Accuracy of neutrophil to lymphocyte and monocyte to lymphocyte ratios as new inflammatory markers in acute coronary syndrome

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Accuracy of neutrophil to lymphocyte and monocyte to lymphocyte ratios as new inflammatory markers in acute coronary syndrome

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Abstract

Background: Inflammation plays a key role in the development of atherosclerosis and in the pathogenesis of acute coronary syndrome (ACS). Leukocytes and leukocytes ratios were recognized as inflammatory markers in predicting the presence and severity of ACS.

Methods: This study aimed to investigate the diagnostic accuracy of neutrophil to lymphocyte ratio (NLR) and monocyte to lymphocyte ratio (MLR) with ACS. One hundred patients admitted to the Cardiac Center who were confirmed to have ACS and 100 healthy controls confirmed not to have ACS were enrolled in this study. ECG and troponin I test were used as gold standards to make sure that the participants with or without ACS. Total Wight blood cells (WBCs) count, NLR, and MLR values were estimated.

Results: Total WBC, neutrophil, and monocyte counts were significantly higher while lymphocyte counts were significantly lower in ACS patients than in the healthy controls (p <0.001). NLR and MLR were significantly higher in ACS patients than in the healthy controls (p <0.001). Among all the studied markers, NLR was found to be the strongest predictor of ACS (OR: 3.3, p <0.001), whereas MLR was non-significant (p >0.05). A cut-off value of 2.9 of NLR had 90% sensitivity and 88% specificity while 0.375 cut-off value of MLR had 79% sensitivity, 91% specificity for predicting presence of ACS.

Conclusions*:* NLR is inexpensive, widely available, and simple inflammatory marker that can take a role in the diagnosis of ACS with a cut-off of 2.9 in our population.

Keywords: Acute coronary syndrome, Monocyte to lymphocyte ratio, Neutrophil to lymphocyte ratio, Yemen.

Background

Cardiovascular diseases (CVD) remain the major cause of death worldwide. Atherosclerosis disease, the underlying process that results in ACS, is responsible for a large rate of CVD. In 2016, out of the 17.7 million cardiovascular deaths, about 85% were due to atherosclerosis disease [1]. Inflammation plays a key role in the development of atherosclerosis and in the pathogenesis of ACS. To show this inflammation, numerous markers such as hs-CRP, fibrinogen, and interleukins have been used [2, 3]. Leukocytes are major mediators of inflammation. Therefore, leukocytes and its subtypes were studied as an inflammatory marker in predicting adverse events in patients with ACS [4-6]. Recently, NLR and MLR have emerged as one of the most important novels widely available, inexpensive and robust inflammatory markers which can aid in the prognosis and diagnosis of ACS [3, 7-11]. Diagnosis of ACS is mainly based on Troponin, electrocardiograph (ECG), and other cardiovascular imaging modalities; however, these investigations are expensive and time consuming. Discovering simple markers becomes important in order to help diagnostic accuracy and to provide prognostic information about this disease.

Since it has been hypothesized that the NLR and MLR reflect ongoing inflammation in ACS, this study aimed to investigate the diagnostic accuracy of NLR and MLR with ACS.

Methods

Study design and definition

This study was testing a test case-control study carried out at Al-Thawra General Hospital in Sana'a city from April 2019 to July 2020. It was conducted on 100 patients who admitted to the Cardiac Center and were confirmed to have ACS (patients group) and 100 healthy individuals (control group).

ECG and troponin I test were used as gold standards to make sure that the participants with or without ACS. The ACS group included ST elevated myocardial infarction (STEMI), non-ST elevated myocardial infarction (NSTEMI) and unstable angina (UA) that were defined based on the criteria formulated by updated guidelines [12]. The control group was defined as healthy individuals matching for age and sex and confirmed not to have ACS by the gold standard tests.

Patients with CVD other than ACS, patients aged lower than 18 years, medical conditions or treatments known to affect the WBCs count were excluded from this study. The study was approved by the local Ethics committee at Sana'a University and informed consent of the patients and controls.

Laboratory analysis

Total WBCs, differential WBC count, mean platelet volume (MPV), and red cell distribution width (RDW) were measured by using an automated analyzer (Mindray Medical International Limited, Shenzhen, China). NLR and MLR were calculated from differential WBC count as ratio of neutrophil and monocyte cell counts to lymphocyte cell counts. The PLR was calculated as the ratio of platelets cells count to lymphocyte cells count. Erythrocytes sedimentation rate (ESR) was performed by Westergren method. Body mass index was calculated by taking the body weight in kilograms (kg) and dividing it by the height in meters (m) squared.

The ECG investigation was conducted and read by a cardiologist at the Heart Center of Al-Thawra General Hospital, where we focused on the following; T-wave, Q waves, and ST-segment. Cardiac troponin I was detected in serum by qualitative, membrane-based immune-assay (Cardiac Troponin I Rapid Test Cassette). Biochemical measurements such as CK-MB, CK, and glucose, were obtained from the patients files.

Statistical analysis

For comparing two groups, a student-t-test or Mann-Whitney U test was used for numerical variables while Chi-square test was used for categorical variables. A correlation between the numerical variables was determined by using Pearson's or Spearman correlation test. The strength of an association of the markers with disease presence was assessed using logistic regression. Receiver operating characteristic (ROC) curve analysis was performed to determine accuracy of NLR and MLR for predicting ACS presence.

Results

The study population comprised 200 participants, 100 apparently ACS (60 males and 40 females) and 100 healthy controls. The ages and genders were matched between the two groups.

Total WBCs, neutrophil, and monocytes counts, were significantly higher while lymphocytes count was significantly lower in ACS patients than in the healthy controls (p <0.001). NLR and MLR were significantly higher in the ACS patients than in the healthy controls (p <0.001). Raised levels of PLR, MPV, ESR, and RDW were also found in ACS patients as compared to healthy controls (p <0.05). The hematocrit was lower in ACS patients than in the healthy controls. The frequency of smoking and

the mean of BMI were significantly higher in the ACS patients than control group ($p < 0.05$) (Table 1).

Correlation of NLR and MLR with inflammatory and myocardial infraction markers:

The correlation analysis observed that NLR and MLR markers were positively correlated with each other, inflammatory cells and **myocardial infraction** (MI) markers. Although the correlation was moderate with inflammatory markers and weak with MI markers, it was a statistically significant ($p < 0.05$) (Table 2).

NLR is the independent predictor for the presence of ACS

The strength of association of the NLR and MLR markers with ACS was assessed by multivariate regression analysis and is presented in table 3. After adjustment for covariates in multivariate regression, NLR was found to be the strongest predictor for ACS (OR: 3.34; $p = 0.014$), followed by MPV, RDW, and ESR, whereas MLR and other markers were not significant ($p > 0.05$) (Table 3).

The accuracy of NLR in detecting ACS

The diagnostic accuracy of the NLR and MLR markers for the diagnosis of ACS was investigated by ROC analysis figure 1. Based on ROC curve, NLR marker exhibited a higher area under curve (AUC: 0.941, $p < 0.001$), followed by MLR (AUC: 0.896, $p < 0.001$). The suitable cut-off value of NLR for the diagnosis of ACS was found to be 2.95 with 90% sensitivity, 88% specificity, 89.7% negative predictive value (NPV), and 88.2% positive predictive value (PPV). The suitable cut-off value of MLR for the diagnosis of ACS was found to be 0.375 with 79% sensitivity, 91% specificity 81.2% NPV and 89.7% PPV (figure1).

Discussion

To the best of our knowledge, this is the first study aimed to investigate diagnostic accuracy of NLR and MLR in detecting the presence of ACS in Yemen. We herewith demonstrate that total WBCs, neutrophil and monocytes counts were higher while lymphocyte was lower in ACS patients than in healthy controls. This increase of total WBCs, neutrophil, and monocytes counts in ACS patients may be due to the inflammatory response during atherosclerosis and a probable cause of lymphopenia include decreased production as a result of increased steroid level due to stress and increased apoptosis triggered by increased inflammation thereby resulting in elevated NLR and MLR markers [13, 14].

NLR is an integrated reflection of two dissimilar yet balancing immune pathways and is better predictive of inflammation in ACS than neutrophils or lymphocytes alone [15, 16]. In our study, we demonstrate that ACS patients had significantly higher NLR and MLR compared to healthy controls. This difference is in agreement with previous studies [9, 17, 18]. Other studies also reported that NLR was significantly higher in the troponin-positive than in the troponin-negative patients [7, 8, 19].

NLR and MLR markers were positively correlated with inflammatory cells and MI markers including CK and CK-MB in patients with ACS. This may indicate that these markers may reflect inflammatory response and myocardial injury during atherosclerosis. These findings have been described by several other studies [9, 10, 16, 20, 21].

The sensitivity of NLR in our study is relatively similar to those reported by Yilmaz *et al*, 2015 and Corriere *et al*, 2018 who found that NLR has 93% and 97% sensitivity for predicting presence thrombus and carotid plaques respectively [4, 22]. Their

results support those in our study that stated that NLR has high sensitivity to reflect ongoing inflammation associated with ACS. Thus, we suggest that NLR may be served as a valuable biomarker for the diagnosis of ACS.

Among all studied markers, NLR was found to be the strongest independent predictor of ACS presence. This result is in agreement with two other studies which reported that NLR was a strong independent predictor of ACS in chest pain patients [23, 24]. Other studies also reported that NLR independently predicted troponin positive in chest pain patients [8, 19]. However we achieved greater degree of accuracy (AUC-0.941; $p < 0.001$; sensitivity: 90%; specificity: 88%) as compared to those reported by previous studies. This is because those studies are different from our study in two main aspects: Firstly, unlike the current study, the control groups were not healthy individuals but included patients with chest pain where the inflammatory response is expected to be absent or less prominent in healthy compared to patients with chest pain. This explains why the specificity of NLR was higher in the current study compared to previous studies. Secondly, the higher frequency of STEMI sub-type among ACS group in our study compared to the previous studies where the inflammatory response is expected to be more prominent in STEMI patients compared to those with NSTEMI/UA [25]. This explains why the sensitivity of NLR was higher in the current study compared to those studies.

Compared to NLR, MLR was a non-significant predictor for ACS ($p > 0.05$) and has a lower accuracy for the diagnosis of ACS. There is no previous study so far that discussed the predictive value of MLR in the discrimination of ACS patients, but there is only a study conducted on patients with stable angina and showed that MLR was significantly independent predictor of thin cap fibrous atheroma, with a sensitivity of 73.7% and a specificity of 61.8% [26].

In addition to NLR marker, the MPV, RDW and ESR were also significantly independent predictors of ACS. These results were consistent with results in previous studies [27-30]. Although these studies differ in some of the methodologies from our study, they support our findings that MPV and RDW were independent predictors of ACS in different populations.

Study limitations

The present study must be interpreted within the context of its potential limitations.

- First, this was a single-center disgned study and further comprative multicenter studies will be needed.
- Second, the patients in STEMI sub-group were higher compared with the NSTEMI/UA subgroups thus equal subgroups of ACS are recommended to be enrolled in future studies
- Third, we could not compare NLR and MLR with inflammatory markers (e.g. Hs-CRP) and pro-inflammatory cytokine (e.g. interleukin-6), because they were not evaluated in our study or routinely assessed in our study population.

Conclusions

The results of this study revealed that NLR was the strongest predictive predictor of ACS, so the study recommends using NLR as a simple, inexpensive, and widely available inflammatory marker which can be an auxiliary biomarker in the diagnosis of ACS. Also further large scale and comprehensive studies are highly recommended.

Declarations

Ethics approval and consent to participate

The study was performed after the approval of postgraduate studies and scientific research council of Sana'a University where written consent form was taken from all participants to be included in the study, and they were informed that participation was voluntary and they can be free to withdraw from the research. All methods were carried out in accordance with relevant guidelines and regulation.

Consent for publication

"Not applicable"

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request

Abbreviations

ACS: Acute coronary syndrome; BMI: Body mass index; CHD: Coronary heart disease; CK: Creatine kinase; CK-MB: Creatine kinase myoglobin-binding; CVD: Cardiovascular diseases; ECG: Electrocardiogram; ESR: Erythrocyte sedimentation rate; Hs-CRP: High-sensitivity C-reactive protein; MI: Myocardial infarction; MLR: Monocyte to lymphocyte; MPV: Mean platelet volume; NLR: Neutrophil to lymphocyte ratio; NSTSEMI: Non–ST-segment elevation myocardial infarction; PLR: platelet to lymphocyte ratio; RDW: Red cell distribution width; STSEMI: ST-segment elevation myocardial infarction; UA: Unstable angina; WBC: Total white blood cells; WHO: World health organization.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

ASH and AMO conceived and designed the study. The manuscript was revised by ASH, AMO and AKM. The manuscript was written by ASH and AMO. All authors read and approved the final manuscript.

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Table 1: Comparison of all variables between ACS patients and controls groups

Variables	Patients (n=100)	Controls (n=100)	P Value
Age (years)	55.5 ± 15	54.1± 15	0.516
Genders (males) (%)	60	60	1.00
Smokers (%)	62	45	0.023
BMI (Kg/m^2)	23.9 ± 3.5	21.4 ± 3.1	<0.001
WBC ×10^3/µL	9.6 ± 2.5	6.8 ± 1.9	< 0.001
Neutrophil ×10^3/µL	7.2 ± 2.4	3.8 ± 1.5	< 0.001
Monocyte ×10^3/µL	0.69 ± 0.28	0.47± 0.22	< 0.001
Lymphocyte ×10^3/Ml	1.3 ± 0.50	2.1 ± 0.62	< 0.001
Platelets ×10^3/µL	249 ± 81	263 ± 52	0.152
NLR	6.5 ± 3.0	1.9 ± 0.9	< 0.001
MLR	0.61 ± 0.29	0.23± 0.10	< 0.001
PLR	211 ± 74	131 ± 42	< 0.001
MPV/ fl	9.8 (8.6)	9.6 (5.9)	0.003
ESR (mm/hours)	47 ± 23	17 ± 9	< 0.001
RDW (%)	14.8 (12.5)	13.4 (14.6)	< 0.001
Hematocrit (%)	37.5± 6.7	41.9 ±5.9	< 0.001

ES R: erythrocyte sedimentation rate**, MLR**: monocytes to lymphocyte ratio, **MPV:** mean platelet volume, **NLR:** neutrophil to lymphocyte ratio, **PLR:** platelet to lymphocyte ratio, **RDW:** red cell distribution width, and **WBC:** white blood cell count.

Table 2: Correlation of NLR and MLR with inflammatory and MI markers among ACS patients

Markers	NLR (95% CI)	P value	MLR (95% CI)	P value
NLR	1	< 0.001 [a]	0.616 (0.493 - 0.715)	< 0.001 [a]
PLR	0.536 (0.385-0.649)	< 0.001 [a]	0.427 (0.209 - 0.555)	< 0.001 [a]
WBC	0.464 (0.249 - 0.655)	< 0.001 [a]	0.205 (0.019 - 0.442)	0.041 [a]
Neutrophil	0.622	< 0.001 [a]	0.234	0.019 [a]
Lymphocytes	-0.774	< 0.001 [a]	-0.647	< 0.001 [a]
ESR	0.106	0.296 [a]	0.222	0.027 [a]
CK-MB	0.341 (0.135 - 0.565)	0.001 [b]	0.307 0.104 - 0.493)	0.003 [b]
CK	0.280 (0.033 - 0.512)	0.032 [b]	0.350 0.059-0.554)	0.007 [b]

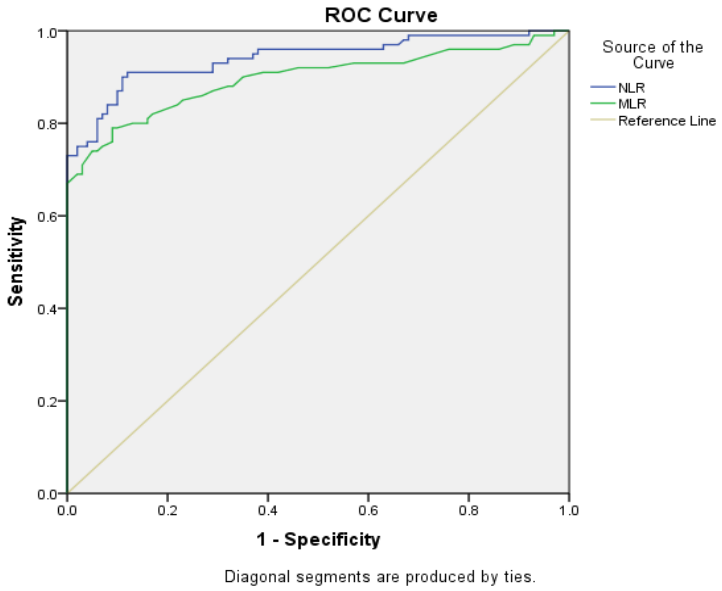
[a] **:** Pearson, [b]**:** Spearman's, **CK:** creatinine kinase، and **CK-MB**: creatinine kinase-myoglobin binding.

Table 3: Multivariate logistic regression analysis for the presence of ACS

Markers	S.E	P Value	Exp (B)	95% CI for Exp (B)	
				Lower	Upper
NLR	0.490	0.014	3.340	1.279	8.722
MPV	0.327	0.006	2.455	1.292	4.664
RDW	0.160	0.040	1.39	1.016	1.901
ESR	0.047	<0.001	1.197	1.090	1.313
MLR	2.679	0.597	4.12		
PLR	0.010	0.313	1.01		
WBC	0.314	0.938	0.976		
Hematocrit	0.066	0.125	1.106		
Smoking	0.740	0.827	1.175		
Constant	8.311	0.001	0.000		

Exp ($_B$): exponentiation of the coefficients/odds ratios of the predictors, CI: confidence interval, **S.E**: sample error

Figure 1: Receiver operating characteristic (ROC) analyzes of NLR and MLR for diagnosis of ACS



Figures

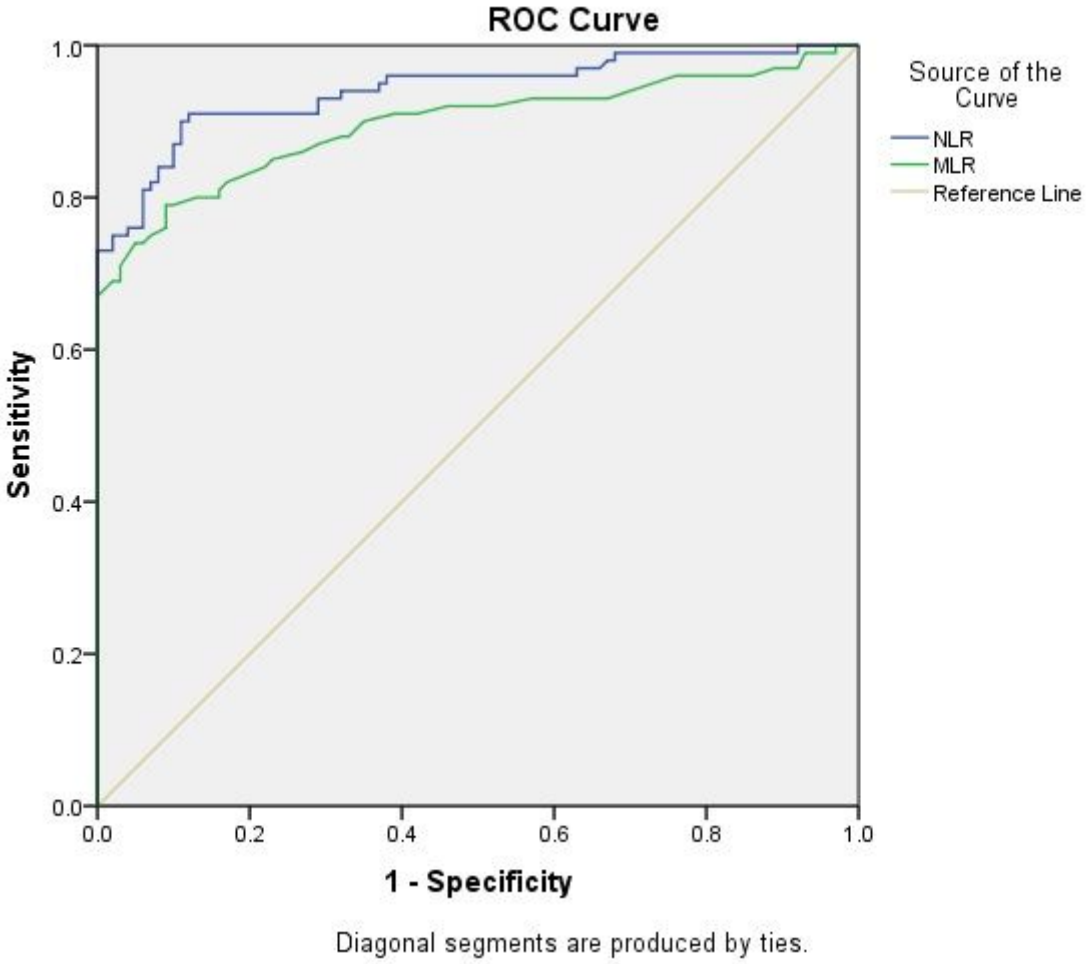


Figure 1

Receiver operating characteristic (ROC) analyzes of NLR and MLR for diagnosis of ACS