# The diagnostic value of anti-citrullinated peptide antibodies in the Albanian patients with rheumatoid arthritis

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## Abstract

**Aim:** Anti-citrullinated peptide antibodies (ACPA) represent a valid marker for rheumatoid arthritis (RA). The aim of this study was to evaluate the diagnostic parameters of ACPA versus the rheumatoid factor (RF) among Albanian patients with RA.
**Methods:** This prospective study was conducted from November 2010 to November 2012. Serum samples analyses were performed in patients with RA (N=126), patients with other non-RA systemic rheumatic disorders (NRA-SRD; N=78), as well as in normal control individuals (N=105). Both ACPA and RF were measured through an ELISA method in both patient group and the control group
**Results:** ACPA positivity was detected in 54% and RF in 44.4% of the patients with RA. The diagnostic specificity related to the normal control group was 96.2% for ACPA and 86.7% for RF. In relation to the NRA-SRD patients, the specificity was 84.6% for ACPA and 32.1% for RF. The presence of both RF and ACPA positivity decreased the sensitivity for the diagnosis of RA up to 35%, but provided a substantial increase in specificity (100%).
**Conclusions:** In our RA patients, the ACPA positivity resulted significantly higher compared to RF. The detection of ACPA provides also a higher specificity than RF for the RA diagnosis. The ACPA testing provides a better marker in order to differentiate RA from NRA-SRD. We also showed that the ACPA and RF prevalence in the Albanian RA patients is lower than in the Western European populations and this finding is probably related to the different immunogenetic background of the studied populations.

**Keywords:** autoantibodies against citrullinated peptides, negative predictive value, non-RA systemic rheumatic disorders, positive predictive value, rheumatoid arthritis, rheumatoid factor.

## Introduction

Rheumatoid arthritis (RA) is a common chronic autoimmune rheumatic disease leading to disability and substantial economic costs (1,2). In order to improve the overall outcome and to prevent irreversible joint damages, early diagnosis and therapy are crucial. However, the initial clinical signs of RA are often non-characteristic and rather resembling undifferentiated arthritis or other diseases (3,4).

The discovery at the recent years of the anti-citrullinated peptide antibodies (ACPA) has added a new disease marker with a significant value for the early diagnosis and prognosis prediction of RA (5,6). ACPA seem to play a role in the pathogenesis of RA and they are sensitive early markers of the disease severity (7,8). However, the studies concerning the ACPA diagnostic parameters for RA, similar to those about the rheumatoid factor (RF), have shown varying results in different populations and it seems evident that these results are related to the genetic background of the populations studied (9-12). Taking these facts into account, it becomes important to define the ACPA diagnostic parameters in a specific population and also to compare these parameters with those of other RA auto-antibody markers. For this reason, we conducted a study in a population of Albanian patients with established RA diagnosis, intending to evaluate the ACPA diagnostic parameters and to compare them with the respective RF data in this population.

## Methods

### *Patients and controls*

In this study, there were included 309 individuals. Of these, 105 were healthy blood donors, who constituted the normal control group. The diseased group included 204 consecutive patients who were sent to our laboratory for serologic testing from the Rheumatology Department, from 2010 to 2012. Overall, 126 patients were with established RA diagnosis and 78 patients with other non-RA systemic rheumatic disorders (NRA-SRD). The main diagnoses in the NRA-SRD group were systemic lupus erythematosus (N=37), scleroderma (N=19), mixed connective tissue disease (N=14), dermatomyositis (N=6) and systemic vasculitis (N=2). The RA and NRA-SRD diagnoses were established by the attending rheumatologists applying the ACR classification criteria (13,14). The presence of RA clinical data, including their specific auto-antibodies, was checked during the entire disease course in all patients' files.

### *ACPA and RF auto-antibody study*

Serum levels of ACPA were measured using an ACPA 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 (15,16). RF auto-antibodies (anti Fc IgG antibodies of IgM, IgG and IgA isotypes) were also measured using an ELISA method (RF screen, Orgentec Diagnostika GmbH, Mainz, Germany). The results were expressed in arbitrary units (U/ml). The cut-off values for both ACPA and RF have been determined as following the respective manufacturer's instructions (anti-CCP IgG <20 U/ml; RF screen <25 U/ml).

### *Statistical analysis*

For statistical analysis, the results of ACPA and RF antibodies were recorded as continuous variables and categorical data. The diagnostic parameters such as sensitivity, specificity and positive/negative predictive values (PPV and NPV) for each assay were determined with respect to both NRA-SRD and the normal control group. Statistical analysis was carried out using the SPSS software, version 18.

## Results

The serum testing of 126 RA patients showed 68 positive ACPA and 56 positive RF results, reflecting a diagnostic sensitivity of 54.0% for ACPA and 44.4% for RF. The testing of 78 NRA-SRD patients led to 12 (15.4%) positive ACPA results and 53 (68%) positive RF results (Table 1).

**Table 1. Age, gender, ACPA and RF seropositivity in the RA patients and in two control groups**

| Patients' characteristics | RA patients | NRA-SRD patients | Normal control group |
|---|---|---|---|
| Mean age mean (SD) | 51.0 (10.6) | 45.8 (11.4) | 42.5 (13.4) |
| female/male (n) | 106/20 | 72/6 | 73/32 |
| ACPA positive (n) (%) | 68 (54%) | 12 (15.4% ) | 4 (3.8%) |
| RF positive (n) (%) | 56 (44.4%) | 53 (68% ) | 14 (13.3% ) |
| ACPA+ and RF+ (n) (%) | 44 (35%) | 0 (0%) | 0 (0%) |
| **Total** | **126** | **78** | **105** |

Therefore, in relation to the NRA-SRD patient group, the diagnostic specificity of ACPA for RA was 84.6% and that of RF was 34.0% (Table 2). The analysis of 105 normal control individuals revealed 4 (3.8%) ACPA and 14 (13.3%) RF positive results, providing an ACPA specificity of 96.2% and a RF specificity of 86.7% for RA, in relation to this control group. The presence of both auto-antibodies (RF and ACPA positivity) decreased the sensitivity for the RA diagnosis up to 35%, but showed a substantial increase in specificity (100%) in comparison to the ACPA specificity alone (Table 2

85.0% and the NPV 53.2%. The same results regarding the normal control group showed an ACPA PPV of 94.4% and a NPV of 63.5%. The RF PPV and NPV values for RA resulted much lower than those of ACPA when we compared them with NRA-SRD patients (51.4% and 26.3%, respectively), or with the normal control group (80.0% and 56.5% respectively).

**Table 2. Diagnostics parameters of ACPA and RF in the Albanian patients with RA, in reference to NRA-SRD patients and the healthy controls**

| Diagnostics parameters relating to NRA-SRD | ACPA | RF Screen | ACPA and RF screen |
|---|---|---|---|
| Sensitivity (%) | 54.0 | 44.4 | 35.0 |
| Specificity (%) | 84.6 | 32.0 | 100 |
| PPV (%) | 85.0 | 51.4 | 100 |
| NPV (%) | 53.23 | 26.3 | 48.75 |
| **Diagnostics parameters relating to normal controls** | | | |
| Sensitivity (%) | 54.0 | 44.4 | 35.0 |
| Specificity (%) | 96.2 | 86.7 | 100 |
| PPV (%) | 94.4 | 80.0 | 100 |
| NPV (%) | 56.2 | 56.5 | 56.2 |

## Discussion

During a long time RF has been used as a unique auto-antibody marker for the confirmation of RA diagnosis. But the specificity of RF for this diagnosis is rather limited, since it is also found in patients with malignancies, in other autoimmune and infectious diseases and to a certain extent also in the healthy population (17-19). The discovery of ACPA as a new and more sensitive and specific RA biomarker, has improved considerably the RA diagnosis and prognosis (20). But, like RF, the studies about the ACPA positivity in RA subjects have provided variable results, depending also on the geographical and genetic backgrounds of the patient populations studied (21,22).

RA patients studied in the Western and Northern European populations such as in Germans, Dutch, Belgian, French or Swedish, the ACPA positivity have been reported with a rate ranging from 64% to 89%, whereas RF positivity in a range of 59% to 79% (23-26). In our study, RF and ACPA positivity data have been found in a relatively lower rate in comparison to these results.

Using ELISA kits of the same manufacturer, we found in our RA patients an ACPA positivity rate of 54.0%, compared to that of 69.5% reported among RA patients of a German study (27). Similar data to our results have been reported in some other RA studies conducted in Southern European and Mediterranean populations. For example, in an Italian study the ACPA positivity has been reported in 49% of RA patients (25). In another report from a RA Greek population, ACPA sensitivity was found at a level of 59.2% (26). 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 (28). For example, the lower RA predisposing DRB1*04 allele frequency rates reported in the South-Eastern European populations in comparison to the Northern and Western European populations, and the reverse correlation between the predisposing DRB1*04 allele frequencies and the protective DRB1*11 allele frequencies reported in the European populations, could influence the ACPA and RF auto-antibody production in RA (29,30). We compared the ACPA specificity for the diagnosis of RA in relation to both the NRA-SRD patients and also to the normal control group. In the NRA-SRD patient group, the RF positivity was more frequently encountered, while ACPA displayed a much lower positivity and with lower titers. These results in our RA patients confirm other studies reporting that ACPA are valid serological markers for differentiating RA from NRA-SRD related polyarthropathy (31).

Our findings confirm that in our Albanian RA patients, similar to the studies conducted in other populations, ACPA are superior to RF for the establishment of RA diagnosis. This serological marker becomes more important when we need to differentiate RA from undifferentiated arthritis or from arthritis in the context of other systemic rheumatic diseases. In these situations, the ACPA positivity can be a warning sign for the later development of RA (32-35). The combination of both biomarkers (RF and ACPA together positive) provide an important diagnostic aid for the RA diagnosis, because they possess a specificity of 100% and thus they are very helpful in the differential diagnosis of RA from undifferentiated arthritis or other NRA-SRD.

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 in the Albanian population.

**Conflicts of interest**: None declared.

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