

## Analysis of rheumatoid factor and anti-cyclic citrullinated peptide in individuals with rheumatological disorders attending a tertiary care hospital

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### Abstract

**Introduction:** Rheumatoid factor (RF) has been routinely used as a diagnostic test for rheumatoid arthritis (RA). RA should be diagnosed early to prevent joint injury before the damages become inevitable. Several new laboratory tests have also been used, the commonest of them being detection of antibodies to cyclic citrullinated peptides (anti-CCP or ACPA). For the diagnosis of individuals with rheumatoid arthritis and also for prognosis these two tests are universally performed. Antibodies produced against cyclic citrullinated peptide (ACPA) have a higher specificity with almost equal sensitivity when compared to Rheumatoid Factor. Hence we proceeded to analyse these two parameters in our Immunology laboratory.

**Aim:** To analyse presence of Rheumatoid factor and ACPA in individuals with Rheumatological disorders.

**Materials and Methods:** RF was tested qualitatively by using latex agglutination. Anti-CCP was tested using mono specific ELISA kit. Our study is a retrospective observational one, performed on analysis of the reports of the samples received and tested in the Immunology laboratory of a tertiary care hospital. 331 samples referred for testing both RF and ACPA during October 2018 to December 2018 were considered to analyse the correlation.

**Results:** Out of 331 samples tested “18.1% of them were both RF and Anti-CCP positive, 9.3% were only Anti-CCP positive and RF negative, 5.4% were only RF positive and Anti-CCP negative and 67% were negative for both”.

**Conclusion:** Anti-CCP is better than RF in terms of veracity of the diagnosis, and when both these are combined the diagnostic accuracy of RA is increased.

**Keywords:** Rheumatological disorders, Rheumatoid factor, Anti-CCP.

### Introduction

In patients with Rheumatoid Arthritis, the earliest autoantibody detected was Rheumatoid factor (RF). RF is not very precise to RA, since it detects autoantibodies to IgG and there are multiple causes for false positive RF. About 20% diagnosed with RA will have a normal RF test, while 5% of normal individuals will show an abnormally heightened RF test.<sup>2</sup> Hence a negative test does not necessarily preclude the disease, and positive results do not substantiate the diagnosis either. The significant values of RF taken into consideration in our setup is above 20 U/ml. RF above this level is also seen in a variety of conditions including “other autoimmune diseases, certain chronic infections, diabetes, endocarditis of bacterial origin, malignancies, normal aging process, vaccinations and transfusions”.<sup>1</sup>

Anti-cyclic citrullinated peptide (anti-CCP) is the second most accepted laboratory test which is done when diagnosis of rheumatoid arthritis is contemplated. Positive test is interpreted when the value is above 5RU/ml. False positives do occur in anti CCP testing also but at a lower rate than RF – hence the test is more specific compared to RF for the diagnosis of RA. When positive, anti-CCP remains so in spite of remission.<sup>3</sup> This test is 97% specific for Rheumatoid arthritis if it consistently remains elevated. Once an individual tests positive for ACPA, it generally persists positive, despite remission.

Among those individuals with rheumatoid arthritis (proved clinically and radiologically), some test negative for

both RF and ACPA and such patients are classified and grouped as seronegative Rheumatoid arthritis, which is approximately 20%. The utility of RF and ACPA in constituting the diagnosis of rheumatoid arthritis (RA) varies across different clinical trials done worldwide.<sup>4</sup> We analysed the prevalence of RF and ACPA in patients with Rheumatological disorders. A number of published research works have proposed diagnostic criteria integrating anti-CCP antibodies to improve sensitivity and specificity for diagnosis.<sup>5</sup> This included detection of various serotypes of RF (IgG, IgA and IgM), each by a specific ELISA instead of only IgM antibody which is detected by RF latex agglutination test.

Since, these two antibodies are proved to be positive preceding the beginning of clinical symptoms by a few years, probably screening for healthy individuals without any clinical symptoms but at an increased possibility of progressing into full blown RA at later date, as those with a family history of RA, could call for enhanced observance and surveillance for the possibility of establishing the diagnosis earlier and intervention before destructive joint involvement occurs. Quite a few research works have established the emergence of anti-CCP antibodies prior to the onset of RA.<sup>5</sup> We included symptomatic arthritic patients attending Rheumatology OPD of our tertiary care centre for our studies and excluded those already on treatment and not willing.

Table 1

	RF- Negative / ACPA –Positive	RF- Positive / ACPA- Positive	RF- Positive / ACPA- Negative	RF -Negative / ACPA- Negative
Total	31	60	18	222
%	9.3	18.1	5.4	67

(n=331)

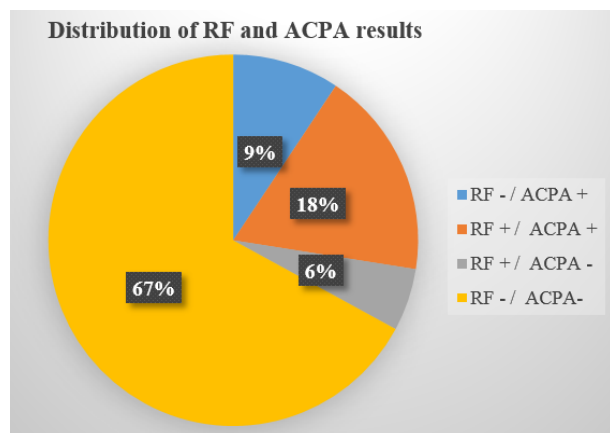


Fig. 1

### Aim

To analyse the Rheumatoid factor and Anti Cyclic citrullinated peptide antibodies (ACPA) in individuals with features suggestive of Rheumatological disorders.

### Materials and Methods

We proceeded with this retrospective observational study with 331 samples that we received in our immunology laboratory for the diagnosis to be established for the Rheumatologic disorders between October 2018 and December 2018. We have performed tests for all the samples from individuals for both RF and ACPA as per the clinicians' request. We did qualitative determination of RF by latex agglutination using RF latex reagent set from Labcare.<sup>6</sup> We used kit instructions for positive control and negative control along with an internal quality control with known samples. We performed tests to detect anti CCP by using mono-specific ELISA using EUROIMMUN kit.<sup>7</sup> We followed the kit instructions to validate the test along with the calibrators and a known internal control. Results were tabulated for analysis.

### Results

As per the kit instructions both the tests were performed meticulously with the serum samples of patients attending Rheumatology OPD with features suggestive of Rheumatologic disorders and the results are: Among the 331 samples that were subjected to necessary laboratory tests as per our study, samples that showed positive results for both RF and ACPA were only 60, samples that tested negative for both RF and ACPA were 222, 31 samples were clearly positive only for ACPA but not for RF and 18 samples of the patients were exclusively positive for RF but not for ACPA. Out of 331 tested 67% of the individuals with rheumatologic disorders had an outcome in laboratory tests

with negative RF as well as ACPA. All these are portrayed in the following Table 1 and Fig. 1.

### Discussion

All patients who attend Rheumatology OPD may not have a specific Rheumatological diseases. Classification criteria allow clinical researchers to novice patients with congruent arthritic expressions (e.g., rheumatoid arthritis or systemic lupus erythematosus) into a diagnostic workup. Response criteria help to resolve whether treatments really work and they actually produce significant clinical amelioration. Classification and Response Criteria have been construed by the American College of Rheumatology (ACR) Subcommittee for the purpose of criteria sets, their development and validation.<sup>8,9</sup> But these criteria must be adopted with caution in developing countries with resource limited settings. Hence it is reasonable to subject the sample only to RF and ACPA for patients with features of Rheumatologic disorders. Bizzaro et al. recorded in their study that a persistent number of patients (n = 79, 41.4%) were documented positive for RF and/or ACPA at baseline in RA patients.<sup>10</sup> The presence of both antibodies in the serum of individuals with features suggestive of Rheumatoid disorders when compared with presence of ACPA alone increases the specificity.<sup>11,5</sup> If a patient tests negative for both Rheumatoid Factor and antibodies against CCP with strong features of Rheumatoid arthritis then they may be grouped and named as seronegative rheumatoid arthritis. It is possible for these patients to possess extremely low levels of antibodies at the time of test, which is not enough for detection. Many such seronegative rheumatoid arthritis patients go on to develop antibodies in later years after their initial diagnosis. After which they change their diagnosis to seropositive rheumatoid arthritis. This is one of the many reasons that a patient can still be diagnosed with rheumatoid arthritis even if they are seronegative.<sup>12</sup> Out of 331 tested in our study 67% were negative for both RF and ACPA. So, they may need to be followed up if the clinical symptoms are consistent and prolonged to detect evaluation of Rheumatoid arthritis at the earliest with these tests.

### Conclusion

The results of our study suggest that the presence of anti-CCP antibodies has a better diagnostic performance than RF in patients with features of Rheumatological disorders. Anti-CCP antibodies correlate with higher positivity and increased specificity in suspected cases of Rheumatoid arthritis.

**Conflict of Interest:** None.

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