ACPA Antibodies Titer at the Time of Rheumatoid Arthritis Diagnosis Is Not Associated with Disease Severity

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ABSTRACT **Background:** Rheumatoid arthritis (RA) is a chronic inflammatory and destructive joint disease with the presence of autoantibodies, rheumatoid factor (RF), and anti-citrullinated protein antibodies (ACPA). The presence of RF or ACPA predicts RA severity. Data on the influence of ACPA titer on RA course are limited.

Objectives: To determine the correlation between ACPA titers at the time of RA diagnosis to RA features and severity during 3 years of follow-up.

Methods: We performed a retrospective study of RA patients treated at our institution during the years 2006–2015 with known ACPA titers at RA diagnosis who completed at least 3 years of follow-up. Patients (N=133) were divided according to ACPA titer: seronegative (< 15 U/ml, n=55), weakly positive (15–49 U/ml, n=18), moderately positive (50–300 U/ml, n=29), and strongly positive (> 300 U/ml, n=31). Patient data, including disease activity score (DAS28), bone erosion on hand and/or foot X-rays, treatments with corticosteroids and disease-modifying-anti-rheumatic drugs (DMARDs), and hospitalizations, were recorded. Chi-square and Mann-Whitney method were used for statistical analysis. $P < 0.05$ was considered as statistically significant.

Results: Male gender, smoking, and RF positivity correlated with ACPA positivity and higher ACPA titers. There was no correlation between ACPA titer and the variables defined as representing RA severity: higher DAS28, bone erosions, hospitalizations, need for corticosteroids, and conventional and biological DMARDs.

Conclusions: Titer of ACPA was not identified as a predictive factor for RA severity.

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KEY WORDS: anti-citrullinated protein antibodies (ACPA), ACPA titer, prognostic factors, rheumatoid arthritis (RA), rheumatoid factor (RF)

Rheumatoid arthritis (RA) is a chronic disease with recurrent episodes of arthritis flare, which leads to joint damage, deformities, and patient disability [1,2]. One of the most prominent autoimmune manifestations of RA is the formation of autoantibodies: rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA)/anti-cyclic citrullinated peptide antibodies (anti-CCP) [1].

About 70–80% of patients with RA are RF-positive. Levels of RF correlate with RA activity, severity, extra-articular manifestations, damage, and prognosis [1,3[. The test for ACPA has the same sensitivity as RF (60–80%), but much higher specificity (95%) [4]. Positivity to RF and/or ACPA and their titer levels are included in the classification criteria for RA [4]. Similar to RF, ACPA positivity serves as a prognostic factor in RA. In early undifferentiated arthritis, positive ACPA predicted future RA with positive predictive values about 78–96% [5-8].

There are other factors for predicting the severity of RA, such as the early appearance of bone erosions and high and persistent RA activity, which is measured by a validated disease activity score based on assessment of 28 joints (DAS28) [9,10]. Uncontrolled rheumatoid inflammation inevitably leads to joints destruction and functional limitation, while control of the disease will restore functional capacity and quality of life and will prevent joint damage and disability. Hence, the treatment target is to achieve RA remission defined as DAS28 < 2.6 or low disease activity defined as DAS28 2.6–3.2 [5,11].

Treatment guidelines for RA include the use of corticosteroids, methotrexate, and/or other conventional synthetic disease modifying anti rheumatic drugs (csDMARD). In cases of csDMARD failure or intolerance, treatment with target molecules or biological DMARD (bDMARD) is justified. Early and aggressive treatment should be considered for patients with high RA activity and for patients with risk factors for severe RA, such as positivity for RF/ACPA or early joint damage [11].

Positive ACPA indicates more severe RA [6, 11]. However, correlation among different ACPA titers and clinical characteristics and severity of RA is still not entirely clear [11,12,13].

The purpose of our study was to check the correlation between ACPA titers at the time of RA diagnosis and the disease severity and progression during 3 years of follow-up.

PATIENTS AND METHODS

This retrospective study was based on the data of a cohort of RA patients who attended the Shine Rheumatology Institute at Rambam Health Care Campus during the years 2006–2015. We used the hospital computerized database (Prometheus). Patient files were retrieved according to the World Health Organization's codes (ICD9): rheumatoid arthritis (7140) and rheumatoid arthritis seronegative (71400). The inclusion criteria were as follows:

- For those who were diagnosed during the years 2006–2010: diagnosis of RA according to 1987 Rheumatoid Arthritis Classification Criteria. For those who were diagnosed during the years 2011–2015: diagnosis of RA according to 2010 Rheumatoid Arthritis Classification Criteria
- Age above 18 years
- Available data on laboratory test for ACPA (negative or positive) close to the time of diagnosis (up to 6 months)
- Available data on at least 3 years of follow-up in the rheumatology clinic

Patients with juvenile chronic arthritis, patients with overlap syndrome with other autoimmune diseases, pregnant women, and patients without complete joint assessment at the time of RA diagnosis were excluded.

Patient demographic, clinical, and laboratory data (RF, ACPA, C-reactive protein [CRP]) were recorded. The severity of RA at the time of diagnosis was classified as either moderate disease activity ($3.2 < DAS28–CRP < 5.2$) or high disease activity ($DAS28–CRP \geq 5.2$). The severity of the RA course during the following 3 years was defined by these factors: presence of bone erosions on hand and/or foot X-rays, the need for joint replacement, the need for corticosteroids in moderate or high doses, the number of csDMARDs used, the number of bDMARDs used, and the number of hospitalizations due to RA flares.

Patients with negative ACPA were included in the control group (group A). Patients with positive ACPA were divided into three groups based on their ACPA titer: weakly positive 15–49 U/ml (group B), moderately positive 50–300 U/ml (group C), and strongly positive > 300 U/ml (group D).

STATISTICAL ANALYSIS

Correlation between the ACPA titers and demographic and clinical data (including DAS28 score and parameters of disease severity) was tested using the chi-square method and Mann-Whitney method. Value of $P < 0.05$ was considered as statistically significant. Statistical analyses were performed using IBM Statistical Package for the Social Sciences statistics software (SPSS, IBM Corp, Armonk, NY, USA).

RESULTS

Overall data of 850 patients with RA were retrieved from the computerized database; 133 patients (mean age 55 years, 65% female) met the inclusion criteria and their data were analyzed. The distribution of the different ACPA titer groups was as follows: group A: 55 patients (41%), group B: 18 patients (14%), group C: 29 patients (22%), and group D: 31 patients (23%). Patient demographic, clinical, and laboratory characteristics are shown in Table 1.

Table 1. Demographic, clinical and laboratory characteristics

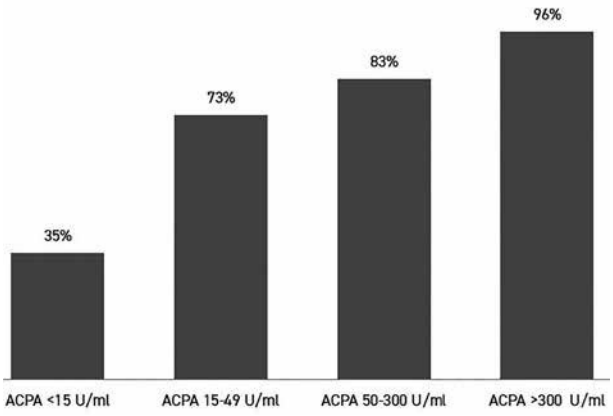
	All Patients	Group A: ACPA <15 U/ml	Group B: ACPA 15–49 U/ml	Group C: ACPA 50–300 U/ml	Group D: ACPA > 300 U/ml
Number of patients	133	55	18	29	31
Age of RA diagnosis [years, median (min, max)]	53 (20–85)	53 (20–83)	50.5 (26–72)	54 (31–85)	54 (25–82)
Gender, female (number of patients, %)	86 (65%)	41(75%)	12 (67%)	18 (62%)	15 (48%)
Ethnicity, Jew (number of patients, %)	70 (53%)	30 (55%)	9 (50%)	9 (31%)	22 (71%)
Smoking/past smoking (number of patients, %)	52 (39%)	16 (29%)	7 (39%)	13 (45%)	16 (52%)
DAS28, median (min, max)	5.4 (2.5–7.9)	5.2 (2.5–7)	5.6 (2.5–7)	5.8 (3.8–7.9)	5.2 (3.4–7)
Patients with DAS28>5.2 (number of patients, %)	44 (54%)	18 (50%)	6 (60%)	11 (58%)	9 (53%)
Patients with at least one cardiovascular risk factor (number of patients, %)	65 (49%)	29 (53%)	6 (33%)	13 (45%)	17 (55%)
RF value, U/ml, median (min, max)	44 (0–1010)	7.8 (0–500)	74 (6.4–409)	94 (0–1010)	107.5 (0–900)
Patients with positive RF (number of patients, %)	71 (64%)	17 (29%)	11 (73%)	20 (83%)	19 (95%)
CRP value, mg/L, median (min, max)	1.8 (0–16.1)	1.7 (0–16.1)	1.1 (0.1–11.6)	2.8 (0.1–11.7)	1.3 (0–7.5)
Patients with positive CRP (number of patients, %)	81 (77%)	35 (78%)	12 (86%)	19 (73%)	15 (75%)
ACPA, U/ml, median (min, max)	27 (0.3–9790)	1.2 (0.3–14.4)	24.4 (17.1–43.9)	121 (50.2–270.4)	479 (300.2–9790)

ACPA = anti-citrullinated protein antibodies, CRP = C-reactive protein, DAS28 = Disease Activity Score 28, RA = rheunatoid arthritis, RF = rheumatoid factor

Most of the characteristics were similar among the groups. The proportion of males was significantly higher in group D (the high ACPA group) compared to the whole study cohort ($P < 0.030$) or to group A (ACPA negative) ($P < 0.015$). The same tendency was seen when patients with moderately high and high ACPA titers (groups C and D) were compared to ACPA negative and ACPA low positive patients (group A and group B, $P < 0.035$). We observed a significant correlation between ACPA titers and positive RF ($P < 0.0001$). Higher ACPA titers were associated with a greater percentage of patients with positive RF [Figure 1]. RF value was not tested for all patients. It was measured for 87% in group A, 83% in group B, 82% in group C, and 77% in group D.

The data on DAS28 were available for 65% patients in group A, 56% in group B, 66% in group C, and 52% in group D. DAS28 was high in all groups without significant differences; over 80% of patients had DAS28 higher than 3.2 and 50-60% had a value higher than 5.2.

Figure 1. Percentage of positive RF in different ACPA titer groups
ACPA = anti-citrullinated protein antibodies, RF = rheumatoid factor



As expected, CRP levels were higher than the standard (> 0.5 mg/L) in most patients; in all groups 73–86% has a positive value defined as CRP > 0.5 mg/L. The data on CRP were available for 82% patients in group A, and 78%, 90%, and 65% patients in groups B, C, and D, respectively.

During the 3-year follow-up, 126 (95%) patients received prednisone with an average daily dose of 14.8 ± 8.9 m), 67 patients (50%) received more than 15 mg prednisone daily. The average number of csDMARD was 2.5 ± 0.73 per patient. Among csDMARD, methotrexate was the most often prescribed: 118 (89%) patients. The average number of bDMARDs per patient was 0.56 ± 0.84. Table 2 shows data on treatments, X-ray findings, and the need for hospitalization according to groups A, B, C, and D.

Data on X-rays for assessment of erosions were available for 67% of patients in group A, 61%, 72%, and 58% of patients in groups B, C, and D, respectively. There were no correlations between negative (group A) or positive ACPA (groups B, C and D) and the variables defined as representing the severity of RA. The percentage of patients with DAS28 > 3.2 ($P = 221$), with DAS28 > 5.2 ($P = 0.914$), patients receiving daily prednisone dose higher than 15 mg/day ($P = 0.563$), patients receiving at least two csDMARDs ($P = 0.952$), patients receiving bDMARD ($P = 0.659$), patients receiving combination therapy of three or more csDMARDs and biological therapy ($P = 0.819$) were not significantly different.

In 87 patients, hand and/or foot X-ray was available. The analysis showed no correlation between ACPA titer and the presence of erosions ($P = 0.781$) during 3 years of follow-up. Finally, there was no correlation between ACPA titers and the number of hospital admissions ($P = 0.929$).

DISCUSSION

In RA, the damage to the joints occurs in the first 2–3 years from the disease onset. There is a narrow window of opportunity for treatment to preserve the joints and thus the patient's quality of

Table 2. Data on treatments and hospitalizations

	All Patients	Group A: ACPA < 15 U/ml	Group B: ACPA 15–49 U/ml	Group C: ACPA 50–300 U/ml	Group D: ACPA >300 U/ml
Number of patients	133	55	18	29	31
Corticosteroids mg/day, median (min, max)	15 (0–55)	15 (0–30)	11.3 (5–40)	10 (0–55)	15 (0–35)
Methotrexate (number of patients, %)	118 (89%)	48 (87%)	15 (83%)	27 (93%)	28 (90%)
csDMARD › 2 (number of patients, %)	121 (91%)	50 (91%)	17 (94%)	26 (90%)	28 (90%)
csDMARD › 3 (number of patients, %)	74 (56%)	28 (51%)	11 (61%)	18 (62%)	17 (55%)
bDMARD (number of patients, %)	49 (37%)	21 (38%)	8 (44%)	8 (28%)	12 (39%)
Erosions on X-ray (number of patients, %)	18 (21%)	7 (19%)	2 (18%)	6 (29%)	3 (17%)
Number of hospitalizations, median (min, max)	2 (0–7)	2 (0–7)	2 (1–6)	2 (1–6)	2 (1–6)

ACPA = anti-citrullinated protein antibodies, bDMARD = biologocal disease modifying antirheumatic drugs, csDMARD = conventional synthetic disease modifying antirheumatic drugs

life and functional capacity. Identifying patients with a high risk for early joint damage is essential in choosing the appropriate treatment strategy. The importance of the ACPA titer in diagnosing RA is reflected in the 2010 Rheumatoid Arthritis Classification Criteria. ACPA positivity was found to correlate with the severity of RA and disease progression [5]. Yet, the significance of ACPA titer in predicting the course of RA and its prognosis has not been clearly assessed [7,8].

We investigated the influence of ACPA titers on clinical manifestations of RA with a focus on risk factors for RA severity. In the patient group, we did not find a correlation between ACPA titers at the time of diagnosis and disease severity in 3 years long-term follow-up, according to defined RA severity criteria.

At the time of RA diagnosis, the DAS28, which represents disease activity, was high in all groups. We hypothesize that the diagnosis of RA occurs in an acute phase of the disease, which is associated with a high DAS28 score regardless of RA severity in the future. Unfortunately, data on DAS28 were not present in all patients, but it was almost equally distributed in all groups.

We did find several differences according to ACPA titers. There was a gender difference. According to our results, male gender is clearly associated with higher ACPA levels, as the proportion of men with ACPA titers elevation was higher. Our cohort was not big enough to determine whether this correlates with RA severity in men.

Our cohort consisted of 39% smokers (present or past). Smoking is one of the factors that activates citrullination of protein that leads to the formation of ACPA antibodies and is a known risk factor for RA [6]. Our data support these facts. The proportion of smokers in the D-high-ACPA-levels-group was 52%, significantly more than in A-ACPA-negative-group ($P < 0.038$).

Finally, we believe that better treatment given in recent years in early stages of RA has contributed to the low number of flairs or hospitalizations and to the low number of patients with erosions on X-ray imaging during 3 years of follow-up. In recent studies, the influence of ACPA status and titers has been shown to be controversial. Some authors reported on positive correlation between ACPA titers and response to treatment, such as Takahashi et al. with regard to bDMARDs [14]. Our data are in agreement with recently published data from a large cohort of patients (N=1826) in a real life setting that showed no correlation between autoantibody titers and response to methotrexate in early RA [15].

LIMITATIONS

Despite the large number of patients in our cohort, the subgroups were still relatively small due to the wide range of ACPA levels, as ACPA titers were very diverse and ranged from negative to thousands (in our definition higher than 300 U/ml). Hence, it was difficult to get significant statistical results. Data on DAS28 were absent in some patients and, in the early 2000s, we did not use the Simple or the Clinical Disease Activity Index that were only introduced in more recent years. However, DAS28 results were very homogenous; therefore, we could suggest that they reflect the whole group situation. Our clinic is a secondary and tertiary center; therefore, it is reasonable that patients with higher RA disease severity or patients with often exacerbations were more represented in our cohort.

CONCLUSIONS

In patients with RA, higher ACPA titers at disease onset are more common in males, smokers, and patients with high RF titers. Despite the fact that ACPA positivity is associated with more severe RA, we did not find a correlation between ACPA titers at early stages of RA and disease activity, progression, or damage. Titers of ACPA did not reflect a need for hospitalizations and treatment escalation. To obtain more strict conclusions, further research with larger groups of patients is warranted.

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Capsule

Coronavirus cross-reactive T cells aid in the fight

There is mounting evidence that immunological memory after infection with seasonal human coronaviruses (hCoVs) contributes to cross-protection against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). **Loyal** and co-authors identified a universal immunodominant coronavirus peptide found within the fusion peptide domain of the coronavirus spike protein. This peptide is recognized by $CD4^+$ T cells in 20% of unexposed individuals, more than 50% of SARS-CoV-2 convalescents, and 97% of patients treated with the Pfizer–BioNTech COVID-19 vaccine. Although ubiquitous, these coronavirus-reactive T cells decreased with age, which may explain in part the increased susceptibility of elderly people to COVID-19.

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Eitan Israeli

Capsule

mRNA-1273 protects against SARS-CoV-2 beta infection in nonhuman primates

B.1.351 is the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variant most resistant to antibody neutralization. **Corbett** and colleagues demonstrated how the dose and number of immunizations influence protection. Nonhuman primates received two doses of 30 or 100 µg of Moderna's mRNA-1273 vaccine, a single immunization of 30 µg, or no vaccine. Two doses of 100 µg of mRNA-1273 induced 50% inhibitory reciprocal serum dilution neutralizing antibody titers against live SARS-CoV-2 p.Asp614Gly and B.1.351 of 3300 and 240, respectively. Higher neutralizing responses against B.1.617.2 were also observed after two doses compared to a single dose. After challenge with B.1.351, there was approximately 4- to 5-$\log_{10}$ reduction of viral subgenomic RNA and low to undetectable replication in bronchoalveolar lavages in the two-dose vaccine groups, with a 1-$\log_{10}$ reduction in nasal swabs in the 100 µg group. These data establish that a two-dose regimen of mRNA-1273 will be critical for providing upper and lower airway protection against major variants of concern.

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Capsule

Environmental allergens trigger type 2 inflammation through ripoptosome activation

Environmental allergens, including fungi, insects, and mites, trigger type 2 immunity; however, the innate sensing mechanisms and initial signaling events remain unclear. **Brusilovsky** and co-authors demonstrated that allergens trigger RIPK1–caspase 8 ripoptosome activation in epithelial cells. The active caspase 8 subsequently engages caspases 3 and 7, which directly mediate intracellular maturation and release of IL-33, a pro-atopy, innate immunity, alarmin cytokine. Mature IL-33 maintained functional interaction with the cognate ST2 receptor and elicited potent pro-atopy inflammatory activity in vitro and in vivo. Inhibiting caspase 8 pharmacologically and deleting murine Il33 and Casp8 each attenuated allergic inflammation in vivo. Clinical data substantiated ripoptosome activation and IL-33 maturation as likely contributors to human allergic inflammation. These findings reveal an epithelial barrier, allergen-sensing mechanism that converges on the ripoptosome as an intracellular molecular signaling platform, triggering type 2 innate immune responses.

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