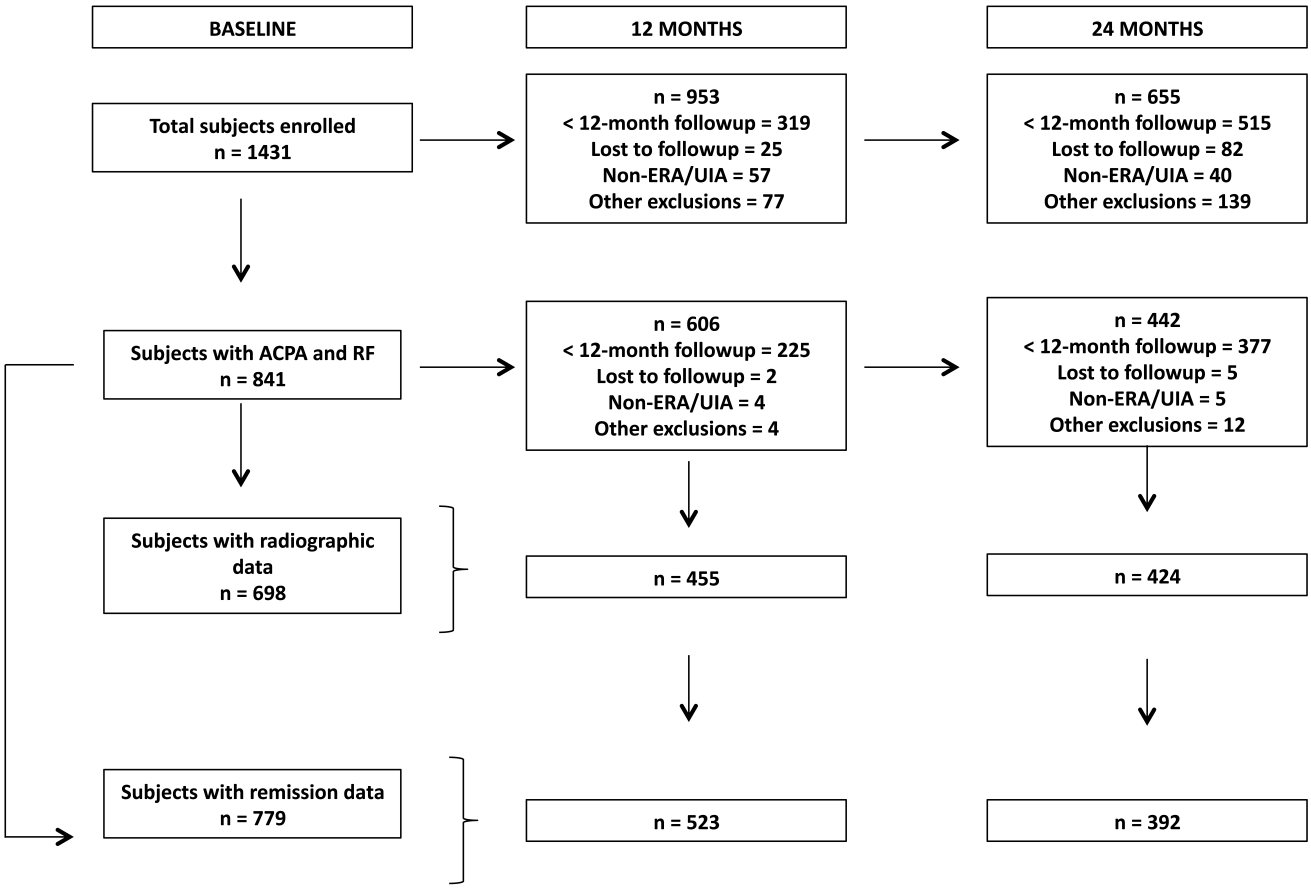

Figure 1. Study sample sizes at baseline, 12, and 24 months. Values are n. ACPA: anticitrullinated protein antibody; RF: rheumatoid factor; ERA: early rheumatoid arthritis (meeting the 1987 ACR or 2010 ACR/EULAR criteria for RA); UIA: undifferentiated inflammatory arthritis (not meeting ACR criteria for any rheumatic condition); ACR: American College of Rheumatology; EULAR: European League Against Rheumatism.

ACPA–RF+ (17%), and 376 were ACPA+RF+ (45%). Baseline characteristics of subjects by antibody status are shown in Table 1 and Table 2. Seronegative patients were older (mean age 57 ± 15 yrs) and more often male (31%) than seropositive subjects (mean age 51 ± 14 yrs and 23% male; p = 0.0001 and p = 0.0225, respectively). History of smoking trended to be more frequent in seropositive subjects (59% vs 52%, p = 0.055). Elevations in inflammatory markers (ESR and CRP) and functional impairment as measured using the HAQ score were similar in the 2 groups. However, other markers of disease severity were significantly increased in seronegative compared to seropositive patients: 28 SJC of 8.8 ± 6.8 versus 6.5 ± 5.6, p < 0.0001; 28 TJC of 9.3 ± 7.2 versus 7.1 ± 6.0, p < 0.0001; DAS28 score of 5.0 ± 1.6 versus 4.8 ± 1.5, p = 0.0493; and 32% versus 24% with radiographic erosive disease, p = 0.0335, respectively. There was no significant difference in the type of joints involved (large or small) for the 2 groups. Despite more severe disease at baseline, seronegative patients had shorter disease duration from symptom onset (166 ± 87 days) than did seropositive patients (192 ± 98 days, p = 0.0007). Initiation of DMARD at or prior to the baseline visit was frequent and not significantly different in both groups (86%–88%).

Seronegative subjects met the American College of Rheumatology (ACR) criteria for RA less often; 179/211 of seronegative subjects (84%) met either the 1987 ACR or the 2010 ACR/European League Against Rheumatism (EULAR) criteria for RA versus 573/615 of seropositive subjects (93%; Table 1). The proportion of subjects meeting the 1987 ACR criteria were 71% and 61% for the seropositive and seronegative groups, respectively (p = 0.007). For the 2010 ACR/EULAR classification criteria, which relies more heavily on antibody status, 86% of seropositive patients met the criteria versus 66% of seronegative subjects (p < 0.0001). Limiting analyses to patients with early RA (meeting the 2010 ACR or 1987 criteria for RA) did not significantly change results (data not shown). However, seronegative subjects meeting the criteria for RA had significantly higher SJC, TJC, ESR, CRP, and DAS28 at baseline compared to seronegative subjects not meeting criteria (Appendix 2).

Characteristics of seropositive patient subgroups are

Table 1. Baseline characteristics of seronegative and seropositive subjects with early inflammatory arthritis. Values are n (%) unless otherwise specified.

Characteristics	Seronegative, n = 216	n	Seropositive, n = 625	n	p
Age, yrs, mean (SD)	57 (15)	216	51 (14)	624	< 0.0001
Male	67 (31)	216	145 (23)	625	0.0225
Symptom duration, days, mean (SD)	166 (87)	216	192 (98)	625	0.0007
Ever smoker	111 (52)	215	369 (59)	624	0.055
SJC28, mean (SD)	8.8 (6.8)	211	6.5 (5.6)	611	< 0.0001
TJC28, mean (SD)	9.3 (7.2)	211	7.1 (6.0)	611	< 0.0001
ESR, mean (SD)	24.9 (22.0)	211	27.3 (22.6)	597	0.6513
CRP, mg/l, mean (SD)	13.8 (18.2)	205	13.4 (16.9)	601	0.7864
Erosions	58 (32)	181	124 (24)	517	0.0335
DAS28, mean (SD)	5.0 (1.6)	203	4.8 (1.5)	576	0.0493
HAQ, mean (SD)	0.65 (0.65)	199	0.59 (0.64)	605	0.2741
1987 ACR RA criteria	128 (61)	211	432 (71)	611	0.007
2010 ACR/ EULAR RA criteria	139 (66)	211	529 (86)	615	< 0.0001
1987 ACR or 2010 ACR/EULAR RA criteria	179 (84)	211	573 (93)	615	0.0007
DMARD	178 (86)	207	534 (88)	607	0.4567
Biologics	5 (2)	207	18 (3)	607	0.8112
Corticosteroids	120 (58)	207	310 (51)	607	0.0859

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

Table 2. Baseline characteristics of ACPA and RF subgroups. Values are n (%) unless otherwise specified. Bonferroni correction for multiple comparisons performed. P value significant if < 0.0167.

Characteristics	Seropositive						
	ACPA+RF–, n = 105	n	ACPA–RF+, n = 144	n	ACPA+RF+, n = 376	n	p
Age, yrs, mean (SD)	47 (14)*	104	55 (13)	144	51 (13)	376	< 0.0001
Males	26 (25)	105	30 (21)	144	89 (24)	376	0.9873
Symptom duration, days, mean (SD)	196 (95)	105	176 (98)	144	202 (108)	376	0.0606
Ever smoker	55 (52.9)	104	80 (55.6)	144	234 (62.2)	376	0.0523
SJC28, mean (SD)	6.5 (6.3)	104	7.1 (5.9)	138	6.2 (5.3)	369	0.3105
TJC28, mean (SD)	8.1 (6.4)	104	6.3 (6.2)	138	7.1 (5.9)	369	0.0776
ESR, mean (SD)	21.8 (19.2)	101	27.7 (24.1)	137	28.6 (22.7)	359	0.0271
CRP, mg/l, mean (SD)	12.3 (17.9)	102	13.3 (16.3)	139	13.8 (17.2)	360	0.7485
Erosions	22 (24.2)	91	28 (22.8)	123	74 (24.4)	303	0.8777
DAS28, mean (SD)	4.6 (1.5)	97	4.7 (1.4)	131	4.8 (1.5)	347	0.5715
HAQ, mean (SD)	0.48 (0.63)	99	0.61 (0.63)	138	0.62 (0.64)	368	0.1488
1987 ACR RA criteria	51 (50)‡	103	81 (58)‡‡	139	300 (81)	369	< 0.0001
2010 ACR/EULAR RA criteria	77 (73)‡	105	120 (83)	144	338 (90)	376	< 0.0001
DMARD	76 (77)‡	99	125 (89)	140	333 (91)	368	0.0005
Biologics	2 (2)	99	5 (3.8)	140	11 (3)	368	0.7466
Corticosteroids	44 (44)	99	86 (61)	140	180 (49)	368	0.8824

*p < 0.0167 for ACPA+RF– versus ACPA–RF+. ‡p < 0.0167 for ACPA+RF– versus ACPA+RF+. ‡‡p < 0.0167 for ACPA–RF+ versus ACPA+RF+. ACPA: anticitrullinated protein antibodies; RF: rheumatoid factor; SJC28: swollen joint count of 28 joints; TJC28: tender joint count of 28 joints; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; DAS28: 28-joint Disease Activity Score; HAQ: Health Assessment Questionnaire; ACR: American College of Rheumatology; RA: rheumatoid arthritis; EULAR: European League Against Rheumatism; DMARD: disease-modifying antirheumatic drug.

shown in Table 2. There were no significant differences in disease activity and severity measures (inflammatory markers, SJC, TJC, DAS28, or HAQ score) between the seropositive groups. However, double-positive patients were more likely to meet the criteria for RA than the single-positive groups (p < 0.0001). The ACPA+RF– group was less frequently treated with DMARD at or prior to the baseline visit (77%) compared to the other 2 seropositive groups (89% and 91%, p = 0.0005).

Patient characteristics at followup. Mean change in disease

severity measures compared to baseline is reported in Table 3. At 12-month followup, seronegative patients had a larger mean decrease in SJC (6.4 ± 6.9) and TJC (6.7 ± 7.4) compared to seropositive patients (4.7 ± 5.5 and 4.9 ± 6.4, p = 0.0017 and p = 0.0104). This difference remained significant at the 24-month followup. Also at 24 months, the mean decrease in CRP was greater for the seronegative (11.0 ± 17.9) than for the seropositive group (6.4 ± 17.5, p = 0.0274), as was the mean decrease in the DAS28 score (2.4 ± 2.0 vs 1.8 ± 1.8, p = 0.0152). The difference in the decrease of disease severity measures between seronegative and seropositive patients was even more substantial when excluding patients not meeting ACR criteria for RA (data not shown). Rates of DMARD use remained high at followup: 92% in seronegative and 94% in seropositive subjects at 12-month followup, and 94% in both groups at 24-month followup. Biologic agents were used in 13% and 18% of seronegative and seropositive patients, respectively, at the 12-month followup; 17% and 21%, respectively, at 24-month followup. There were no significant differences in treatment between the 2 groups.

At the 24-month followup, 93% of all patients met the RA criteria (either 1987 ACR or 2010 ACR/EULAR criteria for RA): 88% in the seronegative group and 95% in seropositive group. Five subjects who did not meet the RA criteria were withdrawn from the study because they were diagnosed with another rheumatic condition (the majority were PsA); the remainder not meeting criteria had undifferentiated inflammatory arthritis at followup. The positive predictive value for RA diagnosis at 12 months in patients positive for either RF or ACPA at baseline was 81%. Results were similar at the 24-month followup. The only other baseline characteristic independently predictive for RA diagnosis in seronegative patients at followup was SJC (OR 1.31, 95% CI 1.11–1.54).

Risk of erosive disease and remission based on baseline RA-associated antibody status. Seronegative subjects were less likely to develop new erosive disease on radiographs at 12 months: 4/43 (9%) versus 44/190 (23%), p = 0.0425. Accounting for potential confounders, ACPA+RF– and ACPA+RF+ patients were significantly more likely to have new erosive disease at 12 months compared to seronegative patients (OR 5.53, 95% CI 1.44–21.20 and OR 3.67, 95% CI 1.13–12.08, respectively; Table 4). Risk of erosions was driven by ACPA status; RF was not independently associated with erosive disease even at high levels [greater than 3 times the upper limit of normal (ULN); data not shown]. At 24 months of followup, there was a trend toward an increased risk of new erosive disease for ACPA-positive subjects, but statistical significance was not reached (Table 5). Baseline antibody status was not significantly associated with remission or DAS28 score at 12- or 24-month followup (Table 4 and Table 5). Associations between antibody status and remission or erosive disease were similar in subjects with high ACPA titers (> 3 × ULN) and in the subgroup of patients meeting criteria for RA (data not shown).

DISCUSSION

We studied a large Canadian prospective cohort of early inflammatory arthritis (EIA) with moderate to severe disease to determine the prognosis of seronegative patients. We also investigated whether there were any differences in disease presentation or outcomes for patients discordant for RF and ACPA positivity. We found that seronegative patients had more severe disease at baseline and were treated similarly to seropositive patients. However, seronegative patients had a better response to treatment and were less likely to have new erosive disease at followup. Differences between seronegative and seropositive patients were driven by ACPA status.

Table 3. Change from baseline in measures of disease severity for seronegative and seropositive subjects at followup.

Measures	Seronegative, Mean (SD)	n	Seropositive, Mean (SD)	n	p
12-mo followup					
SJC28	–6.4 (6.9)	139	–4.7 (5.5)	463	0.0017
TJC28	–6.7 (7.4)	139	–4.9 (6.4)	463	0.0104
ESR	–11.7 (–15.4)	127	–11.5 (–13.6)	405	0.9302
CRP, mg/l	–8.1 (18.9)	118	–8.4 (17.0)	406	0.8392
DAS28	–2.1 (1.9)	118	–1.9 (1.7)	383	0.2649
24-mo followup					
SJC28	–6.9 (7.0)	105	–5.1 (5.9)	334	0.0189
TJC28	–7.1 (8.0)	105	–4.3 (6.6)	334	0.0017
ESR	–12.7 (16.2)	93	–9.5 (21.9)	304	0.1391
CRP, mg/l	–11.0 (17.9)	92	–6.4 (17.5)	308	0.0274
DAS28	–2.4 (2.0)	86	–1.8 (1.8)	291	0.0152

SJC28: swollen joint count of 28 joints; TJC28: tender joint count of 28 joints; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; DAS28: 28-joint Disease Activity Score.

Table 4. Multivariable regression models for new erosive disease or remission at followup. Model for multiple logistic regression included the following variables with $p < 0.1$: baseline antibody group for erosions at 12 months; baseline age, DAS28, HAQ, SJC, CRP for DAS28 remission at 12 months; baseline DAS28 for erosions at 24 months; baseline age, HAQ for DAS28 at 24 months.

Characteristics	ACPA–RF–	n	ACPA+RF–*	n	ACPA–RF+*	n	ACPA+RF+*	n
12 mos								
Erosions	1	43	5.53 (1.44–21.20)	34	1.46 (0.38–5.50)	57	3.67 (1.13–12.08)	99
Remission	1	121	0.84 (0.36–1.94)	64	1.04 (0.51–2.12)	102	0.76 (0.40–1.44)	236
24 mos								
Erosions	1	37	1.21 (0.29–5.05)	25	1.40 (0.44–4.47)	45	1.85 (0.65–5.24)	85
Remission	1	87	0.68 (0.27–1.70)	47	0.83 (0.38–1.80)	79	0.95 (0.49–1.70)	179

*Values are OR (95% CI). DAS28: 28-joint Disease Activity Score; HAQ: Health Assessment Questionnaire; SJC: swollen joint count of 28 joints; CRP: C-reactive protein; ACPA: anticitrullinated protein antibodies; RF: rheumatoid factor.

Table 5. Multiple linear regression model of baseline variables on followup DAS28 scores. Baseline variables: sex, age, smoking history, presence of erosions, DAS28 score, HAQ score, SJC, ESR, CRP, and treatment with DMARD and/or biologic agents. R^2 is the total variance explained in the model. β values are standardized coefficients.

Variables	β	p
12-mo followup		
Seronegative	0.111	0.3811
Baseline HAQ	1.219	< 0.0001
Baseline ESR	0.016	< 0.001
Baseline CRP	–0.009	0.0087
24-mo followup		
Seronegative	–0.121	0.4736
Age	0.014	0.0063
Baseline DAS28	0.243	0.0001
Baseline HAQ	0.896	< 0.0001
Baseline SJC28	–0.045	0.0062
Biologics at baseline	1.162	0.0031
R^2	0.275	—

DAS28: 28-joint Disease Activity Score; HAQ: Health Assessment Questionnaire; SJC28: swollen joint count at 28 joints; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; DMARD: disease-modifying antirheumatic drug.

Our finding that erosive disease was more frequent in seronegative patients at baseline is in contrast with other cohorts of EIA in which seropositivity was associated with erosive disease[13,14,15,16,18,19]. With respect to other measures of disease activity, studies have revealed divergent results. We found higher SJC, TJC, and DAS28 scores in seronegative compared to seropositive patients. These findings suggest that seronegative patients with more severe disease are more frequently referred to rheumatology than seronegative patients with less severe disease, whereas primary care physicians have been shown to refer seropositive patients regardless of disease severity[21].

Other studies have shown either no difference in baseline DAS28, HAQ, and SJC for seropositive versus seronegative subjects[15,16,19,22], or higher levels in seropositive patients[13,14]. Discrepancies in the results may be related to differences in the baseline subject characteristics between studies. In our population, seronegative subjects were significantly older than seropositive patients. Increasing age is known to be a poor prognostic indicator in RA and as expected, in our study, increasing age correlated significantly with higher SJC, TJC, DAS28, HAQ score, and inflammatory markers, and was significantly associated with shorter disease duration. Accounting for differences in age, seronegative subjects continued to have higher SJC, TJC, and shorter disease duration than seropositive subjects at baseline (data not shown).

The CATCH study also differed from other studies in the high rates of DMARD initiation at or just before enrollment (> 85%) regardless of antibody status, reflecting the high disease activity seen in both antibody groups[14,15,22]. In addition, some EIA cohorts had higher rates of UIA (not meeting criteria for RA or other arthritis) than our study, particularly in the seronegative group[13,16,18,22]. UIA can consist of patients with viral, crystal-related, connective tissue disease, or spondyloarthritides, which have different clinical features, and depending on the final diagnosis, may present with milder disease. In CATCH, patients diagnosed with rheumatic conditions other than RA or UIA throughout the course of the study are excluded. In our study, only 7% of all subjects and 12% of seronegative subjects had UIA at followup. Restricting analyses to patients meeting RA criteria did not change results.

Even though the seronegative group had more severe disease at baseline, only 66% met the 2010 ACR/EULAR RA criteria at baseline. However, 83% and 88% met either the 1987 ACR or 2010 ACR/EULAR RA criteria at baseline and followup, respectively, and over 90% remained on DMARD therapy at followup. These findings support the use of both the 1987 ACR and 2010 ACR/EULAR RA criteria, particularly in seronegative patients[23,24].

We found that seronegative patients had a better response to treatment with greater decreases in DAS28, SJC, TJC, and inflammatory markers, which is consistent with prior studies[13,14,15,18]. Better responses to treatment may be explained by the shorter disease duration in seronegative patients. Antibody status was not independently associated

with remission, defined as DAS28 < 2.6. The lack of association between DAS remission and ACPA status has been previously reported[15,16,19,22]. Seropositive patients with RA with > 1 ACPA have been shown to have decreased likelihood of obtaining DAS remission compared to patients expressing only 1 ACPA[18]. In our study, we tested for anti-CCP2 and no other ACPA. In prior studies, the majority of anti-CCP2–positive patients with RA express > 1 ACPA with a mean of 5 different ACPA; therefore, testing for additional ACPA is unlikely to change results[25,26].

It is well established that seropositive patients are at increased risk of erosive disease[13,14,16,18,19,27,28]. We also showed that ACPA, but not RF, was independently associated with new erosive disease with an OR of 2.9. Because erosions were less frequent at baseline in seropositive patients, these patients were experiencing radiographic progression despite treatment with DMARD and biologic agents, whereas new erosive disease was uncommon in seronegative patients (23% vs 9%). This suggests that seropositive patients have a worse response to current management strategies and may require earlier, more aggressive treatment compared to seronegative patients. Alternatively, the seronegative patients may consist of a subgroup with milder stable disease and a subgroup with more severe disease, presenting with early erosions. Another study, however, did not identify subgroups within seronegative subjects[29].

The majority of patients positive for RF are also positive for ACPA and vice versa. We found that 105/841 of patients (12%) positive for ACPA were negative for RF, and 144/841 (17%) positive for RF were negative for ACPA. Little is known about patients discordant for ACPA and RF[13,16]. We found no significant differences in the baseline clinical features and followup rates of remission of patients discordant for ACPA and RF. However, ACPA-positive, RF-negative patients were less likely to be treated with DMARD at study enrollment. Testing for ACPA is not as widely available in Canada as testing for RF. Some patients who had ACPA testing done at time of study enrollment may not have had ACPA testing at the time of treatment decisions that occurred prior to study enrollment. Although treatment was similar for all groups at 12-month followup, ACPA-positive, RF-negative patients had the highest risk of erosive disease, which could be secondary to delays in DMARD initiation.

Our study has some limitations: validated scoring of radiographs was not performed; erosions were determined by experienced musculoskeletal radiologists and reviewed by the treating rheumatologist. Radiographic data were not available in a large proportion of patients, which can introduce bias or reduce generalizability. We found that patients with and without these missing data were not otherwise significantly different.

With respect to ACPA, data was available for about 60% of enrolled subjects; however, > 85% of the subjects included in the analysis were from sites where ACPA was consistently measured, therefore, risk of bias is likely minimal. To test the strength of our results, missing values for antibody status, presence of erosions, and DAS28 scores were replaced using multiple imputations that did not change the results: seronegative subjects had higher disease activity at baseline [DAS28 of 5.03 (SD 1.56) vs 4.86 (SD 1.47), p = 0.001] and a greater improvement of DAS28 at followup [–2.45 (SD 1.9) vs –2.08 (SD 1.82), p = 0.001]. Also, ACPA continued to be associated with erosive disease at 12-month followup (OR 1.57, 95% CI 1.05–2.35 for ACPA+RF–; 2.08, 95% CI 1.47–2.94 for ACPA+RF+; and 1.59, 95% CI 1.04–2.43 for ACPA–RF+ compared to ACPA–RF–), but not with DAS28 remission. Physicians were not blinded to antibody status; however, this is a real-world cohort that best mimics clinical practice and improves generalizability.

We tested for anti-CCP2 and RF, but it is possible that some of the seronegative patients expressed other RA-associated antibodies, such as antihomocitrullinated peptide antibodies (also known as anticarbamylated antibodies). However, based on currently available studies, this likely represents a small proportion (< 15%) of the seronegative population[30,31].

We have shown that patients with seronegative EIA, particularly those meeting criteria for RA, present with severe disease (higher baseline disease activity and more erosions) requiring aggressive management with DMARD. In these patients, the use of both the 1987 ACR and 2010 ACR/EULAR criteria may be warranted. Seronegative patients with RA may be referred to rheumatologists less often when they have a lower disease activity because underrecognition of the disease may occur. Response to treatment was significantly better in seronegative subjects, and they were less likely to have progression of erosive disease, although the majority continued to take DMARD at the 24-month followup. The role of more aggressive treatment of seropositive patients and stepdown therapy in seronegative patients requires further study.

APPENDIX 1.

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APPENDIX 2. Baseline characteristics of seronegative subjects meeting RA criteria compared to those not meeting criteria. Meeting criteria for RA was defined as meeting either the 1987 ACR RA criteria or the 2010 ACR/EULAR RA criteria. Values are n (%) unless otherwise specified.

Characteristics	Seronegative Patients Meeting RA Criteria, n = 179	n	Seronegative Patients Not Meeting RA Criteria, n = 37	n	p
Age, yrs, mean (SD)	58 (14)	179	53 (15)	37	0.1082
Male	53 (30)	179	14 (38)	37	0.3343
Symptom duration, days, mean (SD)	161 (83)	179	196 (102)	37	0.0245
Ever smoker	92 (52)	178	19 (52)	37	1
SJC28, mean (SD)	9.7 (6.7)	179	3.4 (4.3)	32	< 0.0001
TJC28, mean (SD)	10.4 (7.1)	179	3.1 (3.5)	32	< 0.0001
ESR, mean (SD)	26.3 (22.9)	174	18.6 (16.0)	37	0.0176
CRP, mg/l, mean (SD)	15.3 (19.6)	168	7.0 (6.8)	37	0.0119
Erosions	53 (34)	155	5 (19)	26	0.1737
DAS28, mean (SD)	5.2 (1.6)	171	3.6 (1.4)	32	< 0.0001
HAQ, mean (SD)	0.67 (0.66)	164	0.53 (0.56)	35	0.2358
DMARD	154 (87)	177	24 (80)	30	0.3904
Biologics	4 (3)	177	1 (3)	30	0.5467
Corticosteroids	102 (58)	177	18 (60)	30	0.8442

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