



Original Research Article

## Diagnostic Value of Specific Auto-Antibody Markers in Albanian Patients with Rheumatoid Arthritis

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### ABSTRACT

**Objective:** The aim of this study was to determine the diagnostic role of anti-cyclic citrullinated peptide antibodies, rheumatoid factor including RF isotypes and antinuclear antibodies in patients with rheumatoid arthritis.

**Methods:** This prospective study, conducted during the time interval from November 2010 to November 2012, included 126 consecutive patients sent from the Rheumatology Clinic of the University Hospital Center of Tirana. In all the RA patients, ACPA, RF screen, RF IgA, IgM, IgG isotypes and ANA were tested using respectively ELISA and indirect immunofluorescence methods.

**Results:** The age of RA patients ranged from 17 to 78 years and 84% of them were females. The prevalence rates of ACPA, RF and ANA were 54.4 %, 44.4 % and 39.7% respectively. 35% of patients resulted positive for both specific serological markers (ACPA and RF). Positive results with two or three RF isotypes detected together were observed in 38.8% of patients. The RF isotype pattern IgM +/ IgA+ was found in 13.5% of patients, whereas RF isotype patterns IgA+/ IgG+ and IgM+/IgG+ have been detected at a rate of 1.6% respectively. The most frequent pattern (IgA+/IgM+/IgG+) RF was in 22.2% of RF screen positive patients.

**Conclusion:** The RF and ACPA positivity rates in the RA Albanian patients were found lower compared to the results reported in other populations. The ACPA positivity resulted higher compared to RF. An IgA RF positivity, combined with IgM RF positivity, are more frequently found than other RF isotypes. The specific RA markers studied, provide an important support for the diagnosis of RA.

**Key-words:** rheumatoid arthritis, rheumatoid factor, anti-citrullinated peptide antibodies, anti-nuclear antibodies

### INTRODUCTION

Rheumatoid arthritis (RA), is the most frequent systemic inflammatory autoimmune disease in humans with an overall prevalence rate that ranges from 0.5% to 1% depending on the population studied. <sup>(1,2)</sup> The aetiopathogenesis of RA is

still unclear and this disease affects more women than men especially in the age from 40 to 60 years old. <sup>(3,4)</sup> RA is characterized by a chronic inflammation of the joints but it can be associated with damage of other organs or systems that can affect significantly the quality of life of patients

and present an important social and economic burden. <sup>(5,6)</sup> According to the recent knowledge, the irreversible lesions in RA begin in the first two years of the onset of symptoms <sup>(7,8)</sup> and for this reason a rapid and effective therapy should be initiated in the first months of the disease. Therefore, an early and accurate diagnosis is an important issue and in this concern the determination of the specific RA disease bio-markers plays an important role in identifying patients with this diagnosis before the beginning of the irreversible joint and bone lesions. <sup>(9)</sup> The criteria for the diagnosis of RA have been based historically on the clinical signs and on the rheumatoid factor (RF) as a specific marker for this disease. <sup>(10)</sup> RF are auto-antibodies directed to the constant region of immunoglobulin G and they are found in 60-90% of patients with RA. <sup>(11,12)</sup> RF of IgM isotype (IGM-RF) has been described as the most frequently detected RF isotype, but it has been also detected in various other conditions, such as in other autoimmune diseases, in different infections, and in up to 5-10% of healthy individuals. <sup>(13-16)</sup> IgG, IgA, IgE, and IgD RF isotypes have also been observed in different rates. <sup>[17]</sup> Different reports have shown that three RF isotypes (IgM, IgA, and IgG) are detected in up to 52% of RA patients but in fewer than 5% of patients with other connective tissue diseases. <sup>(18,19)</sup> Moreover, the presence of IgA-RF and IgG-RF isotypes in absence of IgM-RF has been reported as more prevalent in patients with connective tissue diseases other than RA, whereas positivity in both IgM and IgA RFs is almost exclusively observed in patients with RA and this combination is a strong indicator of RA. <sup>(20-22)</sup>

The discovery at the recent years of the autoantibodies to the citrullinated peptide (ACPA) has added a new marker with a significant value for the diagnosis and prognosis of RA. <sup>(23)</sup> However, the data

concerning this auto-antibody, like those about the rheumatoid factor, have shown different sensitivity results for the disease which depends also mostly on the genetic background of the populations studied. <sup>(24-27)</sup> Taking these facts into account we conducted a study in a population of Albanian patients with established RA diagnosis aiming at the determination of the positivity frequency rates of RF, ACPA and anti-nuclear antibodies (ANA). Secondly, we wanted to investigate the diagnostic value of the different isotypes (IgA, IgM and IgG) of RF in Albanian patients with RA.

## **MATERIALS AND METHODS**

The study was conducted during the time interval November 2010 to November 2012 and it included 126 consecutive patients with RA who were sent in our laboratory for auto-antibody testing from the Rheumatology Clinic of the University Hospital of Tirana. The RA diagnosis was established as following the 1987 ACR/EULAR Rheumatoid Arthritis Classification Criteria. <sup>(10)</sup>

The specific autoantibodies studied among RA patients ANA testing was carried out using an indirect immunofluorescence assay, using a Hep-2 kit (Orgentec Diagnostika GmbH, Mainz, Germany) and following the manufacturer recommendations. The results are reported as fluorescence intensity evaluated with a qualitative scale of values from + to ++++. Serum ACPA levels were measured using a CCP IgG ELISA method as following the manufacturer's instructions (Anti-CCP high sensitive, Orgentec Diagnostika GmbH, Mainz, Germany). This kit uses mutated citrullinated vimentin as antigen. <sup>(28,29)</sup> RF in U/ml (anti Fc IgG antibodies of IgM, IgG and IgA isotypes) was also measured using an ELISA method (RF screen, Orgentec Diagnostika GmbH, Mainz, Germany) The cut-off values for both ACPA, RF screen

and RF isotypes have been determined as following manufacturer's instructions ( anti-CCP IgG < 20 U/ml; RF screen < 25 U/ml; RF IgA, IgM, IgG < 20 U/ml ).

### Statistics

Age as a continuous variable, was presented in mean and standard deviation. ACPA and RF antibodies, as categorical variables, were presented in absolute numbers and percentages. Hi-square test was used to analyse the differences between categorical data. SPSS 19.0(Statistical Package for Social Science version19.0) was used to analyze data. P value  $\leq 0.05$  was considered as significant.

## RESULTS

A total of 126 patients have been studied, 106 were females and 20 males. The mean age of our patients was  $51.05 \pm 10.59$  years. Out of 126 RA patients, 68 (54.4 %) were ACPA positive, 56 (44.4 %) RF screen positive and 50 (39.7 %) resulted ANA positive. 22 (17.5 %) of all RA patients were positive for the three serological markers (ACPA, ANA, RF screen) concomitantly, while 44 (35%) of them only for both ACPA and RF antibodies. The isolated ANA positivity, was observed in 12.7 % (16) of RA patient (table 1).

Table 1. Age, gender and ACPA, RF and ANA seropositivity rates in RA patients .

Basic Characteristics	Number ( % ) of 126 RA patients
Age in years (mean $\pm$ SD*)	51.05 $\pm$ 10.59
Percentage of female patients	106 (84.0)
ACPA positive	68 (54.4)
RF positive	56 (44.4)
ANA positive	50 (39.7)
ACPA+ and RF+	44 (35.0)
ANA+ and RF+ and ACPA+	22 (17.5)
ANA+ and RF- and ACPA-	16 (12.7)

\*SD, standard deviation

As we expected, the concomitant RF screen and ACPA positivity showed a significant association ( P = 0.001) , but these tests also had independent reactivity in a significant subset of

patients: 34.3% (24) RF screen negative patients showed reactivity to ACPA and 21.4% (12) ACPA negative patients showed reactivity to RF screen (table 2).

Table 2. Relationship between RF and ACPA results among RA patients

RF and ACPA combinations	All N	ACPA (+) N (%)	ACPA (-) N (%)
All RA patients	126	68 (53.9)	58 (46.1)
All RF positive*	56	44 (78.6)	12 (21.4)
All RF negative	70	24 (34.3)	46 (65.7)

\*(Hi-square test=24.5, df=1, p<0.001)

RF isotypes were determined in all RA patients with RF screen positive. The distribution of RF isotypes among the RF screen positive RA patients, showed that the largest subset was IgA+/IgG+/IgM+ (n=28), followed by IgA+/IgM+/IgG- (n=17), IgA-/IgM+/IgG+(n=2), IgA+/IgM-/IgG+ (n=2), IgA+/IgM-/IgG- (n=2), IgA-/IgM-/IgG+ (n=1), IgA-/IgM+/IgG- (n=1). Positive results for two or three RF isotypes were observed in the great majority (38.8%) of patients while 22.2% of them were positive for all three RF isotypes. A combined positivity of IgM-RF + IgA-RF was detected in 13.5% of patients, while the pattern IgA+/IgG+ (1.6%) and IgM+/IgG+ (1.6%) were much lower (table 3).

Table 3. The prevalence rates of single or combined RF isotypes in RF screen positive RA patients

Rheumatoid factor pattern positivity	RA patients N = 126	%
IgM RF only	1	0.8%
IgG RF only	1	0.8%
IgA RF only	2	1.6%
IgM + IgA+	17	13.5%
IgM + IgG+	2	1.6%
IgG + IgA+	2	1.6%
IgM + IgA + IgG+	28	22.2%
(Two or three) RF isotypes	49	38.8%

Among the 56 RA patients with RF screen positivity, three of them resulted negative when tested for the RF isotypes and these patients had low titers of RF screen antibodies.

## DISCUSSION

Rheumatoid arthritis is a chronic disease in which it is clearly proven that the earlier the diagnosis and treatment, the better the prognosis. For many years RF was the only serological marker available for the supportive diagnosis of RA and the introduction of ACPA testing has made possible an earlier RA diagnosis. <sup>(30,31)</sup> As far as concerns the ANA testing, various studies have shown that ANA positivity ranges from 30-50% for RA. <sup>(32,33)</sup> In our study the ANA positivity rate was 39.7 % which is very similar with these studies. ANA testing is highly positive in many systemic rheumatic diseases, so this test cannot be considered reliable for the diagnosis of RA. In our RA patients the RF and ACPA positivity have been found in relatively lower rate compared to the results reported in RA patients studied in Western and Northern European populations where the ACPA positivity have been reported with a rate ranging from 64 to 89% and RF in a range of 59% to 79%. <sup>(30, 34-36)</sup> These different ACPA and RF positivity rates among RA patients of different populations are probably to be attributed not only to methodological issues, but at a significant extent also to the genetic background diversity of these populations. <sup>(37,38)</sup> An important finding in our series is the ACPA positivity rate of 34.3% among RF seronegative RA patients. It is clear that the ACPA testing has significantly increased the sensitivity of RA diagnosis in RF negative patients and in this context we can confirm that ACPA serves as a very good diagnostic marker for this subgroup of RA. These data are of interest and should be taken into consideration because in many laboratories only RF is tested for the routine RA diagnosis. Nevertheless, RF is still the most widely used biomarker of RA and this biomarker must be tested in concomitance with ACPA since we have found a RF

positivity of 21.4 % among the ACPA negative RA patients and these 2 biomarkers were found in 35 % of RA patients. The most frequently found RF isotype is IgM but IgG-RF and IgA-RF are also present in the serum of patients with RA and they may provide additional diagnostic information. In our RA patients, we found that the combination of three (IgM, IgG and IgA) RF isotypes (22.2%) was more present compared to the pattern of two RF isotypes or to the single RF isotype. The combinations of two (IgM + IgA) RF isotypes was clearly more frequently found, while the patterns (IgA+ IgG+) RF and (IgM+ /IgG+) RF were detected in much lower frequency rate. These findings agree with those of another study which reported a positivity of both IgM and IgA RF isotypes in most of RF positive RA patients. <sup>(39)</sup> In agreement with other reports <sup>(20-22,40,41)</sup> we found that IgM+/IgA+ isotypes RF were more frequently found together than the dual IgG+/IgA+ or IgG+/IgM+ RF isotype positivity. In conclusion, we can confirm that although with a lower sensitivity than in other Western or Northern European populations, ACPA in conjunction with RF testing provide a very valuable support for the diagnosis of RA.

*Conflicts of interest:* None declared.

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