Rheumatoid Arthritis Serology in Macedonian Patients with Rheumatoid Arthritis: Rheumatoid Factor, Anti-Cyclic Citrulinated Peptide Antibodies or both?

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Abstract: *Introduction*: In the last 70 years, Rheumatoid Factor (RF) was considered the most useful laboratory marker in patients with rheumatoid arthritis (RA). The next very important milestone for the RA diagnosis was the discovery of anti-citrullinated peptide/protein antibodies (ACPA). The detection of ACPA is usually done with the CCP test of the second generation, CCP2.

Objective: To evaluate the performances of RF and CCP2 tests and to see whether or not the performance of both tests together is better then the performances of either of the tests alone.

Materials and Methods: We performed a cross-sectional study with 380 participants of which 155 RA patients who fulfilled the American College of Rheumatology (ACR) 1987 classification criteria for RA, 120 patients with inflammatory and other connective tissue diseases (Non-RA) and 105 healthy controls (HC), at the Rheumatology Clinic in Skopje, Macedonia. The patients and controls were tested for RF and ACPA.

Results: The mean age of the RA patients was 50 years, and 87 % of the patients were female. The average duration of the disease has been 6 years. In this group of patients, CCP2 test showed identical sensitivity of 0,69 and better specificity than RF (0,95 vs. 0,87 respectively). For the patients who tested positive for both tests, the sensitivity was lower 0,60 and for the patients who were either ACPA or RF positive, sensitivity was slightly higher 0,73. The specificity of 0,88 for both or either of the tests was identical with that of RF. The positive likelihood ratio was 13,8 for the CCP2 test, 5,3 for RF and 5,1 for both tests done together. Ninety four out of 155 RA patients were positive for both ACPA and RF, 20 patients were positive for either one of the antibodies of which 7 (4,5%) RA patients were only anti-CCP positive and 13 (8,3%) patients were only RF positive.

Conclusion: The results from our study showed that the sensitivity and specificity of both tests, done in parallel, does not differ much from the sensitivity of either of the tests alone and from the specificity of RF, respectfully. Still, CCP2 test showed the highest specificity and positive likelihood ratio as was expected. The results from our study support the idea that in countries like Macedonia, which can not afford enough CCP2 antibody kits, we may use RF first, especially in patients who are not likely to meet any clinical criteria for RA. In patients with early undifferentiated arthritis or early RA we may use both antibodies in order to select the patients who will need more aggressive treatment.

Keywords: Anti-citrullinated peptide antibodies (ACPA), Rheumatoid factor (RF), Rheumatoid arthritis (RA).

INTRODUCTION

Until the introduction of anti-citrullinated peptide/protein antibodies (ACPA), rheumatoid factor (RF) was considered the most useful laboratory marker in patients diagnosed with rheumatoid arthritis (RA). The reported sensitivity of RF for RA is pretty high, in a range from 60% to 90%, but the specificity is relatively low, between 70% and 80%. Patients with other rheumatic and connective tissue disease often have positive results for RF and it may be positive in patients with other inflammatory and infective diseases, as well as in 15% of healthy individuals over 65 years of age [1-3].

The explanation of citrullination and the discovery of ACPA in RA patients, have been the most important milestones for the serological diagnosis of patients with RA in the last 20 years [1].

During that time, the tests for the detection of ACPA have emerged as a sensitive and highly specific markers of RA. Currently, there are three generations of the ACPA tests: CCP1, CCP2, CCP 3.1 [4-6].

The data presented in the most of the studies is based on the second-generation immunoassay CCP2, which has improved sensitivity and equivalent specificity relative to the first-generation assays.

As detailed in several recent reviews and publications, there is general agreement about the excellent diagnostic properties of the CCP2 test with the sensitivity of 75-80 % and the specificity of 97–98 % [2-8]. In most side-by-side comparisons, CCP2 was as sensitive as RF and more specific in patients with established RA [4-7].

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The detection of ACPA has become a very valuable tool in the early diagnosis of RA and the performance of the CCP2 test has been reported to be superior to that of rheumatoid factor (RF), especially in the differential diagnosis of RA with other rheumatic and other connective tissue diseases.

ACPA positivity became the predicting factor of the disease severity and clinical outcome. ACPA positive patients tend to have higher number of swollen joints and more aggressive radiographic progression. They will need more aggressive treatment with disease modifying antirheumatic drugs (DMARDS) during the course of the disease [4, 6-8].

If there were no financial restrictions, it would be ideal to use both tests (CCP2 and RF) in parallel, in order to use the positive characteristics of each of the serology markers in the early and accurate diagnosis of RA which would lead to early aggressive treatment of the disease [9].

However, it is well known that up to 90% of ACPA positive patients are also positive for RF and that the co-occurrence of both antibodies was not more specific for RA than occurrence of either antibody alone [4].

The aim of this study is to investigate whether the performance of both tests in parallel is better than the performance of either of the tests alone in patients with RA, who are diagnosed and treated in Macedonia. Macedonia is a very small eastern European country, which finds itself in a very long economical transition, with very limited resources for the diagnosis and treatment of RA.

MATERIALS AND METHODS

We performed a cross-sectional study with 380 participants: 155 RA patients who fulfilled ACR classification criteria from 1987 [10], 120 patients with inflammatory arthritides and other connective tissue diseases (Non-RA) and 105 healthy controls (HC). The patients were randomly selected at the Outpatient Clinic at the Rheumatology Clinic in Skopje, Macedonia. The have signed an informed written consent to be included in this study. The blood samples were taken at the Outpatient Clinic. The sera were stored at −80 °C for no longer than 3 months.

IgM RF was measured by Latex test (Biosystems, Spain) and a level above 30 IU/ml was considered positive, as suggested by the manufacturer. ACPA were measured by Enzyme Linked Immunosorbent Assay CCP2 test (Axis Shields Diagnostics, Dundee, UK) and considered positive above a cut off value of 5 arbitrary units, as suggested by the manufacturer.

SPSS statistical software was used for statistical analysis (SPSS Inc, Chicago, IL). A p value ≤ 0.05 was considered significant. We calculated sensitivity, specificity, positive and negative likelihood ratios for each of the tests and for both tests done in parallel.

RESULTS

The mean age of the RA patients was 50 +/-13,9 years, and 87 % of the patients were female. The average duration of RA was 6 years and ranged from 3 months to 30 years. Thirty out of 155 (19,3%) of the RA patients had early RA with the disease duration of less then one year. The percentage of the early RA patients who were ACPA positive was almost identical with the percentage of patients with established RA who were ACPA positive (65 vs. 66 %), which is why they were included in the patients group. In the Non-RA group, the majority of patients (65 out of 120 i.e. 54%) had Systemic Lupus Erythemathosus (SLE).

The results regarding the presence of anti-CCP antibodies and RF in RA patients and controls are shown in Table **1**.

Table 1: ACPA and RF in RA Patients, Non-RA and Healthy Controls

	RA	Non-RA	Healthy
ACPA +	108	31	1
ACPA -	47	89	104
Total	155	120	105
RF +	107	42	6
RF -	48	78	99
Total	155	120	105

Legend: RA = Rheumatoid arthritis, Non-RA = Patients with other inflammatory and connective tissue diseases, ACPA = Anti- citrullinated peptide/protein antibodies, RF= Rheumatoid Factor.

The CCP2 test showed identical sensitivity of 0,69 and better specificity than RF (0,95 vs. 0,87 respectively). The specificity of CCP2 was 0,99 (95% CI=0,95-0,99) for the HC and 0,91 (95% CI= 0,8-0,95) for the Non-RA patients. RF overall specificity was 0,87. More precisely, RF specificity was 0,80 (95% CI 0,72-0,87) for the HC and 0,94 (95% CI 0,87-0,97) for the Non-RA patients.

The sensitivity was highest when either one of the tests was positive, while it was lowest when both tests were positive. The specificity did not change much, when both test were done, and it was almost identical with the specificity of RF Table **2**.

Table 2: Sensitivity and Specificity of ACPA and RF in RA Patients vs. Non-RA + Healthy Controls: Separately and in Combination

	Sensitivity	Specificity
ACPA positive	0,69 (CI ,61-0,76)	0,95 (CI 0,91-0,97)
RF positive	0,69 (CI 0,61-0,76)	0,87 (CI0,81-0,91)
ACPA or RF positive	0,73 (CI 0,65-0,80)	0,87 (CI 0,81- 0,91)
ACPA and RF positive	0,60 (CI 0,52-0,68)	0,88 (CI 0,81-0,91)

Legend: RA = Rheumatoid arthritis, Non-RA = Patients with other inflammatory and connective tissue diseases, ACPA = Anti-citrullinated peptide/protein antibodies, RF= Rheumatoid Factor.
CI=95% Confidence Interval, PPV=Positive Predictive Value, NPV=Negative Predictive Valu.

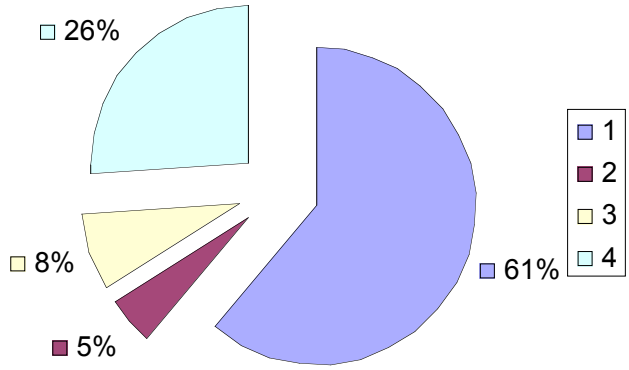
The positive and negative likelihood ratios (LLR) are shown in Table **3**. It can be seen that the positive likelihood ratio of the CCP 2 test was highest, which shows larger and often conclusive increase in the likelihood of RA. The positive LLR for RF, RF or CCP2 and RF and CCP 2 were moderate Table **3**.

Table 3: Positive and negative Likelihood Ratios of ACPA and RF

	ACPA positive	RF positive	ACPA or RF positive	ACPA and RF positive
LLR +	13,8	5,3	5,6	5,1
LLR -	0,32	0,35	0,3	0,45

Legend ACPA = Anti-citrullinated peptide/protein antibodies, RF= Rheumatoid Factor, LLR= Likelihood Ratio.

Ninety four out of 155 RA patients were positive for both ACPA and RF, 20 patients were positive for either one of the antibodies: 7 (4,5%) RA patients were only ACPA positive and 13 (8,3%) patients were only RF positive. The percentages are shown in Graph **1**. These results point out to the fact that CCP2 test has an important diagnostic value in RF negative patients and vice versa.



Graph 1: RA patients serology.

Legend: RA= Rheumatoid Arthritis, 1=Double positive, 2= ACPA positive, 3= Rheumatoid Factor positive, 4=Double negative.

DISCUSSION

We performed a cross-sectional study at the University Rheumatology Clinic in Skopje, Macedonia, in which we have used two tests, RF and CCP2, for the detection of two most important serological markers for the diagnosis of RA, RF and ACPA.

The results from our study are in agreement with the studies of others which showed that the performance of the CCP2 test was superior to that of RF, regarding the specificity [4-7].

However, this does not mean that RF can be replaced with CCP2 in the diagnosis of RA.

For example, out of ACPA negative patients 15% – 20% were RF positive while out of RF negative patients, approximately 10-20% were ACPA positive [7, 8]. These percentages were a little lower in our study. Approximately 30% of RA patients were reported to be negative for both ACPA and RF, which is in agreement with the results of our study [7, 8].

The two tests therefore appear to be complementary, with anti-CCP of particular diagnostic value for the RF negative patients and vice versa.

The combination of a positive ACPA and RF is highly specific for RA (90%-100%) and is associated with an aggressive disease course. Patients with positive ACPA and negative RF results are also likely to have erosive RA. RA is less likely in patients with a positive RF and negative ACPA result, but can not be ruled out and may present with extra-articular manifestations. Negative results on both assays indicate a very low likelihood of RA, but do not exclude

the diagnosis and it is a possibility in 10-20% of the patients [11].

The combination of ACPA and RF appears to provide greater sensitivity than the sensitivity of either assay alone, and is therefore useful in the diagnostic work-up of suspected RA, which is similar to our study [6]. The specificity of the both test together is not much higher than that of the CCP 2, because CCP2 already has very high discriminative abilities. In combination, the two tests appear to be even more powerful with a positive predictive value (PPV) almost a 100%, greater than the PPV of either of the tests alone. In our study, we did not reach that high of a specificity [8, 12, 13].

If we need a cost-benefit approach for early and precise diagnosis of RA, we should refer to the article of Nell and colleagues who have proposed a diagnostic algorithm for autoantibody testing in patients with very early inflammatory arthritis. All patients with early arthritis are first tested for RF. High titer RF (>50 IU/ml) is highly predictive for the diagnosis of RA and for the development of erosive disease, and there is no very important benefit from determining additional auto-antibodies. In patients with low titer (<50 IU/m) or negative RF, ACPA determination helps to identify additional patients with RA who at high risk of developing erosive disease [14].

In the follow up study published in 2010, Nell and coworkers, showed that RF >50 and CCP2 are not interchangeable and can not be substituted by each other. They concluded that it is crucial to use both antibody tests as diagnostic and prognostic tools in the differential diagnosis of very early arthritis and also during follow up, particularly in patients who are negative for these antibodies at baseline [15].

In 2010 the American College of Rheumatology and European League Against Rheumatism published new classification criteria for RA. One of the criteria is serology, which means at least one of test result should be positive, either ACPA or RF, to get some serology points for the diagnosis of RA. Negative RF and negative ACPA score 0 points, low-positive RF or low-positive ACPA score 2 points and high-positive RF or high-positive ACPA score 3 points. The recent addition of ACPA testing in the ACR's updated 2010 RA classification criteria is an acknowledgment of the clinical value of these biomarkers for the diagnosis of RA patients. At the same time it is an acknowledgement of RF, which still can not be replaced by CCP2 test [16].

The results from our study support the idea that in countries like Macedonia, which can not afford enough ACPA antibody kits (sometimes for months), we may use RF first, especially in patients who are not likely to meet any clinical criteria for RA(osteoarthritis, arthralgia etc). However in patients with early undifferentiated arthritis or early RA we may use both antibodies in order to select the patients who will need more aggressive treatment.

CONCLUSION

If the cost of the test is irrelevant, both tests should be used in parallel. The proposed stepwise approach to autoantibody testing in RA may be used in countries in transition where the cost of the tests is very important for the budget of the health organizations.

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