

## A Comparative Analysis of Inflammatory Markers and Disease Activity Score between Seropositive and Seronegative Rheumatoid Arthritis

Amber Khan<sup>1</sup>, Ume Kulsoom<sup>1</sup>, Maham Ghouri<sup>1</sup>, Sehrish Fatima<sup>1</sup>, Saima Saleem<sup>1</sup>, Sitwat Zehra<sup>1\*</sup>

<sup>1</sup>Dr. A. Q. Khan Institute of Biotechnology and Genetic Engineering, University of Karachi, Karachi, Pakistan

**Abstract: Introduction:** Rheumatoid arthritis (RA) is a chronic autoimmune disorder that is characterized by persistent inflammation in the joints and other tissues, leading to significant joint damage irrespective of the serogroups i.e. seropositive and seronegative RA. C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) are commonly used biomarkers to monitor inflammation in RA and to inform therapeutic decision-making. **Methods:** The present study aimed to investigate the relationship between CRP and ESR levels and the severity of disease among seronegative and seropositive patients. ACR/EULAR 2010 criteria were used to recruit RA patients in the study. Clinical assessments were performed to estimate the RF, ACCP antibodies, ESR and CRP. Swollen and tender joints were also taken into account for the calculation of disease activity score-28. **Results:** The results indicated that significantly elevated CRP levels were consistently observed in seropositive RA, in conjunction with seronegative RA patients ( $p=0.036$ ). ESR levels were found elevated between the two groups but were not found to be statistically different ( $p=0.885$ ). DAS28-CRP and DAS28-ESR results showed high severity of disease ( $>5.1$ ) in both seronegative and seronegative RA patients. **Conclusion:** The findings of this study highlight the diagnostic and prognostic significance of ESR and CRP levels in RA among seronegative and seropositive patients, particularly in differentiating seropositive and seronegative cases and in guiding therapeutic decisions.

**Keywords:** Seropositive RA, Seronegative RA, C-reactive protein (CRP), Erythrocyte sedimentation rate (ESR), Disease activity score-28 (DAS-28)

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**\*Correspondence:** Sitwat Zehra, Dr. A. Q. Khan Institute of Biotechnology and Genetic Engineering, University of Karachi, Karachi-75270, Pakistan Tel: +92-3342676512. Email: sitwat.zehra@kibge.edu.pk

### Introduction

Rheumatoid arthritis (RA) is an autoimmune disease with the key characteristic of joint inflammation. This systematic inflammation is present in both seronegative and seropositive forms of the disease [1]. Seronegative arthritis refers to a type of arthritis in which the blood does not contain rheumatoid factor (RF) or anti-citrullinated protein antibodies (ACPAs), which are markers commonly found in the blood of individuals with rheumatoid arthritis [2,3]. In seronegative arthritis, the inflammation is thought to be caused by an immune response to an unknown trigger [4]. Although the main mechanism of seronegative arthritis is still unidentified, it is presumed to be a consequence of a coalescence of genetic and environmental factors [5]. This type of RA is often more challenging to diagnose and may present with different symptoms compared to seropositive RA.



In seropositive, the inflammation is considered to be triggered by an autoimmune reaction in which the immune system inadvertently targets the joints [6,7]. Rheumatoid factor (RF) or anti-citrullinated protein (ACPA) antibodies, or even both, are required for the diagnosis of seropositive rheumatoid arthritis (RA) [8]. These antibodies are considered primary markers for the diagnosis of RA and are present in approximately 75-80% of people with the condition [9]. Seropositive rheumatoid arthritis is associated with higher levels of inflammation than seronegative rheumatoid arthritis. This increased inflammation is thought to contribute to the more aggressive course of RA and greater systemic inflammation seen within seropositive individuals [10].

Common biomarkers for evaluating inflammatory disorders include erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). These markers have been widely utilized in clinical approaches to help with the identification and follow-up of such illnesses [11]. CRP is a clinically relevant biomarker of inflammation that can be utilized to assess the activity of rheumatoid arthritis (RA). In both seropositive and seronegative RA patients, increased CRP levels can be observed in response to the inflammation in joints and other affected tissues [12]. The assessment of disease activity in RA is crucial for guiding therapeutic decisions, and Disease Activity Score 28-joint count (DAS28) is a commonly used tool for this purpose [13,14]. DAS28 scores are calculated based on the number of joints that are tender and swollen, the patient's general health, and a laboratory measure of acute inflammation with the score ranging from 0 to 9.4 [15]. DAS28 can be determined using ESR and CRP as the laboratory measure of acute inflammation. However, there is a lack of consensus on the cutoff values for high or severe disease activity between DAS28-ESR and DAS28-CRP which might result in inconsistent treatment decisions among healthcare providers and health systems [16]. Despite the availability of validated disease activity scores for RA, these scores heavily rely on biomarkers such as CRP and ESR [17]. For the above mentioned reasons, in-depth research on inflammatory marker levels i.e. CRP and ESR and DAS-28 in seropositive and seronegative RA patients is required to gain a deeper understanding of the disease progression.

There is very limited data available on the significance of inflammatory markers over the effectiveness of DAS28 in determining RA activity and severity in Pakistan. Therefore, the objectives of the current study are (i) to measure CRP and ESR levels and (ii) to compare DAS28 between the serogroups of RA patients in order to evaluate the significant contribution of inflammatory markers and disease activity.

## **Methodology**

### **Ethical Approval**

Ethical approval from the Department of Rheumatology at Jinnah Postgraduate Medical Center (JPMC) was taken for RA patient's data collection (NO.F.2-81-IRB/2019-GENL/19855/JPMC). The participants of the current study were selected in accordance with ACR/EULAR 2010 standards and with the guidance of a consultant to ensure proper diagnosis [18]. A total of 126 RA patients were recruited through informed consent.

### **Data Assessment**

Demographic data that include age and gender of patients and duration of disease were collected from patients themselves and otherwise from their attendants. Detailed clinical data that include antibodies against RA factor, ACCP, ESR and CRP were collected after reviewing the patient's medical record.

### **Serological Profiling**

Patients were considered seropositive RA, identified by the presence of either both RF antibodies and anti-CCP antibodies in the blood or anyone while seronegative RA was defined by their absence.



### Disease Activity Score-28

To investigate the severity and aggressive course of RA in both seropositive and negative patients, a more in-depth investigation of the clinical profile was performed. The seropositive and seronegative RA subjects were observed for joint involvement, as the inflammation affects the joints but the pattern of involvement may differ between the two types. For this purpose, DAS28-ESR and DAS28-CRP were calculated by quantifying swollen joints, tender joints (out of the 28), and ESR and CRP lab test results. If a patient's DAS28 score is less than 2.6, they are in remission; if it is between 2.6 and 3.1, it suggests low activity; if it is between 3.1 and 5.1, it shows moderate activity; and if it is 5.1 or more, it indicates high activity.

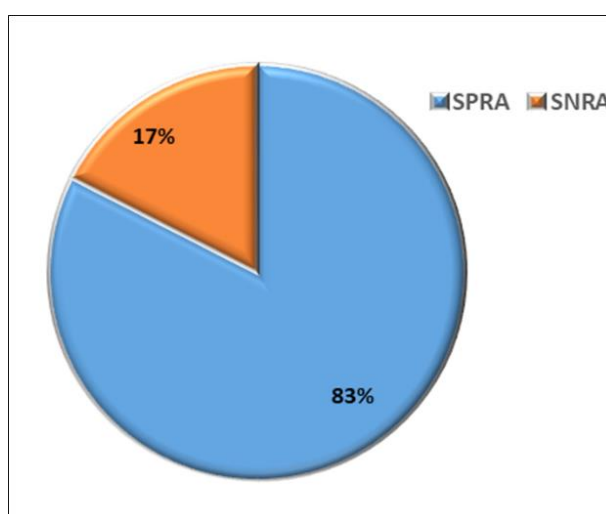
### Statistical Analyses

The study utilized advanced statistical techniques to analyse the data and find possible associations. Frequencies were calculated for the descriptive analyses of each categorical variable. The association of demographic parameters between seropositive and seronegative RA was analysed through the application of the chi-square test. To compare mean ESR and CRP values between the two sero-groups Student's t-test was performed. Scattered plots were used to visually analyse the ranges of DAS-28. Statistical significance was established at a *p*-value less than 0.05, utilizing SPSS version 23 software (SPSS Inc., Chicago, IL, USA).

### Results and Discussion

#### Serology-Based Distribution Analysis of RA Patients

Figure 1 represents the distribution of RA patients on the basis of sero levels. The study found that among 126 recruited RA patients, 104 (83%) were seropositive and 22 (17%) were seronegative. The frequency of seropositive compared to seronegative RA patients was found to be consistent with another study as antibodies for RF and ACCP are present in approximately 75-80% of RA patients [9]. A similar pattern was observed in a Morrocon study with 90% seropositive and 10% seronegative cases [19]. Another study conducted in Korea was found in coherent with 84% seropositive and 16% seronegative RA patients [20]. The rationale behind increased number of seropositive patients might be due to a weak immune response against the symptoms. In general, the underlying causes for the greater incidence of seropositive RA in contrast to seronegative RA are multifactorial. Based on the results obtained from the current study, it can be assumed that combinations of genetic and environmental variables may have an impact in this regard.



**Figure 1.** Pie chart showing the distribution of RA patients on the basis of sero-groups. SPRA: Seropositive rheumatoid arthritis, SNRA: Seronegative rheumatoid arthritis

### Demographic-Based Distribution between Sero-groups

Table 1 represented the demographic data which revealed that female patients were more common in both seropositive (87.5%) and seronegative (81.8%) RA patients compared to males. Sex hormones play a significant part in this gender bias, with estrogens being powerful autoimmune stimulators in females and androgens acting as protective hormones in males [21]. Additionally, the age distribution of subjects showed that the disease was found more common in 31-50 years of age in both the seropositive (62.5%) and seronegative (54.5%) groups. Among the 126 RA patients, 71.4% of them had RA for 1-10 years, approximately 16% of them had it for 11-20 years and only 7.1% had the disease for more than 20 years. It was observed that only seropositive patients had been diagnosed with RA for >1 year (5.5%). This might be due to negligible levels of RF and ACPA antibodies in seronegative patients that lead to late onset of the disease [22]. The association analyses of observed demographic parameters from Table. 1 further revealed that none of the investigated parameters were found statistically linked with seropositive and seronegative RA ( $p>0.05$ ).

**Table 1.** Demographic parameters distribution between seropositive and seronegative RA

| Parameters                  |        | Seropositive RA (%) | Seronegative RA (%) | Chi-square ( $\chi^2$ ) | <i>p</i> -value |
|-----------------------------|--------|---------------------|---------------------|-------------------------|-----------------|
| Gender                      | Male   | 13 (12.5)           | 4 (18.2)            | 0.502                   | 0.478           |
|                             | Female | 91 (87.5)           | 18 (81.8)           |                         |                 |
| Age (years)                 | 11-20  | 3 (2.9)             | 0 (0)               | 1.736                   | 0.784           |
|                             | 21-30  | 19 (18.3)           | 6 (27.3)            |                         |                 |
|                             | 31-40  | 31 (29.8)           | 5 (22.7)            |                         |                 |
|                             | 41-50  | 34 (32.7)           | 7 (31.8)            |                         |                 |
|                             | 51-60  | 17 (16.3)           | 4 (18.2)            |                         |                 |
| Duration of disease (years) | <1     | 7 (6.7)             | 0 (0)               | 3.954                   | 0.266           |
|                             | 1-10   | 73 (70.2)           | 17 (77.3)           |                         |                 |
|                             | 11-20  | 18 (17.3)           | 2 (9.1)             |                         |                 |
|                             | 21-30  | 6 (5.8)             | 3 (13.6)            |                         |                 |

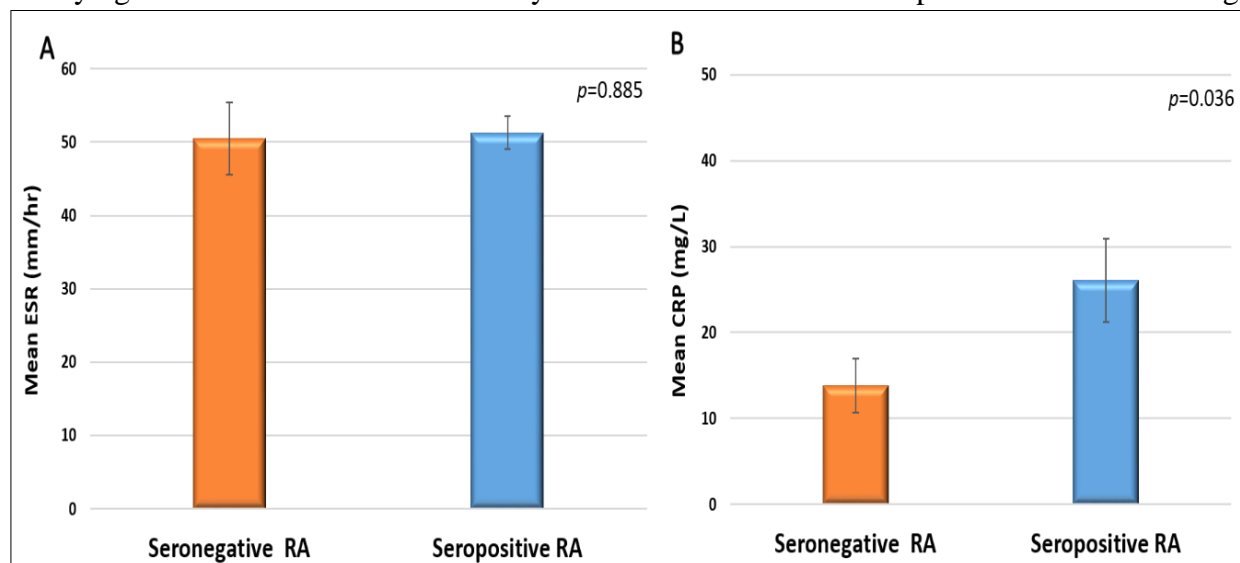
subjects

### Levels of Inflammatory Markers between Sero-groups

The current study revealed a notable increase in CRP levels, a biomarker of acute-phase response, among seropositive patients (Mean  $\pm$  SE: 26.06  $\pm$  49.2 mg/L) compared to seronegative patients (Mean  $\pm$  SE: 13.83  $\pm$  3.13 mg/L) ( $p<0.05$ ) (Fig 2A). The findings revealed a consistent relationship between CRP and the ESR, which aligns with previous observations in the Polish population [23]. The current findings are further in conjunction with another study which reported significant association between CRP levels and RA patient's synovium biopsy samples ( $p<0.0001$ ) [17]. Nevertheless, when comparing the two groups the ESR in serum was observed to exhibit a minor elevation, albeit without statistical significance ( $p>0.05$ ) (Fig. 2B). These elevated levels of inflammatory markers indicated that immune system of seropositive RA patients generate pro-inflammatory RF antibodies, which are a primary contributing factor to inflammation. These antibodies trigger the immune system to produce additional pro-inflammatory substances like cytokines and chemokines, causing chronic inflammation in RA [24]. Another study suggested that CRP plays a role in bone damage through RANKL expression in monocytes, leading to osteoclastogenesis. [25]. In seronegative RA, however, the absence of



RF antibodies and anti-CCP antibodies does not guarantee normal CRP and ESR levels. Thus, increased CRP and ESR levels can still be detected in response to joint inflammation, albeit the underlying cause of this inflammation may differ from that seen in seropositive RA. The findings



highlight the utility of CRP levels in monitoring the response to treatment in both seropositive and seronegative RA. The measurement of CRP levels can provide important information for guiding decisions about adjusting therapy as needed.

**Figure 2.** Mean comparison of (2A) ESR levels and (2B) CRP levels between seronegative and seropositive RA subjects using Students t-test.

### Disease Activity Score Between Sero-groups

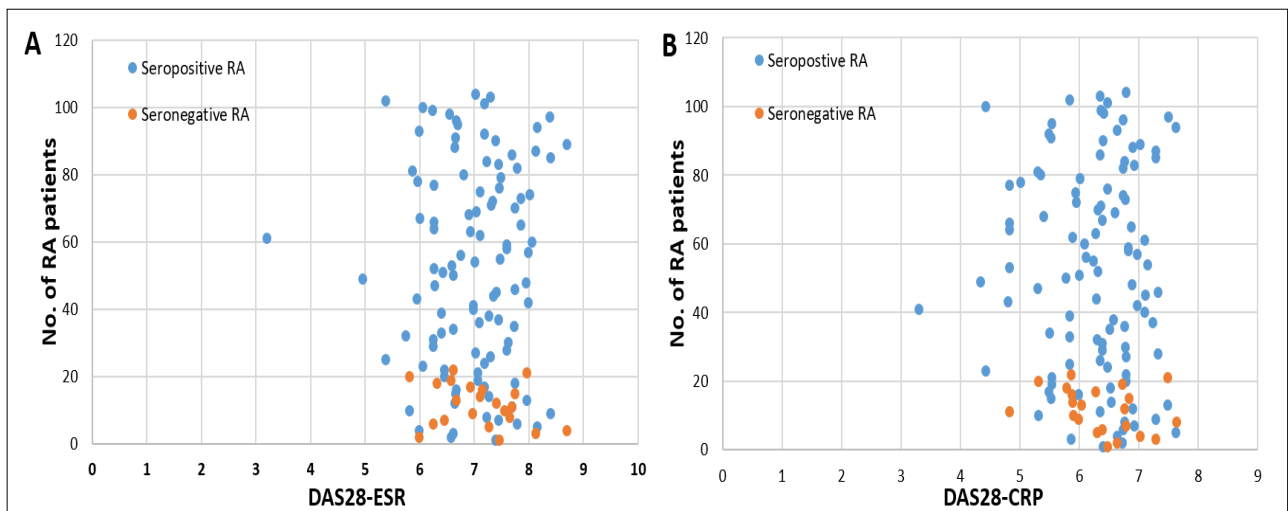
The scatter plot of both sero-groups revealed that DAS28-ESR and DAS28-CRP levels were greater than 5.1, indicating severe or high disease activity. (Fig 3A and 3B). The results suggested that joint involvement was more destructive among both seropositive RA and seronegative RA patients. This persistent inflammation can result in joint damage, pain, and systemic symptoms such as fatigue and fever, commonly observed among the studied subjects [26]. According to the medical record, patients in the study were given disease-modifying anti-rheumatic drugs (DMARDs), however, treatment might have started later due to ignorance or a misdiagnosis. Ultimately, it caused a loss of functionality and a decline in quality of life. As most of the patients from both serological groups have been suffering for more than a decade, it might also be possible that the treatment patients were receiving needs to be revised by the clinicians. Since the main mechanism of joint damage and pain is inflammation. Despite the paucity of research on the subject, it is recommended that DMARDs like NSAIDs, topical capsaicin, weak opioids, and treatments like joint infiltrations or surgery are recommended for the control of RA [27]. In contrast to the current study, in a prospective study negative serotypes of RA patients show remission after treatment and are less erosive [28]. On the contrary, another research revealed that due to late onset and delayed diagnosis, seronegative RA patients miss the opportunity of early treatment and thus are less likely to achieve remission [29]. Therefore, findings from the current study emphasize the significance of timely and effective management for both sero-groups of RA patients to prevent the progression of joint destruction.

### Conclusions:

Elevated inflammatory markers are hallmarks of RA, and can be influenced by several factors including genetics, lifestyle, and disease activity. Although CRP and ESR levels are critical for



the diagnosis and management of RA, the seropositive and seronegative RA subjects in current research did not show any disease-differentiating consequences. These results suggested the future potential of more targeted and efficient treatments for RA. Limitations of the study include (i) sample size that is very small for this kind of retrospective study. Due to lack of epidemiological studies in Pakistan, the number of studied RA cases were calculated using available but old data. (ii) As the study is not prospective in nature therefore the effect of DMARDS on both serological groups of ssRA for a specific period of time has not been studied. On that account, there is a need for a prospective study to find a more in-depth understanding regarding the effect of treatment over a period of time. Additionally, in future elevation of CRP and ESR levels in females with RA could lead to an improved understanding of gender-specific differences in RA and help to develop more personalized and effective management strategies.



**Figure 3.** Scatter plots of (A) DAS28-ESR (B) DAS28-CRP between serology-based RA subjects. Disease severity score:  $<2.6$ : Remission,  $\geq 2.6$ - $<3.2$ : Low,  $\geq 3.2$ - $\leq 5.1$ : Moderate,  $>5.1$ : High

#### ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

#### HUMAN AND ANIMAL RIGHTS

No animals were used in this study. The study on humans was conducted in accordance with the ethical rules of the Helsinki Declaration and Good Clinical Practice.

#### CONSENT FOR PUBLICATION

Not applicable.

#### AVAILABILITY OF DATA AND MATERIALS

None.

#### FUNDING

None.



**CONFLICT OF INTEREST**

The authors declare no conflict of interest, financial or otherwise.

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None.

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