Clinical and Serological Analysis of Patients with Positive Anticyclic Citrullinated Peptide Antibodies Referred Through a Rheumatology Central Triage System

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ABSTRACT. ***Objective.*** Anticitrullinated protein antibodies (ACPA) are a highly specific and sensitive biomarker for the diagnosis of rheumatoid arthritis (RA). Some patients who were found to have a positive ACPA test were referred to our Rheumatology Central Triage (CT; Calgary, Alberta, Canada) for assessment by a rheumatologist. The objectives of our study were to determine the clinical accuracy of ACPA in establishing a diagnosis of RA in a real-time clinical setting.

Methods. Cases that met 3 criteria were included in the study: (1) referred to the CT over 3 calendar years (n = 20,389), (2) reason for referral was a positive ACPA test (n = 568), and (3) evaluated by a certified rheumatologist (n = 314). An administrative serological database was used to retrieve specific ACPA results.

Results. Of patients referred through our CT for evaluation of a positive ACPA test, 57.6% received a diagnosis of RA; the remainder had a variety of other diagnoses, some of which might be considered early RA (9%). The predictive values of ACPA for the diagnosis of RA were increased when rheumatoid factor (RF) results were included in the analysis. When definite and possible RA were combined and the prevalence of moderate/high ACPA was compared to all other individuals, the positive and negative predictive values for moderate/high ACPA for RA were 74.3% and 68.4%, respectively.

Conclusion. About 58% of patients with a positive ACPA referred through a triage system for a rheumatologist opinion received a diagnosis of RA at their first visit. RF provides additional useful information to guide the diagnosis and urgency of referral. (First Release Feb 1 2015; J Rheumatol 2015;42:771–7; doi:10.3899/jrheum.141054)

Key Indexing Terms:
ANTICYCLIC CITRULLINATED PEPTIDE ANTIBODIES
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The 1998 discovery that autoantibodies in sera of patients with rheumatoid arthritis (RA) reacted with citrullinated peptides[1] and the widespread availability of the anticitrullinated protein autoantibody (ACPA) immunoassays were remarkable steps forward in differentiating a wide spectrum of rheumatic conditions that present with joint pain. The value of ACPA was highlighted when it was demonstrated that a positive ACPA test was more specific for RA than the rheumatoid factor (RF) test[2]. This eventually led to the inclusion of the ACPA test in the revised RA classification criteria[3,4]. In addition, ACPA was shown to be pathogenic, identified a subset of patients with RA who tended to have worse disease, was associated with a major histocompatibility genetic marker referred to as the shared epitope, and could be linked to environmental accelerators of the disease such as cigarette smoking[5,6]. In addition, ACPA predates the appearance of clinical symptoms of RA[5,7,8], and studies of ACPA in cohorts of established RA have shown that the patients can be classified into 2 subsets: ACPA-positive and ACPA-negative[5,9]. Although a variety of ACPA immunoassays are available, they tend to consistently demonstrate a specificity of > 90% for RA but a lower sensitivity of about 70%[5,6].

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As evidenced above, ACPA have emerged as one of the most important biomarkers in the early and accurate differentiation of RA from other autoantibody-associated rheumatic diseases (AARD) and a wide spectrum of other conditions that can present with features of joint pain and inflammation[5]. The clinical use of ACPA, particularly by general practitioners, has been facilitated by the wide availability of ACPA tests and the awareness of the prognostic and diagnostic value of ACPA. For example, in the Mitogen Advanced Diagnostics Laboratory (MADL; Calgary, Alberta, Canada) during a 5-year audit period, there was almost a 500% increase in the annual ACPA tests ordered; 52% of these tests were ordered by family practitioners and 40% by rheumatologists (unpublished laboratory audit data). This, in turn, has led to requests for rheumatologic consultations based on presenting symptoms of joint pain and a positive ACPA. These and related observations have raised concerns, and in some jurisdictions the ACPA test has been restricted to rheumatologists. If evidence-based medicine is a guide to the practice of medicine, we considered it important to study the diagnoses that were provided by rheumatologists who were referred patient consultations through our Rheumatology Central Triage (CT) system because of a positive ACPA test. Unlike most studies on the sensitivity and specificity of ACPA, our study is a real-time analysis of clinical associations that attended ACPA testing when the diagnosis was uncertain or not clearly established by primary care and other physicians.

MATERIALS AND METHODS

Ethics. Our study was approved by the University of Calgary Conjoint Health Research Ethics Board (Ethics ID# E-24353). Under the terms of this approval, all patient records and information was anonymized and de-identified prior to analysis, precluding the requirement of written informed consent. All clinical investigation was conducted according to the principles expressed in the Declaration of Helsinki.

Patients, selection criteria, demographic, and clinical information. We used an anonymous administrative database to evaluate the utility of autoantibody testing in the context of triage of referrals to the rheumatology service through a Central Referral and Triage (CReATe) service in the Calgary Health Region (Calgary, Alberta, Canada)[10]. This database included the reason that the patient was referred for consultation, such as abnormal laboratory tests, signs or symptoms thought to suggest a rheumatic disease as well as baseline demographic data, working diagnoses, and wait times. Of particular relevance to our study was the identification of patients referred to the rheumatology service because they had a positive ACPA test (ACPA cohort: ACPA-C). All individuals included in the ACPA-C met the following criteria: (1) referred to Rheumatology Central Triage in a sequential 3-year calendar time frame (n = 20,389); (2) reason for referral was a positive ACPA test (n = 568); and (3) evaluated by a certified rheumatologist at one of our regional referral centers in Calgary. Application of these criteria led to a cohort of 314 patients.

After the ACPA-C patient was seen by the consulting rheumatologist, a form was completed and information expressing the appropriateness of triage and final diagnosis was entered into the database. Final diagnoses were categorized as noninflammatory disorders [e.g., fibromyalgia, osteoarthritis (OA), tendonitis, and bursitis] or inflammatory diseases, including AARD, RA, and other systemic inflammatory arthritis. Accordingly, patients were grouped into 4 main diagnostic categories (RA, possible RA, non-RA, and no rheumatic or autoimmune disease; Table 1). The possible RA group was excluded from certain statistical analyses.

A second anonymous database that contained the serological data was extracted from the MADL master database. The 2 anonymous databases (CReATe and MADL), devoid of unique patient identifiers, were merged using an anchored scrambled unique alphanumeric lifetime identifier to maintain confidentiality.

A descriptive analysis of the combined dataset was performed, including the origin and number of referrals and test results, triage categories, wait times, and any delays in patient care. The number of patients referred because of a positive ACPA and their final diagnoses was tabulated. The primary analysis was the ability of an individual positive ACPA test to predict the diagnosis of RA as recorded by the consulting rheumatologist. This was gauged by determining the sensitivity, specificity, positive predictive value, negative predictive value, and likelihood ratios (LR) for each test in relation to the final diagnosis, as well as the diagnostic categories described above. Secondary outcomes included the ability of individual or combined other autoantibody results to predict the working diagnosis and triage category to assess how these results influenced the triage process as it was currently designed. Agreement between the working diagnosis and the final diagnosis was assessed as a marker of referral quality.

ACPA testing. The MADL performs ACPA tests for the Calgary Health Region and the surrounding catchment areas. ACPA was tested by ELISA (QUANTA Lite CCP3, Inova Diagnostics Inc.) with protocols and cutoff values (absorbance units: AU) as recommended by the manufacturer. RF testing was performed by an immunoturbidimetric assay (Roche Integra Cobas 800) at the regional laboratory, Calgary Laboratory Services.

Statistics. The data were evaluated using the Analyse-it software (Version 1.62, Analyse-it Software Ltd.). Fisher's exact test and the Mann-Whitney U test were used to analyze differences between groups and titers, respectively. P values < 0.05 were considered significant. For statistical analysis, patients were grouped into patients with RA and patients without RA. Individuals in whom a diagnosis was not fully established and verified or in whom RA could not be ruled out were excluded from respective analyses.

RESULTS

The clinical spectrum and referral diagnoses of the 20,389 patients referred to the rheumatology service through CT during the 3-year audit period are shown in Figure 1. A total of 11,634 of the referrals (55.8%) were accepted for assignment to a certified rheumatologist, while 3297 referrals (16.2%) were either redirected to another specialty (e.g., orthopedic surgery) or canceled because of insufficient information. There were 5578 (27.4%) who were directly booked to see the rheumatologist on call. The reasons for referral of the 11,634 accepted referrals are shown in Figure 1 and included 4.9% with a positive ACPA test. By comparison, 28.1% were referred for evaluation of an inflammatory arthropathy, 27.7% for arthralgia/myalgia, and 14.7% for assessment of a previously diagnosed autoimmune disease. The final ACPA-C that met the inclusion criteria accompanied by complete clinical data was composed of 314 of the 568 individual patients (55.3%; Figure 1).

The average age of patients in the ACPA-C was 53.9 years (range 18–88, SD 14.4) and 75.5% were women. About 66% of patients were referred from an urban center and 94.3% were referred by a family physician, while the remainder were referred by subspecialist physicians: general

Table 1. Rheumatologist's diagnosis of 314 ACPA-C patients. Values are n (%) unless otherwise specified.

ACPA-C Clinical Diagnosis	All ACPA	Moderate/High ACPA*#	Low ACPA*$	p
All RA, definite and possible RA^	**209 (66.6)**	191 (60.8)	18 (5.7)	< 0.0001
RA	**181 (57.6)**	173 (67.3)	8 (14.0)	< 0.0001
Possible RA	**38 (12.1)**	18 (5.7)	10 (3.2)	NS
Inflammatory arthritis	**15 (4.8)**	7 (2.7)	8 (14.0)	NS
Palindromic arthritis	**13 (4.1)**	11 (4.3)	2 (3.5)	NS
Non-RA	**73 (23.3)**	50 (15.9)	23 (7.3)	NS
Autoimmune diseases	**15 (4.8)**	10 (3.9)	5 (8.8)	NS
Arthralgia	**6 (1.9)**	4 (1.6)	2 (3.5)	NS
Psoriatic arthritis	**1 (0.3)**	1 (0.4)	0 (0)	NS
Ankylosing spondylitis	**1 (0.3)**	1 (0.4)	0 (0)	NS
Osteoarthritis	**29 (9.2)**	22 (8.6)	7 (12.2)	NS
Other rheumatic conditions**	**21 (6.7)**	12 (4.7)	9 (15.8)	NS
No rheumatic or autoimmune disease	**32 (10.2)**	16 (6.2)	16 (28.1)	< 0.0001
All controls^	**105 (33.4)**	66 (21.0)	39 (12.4)	NA
Total	**314 (100)**	257 (81.8)	57 (18.2)	NA

Entire disease cohorts are in bold face. * Moderate/high ACPA > 40 AU, low ACPA 20–39 AU, and normal results < 20 AU. ** Other: fibromyalgia, gout, degenerative disc diseases, polyarthralgia, Raynaud phenomenon, tendonitis. # Percent of total moderate to high ACPA (n = 257). $ Percent of total low ACPA (n = 57). ^ The positive and negative predictive values for moderate/high ACPA for RA were 74.3% and 68.4%, respectively. ACPA: anticitrullinated protein antibodies; ACPA-C: ACPA cohort; RA: rheumatoid arthritis; AU: absorbance units; NS: not significant; NA: not applicable.

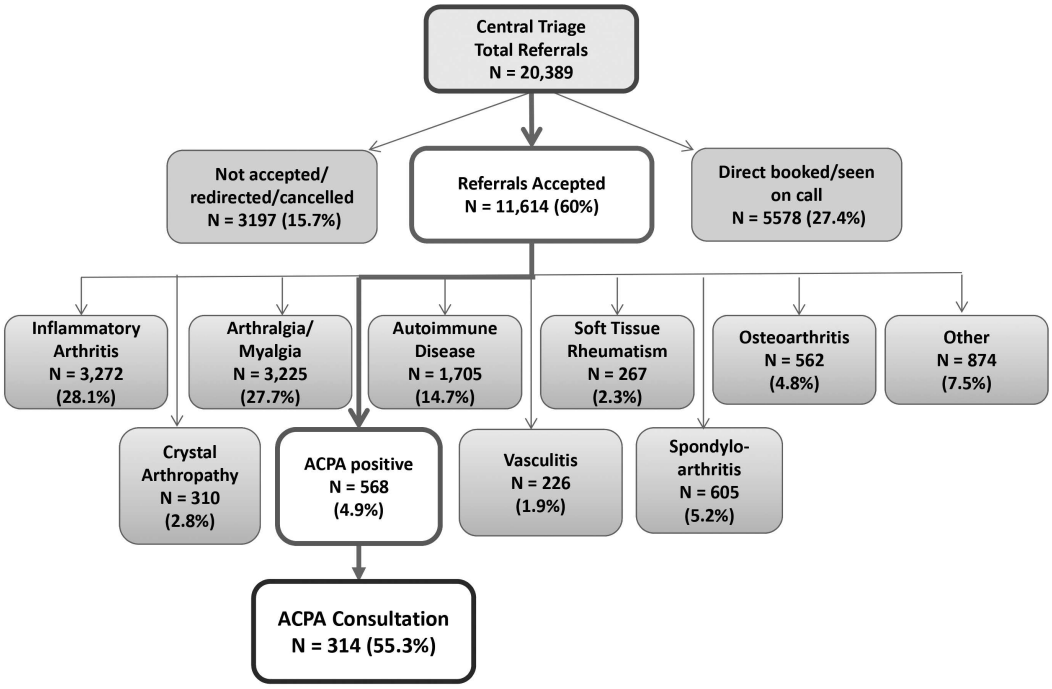

Figure 1. Derivation of the 314 patients in the ACPA-C and the diagnostic profile of 20,389 patients referred to rheumatology central triage in a 3-year audit period. Percent values are based on the group size compared to the superordinate group. ACPA: anticitrullinated protein antibody; ACPA-C: ACPA cohort.

internal medicine (4.1%), orthopedic surgery (0.6%), emergency medicine (0.3%), neurology (0.3%), and gastroenterology (0.3%). The mean interval from date of referral to when the patient was seen by the consultant was 116.8 days (range 4–481 days, SD 237 days). Of the ACPA-C cohort, 257/314 (81.8%) had moderate or high ACPA (> 40 AU) and 57/314 (18.2%) had low ACPA levels (20–39 AU; Table 1). The referring physicians classified 75/314 patients as having RA (23.9%), 225/314 patients as unresolved (71.7%), and 14/314 as not having RA (4.5%).

The consulting rheumatologist's diagnosis of the 314 ACPA-C patients is summarized in Table 1: 57.6% had a

diagnosis of RA (n = 181), followed by an unspecified inflammatory arthritis in 4.8% (n = 15) and palindromic arthritis in 4.1% (n = 13). The remainder had a variety of other diagnoses including 4.8% autoimmune disease (n = 15), 9.2% OA, and a variety of other rheumatic conditions in 6.7%. There were 10.2% who did not have clinical evidence for a rheumatic or autoimmune condition (n = 32). RA was diagnosed in subsets that were high to moderately ACPA-positive and low ACPA-positive, although the frequency of RA in the high-moderate titer group (67.3%) was remarkably higher than in the low-positive group (14.0%, $p < 0.0001$). Conversely, the frequency of a non-RA diagnosis was higher in the low-positive group than in the high-positive ACPA group (Table 1). For example, with particular attention to low-positive ACPA results, 8/57 had a diagnosis of RA (14.6%), 10 had an inflammatory arthritis or palindromic arthritis (17.5%), 7 had OA (12.2%), and 16 had no evidence for a rheumatic or autoimmune disease (28.1%), while the remainder had a variety of other conditions.

Next we combined definite and possible RA and compared the prevalence of moderate to high ACPA positivity against all other individuals. Of the 209 patients with RA, 191 (91.4%) had moderate ACPA and 66/105 had high ACPA (62.9%, $p < 0.0001$). The positive and negative predictive values for moderate/high ACPA for RA were 74.3% and 68.4%, respectively.

When the final diagnosis was compared to the working diagnosis, moderate agreements were observed. The referring physician identified patients with RA with a sensitivity and specificity of 33.7% and 92.5%, respectively. Three-way and 2-way analyses are shown in Table 2.

When the ACPA titers were compared between patients with RA, probable RA, and non-RA, median titers of 83.0, 40.0, and 22.5 units were observed, respectively (Figure 2). The difference of RA versus non-RA ($p < 0.0001$) reached significance, but not RA versus probable RA ($p = 0.24$) or probable RA versus non-RA ($p = 0.12$). When the ability of the ACPA assay to differentiate RA and non-RA was analyzed using receiver-operation characteristic (ROC) curve analysis, a titer-dependent level of discrimination was found (Supplementary Figure 1, available online at jrheum.org). The best descrimination between the 2 groups of our cohort was found at a cutoff of 115 units (based on LR), resulting in a sensitivity of 78.5% (95% CI 71.7–84.2) and a specificity of 71.7% (95% CI 62.1–80.0).

RF test results were available for 233 patients and showed significant quantitative correlation with the ACPA titers (rho = 0.27, 95% CI 0.14–0.38, $p < 0.0001$) and the ACPA titers were higher in RF-positive (median 142.0 units) versus RF-negative patients (median 91.0 units, $p < 0.0001$; Figure 3). When the RF results were analyzed in relation to the diagnosis, high predictive values were observed. The highest OR was found at a cutoff of 11 U/l, reaching an OR of 34. Similar to ACPA, the ability of RF to discriminate the RA from non-RA was titer dependent (Supplementary Figure 2, available online at jrheum.org). Combined ACPA and RF either as double-positive or single-positive at 3× the upper limit of normal did not significantly improve the distinction between the 2 groups.

Table 2A. Comparison of referring physician opinion to consulting rheumatologist's primary diagnosis (3-way analysis).

Referring Physician	Rheumatology Consultant			
	RA	Unresolved	Non-RA	Total
RA	61	2	12	75
Unresolved	114	25	86	225
Non-RA	6	0	8	14
Total	181	27	106	314
Observed agreement	0.299			
Expected agreement	0.673			
κ statistic	0.11			
95% CI, normal approximation	0.07	to 0.15		
SE	0.021			

Table 2B. Comparison of referring physician opinion to consulting rheumatologist's primary diagnosis (2-way analysis).

Referring Physician	Rheumatology Consultant, n = 87		
	RA	Non-RA	Total
RA	61	6	67
Non-RA	12	8	20
Total	73	14	87
Observed agreement	0.793		
Expected agreement	0.683		
κ statistic	0.35		
95% CI, normal approximation	0.11	to 0.58	
SE	0.121		
SE_0	0.105		
Z statistic	3.32		
2-tailed p, normal approximation	0.0009		

RA: rheumatoid arthritis.

DISCUSSION

The primary goal of our study was to determine the clinical utility and accuracy of ACPA in a CT system where patients were referred for a rheumatology consultation. In this sense, our study is unlike most studies that evaluated the sensitivity and specificity of ACPA in established RA cohorts or followed cohorts to determine when in their clinical course they developed RA[2,5,6,7,8]. In further detail, we conducted a real-time analysis of clinical associations that attended ACPA testing when the diagnosis was uncertain or not clearly established by primary care and other physicians. We observed that the majority of patients (58%) referred through our CT system because of a positive ACPA test

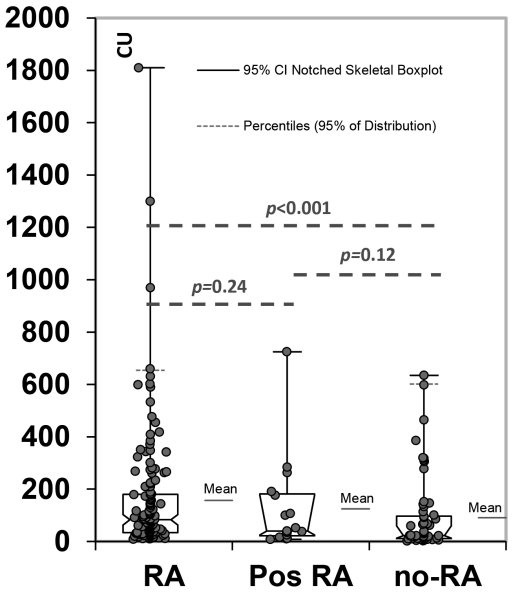

Figure 2. ACPA in patients with RA, possible RA, and non-RA. The median titers of ACPA were 83.0 units in RA, 40.0 units in possible RA, and 22.5 units in non-RA. The difference between RA versus non-RA ($p < 0.0001$), but not RA versus probable RA ($p = 0.24$) or probable RA versus non-RA ($p = 0.12$), reached significance. ACPA: anticitrullinated protein antibody; RA: rheumatoid arthritis.

were diagnosed as RA by a certified rheumatologist at the first visit and another proportion (9%) could conceivably have early RA. Another significant proportion of patients did not have RA, but had another systemic autoimmune rheumatic disease (SARD) while others had conditions that were not typically associated with a positive ACPA. Taken together, the posttest probability of RA using ACPA in this setting was about 60%. However, if the 10% of ACPA-positive patients who were diagnosed with an inflammatory polyarthropathy or palindromic arthritis evolved to have sufficient criteria to fulfill the classification of RA[3,4], the posttest probability would increase to about 70%. Of interest, the posttest probability of 60% is in keeping with published sensitivity of ACPA in patients with early arthritis[11]. In addition, the observation that about 10% of the ACPA-C did not have a rheumatic disease, but had a condition such as OA, which is not normally associated with a positive ACPA, emphasizes the importance of ongoing rheumatologic evaluation of ACPA-positive patients. Certainly, reevaluation of patients in longitudinal followup studies is important because it has been shown that a positive ACPA can predict the onset of RA before fully developed clinical features are present[5,12].

Since about 60% of ACPA-positive patients were diagnosed with RA at the first consultation visit, our data suggest that even if family physicians are suspected of being indiscriminate in their ordering of ACPA tests, they do have some discrimination in those patients who are subsequently referred for a rheumatologist's opinion. Of significant interest and in keeping with previous investigations, the ACPA titer has a significant effect on the positive predictive value and the LR of a patient with RA and therefore should prompt urgent referral with the goal of early, appropriate, and effective therapeutic intervention[13,14]. This relationship between ACPA titers and the probability of RA is now acknowledged in the recent RA classification criteria in that high titer of ACPA or RF contribute 3 points and low titer of ACPA or RF contribute only 2 points to the classification criteria of RA[3]. In contrast, our ROC analyses showed no added value of combining RF and ACPA over the ACPA titers.

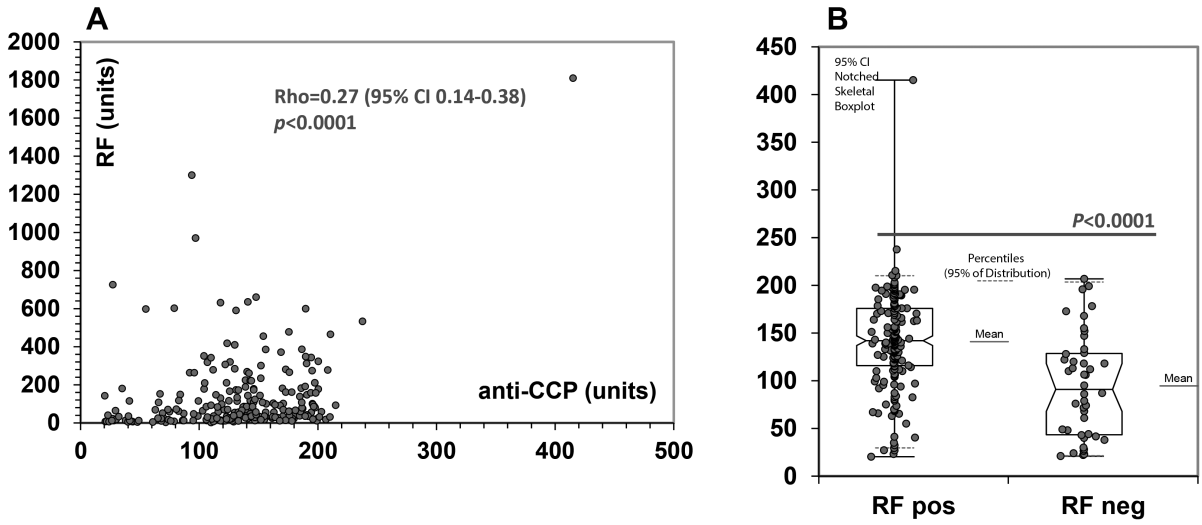

Figure 3. Association of ACPA and RF. (A) The titers of ACPA and RF were significantly correlated according to the Spearman equation. (B) ACPA titers were significantly higher in RF-positive versus RF-negative patients. ACPA: anticitrullinated protein antibody; RF: rheumatoid factor; CCP: cyclic citrullinated peptide.

Distinct and different perspectives of the sensitivity and specificity of disease-specific or disease-related autoantibodies can be quite different in cohorts of patients that are referred with positive autoantibody tests[10]. This has been recently documented in a study by Bossuyt, *et al*[15], where the time-honored sensitivity and specificity of anti-dsDNA and antinucleosome antibodies for systemic lupus erythematosus (SLE) were seen in a different light when the diagnoses of such patients referred through a diagnostic laboratory included patients with inflammatory bowel disease, RA, Sjögren syndrome, and other non-SLE SARD. Intriguingly, in that study, a significant proportion of the antinucleosome-positive and anti-dsDNA–negative patients were being treated with tumor necrosis factor-α inhibitors (i.e., infliximab). In a recent study of patients referred through our CT because of a positive antinuclear antibody test[10], there was a similar mismatch of disease-specific autoantibodies to the final diagnosis, suggesting that real-time clinical application of the sensitivity and specificity of certain autoantibodies may not hold true.

Our study was a cross-sectional study without patient followup to determine the longer term outcome of ACPA-positive patients, especially those who did not have a diagnosis of RA. However, the primary goal of the study was to determine the clinical accuracy of ACPA in a CT setting where patients were referred to a rheumatologist for the first time. Clearly, as discussed, an early and accurate diagnosis of RA is highly desirable and ACPA has already proven its value in predicting disease. A recent study suggested a model to identify patients who progressed from a positive ACPA to clinically diagnosed RA based on clinical features, ultrasonography of joints, and serology (i.e., high positive ACPA)[8].

The utility of ACPA testing in diagnostic algorithms, such as a triage or a referral setting, is complicated by a number of factors. As discussed, most notable is the appreciation that certain autoantibodies often precede symptoms, overt evidence of disease, or sufficient classification criteria[7,8,12]. While the ability of autoantibodies to predict certain diseases is well established in medical literature, many physicians have yet to embrace the concept in the clinical evaluation or counseling of patients. To help understand the use of autoantibodies as predictors of diseases, the ACPA-C is being followed for the evaluation of longterm clinical outcomes.

In addition, our study did not assess the economic effects of ACPA testing in the referral system. The cost-effectiveness of ACPA testing and its effective application to the clinical setting is clearly required[16], and if there were more effective ways to triage patients who will never develop RA or a related SARD, cost savings could be significant. To date, rather than a holistic cost-benefit analysis, cost analyses have been focused on the cost of the autoantibody test itself and related algorithms[17,18]. Of note, it has already been suggested that the detection of anti-DFS70 autoantibodies may result in meaningful cost savings[19] by reducing the number of ancillary tests in search of establishing the diagnosis of an AARD.

With the emergence of additional novel biomarkers for RA, such as antibodies to carbamylated proteins[20], it is thought that disease prevention and morbidity amelioration by establishing an early and accurate diagnosis will likely not rely on a single or any 1 class of biomarker (i.e., autoantibody, genetic, metabolomic), but on multiplexed array analyses including autoantibody, genetic, and metabolomic profiles[21]. Our study suggests that ACPA is an important biomarker in deciding whether an urgent referral is needed. The high posttest probability confirms the appropriateness of APCA testing in a primary care and triage setting.

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ONLINE SUPPLEMENT

Supplementary data for this article are available online at jrheum.org.

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