

# Beyond Rheumatoid Factor and Anticyclic Citrullinated Peptide: Role of 14-3-3 $\eta$ Protein in the Diagnosis of Rheumatoid Arthritis and the Evaluation of Disease Severity



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Visual Abstract

## ABSTRACT

**Background:** Rheumatoid arthritis (RA) is known to cause chronic inflammation of the joints, resulting in their destruction. Given the limitations of serological markers such as anti-cyclic citrullinated peptide antibodies (anti-CCP) and the autoantibody Rheumatoid Factor (RF), there is a need for additional markers to diagnose RA. A new promising marker for RA has been identified, known as 14-3-3 $\eta$  (eta) protein.

**Objective:** To investigate the 14-3-3 $\eta$  protein's role in the diagnosis of RA, to analyze its role in disease severity, and to distinguish between seropositive vs seronegative RA and early vs chronic RA.

**Materials and methods:** A total of 170 clinically suspected cases of RA met the inclusion criteria and were considered in this study. Data such as demographic details, clinical and laboratory findings, and DAS28 scores were collected from all participants. 14-3-3 $\eta$  levels were estimated. Statistical analysis was done using R software.

**Results:** Out of 170 participants, 79 were definite RA, and 91 were disease controls, as per the defined criteria. At a cutoff value of 4.15 ng/mL, 14-3-3 $\eta$  was 58% sensitive and 90% specific. There was a significant difference in 14-3-3 $\eta$  positivity between RA cases and disease controls ( $p < 0.05$ ). Likewise, the difference in 14-3-3 $\eta$  positivity between SNRA cases and disease controls was also significant ( $p = 0.0071$ ). The association of 14-3-3 $\eta$  positivity with DAS28 score was statistically significant ( $p < 0.001$ ). When 14-3-3 $\eta$  was used in combination with anti-CCP and RF, the sensitivity of 14-3-3 $\eta$  increased from 58% to 87%, but the specificity decreased from 84% to 63%.

**Conclusion:** The novel biomarker 14-3-3 $\eta$  can be used as a diagnostic tool for assessing disease severity in RA and can distinguish definite cases and SNRA cases from disease controls. Also, adding this 14-3-3 $\eta$  to the traditional markers enhances their diagnostic capacity.

**Keywords:** 14-3-3 $\eta$ , Anticyclic citrullinated peptide antibodies, Disease severity, Rheumatoid arthritis, Rheumatoid factor.

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## INTRODUCTION

Rheumatoid arthritis (RA) is known to cause chronic inflammation of the joints, resulting in their destruction.<sup>1</sup> According to WHO, in 2019, 18 million people in the world were living with RA, of whom 13 million had severe or moderate levels of disease, who could benefit from rehabilitation.<sup>2</sup> RA can affect men, women, and children at any age, but globally, 70% of people affected are women, and among them, 55% are older than 55 years.<sup>2</sup> In India, it is estimated that 0.7% of people have RA.<sup>3</sup>

Early and accurate diagnosis is important for RA for its proper management and also to prevent long-term damage. Early RA is the initial stage of the disease, with a duration of less than 1 year, while established RA is with symptoms that have lasted for more than one year,<sup>4</sup> which includes pain, tenderness, swelling, and stiffness, especially in the hands, wrists, and feet joints.<sup>2</sup> The 2010 classification criteria developed by the American College

of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) identify anti-cyclic citrullinated peptide antibodies (anti-CCP) and an autoantibody, Rheumatoid Factor (RF), as key serological markers for diagnosing RA.<sup>5</sup> However, there are limitations to the two markers. The sensitivity of RF ranges between 60% and 80%, with a specificity of about 85%.<sup>6</sup> Also, RF can be present in other autoimmune diseases and even in healthy individuals (about 5% of cases<sup>7</sup>), leading to false positive results.<sup>5</sup> The anti-CCP has also been found to be equally sensitive at about 67%, but has a better specificity of about 98%.<sup>6</sup> However, anti-CCP is positive only in a set of RA patients.<sup>8</sup> This seronegativity observed in both established and early RA cases highlights the need for additional biomarkers to improve the diagnostic sensitivity, ensure accurate detection across all RA patients, and provide them with timely, accurate treatment. When either of the two tests, anti-CCP and RF, is positive, it is known as seropositive RA (SPRA), while in RA cases when both anti-CCP and RF

are negative, it is classified as seronegative RA (SNRA).<sup>9</sup>

Serum 14-3-3 $\eta$  (eta) is an emerging marker in RA. The family of 14-3-3 proteins has 7 isoforms:  $\beta$ ,  $\gamma$ ,  $\epsilon$ ,  $\eta$ ,  $\sigma$ ,  $\theta$ , and  $\zeta$ , out of which 14-3-3 $\eta$  plays an important role in the onset and progression of RA.<sup>5</sup> Serum 14-3-3 $\eta$  was seen significantly higher in synovial fluid and serum of patients with arthritis as compared to healthy individuals. Studies have shown that 14-3-3 $\eta$  may play a role in cartilage destruction through regulation of matrix metalloproteinases (MMPs). Also, studies have shown that combining 14-3-3 $\eta$  protein with anti-CCP and/or RF, the diagnostic rate of RA is increased.<sup>5</sup>

While many Indian studies have focused on the role of 14-3-3 $\eta$  protein in diagnosing RA, to the best of our knowledge, this is the first Indian study that analyses its significance in disease severity, distinguishing seropositive vs. seronegative RA, and early vs. established RA.

## MATERIALS AND METHODS

This was an observational study that was conducted over 18 months, from May 2023 to October 2024, in the Department of Microbiology at a tertiary care hospital in North India. The study was approved by the Institute's Ethics Committee. (IEC No. 12/23, dated 8/5/2023). The sample size was calculated based on a previously published study by Shovman et al.<sup>10</sup> Adding 10% loss

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of data, we got an optimum sample size of 90 patients. Patients attending the Rheumatology OPD in the Internal Medicine Department for the first time during the study period with chief complaints suggestive of RA (joint pain, swelling, tenderness, and stiffness, especially in the hands, wrists, and feet) and consenting to participate were enrolled in the study.

### Exclusion Criteria

Follow-up RA patients, patients who are diagnosed with non-RA conditions like osteoarthritis, ankylosing spondylitis, systemic sclerosis, chronic kidney disease, subjects not consenting to participate, and repeat samples from the same patient were excluded from the study.

### Data Collection

After the enrollment of participants, their demographic, clinical, and laboratory data were collected, such as age, gender, and duration of disease from the initial appearance of symptoms. Tender joint count (TJC) and swollen joint count (SJC), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) levels were collected to calculate the DAS28 score, which is derived from assessing 28 joints. The serostatus of RF autoantibodies and anti-CCP antibodies was collected. The ACR criteria of each patient were recorded according to the 2010 ACR/EULAR criteria. This criterion assigns a score ranging from 0 to 10, with a score of 6 or higher indicating RA.<sup>11</sup> Those with a score of <6 are classified as non-RA patients. Depending on the presence/absence of joint erosions as seen in radiographic evaluations, RA was further classified into erosive and non-erosive types.

### Sample Collection

Two mL of peripheral blood was drawn from each patient. The serum was separated from each blood sample and aliquoted at -20°C for further analysis. The serum concentration of 14-3-3 $\eta$  protein was quantified by enzyme-linked immunosorbent assay (ELISA) using

the Human YWHAH (14-3-3 protein eta) ELISA kit, Fine Biotech, Wuhan, China. The ELISA tests were performed according to the manufacturer's instructions.

### Statistical Analysis

R software version 4.3.1 was used for Statistical analysis. Receiver operating characteristic (ROC) analysis was performed to evaluate the diagnostic accuracy of the indexes, and the results were presented as the area under the curve (AUC) along with 95% confidence intervals (CI). The optimum cutoff value and diagnostic accuracy criteria were determined using Youden's index.

## RESULTS

In total, 170 patients presenting with clinical suspicion of RA were included. Of these, 79 study participants were categorized as definite RA as they fulfilled the ACR criteria, and the remaining 91 participants were non-RA patients and were taken as disease controls.

### Patients' Disease Characteristics

Out of the 79 definite RA cases, females were the majority (64 cases, 81.01%), while males accounted for only 15 cases (18.98%). The median age of the patients was 45 years, with a range of 19 to 71 years. The disease activity was moderate to severe in 63% of the patients, and the mean DAS28 score

was 5.13. The median duration of RA was 1.02 years. Out of all the definitive RA cases, 30 were negative for anti-CCP, whereas 49 tested positive. Additionally, 36 patients tested RF positive, while 43 were RF negative. SPRA was identified in 56 patients, whereas 23 patients had SNRA.

### Diagnostic Value of 14-3-3 $\eta$

According to the ROC curve, using ACR criteria as the gold standard, the AUC for 14-3-3 $\eta$  was 0.758, with a Youden's index of 0.483 and a 95% CI of 0.684–0.832. At a cut-off value of 4.15 ng/mL, 14-3-3 $\eta$  was 58% sensitive and 90% specific (Fig. 1)

### Correlation of 14-3-3 $\eta$ with Clinicopathological Characteristics of RA

The median 14-3-3 $\eta$  in the study was 2.53 ng/mL. No significant correlation was found between 14-3-3 $\eta$  expression with age or sex. The correlation of 14-3-3 $\eta$  with the clinicopathological characteristics is detailed in Table 1. Correlation of demography with various biomarkers is summarized in Table 2.

**Table 1:** Comparing the median values of 14-3-3 $\eta$  and their significance values

| Condition         | Median value of 14-3-3 $\eta$ (ng/mL) | p-value |
|-------------------|---------------------------------------|---------|
| Definite RA       | 4.6                                   | 0.001   |
| Disease control   | 1.7                                   |         |
| Early RA          | 3.125                                 | 0.972   |
| Established RA    | 2.175                                 |         |
| Anti-CCP positive | 4.16                                  | 0.184   |
| Anti-CCP negative | 4.64                                  |         |
| RF positive cases | 4.7                                   | 0.832   |
| RF negative cases | 4.42                                  |         |
| SPRA              | 5.1                                   | 0.758   |
| SNRA              | 4.312                                 |         |
| Erosive           | 24                                    | 0.132   |
| Nonerosive        | 8.85                                  |         |

SPRA, seropositive RA; SNRA, seronegative RA

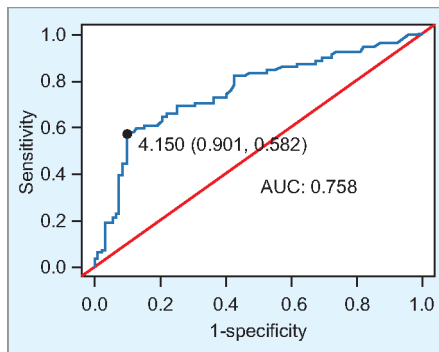


**Fig. 1:** ROC curve showing 14-3-3 $\eta$  sensitivity and specificity for diagnosing RA

**Table 2:** Correlation of demography with biomarkers

| Group, (n)               | Mean age (years) | Sex (% female) | Median 14-3-3 $\eta$ (ng/mL) | 14-3-3 $\eta$ positive | Anti-CCP positive | RF positive |
|--------------------------|------------------|----------------|------------------------------|------------------------|-------------------|-------------|
| Total cases (n = 170)    | 44               | 134 (78%)      | 2.53                         | 55 (32%)               | 71 (41.4%)        | 50 (29.4%)  |
| RA (n = 79)              | 45               | 64 (81%)       | 4.6                          | 46 (58.2%)             | 49 (62%)          | 36 (45.5%)  |
| Disease control (n = 91) | 42               | 70 (77%)       | 1.7                          | 9 (9.89%)              | 22 (24%)          | 14 (15%)    |
| Early RA (n = 51)        | 45               | 38 (74.5%)     | 3.125                        | 32 (62.7%)             | 27 (53%)          | 17 (33.3%)  |
| Established RA (n = 28)  | 45.5             | 26 (93%)       | 2.175                        | 14 (50%)               | 22 (78.57%)       | 19 (68%)    |
| SPRA (n = 56)            | 44.5             | 45 (80%)       | 5.1                          | 33 (59%)               | 49 (87.5%)        | 36 (64.2%)  |
| SNRA (n = 23)            | 50               | 19 (82.6%)     | 4.312                        | 13 (56.5%)             | 0                 | 0           |

SPRA, seropositive RA; SNRA, seronegative RA

**Table 3:** 14-3-3 $\eta$  positivity and value of significance in different categories

| Categories                             | Total 14-3-3 $\eta$ positive | p-value |
|----------------------------------------|------------------------------|---------|
| Female                                 | 64                           | 0.46    |
| RA                                     | 46                           | 0.0001  |
| Disease controls                       | 9                            |         |
| High DAS28 score (>5.1)                | 37                           | 0.007   |
| Moderate-low DAS28 score ( $\leq$ 5.1) | 9                            |         |
| Early                                  | 32                           | 0.38    |
| Established RA                         | 14                           |         |
| SPRA                                   | 31                           | 0.9     |
| SNRA                                   | 13                           |         |
| SNRA                                   | 13                           | <0.001  |
| Disease controls                       | 9                            |         |

SPRA, seropositive RA; SNRA, seronegative RA

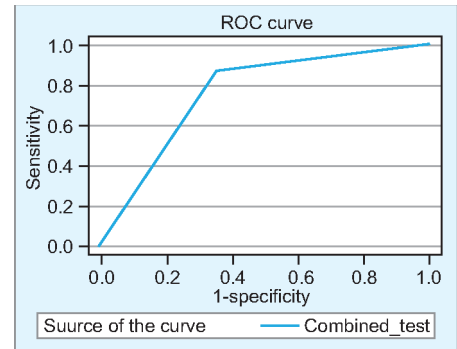

**Fig. 2:** The ROC curve analysis showing the sensitivity, specificity, and AUC for the combination of 14-3-3 $\eta$ , RF, and anti-CCP

**Table 4:** The results of the diagnostic value of RF, anti-CCP, 14-3-3 $\eta$  alone, and in combination with 14-3-3 $\eta$ 

| Biomarker tested (s)                    | Sensitivity(95%CI) | Specificity(95%CI) | PLR  | NLR   | DOR   |
|-----------------------------------------|--------------------|--------------------|------|-------|-------|
| 14-3-3 $\eta$                           | 0.58 (0.46–0.69)   | 0.90 (0.82–0.95)   | 5.9  | 0.463 | 12.74 |
| RF                                      | 0.45 (0.34–0.57)   | 0.84 (0.75–0.91)   | 3    | 0.64  | 4.68  |
| Anti-CCP                                | 0.62 (0.46–0.75)   | 0.75 (0.62–0.86)   | 2.6  | 0.500 | 5.2   |
| 14-3-3 $\eta$ or/and RF                 | 0.77 (0.67–0.86)   | 0.76 (0.65–0.84)   | 3.2  | 0.30  | 10.66 |
| 14-3-3 $\eta$ or/and Anti-CCPs          | 0.84 (0.77–0.93)   | 0.68 (0.57–0.77)   | 2.6  | 0.23  | 11.30 |
| 14-3-3 $\eta$ or/and RF or/and anti-CCP | 0.87 (0.77–0.93)   | 0.63 (0.52–0.73)   | 2.35 | 0.21  | 11.19 |

PLR, positive likelihood ratio; NLR, negative likelihood ratio; DOR, diagnostic odds ratio

### Positivity of 14-3-3 $\eta$ in RA and Disease Controls (n = 170)

Among the 170 cases, 14-3-3 $\eta$  was positive in 46 RA cases (58.2%) and negative in 33 RA cases (41.8%). The disease control group had 82 cases (90.1%) with 14-3-3 $\eta$  being negative, and 9 cases (9.9%) with it being positive. The difference in 14-3-3 $\eta$  positivity between RA patients and disease controls was significant ( $p < 0.05$ ) (Table 3).

### 14-3-3 $\eta$ Levels and Disease Activity

Out of the 79 definite RA patients, the DAS28 score was mild to moderate in 28 cases, and high in 51 cases. 14-3-3 $\eta$  was positive in 37 (72.5%) and negative in 14 (27.5%) of the 51 patients with a high DAS28 score. Among those with a mild to moderate DAS28 score, 14-3-3 $\eta$  was positive in 9 cases and negative in 19 cases. The association between 14-3-3 $\eta$  positivity and DAS28 score was highly significant ( $p = 0.0071$ ) (Table 3).

### 14-3-3 $\eta$ in Early vs Established RA (n = 79)

Out of 51 cases of early RA, 14-3-3 $\eta$  was tested positive in 32 cases (62.7%) and negative in 19 cases (37.3%). It was also positive in 14 of the 28 cases of established RA and negative in 14 of the cases. The difference in 14-3-3 $\eta$  positivity between early and established RA was not statistically significant ( $p > 0.05$ ) (Table 3).

### Correlation of 14-3-3 $\eta$ Positivity with SPRA and SNRA (n = 79)

Among the 79 definite RA cases, 14-3-3 $\eta$  was positive in 26 anti-CCP-positive cases ( $n = 49$ ) and negative in 10 anti-CCP-negative cases ( $n = 30$ ). Similarly, 14-3-3 $\eta$  was positive in 20 RF-positive cases ( $n = 36$ ) and negative in 17 RF-negative cases ( $n = 43$ ). Out of the 56 SPRA cases, 14-3-3 $\eta$  was positive in 33 cases, while out of the 23 SNRA cases, it was positive in 13 cases. The prevalence of this marker in the seropositive population was found to be 59%, and in the seronegative population, it was 57% (Table 2).

### Comparison of 14-3-3 $\eta$ Positivity in SNRA and Disease Controls

Of the SNRA cases, 13 had a positive 14-3-3 $\eta$  and 10 had a negative 14-3-3 $\eta$ . In the disease control group, the majority were 14-3-3 $\eta$  negative (82 cases), and only 9 cases were positive. The difference in 14-3-3 $\eta$  positivity between SNRA cases and disease controls was highly significant ( $p < 0.001$ ) (Table 3).

### Diagnostic Value of Combining 14-3-3 $\eta$ with RF and Anti-CCP

The sensitivity increased from 45% to 77.26% when 14-3-3 $\eta$  was combined with RF, but the specificity decreased from 84% to 76.25%. Similarly, combining 14-3-3 $\eta$  with anti-CCP raised sensitivity from 62% to 84.14%, with a reduction in specificity to 68.32% (Table 4).

### Use of 14-3-3 $\eta$ in Combination with RF and/or Anti-CCP

The combination of 14-3-3 $\eta$  with RF and anti-CCP increased the sensitivity from 58% to 87%, and the specificity was 63% (Table 4 and Fig. 2)

## DISCUSSION

This study evaluated the significance of 14-3-3 $\eta$  among various subgroups of RA and disease controls to explore its clinical relevance and potential diagnostic utility. The median 14-3-3 $\eta$  was significantly higher in definite RA cases (4.6 ng/mL) when compared to disease controls (1.7 ng/mL). This is similar to the findings from Yarlagadda et al.,<sup>12</sup> where RA patients exhibited higher median 14-3-3 $\eta$  levels than individuals with other autoimmune diseases. Yarlagadda et al.<sup>12</sup> also studied the activity of 14-3-3 $\eta$  in the Indian population, focusing on its use as a biomarker for RA. Additionally, no association between 14-3-3 $\eta$  and age or sex was found in our study.

In this study, the median value of 14-3-3 $\eta$  did not show any significant differences between early and established RA. This contrasts with findings from Gong et al.,<sup>13</sup> where median levels of 14-3-3 $\eta$  were higher in early RA group. This result could be because of a smaller number of early RA cases in our study. In our study, the ROC curve of 14-3-3 $\eta$  differentiating RA cases from disease controls exhibited a sensitivity and specificity of 58%

and 90%, respectively, with an AUC of 0.758 at a cutoff of 4.15 ng/mL. Whereas in a study done by Tu et al.<sup>14</sup> where 14-3-3 $\eta$  was compared between RA cases with osteoarthritis (OA), at a cutoff of 0.656 ng/mL, 14-3-3 $\eta$  showed a sensitivity of 60% and specificity of 84.4%.

While higher 14-3-3 $\eta$  positivity was seen in anti-CCP and RF-positive cases in our study, the association was statistically insignificant. The study conducted by Tu et al.<sup>14</sup> also reported a positive association of serum 14-3-3 $\eta$  levels with both RF and anti-CCP.<sup>14</sup> This is because all three markers release proinflammatory factors, such as IL-1 $\beta$  and IL-6.<sup>13</sup> The prevalence of 14-3-3 $\eta$  was only marginally higher in SPRA (59%) when compared to SNRA (57%) in our study. Whereas, in a study done by Yarlagadda et al., the prevalence of 14-3-3 $\eta$  was 71% in SNRA and 78% in SPRA cases.<sup>12</sup> Our study also analyzed the median 14-3-3 $\eta$  levels in SPRA and SNRA, which were 5.1 and 4.31, respectively, showing no significant difference.

Although 14-3-3 $\eta$  could not differentiate early from established RA in this study ( $p$ -value = 0.389), its prevalence was higher in early RA cases (62.7%) compared to established RA cases (50%). Conversely, Gong et al. reported a significantly higher incidence of 14-3-3 $\eta$  positivity in the established RA group compared to the early RA group.<sup>13</sup> In another study done by Maksymowych et al.,<sup>15</sup> 14-3-3 $\eta$  could distinguish early and chronic RA cases from those of the healthy population and disease controls.

In our study, a significant association was observed between 14-3-3 $\eta$  and disease activity, with a  $p$ -value of 0.0071. The study by Gong et al.<sup>13</sup> stated that 14-3-3 $\eta$  positive cases in RA showed significantly higher DAS28 scores when compared to 14-3-3 $\eta$  negative cases. Research has shown that 14-3-3 $\eta$  positivity is associated with worse outcomes or higher disease severity because elevated expression of 14-3-3 $\eta$  promotes the overproduction of RANKL, MMP-1, MMP-3, IL-1 $\beta$ , and IL-6, which exacerbates inflammation and contributes to cartilage and bone destruction, ultimately leading to higher disease activity.<sup>14</sup> Kilani et al.<sup>16</sup> were the first to establish a correlation between 14-3-3 $\eta$  and RA in 2007, demonstrating that 14-3-3 $\eta$  was elevated in both serum and synovial fluid of RA patients. They also found a positive association between 14-3-3 $\eta$  and matrix metalloproteinases (MMPs).<sup>16</sup> MMPs cause structural erosions that contribute to progressive joint destruction and eventually lead to increased disease activity in RA.<sup>16</sup>

In the present study, 14-3-3 $\eta$  levels were markedly elevated in RA cases than in disease controls with a significant  $p$ -value of <0.0001.

These findings are similar to those of a study by Huang et al.,<sup>17</sup> which showed elevated 14-3-3 $\eta$  in RA cases when compared to other non-RA cases and even in healthy controls, suggesting its role in differentiating RA cases from non-RA cases. Another study by Tu et al.<sup>14</sup> also reported that 14-3-3 $\eta$  can differentiate RA from osteoarthritis (OA) and healthy controls (HCs), potentially due to its role in upregulating pro-inflammatory cytokines. A study by Salman et al.<sup>18</sup> reported a significant positive association between 14-3-3 $\eta$  and SNRA when compared to healthy controls. Similarly, our study also found a significant association with 14-3-3 $\eta$  positivity in SNRA and disease controls, yielding a  $p$ -value of <0.0001. This finding is of much significance as many SNRA cases can be misdiagnosed as non-RA, leading to wrong treatment and thus increased morbidity.

Multiple studies have evaluated the diagnostic utility of 14-3-3 $\eta$  in RA.<sup>10,13,15</sup> 14-3-3 $\eta$  showed a PLR of 5.9 and an NLR of 0.46, suggesting that 14-3-3 $\eta$  is clinically useful in making an appropriate diagnosis (Table 4). An increased DOR indicates better diagnostic accuracy, meaning the test is more effective at differentiating between individuals with and without the condition. With a DOR of 15.90, 14-3-3 $\eta$  indicates high diagnostic accuracy. This suggests that the test is quite effective in distinguishing between the presence and absence of RA. When 14-3-3 $\eta$  was combined with RF, the DOR was 10.66, which was greater than that of RF alone (DOR was 4.68). Likewise, when combining 14-3-3 $\eta$  to anti-CCPs, DOR improved from 5.2 to 11.30. These findings indicate that the combination of 14-3-3 $\eta$  with RF or anti-CCP improves diagnostic accuracy over using a single marker. Although the combined DOR of all three markers (11.19) is higher than those of individual RF and anti-CCP, it is similar to the DOR of 14-3-3 $\eta$  alone and its combination with anti-CCP. This indicates that while the combined use of markers enhances diagnostic performance relative to RF and anti-CCP alone, the improvement is marginal when compared to 14-3-3 $\eta$  and its combinations; thus, testing any of the two traditional markers (Anti-CCP or RF), preferably anti-CCP (DOR 11.30) with 14-3-3 $\eta$  will be sufficient to support a diagnosis of RA. According to Maksymowych et al.,<sup>15</sup> the addition of 14-3-3 $\eta$  provides incremental benefits in diagnosing RA compared to using a single indicator (RF or anti-CCP). However, the study by Wu et al.<sup>19</sup> stated that adding 14-3-3 $\eta$  does not provide any added advantage in diagnosing RA. Few studies have shown that the combined specificity of the three markers (14-3-3 $\eta$ , anti-CCP and RF) increases significantly,<sup>12,20</sup>

unlike our study, which has shown a significant increase in sensitivity of the three markers but a slight decrease in specificity of the three markers combined. Our study has shown an overall increased diagnostic accuracy on combining 14-3-3 $\eta$  with either anti-CCP or RF, thus making the testing cost more reasonable (using two tests as compared to combining three tests).

This study has certain limitations. It lacks a follow-up period to assess the correlation of 14-3-3 $\eta$  with treatment response. Also, this study did not compare the levels of 14-3-3 $\eta$  in RA patients and healthy controls.

## CONCLUSION

In conclusion, the serum of RA patients has been confirmed to contain elevated levels of 14-3-3 $\eta$ , establishing it as a promising new biomarker for diagnosing RA. 14-3-3 $\eta$  in serum can also be employed for assessing the disease severity. Additionally, we have demonstrated that 14-3-3 $\eta$  can effectively distinguish definite cases of RA from disease controls. Furthermore, adding the 14-3-3 $\eta$  alongside anti-CCP increases the positive likelihood ratio and diagnostic odds ratio, leading to a more accurate and reliable diagnosis of RA.

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## ANNOUNCEMENT

### ANNUAL CONFERENCE OF ASSOCIATION OF PHYSICIANS OF INDIA (EAST ZONE)

Organised by: API TRIPURA STATE BRANCH

Date: 08/09/10th January, 2027

Venue: Rabindra Shatabarshiki Bhavan, Agartala, West Tripura

Organising Chairman : Prof. Dr Pradip Bhaumik

Organising Secretary : Dr Saumen Chaudhuri