STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1	The utility of NLR determined at initial diagnosis in predicting disease stage and discriminating between active and stable disease in patients with sarcoidosis : Cross-sectional study.
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Abstract Section	Objective: To evaluate the utility of neutrophil-lymphocyte ratio (NLR) determined at initial diagnosis in predicting advanced disease stage and discriminating between active and stable disease in sarcoidosis Methods: A total of 465 patients with biopsy-proven sarcoidosis (age: 47 years, 70.5% females) were included in this retrospective cross-sectional study. Data on patient demographics, sarcoidosis stage, clinical status (stable and active), anti-inflammatory treatments, complete blood count, and inflammatory markers including erythrocyte sedimentation rate (ESR), C-

reactive protein (CRP), neutrophil-to-lymphocyte ratio (NLR) and platelet/mean platelet volume (MPV) ratio were recorded. NLR values were compared by subgrouping the patients according to the stage of sarcoidosis and clinical status, while the receiver operating characteristics (ROC) curve was plotted to determine the role of NLR in the identification of disease activity with the calculation of area under the curve (AUC) and cut-off value via ROC analysis. Results: Overall, active, and stable disease was evident in 36 (7.8%) and 427 (92.2%) patients, respectively. Median NLR values were significantly higher in patients with active disease compared with stable disease (3.31 (2.34-4.31) vs. 2.29 (1.67- 3.23), p=0.005). Advanced sarcoidosis stage was associated with significantly higher NLR values at stages 0, I, II, III and IV, respectively (p=0.001). ROC analysis revealed a NLR cut-off value of ≥2.39 (AUC (95% CI):

				0.70(0.62 - 0.79), p<0.001) to discriminate between active and stable clinic with a sensitivity of 72.0% and specificity of 52.0%. The significantly higher percentage of patients with active vs. stable disease had NLR values ≥ 2.39 (74.0 vs. 47.0%, p=0.002). Conclusion: Our findings indicate the potential utility of on-admission NLR values to predict the risk of advanced disease stage and to discriminate between active and stable disease in sarcoidosis. Measured via a simple, readily available, and low-cost test, NLR seems to be a valuable marker for monitoring disease activity and progression.
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	1	Sarcoidosis, an inflammatory granulomatous disease of unknown etiology, is characterized by pulmonary involvement in most cases along with a variable clinical course and unpredictable natural history and prognosis (1-3).
Objectives	3	State specific objectives, including any prespecified hypotheses	1-2	Although progression to fibrosis

and risk of permanent organ impairment is evident in one-third of patients (4,5), sarcoidosis has no specific treatment modality or pathognomic markers of clinical outcome due to its unknown etiology (1,5-8). Hence, identification of potential markers for disease activity and progression is considered critical for better management of these patients (1,5,6,9,10). Neutrophil-lymphocyte ratio (NLR) has been recently emerged as a cost-effective and practical inflammatory marker with the diagnostic and prognostic value shown in several respiratory and cardiac diseases (11-15). In accordance with consideration of sarcoidosis as a systemic inflammatory disease with the formation of granulomas in the affected organs (1,5,11), the increase of NLR in sarcoidosis compared with the control group has also been demonstrated by several studies (7,10,16,17). However, while a need for

				objective disease-specific biomarkers that can predict clinical course, severity, and prognosis of sarcoidosis has long been recognized (4,7,9), the relation of NLR with severity or progression of sarcoidosis remains unknown since limited data are available on the role of NLR in the monitoring of sarcoidosis patients (6,7,10,11,16,18-20). This retrospective study was therefore designed to evaluate the utility of NLR determined at initial diagnosis in predicting advanced disease stage and discriminating between active and stable disease in patients with biopsy-proven sarcoidosis.
Methods				
Study design	4	Present key elements of study design early in the paper	2	Retrospective , Cross-sectional study
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	2-3	Study population Of 1198 patients followed up with the diagnosis of sarcoidosis (ICD-10-D86 and subcodes) at Sarcoidosis out patient clinic in tertiary care chest diseases hospital between January 2016 and July 2017, 465 patients with

biopsy-proven sarcoidosis (mean(SD) age: 47.0(12.0) years, 70.5% were females) were included in this retrospective cross-sectional study. Age over 18 years, presence of histopathological diagnosis, and complete blood count findings on initial admission were the inclusion criteria of the study. Patients with co-morbid silicosis, tuberculosis, malignancy, or rheumatologic disease were excluded from the study.

EXPOSURE: NLR values obtained during initial diagnosis of sarcoidosis.

No follow-up.

Data collection:

Data on patient demographics (age, gender), sarcoidosis stage (0-IV), clinical status (stable disease and active disease), anti-inflammatory treatments, complete blood count (CBC), and inflammatory markers including erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), neutrophil-to-lymphocyte ratio (NLR) and platelet/mean

platelet volume (MPV) ratio were retrieved from hospital information system (HIS). NLR values were compared by subgrouping the patients according to the stage of sarcoidosis and clinical status, while receiver operating characteristics (ROC) curve was plotted to determine the role of NLR in the identification of disease activity with the calculation of area under the curve (AUC) and cut-off value via ROC analysis.

Active disease was considered for patients with an increase in dyspnea from clinical complaints on admission, and patients with radiological progression. Stable disease was defined as the absence of any of the findings explained above, regardless of whether the patient received treatment.

Hematological analysis

CBC analysis was performed via the method of flow cytometry (Beckman Coulter LH 780 Analyzer; Beckman Coulter Inc., Miami, FL, USA). Serum CRP levels were

				determined by the turbidimetric method (Toshiba ACCUTE TBA-40FR; Toshiba Medical Systems, Tokyo, Japan). NLR was calculated as the ratio of neutrophil to lymphocyte counts, and the ratio of the platelet count to MPV was also calculated.
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	2-3	Cross-sectional: Of 1198 patients followed up with the diagnosis of sarcoidosis (ICD-10-D86 and subcodes) at our clinic between January 2016 and July 2017, 465 patients with biopsy-proven sarcoidosis (mean(SD) age: 47.0(12.0) years, 70.5% were females) were included in this retrospective cross-sectional study. Age over 18 years, presence of histopathological diagnosis, and complete blood count findings on initial admission were the inclusion criteria of the study. Patients with co-morbid silicosis, tuberculosis, malignancy, or rheumatologic disease were excluded from the study. Study was also summarized in figure 1(flowchart).

		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case		

Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	2-3	Exposure: NLR
				Out come:
				  1. Discrimination between active and stable disease in patients with sarcoidosis
				  2. Predicting disease stage
				Confounder: Age, gender.
				Biopsy proven Sarcoidosis cases were included in study.

Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*2-3*	*Data on patient demographics (age, gender), sarcoidosis stage (0-IV), clinical status (stable disease and active disease), anti-inflammatory treatments, complete blood count (CBC), and inflammatory markers including erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), neutrophil-to-lymphocyte ratio (NLR) and platelet/mean platelet volume (MPV) ratio were retrieved from hospital information system (HIS). NLR values were compared by*

subgrouping the patients according to the stage of sarcoidosis and clinical status, while receiver operating characteristics (ROC) curve was plotted to determine the role of NLR in the identification of disease activity with the calculation of area under the curve (AUC) and cut-off value via ROC analysis.

Active disease was considered for patients with an increase in dyspnea from clinical complaints on admission, and patients with radiological progression. Stable disease was defined as the absence of any of the findings explained above, regardless of whether the patient received treatment.

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			calculated as the ratio of neutrophil to lymphocyte counts, and the ratio of the platelet count to MPV was also calculated.
			Statistical analysis
			Statistical analysis was made using IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY). Fisher's exact test and Pearson chi-square analysis were performed for categorical variables. Mann-Whitney U test was used to analyze non-normally distributed numerical data, while Student t-test was used for normally distributed data. ROC curve was plotted to determine the role of NLR in the identification of disease activity with the calculation of AUC and cut-off value via ROC analysis. Data were expressed as "mean (standard deviation; SD)", "n (%)" and "median (minimum and maximum)" values, where appropriate. p<0.05 was considered statistically significant.
Bias	9	Describe any efforts to address potential sources of bias	Data was obtained electronically from hospital data base system.

				Restrospective, cross-sectional study.
Study size	10	Explain how the study size was arrived at	2	Of 1198 patients followed up with the diagnosis of sarcoidosis (ICD-10-D86 and subcodes) at our clinic between January 2016 and July 2017, 465 patients with biopsy-proven sarcoidosis (mean(SD) age: 47.0(12.0) years, 70.5% were females) were included in this retrospective cross-sectional study

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Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	2-3	Patients group according to diseases state as active or stable (active disease was considered for patients with an increase in dyspnea from clinical complaints on admission, and patients with radiological progression. Stable disease was defined as the absence of any of the findings explained above, regardless of whether the patient received treatment) and Sarcoidosis stage (according to chest X ray)
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	2-3	Statistical analysis was made using IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY). Fisher's exact test and Pearson chi-square analysis were performed for categorical variables. Mann-Whitney U test was used to analyze non-normally distributed numerical data, while Student t-test was used for normally distributed data. ROC curve was plotted to determine the role of NLR in the identification of disease activity with the calculation of AUC and cut-off value via ROC analysis. Data were expressed as "mean (standard deviation; SD)", "n (%)" and "median (minimum and maximum)" values, where

				appropriate. p<0.05 was considered statistically significant. Age , gender was determined.
		(*b*) Describe any methods used to examine subgroups and interactions		NA
		(*c*) Explain how missing data were addressed	2-3	No missing data , retrospective study designed. Patients without complete blood count were excluded.
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy		Consecutive sampling. Retrospective cross sectional.
		(*e*) Describe any sensitivity analyses	3	ROC curve was plotted to determine the role of NLR in the identification of disease activity with the calculation of AUC and cut-off value via ROC analysis
Results				
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Figure 1	Flow chart (Consort diagram) was done.
		(b) Give reasons for non-participation at each stage		NA
		(c) Consider use of a flow diagram		
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders		NA
		(b) Indicate number of participants with missing data for each variable of interest		NA
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)		
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time		
		Case-control study—Report numbers in each exposure category, or summary measures of exposure		
		Cross-sectional study—Report numbers of outcome events or summary measures	*3-4*	*Demographic characteristics and disease activity* *The mean patient age was 47.0(SD*

*12.0) years, and 328(70.5%)
patients were female patients.
Overall, active and stable disease
was evident in 36 (7.8%) and 427
(92.2%) patients, respectively.
CBC findings and inflammatory
markers according to disease
activity
Active vs. stable disease was
associated with significantly higher
leukocyte (median(min-max) 8.16 (
6.70- 11.6) vs. 6.70 (5.80- 8.00) 109
/ L, p=0.005), neutrophil (5.25
(4.30- 8.85) vs. 4.00 (3.30- 5.00)
109 / L, p=0.001) and monocyte
(0.63 (0.50- 0.90) vs. 0.53 (0.43-
0.70)%, p=0.016) counts, RBC
levels (4.98(4.80-5.50) vs. 4.85
(4.52-5.18) 109 / L, p=0.005) and
RDW (14.70 (13.70-16.05) vs.
14.07 (13.40- 15.00), p=0.035),
whereas with lower eosinophil
counts (1.56 (0.45- 2.89) vs. 2.39
(1.50- 3.80)%, p=0.008) (Table 1).
Median(min-max) NLR values were
significantly higher in patients with
active disease compared to those
with stable disease (3.31 (2.34-
4.31) vs. 2.29 (1.67- 3.23),
p=0.005) (Table 1).
CBC findings and inflammatory
markers according to the stage of*

				sarcoidosis
				Advanced sarcoidosis stage was associated with significant increase in serum leukocyte (p=0.024), neutrophil (p=0.005), monocyte (p=0.002), CRP (p=0.026) levels (Table 2).
				Advanced disease stage was also associated with significantly higher NLR values (median (min-max) 1.95(1.58-2.59), 2.27(1.65-3.26), 2.56(1.84-3.73), 2.29(1.83-3.81) and 4.83(2.94-6.71) at stages 0, I, II, III and IV, respectively, p=0.001) (Table 2).
				ROC analysis
				ROC analysis revealed a NLR cut-off value of ≥2.39 (AUC (95% CI): 0.70(0.62 - 0.79), p<0.001) to discriminate between active and stable clinical status with a sensitivity of 72.0% and specificity of 52.0% (Figure 2).
				Significantly higher percentage of patients with active vs. stable disease had NLR values ≥ 2.39 (74.0 vs. 47.0%, p=0.002) (Table 3).
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included		NA
		(*b*) Report category boundaries when continuous variables were categorized	4	Active vs. stable disease was

associated with significantly higher leukocyte (median(min-max) 8.16 (6.70- 11.6) vs. 6.70 (5.80- 8.00) 109 / L, p=0.005), neutrophil (5.25 (4.30- 8.85) vs. 4.00 (3.30- 5.00) 109 / L, p=0.001) and monocyte (0.63 (0.50- 0.90) vs. 0.53 (0.43-0.70)%, p=0.016) counts, RBC levels (4.98(4.80-5.50) vs. 4.85 (4.52-5.18) 109 / L, p=0.005) and RDW (14.70 (13.70-16.05) vs. 14.07 (13.40- 15.00), p=0.035), whereas with lower eosinophil counts (1.56 (0.45- 2.89) vs. 2.39 (1.50- 3.80)%, p=0.008) (Table 1). Median(min-max) NLR values were significantly higher in patients with active disease compared to those with stable disease (3.31 (2.34- 4.31) vs. 2.29 (1.67- 3.23), p=0.005) (Table 1).

CBC findings and inflammatory markers according to the stage of sarcoidosis

Advanced sarcoidosis stage was associated with significant increase in serum leukocyte (p=0.024), neutrophil (p=0.005), monocyte (p=0.002), CRP (p=0.026) levels (Table 2).

Advanced disease stage was also associated with significantly higher

		NLR values (median (min-max) 1.95(1.58-2.59), 2.27(1.65-3.26), 2.56(1.84-3.73), 2.29(1.83-3.81) and 4.83(2.94-6.71) at stages 0, I, II, III and IV, respectively, p=0.001) (Table 2). ROC analysis ROC analysis revealed a NLR cut-off value of ≥2.39 (AUC (95% CI): 0.70(0.62 - 0.79), p<0.001) to discriminate between active and stable clinical status with a sensitivity of 72.0% and specificity of 52.0% (Figure 2). Significantly higher percentage of patients with active vs. stable disease had NLR values ≥ 2.39 (74.0 vs. 47.0%, p=0.002) (Table 3).
	(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA, Cross-sectional

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Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses		NA

Discussion

Key results	18	Summarise key results with reference to study objectives	5	Our findings revealed the association of higher NLR values detected on initial admission with a more advanced disease stage and an active clinical status in patients with biopsy-proven sarcoidosis, while a NLR cut-off value of $\geq$2.39 (72.0% sensitivity and 52.0% specificity) was determined to discriminate between active and stable disease.
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	8	The major limitations of this study seem to be the retrospective single-center design and relatively low sample size due to the rarity of the disease, which prevents generalization of our findings to the overall sarcoidosis patient population
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	5-8	Although elevated NLR is an expected and previously demonstrated finding in patients with a systemic inflammatory disease such as sarcoidosis when compared to control subjects (7,10,16,17), our findings indicate the likelihood of further elevation of NLR ($\geq$2.39) to signify an increased risk of advanced stage and active clinical state in

sarcoidosis patients. Likewise, in a past study among sarcoidosis patients, NLR values were reported to be significantly higher in those with parenchymal involvement (stage 2,3,4) compared to those without parenchymal involvement (stage 0,1), while a NLR cut-off value of 2.4 (87% sensitivity and 58% specificity) was shown to discriminate between advanced and milder disease stage (17). In addition, in the past a study with 116 sarcoidosis patients and 56 healthy individuals, a NLR cut-off value of ≥2 (80% sensitivity and 59% specificity) was reported to discriminate between sarcoidosis patients and healthy controls, along with an increased likelihood of higher NLR values in patients with extrapulmonary involvement (10). In a past study with 122 sarcoidosis patients, NLR was reported to significantly differ with respect to radiological stages (mean 1.28 in stage 0 and 8.48 in stage 4) and parenchymal involvement (mean 1.63 and 5.46 in total HRCT score group 1 and group 4, respectively) (7). The authors also noted the association of NLR with more

severe parenchymal involvement and thus its potential role to predict the radiological extent of pulmonary sarcoidosis (7). In a past study with 40 sarcoidosis patients, NLR values in sarcoidosis cases of stages 2,3, and 4 were reported to be significantly higher when compared to the cases of stage 1, while high NLR values were also reported to be significantly correlated with one-year disease progression (20). The authors indicated a NLR cut-off value of 3.20 (80.0% sensitivity and 76.7% specificity) to predict the disease progression in sarcoidosis patients (20).

Hence, the association of high NLR determined at the initial diagnosis with an advanced disease stage, and the active clinical status in our sarcoidosis patients seems notable given that both stage III disease and extra-pulmonary involvement were reported to be associated with the chronic and progressive course and increased likelihood of relapse (21-23).

Nonetheless, in a past study with 75 sarcoidosis patients, NLR values were reported to be significantly

higher at stage-2 and stage-3 than at stage -1 and stage -4, while the authors also noted no significant association of high NLR with pulmonary PH, spontaneous remission, response to treatment or prognosis (6).

We have previously reported in a study with 1300 sarcoidosis patients that 73.0% of patients had NLR $\geq$ 2, while 27.0% had NLR < 2 at the time of presentation to the hospital, along with correlation of NLR values with inflammatory markers such as PLT/MPV, ESR and CRP (19). Similarly, a NLR cut-off value of > 3.5 (sensitivity: 50%, specificity: 78%) was reported to be associated with a more intense inflammatory response and thus increased likelihood of sarcoidosis to be accompanied with pulmonary hypertension, while higher NLR also remained an independent predictor of pulmonary hypertension in multivariate analysis (11). In addition, in a retrospective past study with 50 patients with chronic hypersensitivity pneumonia (HP), 20 patients with acute HP and 70 control subjects, NLR cut-off

values of ≥2.76 and ≥2.15 were reported to discriminate between patients and controls and between acute and chronic HP, respectively (18).

In a retrospective analysis of bronchoalveolar lavage (BAL) samples from the 167 interstitial lung disease (ILD) patients, including those with sarcoidosis, HP, and idiopathic pulmonary fibrosis (IPF), the authors reported a NLR threshold value of 0.48 (73% sensitivity and 63% specificity) to discriminate between sarcoidosis and other ILDs, while NLR was also correlated negatively with forced vital capacity (FVC) and forced expiration volume in 1 second (FEV1) percentages and positively with composite pulmonary index (CPI) score (24).

In another study, the mean NLR value was reported to be higher in tuberculosis cases compared to the sarcoidosis cases, while a NLR cut-off value of ≥2.55 (79% sensitivity and 69% specificity) was reported to discriminate between sarcoidosis and tuberculosis (16).

In the case of HP, NLR was reported to decrease in the chronic

period compared to the acute period, and this was related to the gradual decrease in the granulomas and inflammation in the lung and their replacement by fibrosis (chronic / fibrotic HP) as the disease progresses to chronic HP (18,25). Notably, NLR was also reported to predict the exacerbations and severity of COPD (26), while the increase in NLR was reported to be related to all-cause mortality in acute pulmonary embolism (27). Accordingly, in line with previous studies that indicated the utility of NLR in predicting the risk of parenchymal involvement, radiological extent and progression of disease (7,10,11,17,20), as well as the development of pulmonary hypertension (11) and hypersensitivity pneumonia (18) in sarcoidosis patients, our findings revealed the utility of NLR as a simple readily available and low-cost biomarker in predicting the risk of advanced disease stage and active clinical status in patients with sarcoidosis.

Notably, RDW values were also significantly higher in patients with

			active vs. stable sarcoidosis in the current study, while there was a non-significant tendency for higher RDW values in the case of the advanced disease stage. These findings seem notable given that in the past study with 138 sarcoidosis patients, baseline, and follow-up values of RDW were reported to be significantly higher in patients with stage 4 than other stages, while significant increase in RDW levels from baseline was noted in follow up of patients with progressive disease but not in follow up those with stable disease (28).
Generalisability	21	Discuss the generalisability (external validity) of the study results	Sarcoidosis is rare chest diseases, due to the rarity of the disease, which prevents generalization of our findings to the overall sarcoidosis patient population. However, study was designed in a tertiary care teaching hospital especially established for chest diseases hospital. All over the country from different cities , sarcoidosis cases were referred to our hospital.
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	NA

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.