

## Research Article

## Neutrophil CD64 expression in radiographic axial spondyloarthritis activity; is it valuable?

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DOI: 10.21608/MJMR.2023.207247.1380

### Abstract

**Background:** In a number of inflammatory diseases, neutrophils that express CD64 may be utilized as a biomarker of inflammation. **This research examined** individuals with radiographic axial spondyloarthritis (axial SPA) for neutrophil CD64 expression as a possible indicator of disease activity. **Methods:** There were 32 patients with radiographic axial SPA who were matched for age and gender with 15 healthy controls. The Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and neutrophil CD64 expression were evaluated. The patients with radiographic axial SPA were then separated into two groups based on their BASDAI scores: those with active disease (BASDAI > 4) and those with inactive disease (BASDAI < 4). Analysis was done on the expression of neutrophil CD64, ESR, CRP, and disease activity. **Results:** Active radiographic axial SPA patients, inactive radiographic axial SPA patients, and controls did not significantly vary in neutrophil CD64 expression. Between patients with active and inactive radiographic axial SPA, there is a statistically significant difference in ESR and CRP (P0.0001, 0.001, respectively). **Conclusion:** neutrophil CD64 expression is not a useful diagnostic for assessing disease activity in radiographic axial SPA, which is a conclusion that can be drawn from the data.

**Keywords:** Axial SPA, neutrophil CD64 expression, and BASDAI

### Introduction

Braun and Sieper (2007) state that the most common subtype of spondyloarthropathies is radiographic axial SPA.

The primary sites of involvement for radiographic axial SPA as a rheumatic condition, are the axial skeleton and the sacroiliac joints. It is a chronic, inflammatory, persistent illness. Enthesopathy, anterior uveitis, and oligoarthritis of the hips and shoulders are examples of common disorders that may proceed to generate significant functional limits that lower quality of life, according to Bascherini et al., (2017).

The Bath Ankylosing Spondylitis Illness Activity Index (BASDAI), a patient-based questionnaire, is often used to assess the activity

of radiographic axial SPA illness (Garrett et al., 1994)

Only about 60% of clinically active radiographic axial SPA patients had elevated levels of C-reactive protein (CRP) or erythrocyte sedimentation ratio (ESR), according to Poddubnyy et al., (2014).

Few circulating polymorphonuclear leukocytes (PMN) carry CD64 on their surface, despite the fact that neutrophil CD64 expression surges within four to six hours in response to microbial wall components complement split products, and a variety of proinflammatory cytokines. Qureshi et al., (2001); Simms and D'Amico (1994); Hoffmeyer et al., (1997).

Mediators that are crucial in both infection and

autoimmune inflammation causes an increase in neutrophil CD64. Allen and others in 2002

The intensity of neutrophil CD64 positivity in the absence of infection may be due to poorly defined responses to trauma or an inflammatory insult; these non-infectious stimuli may be to blame for the high neutrophil CD64. (2002) Qureshi and company.

Comparing active autoimmune disease to non-inflammatory sickness and healthy controls, active autoimmune disease exhibited a greater incidence of CD64 positive PMN. Urete et al., (2005); Allen et al.,

The two frequent markers of an acute phase response (ESR and CRP) have been used for assessment, however they may not always reflect the patient's symptoms or how the radiological picture developed. It is challenging and complex to clinically assess disease activity and therapy response in radiographic axial SPA. Finding a new monitoring marker that can detect disease activity is crucial. In radiographic axial SPA patients, the expression of neutrophil CD64 during activity may be advantageous.

### Patients and Methods

32 radiographic axial SPA patients 12 males and 20 female their ages ranged from (18– 49) years old who were matched with 15 healthy participants 4 males and 11 females their ages ranged from (22 – 41) years and attending the rheumatology outpatient clinic of the rheumatology, rehabilitation, and physical medicine department of Minia University hospital was included in the current study using modified 1984 New York criteria (van der Linden et al., 1984). Between March 22, 2021, and April 1, 2023, the investigation was conducted. Before participating in the research, everyone was required to sign a written permission form, and each patient was informed of the examination procedures. The Minia University Postgraduate Studies and Research Ethical Committee approved the research with permission number 15/2021.

And exclude the patients with history of any orthopedic or medical condition that may cause chronic back pain other than radiographic axial

SPA patients (i.e. spine fractures, spinal infection, prior back surgery, history of trauma or malignancy), patients age < 16 years, other seronegative SPA (e.g., psoriatic arthritis, enteropathic arthritis, reactive arthritis), malignancy, other rheumatic diseases (e.g. rheumatoid arthritis/SLE/scleroderma/.....), and Presence of infectious disease or inflammatory bowel disease.

The Bath Ankylosing Spondylitis Disease Activity Index score was used to measure activity of disease. Additionally, ESR and CRP laboratory findings were gathered, and Neutrophil CD64 expression was assessed using flow cytometry. An in vitro diagnostic test kit (Leuko64, Trillium Diagnostics, Brewer, Maine) has been approved by the CE for use in clinical settings. According to Davis et al., (1995), this test enables an efficient internal quality control. Leuko64™ kit, a whole-blood lysed no-wash method with internal calibration beads for quantification, was used to measure CD64 expression on neutrophil granulocytes after blood samples (50 µl EDTA-anticoagulated blood) were collected. Data analysis was carried out using the unique CD64 program. This kit uses fluorescein-labeled calibration beads to report leukocyte expression of CD64 and CD163 as an index, according to Qureshi et al., (2001).

Erythrocyte sedimentation rate (ESR) and CRP were also identified as active inflammatory indicators (by ELISA). Plain X-rays for sacroiliac joints and lumbarsacral spine.

Two groups of patients were created based on their BASDAI scores. Patients with scores of four or higher were considered to be demonstrating active disease, whilst those with scores of four or below were considered to be displaying inactive or moderate disease activity. The BASDAI is a composite measure on a scale of 0 (no symptoms) to 10 (the most severe symptoms). It is a patient-based questionnaire that asks about symptoms such as morning stiffness, widespread body pains, peripheral joints pain, enthesitis. Scores on the BASDAI scale that are equal to or higher than the cut-off point of 4 indicate that the illness is more active, the range of BASDI (0-3.5) in the inactive group, and the range of BASDI (4-9.5) in the active group.

### Statistical analysis

With the help of the SPSS application for Windows, version 24, data were statistically analyzed. The p value—considered significant at 0.05—showed the statistical difference between the groups. Normality of the data was tested using the Kolmogorov-Smirnov test, Data were expressed as median (IQR) for non-parametric quantitative data, in addition to both number and percentage for qualitative data, Kruskal Wallis test was done for non-parametric quantitative data between the three groups followed by Mann Whitney test between each two groups, the Chi square test or Fisher's exact test were used to compare categorical variables, spearman's rank correlation was done for non-parametric data, a p-value less than 0.05 was considered significant.

### Results

There is no significant difference in neutrophil CD64 expression between active and inactive radiographic axial SPA patients and between active, inactive radiographic axial SPA patients and controls.

There is statistically significant difference in ESR and CRP between active and inactive radiographic axial SPA patients ( $P < 0.009$ ,  $< 0.033$ ) respectively, between active group and control ( $P < 0.0001$ ) and between three groups ( $p < 0.001$ ,  $< 0.001$ ) respectively, while there was statistically significant difference between active patients and control regarding ESR only ( $p < 0.038$ ).

**Table 1: comparison between group I, group II and group III regarding Neutrophil CD64 expression, ESR and CRP:**

	Inactive group I N= 15	Active group II N= 17	Control group III N= 15	P value			
	Median (IQR) Mean $\pm$ SD (Range)	Median (IQR) Mean $\pm$ SD (Range)	Median (IQR) Mean $\pm$ SD (Range)	Within 3 groups	I vs II	I vs III	II vs III
Neutrophil CD64 expression %	17.0 (13.2 – 30.5) $28.6 \pm 24.7$	28.2 (14.8– 33.5) $25.9 \pm 10.2$	19.0 (13.8 – 29.7) $21.6 \pm 9.7$	0.515			
ESR	14.0 (13.0 – 15.0) $13.8 \pm 1.2$	54.0 (16.0– 80.0) $51.4 \pm 29.9$	10 (5.0 – 23.0) $15.1 \pm 14.6$	$< 0.0001^*$	0.009*	0.038*	$< 0.0001^*$
CRP	0 (0 – 8.0) $3.6 \pm 4.6$	6.0 (5.0 – 29.5) $18.8 \pm 25.9$	1.0 (0 – 4.0) $2.4 \pm 3.4$	$< 0.001^*$	0.033*	$> 0.99$	$< 0.0001^*$

- Kruskal Wallis test for non-parametric quantitative data between three groups followed by Mann-Whitney between each two groups.

- \*: Significant level at p value  $< 0.05$

ESR: Erythrocyte sedimentation rate

CRP: C reactive protein

### Discussion

The main results of this study show that, although there was no significant difference in CD64 expression between radiographic axial SPA patients and healthy controls, ESR& CRP were significantly higher in active radiographic axial SPA patients compared to inactive disease

patients and active disease patients compared to healthy controls and between 3 groups.

The demographic data from the study's patients did not reveal any significant variations from those of the healthy control volunteers. This demonstrated the good matching between the

patients and controls and the limited impact of any possible confounding factors on the findings.

Our research focused on neutrophil CD64 expression and radiographic axial SPA activity. We observed that there were no discernible changes between the active and inactive groups in either radiographic axial SPA patients or healthy controls.

The presence of disorders like Behçet's Disease, however, has been shown to significantly boost neutrophil CD64 expression in several investigations on various autoimmune inflammatory illnesses (Ureten et al., 2005).

A different investigation on the systemic inflammatory response syndrome (SIRS) found that people with SIRS also had higher than average frequencies of PMNs bearing CD64 and greater levels of CD64 molecules expressed on their PMN surfaces. Given that sepsis predominated among the subjects of these investigations who had SIRS (Qureshi et al., 2001).

There were some limitations to this study. This was a cross-sectional case-control study in a single centre, and the sample was smaller than samples used in previous studies, which did not permit the possible effects of treatment on the CD64 expression not considered due to incomplete or unavailable treatment records.

## Conclusion

The neutrophil CD64 expression there was no difference between the active and inactive groups. Even though there was no statistically significant difference in neutrophil CD64 expression between radiographic axial SPA patients.

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