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Original Research Article

# Association between Neutrophil to Lymphocyte Ratio and Severity of Coronary Artery Disease

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**Conflict of interest: Nil**

**Abstract**

**Introduction:** Neutrophil/lymphocyte ratio (NLR) as a biomarker that can add predictive value in evaluation of severity of coronary Artery Disease (CAD).

**Objective:** To study the Association between Neutrophil to Lymphocyte Ratio and Severity of Coronary Artery Disease in patients above 18 years of age who will undergo coronary angiography for CAD in large cohort of patients.

**Materials and Methods:** In this single-centre, prospective, observational study, 2000, eligible patients who had undergone coronary angiography for suspected or known coronary atherosclerosis. NLR was calculated from the absolute values of neutrophils and lymphocytes from the complete blood count. The association between NLR and CAD severity was assessed by comparing with SYNTAX score.

**Results:** The study population was divided into 3 groups according to the NLR value (<2, 2-3, and >3). Receiver operating curve (ROC) curve of the NLR for the prediction of severity of CAD w.r.t SYNTAX Score, revealed a cutoff point of 2.48 for the NLR with an AUC of 0.631 (95% CI: 0.54–0.74; P=0.02). An NLR of greater than 2.48 had 53.6% sensitivity and 74.9% specificity in predicting severity of CAD w.r.t SYNTAX Score.

**Conclusion:** NLR is independently associated with CAD severity especially Acute coronary Syndrome cases and provides additive predictive value to conventional risk factors and commonly used biomarkers.

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## Introduction

Atherosclerosis as a complex inflammatory disease with White blood cell count and its subtypes as inflammatory biomarkers to predict adverse cardiovascular outcomes has been studied [1-4] Neutrophil to lymphocyte ratio (NLR) has emerged as new prognostic marker of severity of coronary artery disease in patients with stable and unstable CAD.[4-9]

The two most common scoring system used in various studies to describe the severity of CAD - SYNTAX Score and Gensini Scoring system.[13,14] In the present study, we evaluated the association between NLR and severity of coronary atherosclerosis in patients undergoing coronary angiography using SYNTAX SCORE.[13]

NLR is an inexpensive and easily available marker that provided an additional level of risk indicator in predicting the severity of coronary artery stenosis. After excluding the etiology of infection, inflammation and cancers, physicians should pay more attention to the CAD patients with high NLR levels. [15-21]

There are very less data available with severity of coronary atherosclerosis measured among Indian population and particularly local Odisha population. In a study of western Indian population by Sharma et el in 2017, the NLR showed strong predictive value but it had limited small sample size.[22] With increasing cost of diagnostic procedures in a developing country like India ,

NLR can be a simple, cost effective , feasible ,acceptable modality to predict severity of CAD.

Primary objective was to study the Association between Neutrophil to Lymphocyte Ratio and Severity of Coronary Artery Disease in patients above 18 years of age who will undergo coronary angiography for CAD. Secondary objectives were Study the association of gender, religion, age, diabetes, obesity, family history of CAD, hypertension, smoking/tobacco use, alcohol intake history, ejection fraction, lipid profile, renal function with respect to severity of CAD.

**Materials and Methods**

The present study was conducted from February 2023 to MAY 2024 in a single-centre[ S C B MEDICAL COLLEGE, Cardiology Department], prospective, observational study consisting of 2000, eligible patients who had undergone coronary angiography for suspected or known coronary atherosclerosis as determined with respect to previous studies for adequacy of sample size. All participants were enrolled with an informed consent and the study was approved by Institutional ethics committee.(IEC Clearance Certificate Attached)

Standard Clinical Variables definitions for the study:

Arterial hypertension: Hypertension was considered in patients with repeated (at least 3 times) blood pressure measurements ≥ 140/90mmHg in or active use of antihypertensive drugs. Diabetes mellitus was defined as per American diabetes association guidelines [42]. Smoking was defined as per current smoking status. The family history of CAD was considered as a history of CAD or sudden cardiac death in a first degree relative before the age of 55 years for men and 65 years for women.

Acute coronary syndrome (ACS) and Chronic Coronary Syndrome (CCS), Stable angina was defined as presentation with symptoms of ischemia in association with qualifying electrocardiographic changes or positive cardiac enzymes as per AHA/ESC Guidelines. [43-46 ]

Biochemical and Haematological Parameters: Peripheral venous blood samples was drawn after patients overnight fasting or at time of Hospitalization. Total and differential leukocyte counts was measured by an automated haematology analyser (SYSMEX XN 1000 Automated Haematology Analyzer 6 Part; serial number-47927, Regional Diagnostic Centre, SCB MCH) used for majority of patients of our institution. Absolute cell counts was used in the analyses. NLR values was calculated as ratio of Absolute Neutrophil Count to Absolute Lymphocyte Count. Biochemical parameters like Total and high-density lipoprotein (HDL) and LOW Density Lipoprotein (LDL) cholesterol, triglycerides, and fasting glucose etc. was measured (using the CANON TBA 120FR Pearl, Automated clinical chemistry analyser; Regional Diagnostic Centre, SCB MCH).Serum electrolytes was measured using HDC LYTE Electrolyte analyser Regional Diagnostic Centre, SCB MCH

Echocardiography for Left Ventricular Ejection Fraction (LVEF):

LVEF was measured by biplane method using-General Electric (GE) VIVID T 8 Echocardiography USG Machine Or Philips EPIQ CVX CV 350 Ultrasound Machine For Echocardiography, AT ECHO LAB, Department of Cardiology , SCBMCH

Coronary Angiography and Severity of Coronary Atherosclerosis: Coronary angiography was performed through femoral artery/radial artery access using single plane PHILIPS AZURION 7 M20 (Philips, Amsterdam, The Netherlands) Angiographic digital X- Ray system, Department Of Cardiology, SCBMCH. CAD was defined as the presence of at least 50% luminal stenosis of the vessel diameter, in any of the major epicardial coronary arteries, in accordance with the American College of Cardiology/American Heart Association lesion classification.[11] SYNTAX SCORING (I) of coronary artery disease was used to classify severity as per figures and table below .[12,13]

After obtaining the SYNTAX SCORE, CAD was graded with respect to severity of Coronary Artery Disease as per table 1 below

**Table 1: SYNTAX SCORE GRADING and CAD**

| CAD Severity | SYNTAX SCORE |
|---|---|
| Mild | 1-22 |
| Moderate | 23-32 |
| Severe | ≥33 |

**NLR Classification:** The study results were classified into 3 categories as below with respect to coronary artery severity as per SYNTAX SCORE and will be discussed in relevant sections as below –

**Table 2: NLR Grading and CAD**

| Category | NLR ratio |
|---|---|
| 1. Low | <2 |
| 2. Intermediate | 2-3 |
| 3. High | >3 |

**Inclusion Criteria:** All patients above 18 years of age who will undergo coronary angiography for CAD.

**Exclusion Criteria:** Patients undergoing coronary angiography for Valvular Heart Disease surgery or reasons other than CAD. Patients of Septicemia, Autoimmune diseases, neurodegenerative diseases, Chronic Kidney Disease, Hepatic Cirrhosis.

**Statistical Methods**

Continuous variables were measured by means + standard deviation; categorical variables were given as percentages. The Kolmogorov-Smirnov test was used to verify the normality of distribution of continuous variables. The independent sample t test or the Mann-Whitney U test were be used for the continuous variables and the chi-square test for categorical variables. One-way analysis of variance or Kruskal-Wallis tests was used to compare 3 groups. Correlations was assessed using Spearman rank test. Receiver–operating characteristic (ROC) curve analysis was used to determine the optimum cutoff levels of NLR to predict the severity of CAD. Multivariate logistic regression analysis was performed to assess the independent predictors of severe atherosclerosis. All variables which were found significant in univariate analysis will be included in the model of logistic regression model and results were shown as an odds ratio (OR) with 95% confidence intervals (CIs). Statistical analyses were performed using SPSS 23 (SPSS Inc, Chicago, Illinois). A 2-tailed P < .05 will be considered as statistically significant. [47] After completion of the observational study period from February 2023 to May 2024, relevant data of 2000 patients compiled in Microsoft excel sheet and analysed using SPSS software v23 (IBM, New york). Data normality and distribution was assessed by Kolmogorov –Smirnov test. Population characteristics were as shown in the table number 3.

**Results**

The study population of 2000 patients consisting 1618 men (80.9%) and 382 female patients (19.1%). The mean SYNTAX score was 20.71±9.86. Regarding the distribution of the SYNTAX score subgroups, low, intermediate, and high SYNTAX scores were present in 32.25%, 46.2%, and 21.55 % of the study patients, respectively.

Table 3 shows of baseline characteristics, angiographic and hematological findings between the 3 SYNTAX subgroups. Demographic information and cardiovascular risk factors were similar in the 3 subgroups. The left ventricular ejection fraction was significantly different in the 3 subgroups [55(±7) vs 45(±6) vs 40(±8) ]in the low-, intermediate-, and high-risk subgroups, respectively; P=0.05).

**Table 3: Baseline Characteristics of Study Population w.r.t SYNTAX SCORE**

| Characteristics | LOW SS (1-22) N=645 | MEDIUM SS (23-32 ) N=924 | HIGH SS (≥33) N=431 | p value |
|---|---|---|---|---|
| Age In Years | 58.46±10.90 | 61.98±10.91 | 66.50±14.59 | 0.03 |
| Gender Male[Female] | 537 [108] | 745 [179] | 336 [95] | |
| Weight (kg) | 63.9(±4.2) | 67.9((±5.2) | 70.02(±5.9) | 0.57 |
| RELIGION(H/M/C) | 578/64/03 | 814/103/07 | 384/42/05 | |
| BMI (KG/Mt$^2$) | 24.6(±2.3) | 26.2(±2.2) | 26.9(±2.8) | 0.05 |
| FBS/RBS (gm/dl) | 117.4(±41.5) | 127.5(±44.4) | 145.3(±47.8) | 0.04 |
| Diabetes Mellitus (%) | 146 (22.6) | 269 (29.1) | 123(28.5) | 0.05 |
| HbA1c | 6.1 (±0.3) | 6.9 (±0.4) | 6.9 (±0.3) | 0.04 |
| Hypertension (%) | 158(24.5) | 285(30.1) | 144(33.4) | 0.03 |
| Smoking (%) | 145(22.5) | 256 (27.7) | 109(25.3) | 0.09 |
| TOBACCO CHEWING (%) | 88(13.6) | 154(16.6) | 67(15.5) | 0.75 |
| Family History Of CAD (%) | 44(6.8) | 98(10.6) | 48(11.1) | 0.26 |
| Total Cholesterol, Mg/dl | 167± 44.4 | 186± 42.9 | 188± 45.8 | 0.21 |
| LDL, Mg/dl | 101±31.6 | 112±34.6 | 109±37.8 | 0.04 |
| HDL, Mg/dl | 35.4±8.8 | 34.6±9.6 | 34.7±9.2 | 0.12 |
| Triglyceride, Mg/dl | 148.8 ± 78.6 | 144.2 ± 78.6 | 146.1±64.0 | 0.20 |
| Creatinine, Mg/dl | 0.7±0.2 | 0.9±0.2 | 1.0±0.2 | 0.46 |
| Ejection Fraction (%) | 55(±7) | 45(±6) | 40(±8) | 0.05 |

| | | | | |
|---|---|---|---|---|
| Haemoglobin(gm/dl) | 12.4(±1.2) | 13.8(±1.1) | 12.7(±1.2) | 0.80 |
| Platelet Count($10^9$/L) | 284 ±61 | 279 ± 68 | 258± 55 | 0.48 |
| WBC Count($10^9$/L) | 8.6 ± 3.1 | 11.9 ± 3.2 | 9.5 ±2.9 | 0.83 |
| Neutrophil ($10^9$/L) | 5.3±2.7 | 9.1 ± 3.1 | 6.6 ± 1.3 | <0.001 |
| Lymphocytes ($10^9$/L) | 2.9± 1.2 | 2.3± 0.8 | 2.4 ± 0.9 | <0.001 |
| NLR | 1.9 ±1.2 | 4.12±2.35 | 3.4± 3.7 | <0.001 |
| Type Of CAD | LOW SS (1-22) N=645 | MEDIUM SS (23-32 ) N=924 | HIGH SS (≥33) N=431 | p value |
| STEMI (%) | 301(46.7) | 516(55.9) | 257(59.6) | 0.04 |
| NSTE- ACS (%) | 220(34.1) | 258(27.9) | 93(21.6) | 0.05 |
| CCS (%) | 124(19.2) | 150(16.2) | 81(18.8) | 0.09 |
| INFARCT RELATED ARTERY | LOW SS (1-22) N=645 | MEDIUM SS (23-32 ) N=924 | HIGH SS (≥33) N=431 | |
| LMCA (%) | 09(1.4) | 86(9.3) | 42(9.7) | |
| LAD (%) | 358(55.5) | 489(52.9) | 198(45.9) | |
| LCX (%) | 72(11.2) | 178(19.3) | 104(24.1) | |
| RCA (%) | 206(31.9) | 278(30.1) | 148(34.3) | |
| Number Of Arteries Involved | LOW SS (1-22) N=645 | MEDIUM SS (23-32 ) N=924 | HIGH SS (≥33) N=431 | |
| Single Vessel Disease (%) | 462(71.6) | 487(52.7) | 56(13) | |
| Double Vessel Disease (%) | 169(26.2) | 261(28.2) | 95(22.1) | |
| Triple Vessel Disease (%) | 14(2.2) | 176(19.1) | 280((64.9) | |

**Table 4: Patient Characteristics and Comparison with NLR**

| **Characteristics** | **LOW NLR (<2) N=627** | **INTERMEDIATE NLR (2-3 ) N=864** | **HIGH NLR (>3) N=509** | **p value** |
|---|---|---|---|---|
| Age In Years | 57.76±11.10 | 62.78±10.78 | 65.58±17.74 | 0.03 |
| Gender Male[Female] | 511 [116] | 703 [161] | 404 [105] | |
| Weight (kg) | 65.8(±4.6) | 66.9((±4.8) | 71.02(±5.8) | 0.57 |
| RELIGION(H/M/C) | 561/63/03 | 756/102/06 | 412/92/05 | |
| BMI (KG/$Mt^2$) | 24.8(±2.1) | 26.6(±2.2) | 27.9(±2.8) | 0.05 |
| FBS/RBS (gm/dl) | 118.4(±40.4) | 127.5(±45.4) | 147.3(±49.8) | 0.03 |
| Diabetes Mellitus (%) | 139 (22.20) | 249 (28.80) | 163(32.02) | 0.05 |
| HbA1c | 6.2(±0.3) | 6.8 (±0.4) | 6.9 (±0.3) | 0.04 |
| Hypertension (%) | 149(23.8) | 284(32.9) | 168(33.1) | 0.04 |
| Smoking (%) | 155(24.7) | 246 (28.5) | 128(25.1) | 0.09 |
| Tobacco Chewing (%) | 98(15.6) | 152(17.6) | 88(17.3) | 0.75 |
| Family History Of CAD (%) | 43(6.8) | 108(12.5) | 58(11.4) | 0.26 |
| Total Cholesterol, Mg/dl | 168± 43.5 | 184± 43.3 | 190± 44.8 | 0.21 |
| LDL, Mg/dl | 102±31.3 | 113±33.6 | 111±36.8 | 0.06 |
| HDL, Mg/dl | 35.8±8.9 | 35.2±8.9 | 33.7±8.9 | 0.12 |
| Triglyceride, Mg/dl | 147.8±77.6 | 146.2±78.8 | 148.1±64.0 | 0.20 |
| Creatinine, Mg/dl | 0.7±0.2 | 0.9±0.2 | 1.0±0.2 | 0.46 |
| Ejection Fraction of LV (%) | 54(±7) | 44(±6) | 41(±8) | 0.02 |
| Haemoglobin(gm/dl) | 12.8(±1.3) | 13.6(±1.2) | 12.9(±1.3) | 0.80 |
| Platelet Count($10^9$/L) | 282 ±62 | 284 ± 66 | 278± 64 | 0.48 |
| SYNTAX SCORE | 14.98±9.12 | 21.44±11.02 | 25.32±10.96 | <0.001 |
| Type Of CAD | LOW NLR (<2) N=627 | MEDIUM NLR (2-3 ) N=864 | HIGH NLR (>3) N=509 | p value |
| STEMI (%) | 293(46.7) | 486(55.3) | 278(54.6) | 0.04 |
| NSTE- AC S (%) | 198(31.6) | 246(28.5) | 118(23.2) | 0.05 |
| CCS (%) | 136(21.7) | 132(15.2) | 113(22.2) | 0.09 |
| INFARCT RELATED ARTERY | LOW NLR (<2) N=627 | MEDIUM NLR (2-3 ) N=864 | HIGH NLR (>3) N=509 | p value |
| LMCA (%) | 10(1.6) | 56(6.5) | 51(10.0) | 0.43 |
| LAD (%) | 348(55.5) | 458(53.0) | 258(50.7) | 0.01 |
| LCX (%) | 68(10.8) | 168(19.4) | 116(22.8) | 0.14 |
| RCA (%) | 196(31.3) | 255(29.5) | 158(31.0) | 0.12 |

| Number Of Arteries Involved | LOW NLR (<2) N=627 | MEDIUM NLR (2-3 ) N=864 | HIGH NLR (>3) N=509 | p value |
|---|---|---|---|---|
| Single Vessel Disease (%) | 450(71.8) | 447(51.7) | 69(13.6) | 0.05 |
| Double Vessel Disease (%) | 159(25.4) | 234(27.1) | 156(30.6) | 0.06 |
| Triple Vessel Disease (%) | 18(2.8) | 183(21.2) | 284(55.8) | 0.02 |

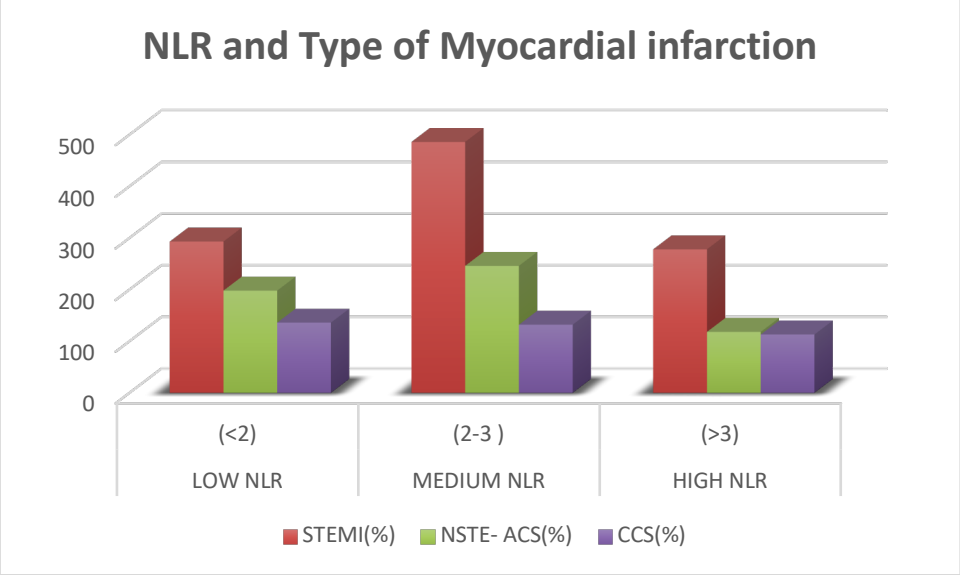


**Figure 1: NLR and Type of Myocardial Infarction**

From the result table-3, Patients in the higher-risk SYNTAX score group were more likely to experience STEMI AS COMPARED TO NSTE- ACS, CCS. The post hoc analysis also demonstrated that the difference in the platelet count and neutrophil count was significant between the high and low SYNTAX subgroups.

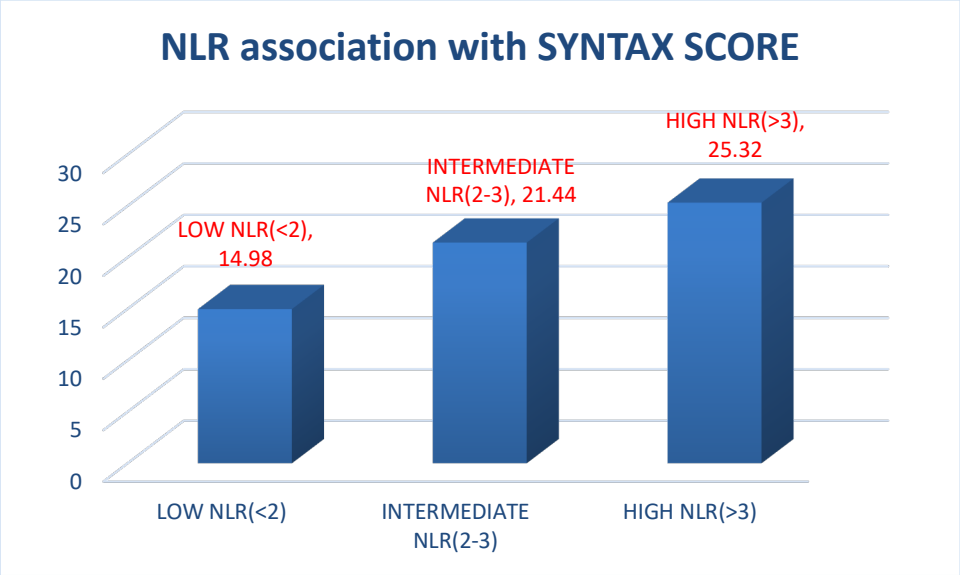


**Figure 2: NLR and its association with SYNTAX SCORE**

**Association of NLR with SYNTAX score and CAD:** Table 4 shows the results of the univariate linear regression analysis of NLR and other covariates, with SYNTAX score in patients of CAD. Higher NLR was significantly associated with higher SYNTAX score (beta = 0.152, *P* = 0.001).

Also, older age, having hypertension, higher HbA1c, FBS/RBS, Diabetes Mellitus and lower Ejection fraction on echocardiographic examination were significantly associated with higher NLR as illustrated in table 4. In multivariate linear regression analysis, NLR was significantly contributed to predicting SYNTAX score after adjustment for age, hypertension, DM, BMI, (beta = 0.142, *P* = 0.002). Figure 2 depicts the graphical relationship between NLR and SS. Out of 2000 patients, STEMI accounted for 1057(52.85%), NSTE-ACS were 562(28.1%) and CCS were

381(19.05%). Higher NLR is significantly associated in STEMI patients as compared to NSTE-ACS or CCS patients (p=0.04) demonstrating severe inflammatory interactions in STEMI [figure 1] [table 4]. Similar trend is observed in SYNTAX score as well [table 3]. Out of 2000 patients , Left main coronary artery(LMCA) was involved in 117(5.85%), Left Coronary Artery(LAD) involved in 1064(53.2%), Left Circumflex artery (LCX) in 352(17.6%) and Right coronary Artery(RCA) was involved in 609(30.45%) cases as depicted in figure 6. NLR was raised significantly in cases involving LAD as compared to other coronary arteries. [Table 4]

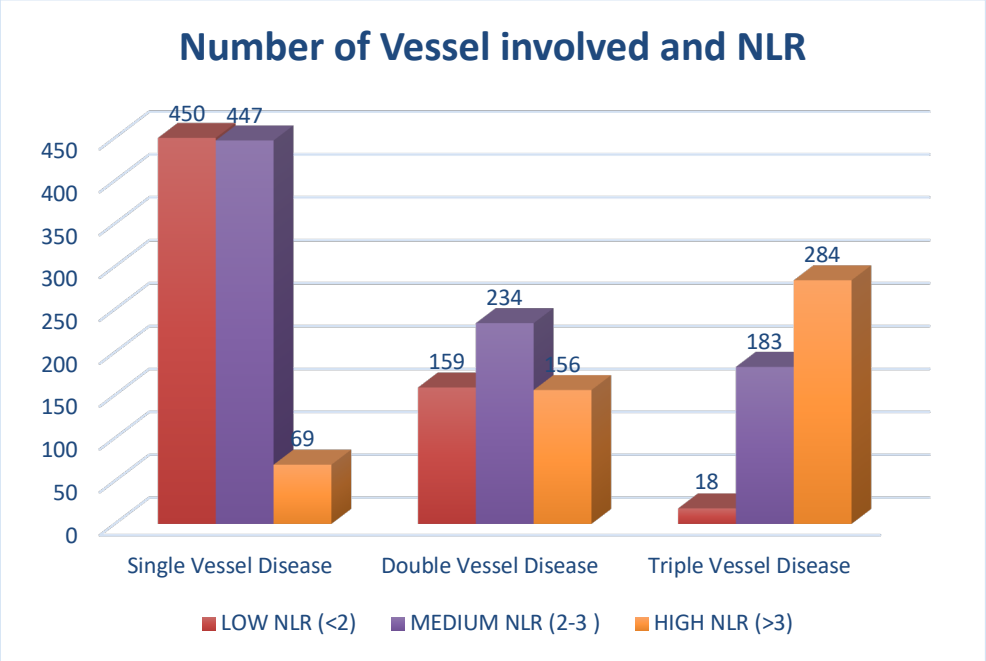


**Figure 4: NLR and Number of Coronary Vessels Involved**

Out of total patients, single vessel disease was 966(48.3%), double vessel disease were 549 (27.45%) and Triple Vessel disease were (24.25%) as depicted in figure 4. Higher NLR was associated with triple vessel disease.

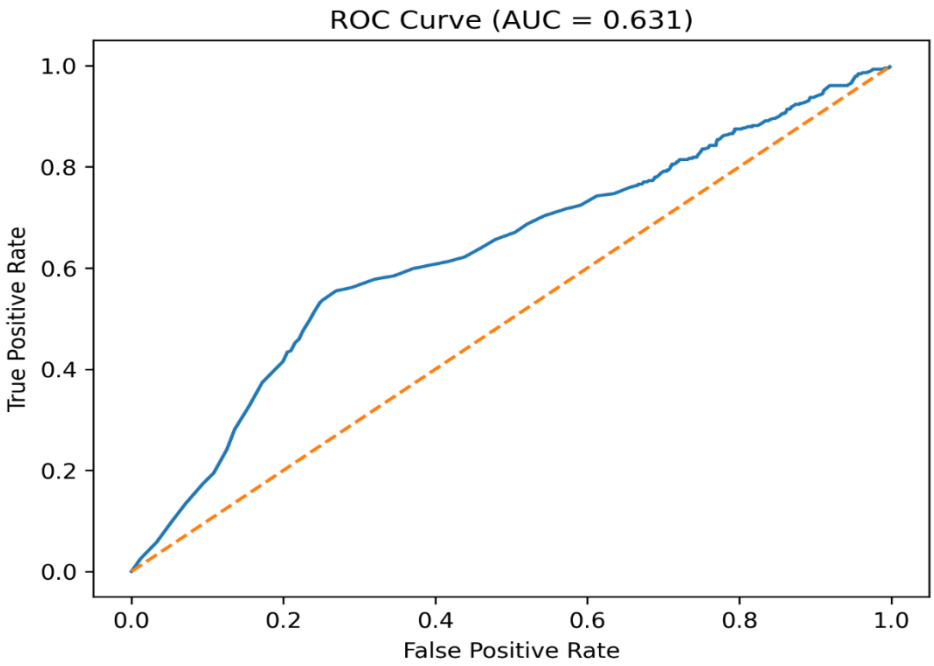


**Figure 4: ROC Curve of NLR with SYNTAX SCORE**

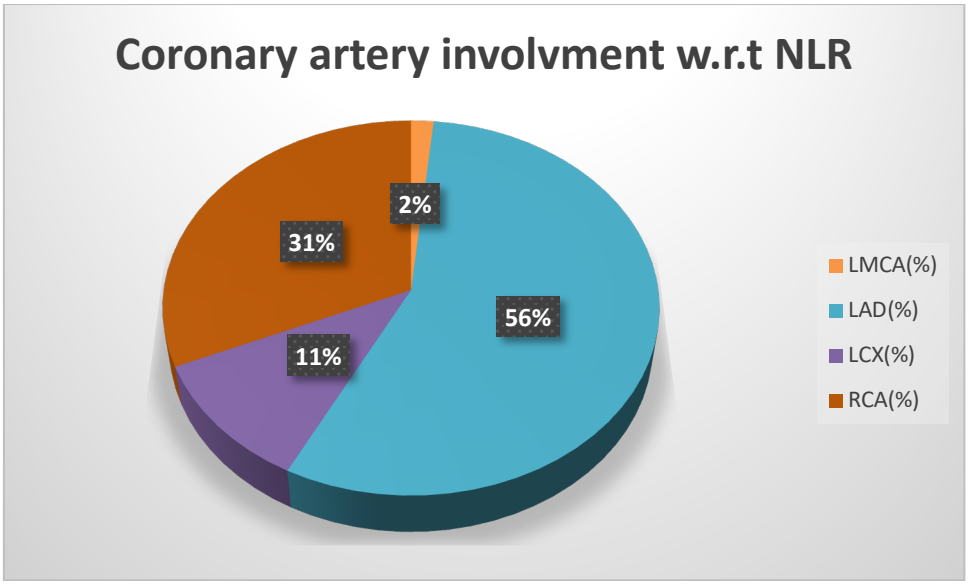


**Figure 5: Different Coronary artery involvement w.r.t NLR**

Figure 4 illustrates the ROC curve of the NLR for the prediction of severity of CAD w.r.t SYNTAX Score. The ROC analysis revealed a cutoff point of 2.48 for the NLR with an AUC of 0.631 (95% CI: 0.54–0.74; P=0.02). An NLR of greater than 2.48 had 53.6% sensitivity and 74.9% specificity in predicting severity of CAD w.r.t SYNTAX Score.

## Discussion

From the results of our study which clearly illustrates the strong association of NLR with SYNTAX SCORE ($p<0.001$). Previous studies like, Karabağ et al [48] reported an independent association between the presentation NLR and severity of CAD with STEMI. In another study, Lee et al reported an association between the presentation NLR and SYNTAX SCORE. [49] In a study by Sahin et al the NLR was an independent predictor of the SYNTAX score in patients with STEMI who underwent primary PCI.[25] In a univariate analysis, they also found an association between a higher SYNTAX score and WBC, neutrophil, and lymphocyte counts, but not with the platelet count. In our study NLR is significantly raised in STEMI patients as compared to NSTE-ACS or CCS patients ($p=0.04$) demonstrating severe inflammatory interactions in STEMI [Figure 1] [table 4]. Similar trend is observed in SYNTAX score as well [table 3].

NLR is also significantly raised in cases where Left Coronary Artery (LAD) involved as compared to Left Circumflex artery (LCX) or Right coronary Artery(RCA) or Left main artery(LMCA).[Table 4] NLR is also significantly more in Triple vessel disease as compared to single of multi vessel disease [Figure 5] In this study, we also found a positive association between presentation NLR and SYNTAX score in patients with NSTE-ACS. As shown in our study, in patients with NSTE-ACS, high presentation NLR was associated with high angiographic SYNTAX score. Altun et al, found NLR as an independent predictor of high SYNTAX score group in patients with NSTE-ACS.[50] In another study, Kurtul et al compared NSTEMI patients with low SYNTAX scores to patients with intermediate or high SYNTAX scores in univariate analysis and found lower NLR in the group with low SYNTAX score.[51] Soyla et al studied three NLR tertiles in patients with NSTE-ACS, found that a positive correlation was present only in patients of the high NLR group and not in the low or intermediate group.[52] Zuin et al found a positive association of NLR and SYNTAX score in patients with NSTEMI in a univariate analysis and found a higher SYNTAX score in patients of higher NLR tertile group.[53]

There are very few studies that explored association of NLR with Stable Coronary artery disease. In a study by Papa A et al, 422 consecutive ischemic patients with angiographically documented stable CAD, neutrophil to lymphocyte ratio (N/L) emerged as independent predictor of cardiac death (HR 8.13; $p=0.02$) together with CRP, left ventricular ejection fraction (LVEF), FG, HDL.[25] but in our study NLR association with stable CAD or CCS was non-Significant ($p= 0.09$).[54]

## Limitations

There are some limitations to this study that should be mentioned. Our study is a single-centre observational research. In addition, NLR was measured only on patient presentation, and information about the serial changes of NLR value was not available. No follow up done. While patients with various diseases that might affect the inflammatory markers were excluded from this study, there was the possibility of the presence of

other unknown inflammatory conditions that might influence the results. We only included patients who presented to our tertiary cardiovascular centre with STEMI, NSTE-ACS and CCS, did not have access to the information regarding the out-of-hospital deaths due to MI.

## Conclusion

Our study illustrates that higher NLR is significantly associated with higher SYNTAX score in patients with STEMI, NSTE-ACS but not CCS and can be a good candidate for prognostic marker for severity of CAD. In a developing country like India and state like Odisha, NLR can be a affordable, accessible and diagnostic parameter for innumerable CAD patients for risk stratification as well as prediction of severity

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