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Red Cell Distribution Width and NeutrophilLymphocyte Ratio as a New Biomarker for Severity in Rheumatoid Arthritis and itsComparison with DAS 28 ESR Score

Sarah Azam Shah, Taqdees Khaliq, Khadija Iftikhar, Saad Saleem

Department of Rheumatology, Federal Government Polyclinic Hospital, Islamabad Pakistan

ABSTRACT

***Objective*:** To determine the association of red cell distribution width (RDW)and neutrophil-lymphocyte ratio (NLR)with the severity of rheumatoid arthritis by using DAS28 ESR. Additionally, toxic granulations were assessed as an indicator of disease severity.

***Study Design*:** Cross-sectional study.

***Place and Duration of Study*:** Rheumatology Outpatient Department, Federal Government Polyclinic Hospital, Islamabad Pakistan, from Dec 2020 to Jun 2021.

***Methodology*:** Two hundred ninety rheumatoid arthritis patients were included. DAS 28 ESR was calculated using the DAS 28 ESR calculator, and RDW and NLR were extracted from the complete blood picture and peripheral smears.

***Results*:** There were 238(82.1%) female and 52 17.9%) male study participants. The mean age of patients was 45.2±12.8. The mean RDW, NLR and DAS 28 ESR in the study were 45.67±4.25, 2.93±1.35 and 4.29±1.25, respectively. Toxic granulations on peripheral smears were also found in 188(64.8%) patients. A significant positive association was found using the Spearman rho correlation between the DAS28 ESR score, RDW and NLR (*p*-value 0.024 and <0.001, respectively).

***Conclusion*:** Red cell distribution width and neutrophil-lymphocyte ratio, along with toxic granulations, can be used as reliable biomarkers of inflammation in rheumatoid arthritis patients. Values higher than the reference range indicate higher disease severity.

Keywords: Disease activity score (DAS 28 ESR), Neutrophil to lymphocyte ratio (NLR), Red cell distribution width (RDW), Spearman correlation, Toxic granulations.

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INTRODUCTION

Rheumatoid arthritis is one of the most com-mon autoimmune disorders in rheumatology clinics and in the primary care physicians' practice, either as its first presentation or with any of its complications. Untreated rheumatoid arthritis is a disabling disease which affects longevity and quality of life.[1] Erythrocyte sedimentation rate and C-reactive protein are the commonly used para-meters to assess the disease activity in routine.[2] The Disease activity scoring 28 (DAS-28) and the inflammatory markers like ESR and CRP are used as a severity index tool to monitor the disease and modify treatment accordingly.[3,4]

Neutrophils are raised in active RA patients, and they increase the activity of the disease by secreting chemical agents like protein-breaking enzymes, i.e. proteases, reactive oxygen species and prostaglandins in the joint space and also by promoting secretion of different cytokines like B lymphocyte stimulator for immunoglobulin pro-duction, joint destruction causing cytokines like tumour necrosis factor and interleukin 17(IL-17) and other inflammatory cytokines, all of which constitute the inflammatory pathway in RA.[5,6] Dividing the percentage of neutrophils by the percentage of lymphocytes we calculate the NLR in blood samples drawn for complete blood picture .[7,8] NLR a simple yet very effective tool has been previously used for the evaluation of disease activity in many other inflammatory disorders like familial Mediterranean fever (FMF)8, COPD, inflammatory bowel diseases like ulcerative colitis & even malignancies like oesophagal cancers.[9,10]

Our study aimed to assess the association between the red cell distribution width & neutrophil-lymphocyte ratio with the disease severity of RA in our region.

METHODOLOGY

The cross-sectional study was conducted at Federal Government Polyclinic Hospital, Islamabad, from December 2020 to June 2021, after approval from the Ethical Committee (No.FGPC.1/12/2020). The WHO calculator was used to calculate the sample size, taking the overall prevalence of rheumatoid arthritis in the world as 0.5-1%.[9]

Correspondence: Dr Sarah Azam Shah, Department of Rheumatology, Federal Government Polyclinic Hospital, Islamabad Pakistan
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Inclusion Criteria: Rheumatoid arthritis patients of either gender, diagnosed on the EULAR/ACR criteria and on treatment of rheumatoid arthritis were included.

Exclusion Criteria: All the patients who had any of these conditions were not included in the study, i.e., chronic diseases of the kidney, ischemic heart disease, malignancies including haematological and other solid organs, hemolytic and nutritional deficiency anaemias, infections, pregnancy, tuberculosis, sarcoidosis or any other chronic ailments.

Informed written consent was obtained from the participants of the study. The purpose, process and benefits of research were told to all patients included in the study, i.e. the impact of the study on the management of their disease. The research anonymity and confidentiality of the study participant's response was maintained.

The joint examination and assessment for calculating the DAS 28 include examining 28 joints, i.e., small and large joints of the hands (wrists, proximal interphalangeal and metacarpophalangeal joints), elbows, shoulders and knees and excluding the hips, ankles and small joints of the feet were done by the doctor. A complete blood profile was done to see the cell counts and to calculate the NLR and RDW-SD. Toxic granulation was also looked for on peripheral film, as it is also recognized as a marker of the severity of the disease. However, this was only one of our targets. Other laboratory investigations included ESR, which was needed to calculate the DAS-28 score of each participant. The variables used in this study were gender, age, RDW, NLR, ESR, DAS 28 Score, and toxic granulations on the peripheral smear.

DAS 28 ESR is the gold standard in rheumatology to assess the clinical disease severity score. It is classified into the following categories based on the severity. Red cell distribution width measures changes in the size of erythrocytes with degree of inflammation. The normal range is 24.2-42fl according to the local laboratory parameter of our population, and a value >42 would be considered abnormal. The neutrophil to lymphocyte ratio is measured by dividing neutrophil percentage by lymphocytes, and the ratio of 1-3 is considered normal and >3 abnormal.[11]

Statistical Package for Social Sciences (SPSS) version 23.0 was used for the data analysis. Quantitative variables were expressed as Mean±SD and qualitative variables were expressed as frequency & percentages. Chi-square test was applied to explore the inferential statistics. Median values of RDW and NLR were compared with their cut-off values using the Wilcoxon Signed rank Test. Spearman correlation analysis was done to find the significance and strength of the relationship between abnormally distributed data among groups. The *p*-value of ≤ 0.05 was considered statistically significant.

RESULTS

Overall, 290 patients with rheumatoid arthritis were included in the study. Of these, 238 patients (82.1%) were females and 52(17.9%) were males, with a mean age of 45.2±12.8 years. The mean RDW, NLR and DAS28-ESR were 45.67±4.25, 2.93±1.35 and 4.29±1.25, respectively. The presence of toxic granulations on peripheral smear was also found in 188(64.8%) of the study population (Table-I).

Table-I: Baseline Demographics of the study population (n=290)

Variables	Minimum	Maximum	Mean±SD
Age (years)	16	75	45.02±12.5
DAS28-ESR	1.20	8.34	4.29895±1.26
ESR(mm/hr)	5	110	33.75±20.6
WBC (4000-11000/mm3)	3700	16510	8630.48±2342.45
Hemoglobin(g/dl)	9.50	16.60	12.41±1.38
Platelets (150,000-400,000/mm3)	120000	651000	310620.83±99183.17
Red cell distribution width(fl)	35.70	59.10	45.67±4.25
Neutrophils (40-70%)	40.00	87.0	66.84±9.03
Lymphocytes (20-40%)	1.7	48.00	25.96±8.10
Neutrophil lymphocyte ratio	0.91	8.30	2.93±1.35

Table-II: Table showing association between DAS28-ESR and Red cell Distribution Width, Neutrophil Lymphocyte Ratio and Toxic Granulation (n=290)

Baseline Characteristics	DAS28-ESR		*p*-value
	<3.2 Inactive RA (Remission and Low Disease Activity) (n=66)	>3.2 Active RA (Moderate and High Disease Activity) (n=224)	
RDW, n(%)			
<42	18(27.3%)	34(15.2%)	0.024*
>42	48(72.7%)	190(84.8%)	
NLR, n(%)			
<3	55(83.3%)	125(55.8%)	<0.001***
>3	11(16.7%)	99(44.2%)	
Toxic Granulation, n(%)			
Present	15(22.7%)	173(77.2%)	<0.001***
Absent	51(77.3%)	51(22.8%)	

Out of the total study population of 290, 238 (79.8%) had raised RDW value (more than 42), of which 190(65.52%) had moderate to high Disease Activity Scores. Ninety-nine out of 110 patients having NLR above 3 had moderate to high disease activity scores, and 188 out of 238 patients showed the presence of toxic granulations, out of which 173 had moderate to high disease activity. According to the results shown in Table-II, DAS28-ESR was found to be significantly associated with RDW (p<0.05), NLR (p< 0.001) and the presence of toxic granulation (p<0.001).

The results shown in Table-III represent a significant positive correlation of DAS28 RA activity score with both RDW AND NLR with p-values of 0.024 and <0.001, respectively. There was statistically significant difference between RDW and NLR observed values in Rheumatoid Arthritis patients with their cut-off values. The values of RDW were significantly higher than the cut-off values (Median=45.00, IQR=5.13), & the values of NLR were significantly lower (Median=2.67, IQR=1.60) than the cut-off values (p<0.001) (Table-IV).

Table-III: Table showing correlation of DAS28-ESR with Red cell Distribution Width and Neutrophil Lymphocyte Ratio (n=290)

Factors		Spearman's Rho Correlation Co-efficient (r) and p-value
Neutrophil lymphocyte Ratio	Spearman's rho Correlation Co-efficient	0.238
	p-value	<0.001
Red cell distribution width(fl)	Spearman's rho Correlation Co-efficient	0.132
	p-value	0.024
DAS 28 ESR	Spearman's rho correlation coefficient	1.00
	p-value	-

Table-IV: Difference of Red Cell Distribution Width and Neutrophil Lymphocyte Ratio with their cut-off values (n=290)

Parameters	Median (IQR) (n=290)	Cut-off Value	p-value
Red cell Distribution Width	45.00 (5.13)	42	<0.001
Neutrophil Lymphocyte Ratio	2.67 (1.60)	3	

DISCUSSION

In our study we found out that out of the 290 rheumatoid arthritis patients, 9% were in remission of disease, 10% had low disease activity, 25.2% had moderate activity while 55.9% had high disease activity based on the DAS28 score. 82.1% of all the RA patients had an RDW> 42, while 17.9% had normal RDW, suggesting that overall, the RDW in RA patients is high, as was proven in other studies in the literature. The mean NLR in the remission and low disease activity group was 2.25±1.2 while that in the moderate and high disease activity group was 3.13±1.32, which was statistically significant. Rheumatoid arthritis (RA) is a group of autoimmune disorders of systemic inflammation involving synovial joints.[11,12] Al-Rawi *et al*. in Iraq in 2018 observed that RDW was raised significantly in patients with RA (14.5±2.8%) compared to controls (12.4±1.1 %).[13] A study done in Mexico in 2019 evaluated 699 patients with different rheumatological diseases, and it was found that in patients with joint pain, RDW was a good and simple tool to differentiate between different etiologies of diseases of the joints, i.e., inflammatory vs inflammatory. Hence, RDW can be utilized as a surrogate to CRP in such cases.[14] Similar findings were observed in a study done by Lin *et al*.[15]

We found a positive and significant association between the rheumatoid arthritis activity and the variations in the RDW values, with a sensitivity of 79.8% for RDW and 90.4% for ESR, suggesting that RDW can be used as a surrogate marker for ESR. Results from our study are consistent with the literature. Yunchun *et al*. in China found that RDW value was raised in those with high activity of disease, i.e., RA with erosions, than in those who had inactive disease with low scores on DAS 28 score (16.5±3.2 vs 13.9±1.5%, p<0.01).[16] In yet another study done by He *et al*., the value of RDW was found to be raised in patients with RA who had active disease and correlated with the cytokines released in inflammation in RA patients compared to those who did not have rheumatoid arthritis in control gorup.[17] Tecer *et al*. also supported the fact that RA patients had a higher RDW and that RDW was equivalent to ESR and CRP to suggest inflammation.[18] In our study, RDW correlated with DAS28, i.e., RDW >42 was observed in 238(82%) of the study population and out of the 238, 190(79.8%) had higher DAS activity, thus supporting a positive correlation of the RDW and DAS28 with a p-value of 0.024 on spearman rho correlation.

Neutrophils are increased in infections and inflammation, a higher number is related to poor outcomes, and the mortality rate is increased.[19,20] A review of literature from our region showed that a study done by Chandrashekara et al. in India in 2017 showed that

NLR is a very simple, inexpensive and easy tool for assessment of the severity of inflammatory process and, thus, the activity of the disease in rheumatoid arthritis.[21] A previous study found that NLR was correlated with disease activity, i.e., patients who had a greater DAS 28 score had an NLR of 3.27±2.81 while NLR was 2.3±0.84 in those patients who had the low activity of disease or remission in accordance with DAS 28 score (p=0.05).[22]

In our study, we also assessed the presence of toxic granulations on the peripheral smears in patients having rheumatoid arthritis and its association with the disease activity was evaluated, which was found to have 92% sensitivity as a marker of active disease; hence, this study can be used as a benchmark study in this regard in literature.

LIMITATIONS OF STUDY

The patients' data was collected on a time visit. However, future studies can be done by observing the variation in RDW and NLR by modifying treatment and assessing the response at an interval. Other parameters like the PLR and MPV, which had also been shown in a few other studies to correlate with disease activities, were not included, although they can be used in future studies.

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CONCLUSION

We conclude that NLR and RDW are useful biomarkers to determine and estimate the inflammatory activity in rheumatoid arthritis and can be used as surrogate markers to conventional inflammatory markers like ESR while assessing the disease activity. The NLR and RDW had higher values in those with high disease activity.

Authors Contribution

Conflict of Interest: None.

Following authors have made substantial contributions to the manuscript as under:

SAS: & TK: Study design, drafting the manuscript, data interpretation, critical review, approval of the final version to be published.

KI: & SS: Concpet, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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