

BMJ Open Prognostic utility of the combination of monocyte-to-lymphocyte ratio and neutrophil-to-lymphocyte ratio in patients with NSTEMI after primary percutaneous coronary intervention: a retrospective cohort study

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ABSTRACT

Objectives This study aimed to evaluate prognostic value of the combination of monocyte-to-lymphocyte ratio (MLR) with neutrophil-to-lymphocyte ratio (NLR) for predicting long-term major adverse cardiac events (MACE) in patients with non-ST elevated myocardial infarction (NSTEMI) who underwent primary percutaneous coronary intervention (PCI).

Design Retrospective cohort study.

Setting Civil Aviation General Hospital, Beijing, China.

Participants 678 patients with NSTEMI undergoing primary PCI between July 2010 and July 2015 were enrolled.

Main outcome measures The main outcomes were MACE. The cumulative MACE-free survival rates were calculated by Kaplan-Meier analysis and the independent predictors of MACE were assessed by Cox regression analysis.

Results According to the cut-off values of MLR 0.36 and NLR 2.15, the study population was classified into four groups: low MLR + low NLR group (n=319), low MLR + high NLR group (n=126), high MLR + low NLR group (n=102) and high MLR + high NLR group (n=131). The high MLR + high NLR group had a lower MACE-free survival rate than the other three groups (p logrank <0.001). Both MLR (HR 2.128, 95% CI 1.458 to 3.105) and NLR (HR 1.925, 95% CI 1.385 to 2.676) were independent predictors of long-term MACE. Moreover, the patients in the high MLR + high NLR group had an HR of 4.055 (95% CI 2.550 to 6.448) for long-term MACE, with the low-MLR + low-NLR group as reference. Comparisons of receiver operating characteristic curves revealed that the combination of MLR with NLR achieved better performance in differentiating long-term MACE, compared with MLR, NLR, high-sensitivity C reactive protein and brain natriuretic peptide alone, and had similar performance to all other pairwise combinations of the four biomarkers.

Conclusions Elevated levels of MLR and NLR were independent predictors of long-term MACE in patients with NSTEMI. Moreover, the combination of MLR and NLR could improve the prognostic value in predicting long-term MACE.

Strengths and limitations of this study

- This is the first study to report the use of monocyte-to-lymphocyte ratio (MLR) in combination with neutrophil-to-lymphocyte ratio (NLR) to predict major adverse cardiac events (MACE) in patients with non-ST elevated myocardial infarction (NSTEMI) undergoing primary percutaneous coronary intervention.
- Receiver operating characteristic curves were used to extensively explore and compare the diagnostic efficacies of MLR alone, NLR alone, high-sensitivity C reactive protein alone, brain natriuretic peptide alone and their pairwise combinations in differentiating MACE in patients with NSTEMI.
- MLR and NLR, that are non-invasive, simple, economical and feasible biomarkers, possess practical clinical utility in the prediction of prognosis of NSTEMI.
- These findings need to be validated in multi-institutional studies with larger sample sizes.

INTRODUCTION

Previous studies have verified that inflammatory response plays a vital role in the development of atherosclerosis and cardiovascular diseases.^{1 2} White blood cells and its subtypes including neutrophils, monocytes and lymphocytes are important immune cells involved in the initiation, formation and destabilisation of atherosclerosis.³ The neutrophil-to-lymphocyte ratio (NLR) has been established as a cost-effective, feasible and reproducible inflammatory biomarker in many cardiovascular disorders, including acute coronary syndrome (ACS), angina pectoris and heart failure.⁴⁻⁶ Elevated NLR has been reported as an independent predictor of major adverse cardiac events (MACE) in patients with ACS.⁷ Monocytes can recruit to the artery

wall, differentiate into macrophages and stimulate the activation of proinflammatory cytokines which play a crucial role at every level of the atherosclerotic process.⁸ The monocyte-to-lymphocyte ratio (MLR) has emerged as a novel systematic inflammatory marker related to increased cardiovascular risk.⁹ Recently, MLR has been reported to be associated with adverse clinical outcomes in various cardiovascular diseases.^{10–12} However, the prognostic value of the combined usefulness of MLR and NLR in non-ST elevated myocardial infarction (NSTEMI) has not been evaluated. The aim of the present study was to investigate the combined usefulness of MLR and NLR in predicting MACE in patients with NSTEMI who underwent primary percutaneous coronary intervention (PCI).

MATERIALS AND METHODS

Study design, setting and participants

This retrospective longitudinal study was performed in the Civil Aviation General Hospital, Beijing, China. A total of 818 consecutive patients with NSTEMI who presented to the emergency department and underwent primary PCI from July 2010 to July 2015 were selected for participation in this study. NSTEMI was defined by typical ischaemia symptoms, elevated level of cardiac troponin-I or creatine kinase-MB and no evidence of ST segment elevation in ECG. We excluded patients who had serious heart failure (New York Heart Association (NYHA)¹³ class III or IV), rheumatic heart disease, valvular heart disease, congenital heart disease, pulmonary heart disease, active or chronic inflammatory conditions, acute infection, haemodynamic disorders, malignancies, severe renal (estimated glomerular filtration rate (eGFR) <30 mL/min/1.73 m²) or hepatic (alanine aminotransferase >40 U/L) disease, steroid therapy in the preceding 3 months, history of cerebrovascular events, or incomplete blood cell count or medical records. Hypertension was defined as current use of an antihypertensive medication or, a systolic blood pressure \geq 140 mm Hg and/or a diastolic blood pressure \geq 90 mm Hg. Diabetes mellitus was defined as active use of an antidiabetic agent, or fasting plasma glucose level \geq 7.0 mmol/L or casual plasma glucose level \geq 11.1 mmol/L.

Informed consent was obtained from all patients.

Study procedures and laboratory analysis

At the time of admission, venous blood samples were collected from each patient. All haematological and biochemical analyses were performed on fresh whole blood/plasma. Plasma was obtained by centrifuging whole blood samples at 3000 rpm for 5 min. Complete blood counts and biochemical indicators were measured by the core laboratory of the Civil Aviation General Hospital. Complete blood counts were performed using a SYSMEX XE-2100 automated cell counter (Sysmex Corporation, Kobe, Japan). Complete blood counts included haemoglobin, leucocytes, neutrophils, monocytes, lymphocytes and platelets. Biochemical indicators (total

cholesterol, triglycerides, creatinine, low-density lipoprotein (LDL), high-density lipoprotein (HDL), creatinine, high-sensitivity C reactive protein (hs-CRP), brain natriuretic peptide (BNP) and Troponin I) were determined using a Hitachi7600 automatic biochemistry analyser (Hitachi, Tokyo, Japan). eGFR was calculated using the Chronic Kidney Disease Epidemiology (CKD EPI) creatinine equation¹⁴. MLR was calculated as the ratio of monocyte counts to lymphocyte counts, and NLR was calculated as the ratio of neutrophil counts to lymphocyte counts.

All patients received a loading dose of aspirin (300 mg) and clopidogrel (300 mg) at least 6 hours before PCI, and an intravenous dose of heparin (70–100 U/kg) to maintain an activated clotting time (250–300 s) during the procedure. Primary PCI was performed according to standard clinical practice by experienced cardiologists. A successful PCI was defined as a residual stenosis less than 30% and final thrombolysis in myocardial infarction (MI) II or III flow in the treated artery. Angiographic characteristics were collected for all the patients.

Clinical outcomes

The main outcomes were MACE that happened in-hospital and during the follow-up period, which were defined as a composite of all-cause mortality, cardiac death, stroke, non-fatal MI, target lesion revascularisation (TLR) and target vessel revascularisation (TVR) according to the Academic Research Consortium definition.¹⁵ Cardiac death was defined as death resulting from any cardiac-related causes (eg, MI, heart failure, lethally cardiac arrhythmia). Non-fatal MI was defined based on the European Society of Cardiology, American Heart Association, American College of Cardiology and World Heart Federation definitions.¹⁶ TLR was defined as repeat revascularisation caused by \geq 50% stenosis within the stent or within 5 mm proximal or distal to the stent. TVR was defined as repeat coronary angioplasty or surgical bypass performed within the coronary artery containing the target lesion. Follow-up data were obtained by review of electronic medical records and/or telephone interview with the patients or patients' primary caregiver.

Statistical analysis

The Kolmogorov-Smirnov test was employed to test the normality of the continuous variables in each group. Continuous variables distributed normally were expressed as mean \pm SD, while categorical data were expressed as numbers and percentages. We initially used receiver operating characteristic (ROC) curves to determine the ability of MLR and NLR to differentiate MACE. Subsequently optimal cut-off values, and specificity and sensitivity were derived. Based on the optimal cut-off values, participants were assigned to four groups: low MLR + low NLR group, low MLR + high NLR group, high-MLR + low NLR group and high MLR + high NLR group. Continuous data differences between the four groups were compared using one-way analysis of variance followed by Tukey's post hoc tests, while categorical data were compared by χ^2 tests.

The MACE-free survival rates according to the cut-off values of MLR and NLR were estimated by the Kaplan-Meier analysis and statistical differences were carried out using the logrank test. Univariate and multivariate Cox regression analyses were carried out to identify the independent predictors of MACE. Variables with $p < 0.10$ in univariate analysis were selected for multivariate Cox regression analysis. We constructed two Cox regression models (model 1 and model 2) with MACE as the dependent variable to investigate the efficacy of MLR and NLR in predicting MACE. Model 1 was to estimate the HR of MLR (low MLR=0 (reference category), high MLR=1) and NLR (low NLR=0 (reference category), high NLR=1) for MACE. Model 2 was to estimate the HR of MLR in combination with NLR for MACE (low MLR + low NLR=0 (reference category), low MLR + high NLR=1, high-MLR + low NLR=2, high MLR + high NLR=3). The effect sizes were expressed as HRs and their 95% CIs. Afterwards, we used ROC curves to evaluate the diagnostic performance of individual biomarkers and their pairwise combinations in predicting long-term MACE. The areas under the curves (AUCs) were compared by Delong's tests. The statistical significance was considered

as a two-tailed $p < 0.05$. Statistical analyses were performed using SPSS V.22.0 (SPSS, Chicago, Illinois, USA).

Patient and public involvement

Patients and public were not involved in the design, recruitment or conduct of this study. There is no plan for the study results to be disseminated directly to participants.

RESULTS

Eight hundred and eighteen patients were screened for inclusion for this study, while 91 (11.12%) patients were excluded because of the exclusion criteria and 49 (6.00%) patients were lost to follow-up. Therefore, a total of 678 (82.89%) patients were included into the analysis, and the median follow-up period was 26 (range: 1–30) months. [Figure 1](#) depicts the clinical layout of the study cohort.

Baseline clinical characteristics

A MLR cut-off value of 0.36 had a sensitivity of 54.74% and a specificity of 73.57%, while an NLR cut-off value of

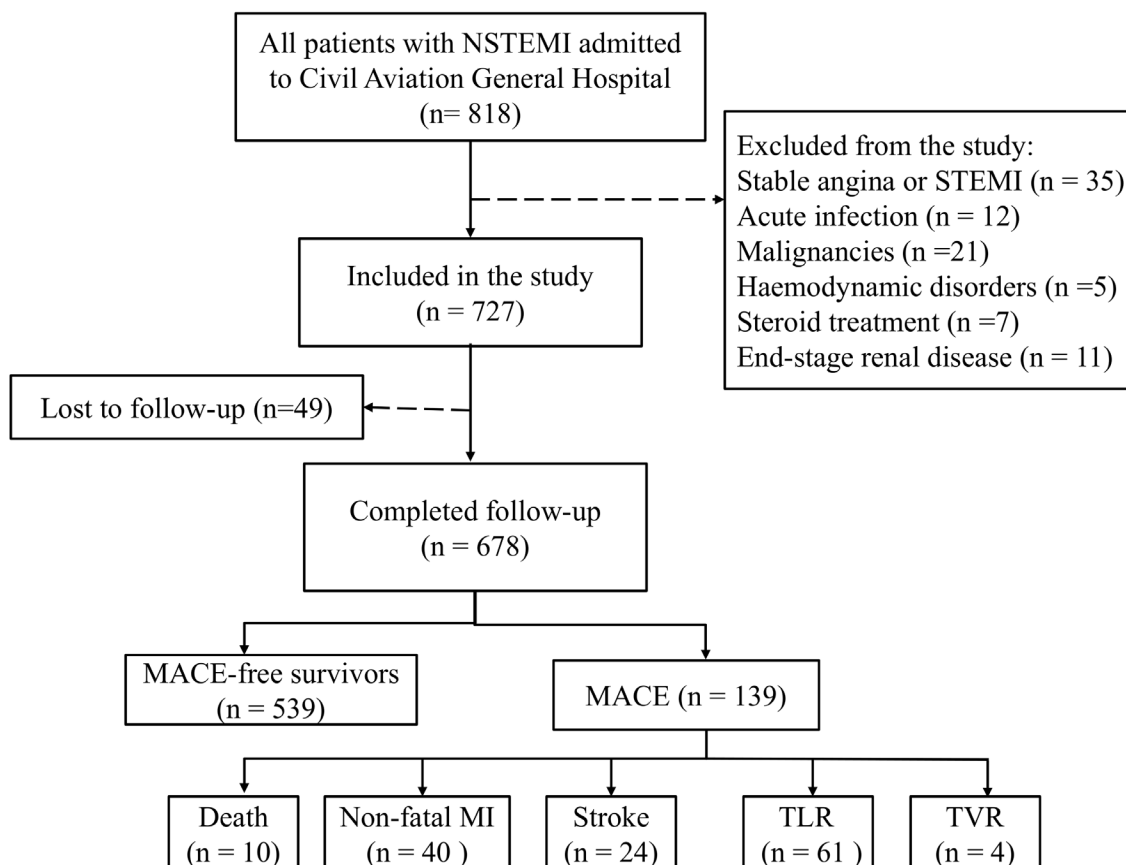


Figure 1 Flow chart of the study cohort. The flow chart presents the selection criteria of the study and the clinical layout of the study population. MACE, major adverse cardiac events; MI, myocardial infarction; NSTEMI, non-STEMI, STEMI, ST elevated myocardial infarction; TLR, target lesion revascularisation; TVR, target vessel revascularisation.

Table 1 Baseline clinical characteristics between the four groups based on the cut-off values of MLR and NLR

Variable	Low MLR + low NLR (n=319)	Low MLR + high NLR (n=126)	High MLR + low NLR (n=102)	High MLR + high NLR (n=131)	P values
Age, years	61.10±14.38	63.63±15.05	64.05±15.15	65.23±16.34*	0.036
Male, n (%)	209 (65.52)	71 (56.35)	65 (63.73)	90 (68.70)	0.188
Family history, n (%)	33 (10.34)	17 (13.49)	11 (10.78)	20 (15.27)	0.463
Hypertension, n(%)	221 (69.28)	86 (68.25)	73 (71.57)	105 (80.15)	0.100
Diabetes mellitus, n(%)	125 (39.18)	56 (44.44)	39 (38.24)	59 (45.04)	0.522
Dyslipidaemia, n (%)	114 (35.74)	51 (40.48)	35 (34.31)	49 (37.40)	0.758
Current smoker, n (%)	108 (33.86)	45 (35.71)	33 (32.35)	51 (38.93)	0.702
Killip class (>I)	192 (60.19)	86 (68.25)	69 (67.65)	99 (75.57)*	0.015
Ejection fraction (%)	65.80±10.05	66.52±10.11	65.26±10.23	62.98±10.01*	0.044
Laboratory parameters					
Leucocyte, ×10 ⁹ /L	6.67±1.72	6.77±2.03	7.06±1.98*	7.25±2.11*	0.027
Neutrophil, ×10 ⁹ /L	4.26±1.07	4.69±1.19*	4.56±1.15*	5.08±1.27*†‡	<0.001
Lymphocyte, ×10 ⁹ /L	2.31±0.53	1.98±0.51*	2.37±0.67†	2.02±0.64*‡	<0.001
Monocyte, ×10 ⁹ /L	0.18±0.09	0.22±0.11*	0.49±0.19*†	0.46±0.13* †	<0.001
Haemoglobin, g/L	136.7±30.38	129.6±27.57*	133.7±39.76	127.4±36.01*	0.039
Total cholesterol, mmol/L	5.70±1.47	5.95±1.49	5.52±1.35	5.69±1.36	0.130
Triglycerides, mmol/L	1.67±0.56	1.63±0.54	1.77±0.59	1.61±0.54	0.109
LDL, mmol/L	2.86±0.95	3.10±1.08*	2.93±1.12*	3.35±1.06*†‡	<0.001
HDL, mmol/L	1.31±0.51	1.43±0.57	1.37±0.49	1.44±0.51	0.059
hs-CRP, mg/dL	1.85±0.71	2.14±0.98*	2.88±0.77* †	3.13±1.02*†‡	<0.001
Creatinine, umol/L	112.38±29.2	106.49±30	112.72±37.44	107.62±34.02	0.203
eGFR, ml/min/1.73 m ²	85.71±29.12	81.85±28.71	78.92±26.31	81.28±27.09	0.101
BNP, pg/mL	265.12±85.39	245.58±79.28*	269.71±76.56* †	298.73±76.56* †‡	<0.001
Troponin I, ng/mL	5.89±2.51	7.52±3.52*	7.65±3.79*	11.08±4.18* †‡	<0.001
Medical treatment					
Aspirin, n(%)	309 (96.87)	119 (94.44)	96 (94.12)	129 (98.47)	0.202
Anticoagulant, n(%)	305 (95.61)	117 (92.86)	93 (91.18)	124 (94.66)	0.340
Statin, n(%)	292 (91.54)	112 (88.89)	90 (88.24)	121 (92.37)	0.589
ACEI or ARB, n(%)	186 (58.31)	71 (56.35)	61 (59.8)	84 (64.12)	0.602
Beta-blocker, n(%)	259 (81.19)	98 (77.78)	86 (84.31)	105 (80.15)	0.654
Calcium-channel blockers, n(%)	67 (21.00)	30 (23.81)	26 (25.49)	28 (21.37)	0.767
Nitrate drugs, n(%)	257 (80.56)	96 (76.19)	75 (73.53)	106 (80.92)	0.369
Angiographic findings					
Number of diseased vessels					0.145
one vessel, n(%)	182 (57.05)	65 (51.59)	54 (52.94)	57 (43.51)	
two vessels, n(%)	76 (23.82)	39 (30.95)	32 (31.37)	42 (32.06)	
three vessels/left main, n(%)	61 (19.12)	22 (17.46)	16 (15.69)	32 (24.43)	
Number of implanted stents	1.95±0.79	2.01±0.85	2.11±0.94	2.15±1.01	0.125
Total stent length, mm	39.6±24.1	37.2±21.9	35.8±26.3	41.9±22.2	0.190
Stent diameter, mm	2.59±1.33	2.85±1.09	2.84±1.27	2.68±1.16	0.114
Moderate or severe tortuosity, n(%)	27 (8.46)	16 (12.70)	11 (10.78)	14 (10.69)	0.575
Moderate or severe calcification, n(%)	29 (9.09)	14 (11.11)	13 (12.75)	15 (11.45)	0.706

*Compared with the low MLR + low NLR group, p<0.05.

†Compared with the low MLR + high NLR group, p<0.05.

‡Compared with the high MLR + low NLR group, p<0.05.

ACEI, ACE inhibitor; ARB, angiotensin receptor blocker; BNP, brain natriuretic peptide; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C reactive protein; LDL, low-density lipoprotein; MLR, monocyte-to-lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio.

Table 2 Clinical outcomes between the four groups based on the cut-off values of MLR and NLR

Variable	Low MLR + low NLR (n=319)	Low MLR + high NLR (n=126)	High MLR + low NLR (n=102)	High MLR + high NLR (n=131)	P values
Follow-up 2 years, n(%)	30 (9.40)	31 (24.60)*	28 (27.45)*	50 (38.17)* †‡	<0.001
All-cause death, n(%)	2 (0.63)	1 (0.79)	1 (0.98)	6 (4.58)*	0.010
Cardiac death, n(%)	1 (0.31)	1 (0.79)	1 (0.98)	5 (3.82)*	0.018
Non-fatal MI, n(%)	9 (2.82)	8 (6.35)	9 (8.82)*	14 (10.69)*	0.006
Stroke, n(%)	4 (1.25)	6 (4.76)*	5 (4.9)*	9 (6.87)*	0.017
TLR, n(%)	14 (4.39)	16 (12.70)*	12 (11.76)*	19 (14.50)*	0.001
TVR, n(%)	1 (0.31)	0 (0)	1 (0.98)	2 (1.53)	0.335

*Compared with the low MLR + low NLR group, $p < 0.05$.

†Compared with the low MLR + high NLR group, $p < 0.05$.

‡Compared with the high MLR + low NLR group, $p < 0.05$.

MI, myocardial infarction; MLR, monocyte-to lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio; TLR, target lesion revascularisation; TVR, target vessel revascularisation.

2.35 had a sensitivity of 77.37% and a specificity of 55.08% for differentiating long-term MACE, via ROC analyses. According to the optimal cut-off values of MLR 0.36 and NLR 2.35, participants were classified into four groups: low-MLR+lowNLR group (MLR<0.36, NLR<2.35, n=319), low MLR + high NLR group (MLR <0.36, NLR \geq 2.35, n=126), high MLR + low NLR group (MLR \geq 0.36, NLR <2.35, n=102) and high MLR + high NLR group (MLR \geq 0.36, NLR \geq 2.35, n=131). The clinical characteristics were summarised in [table 1](#). The distribution of prior medications and angiographic findings were similar between the four groups. However, patients in the high MLR + high NLR group were older, with higher Killip class and lower ejection fraction, and showed higher levels of white blood cells, monocytes, neutrophils, LDL, hs-CRP, BNP and troponin I, whereas they had lower levels of lymphocytes and haemoglobin.

Clinical outcomes

During the median follow-up period of 26 months, long-term MACE were observed in 139 (20.50%) patients. Ten (1.47%) patients died, 40 (5.90%) patients had a non-fatal MI, 24 (3.54%) patients experienced stroke, 61 (9.00%) patients underwent TLR and 4 (0.59%) patients underwent TVR. Overall, the patients in the high MLR + high NLR group had higher MACE rate, compared with the other three groups. The mortality, non-fatal MI, stroke and TLR were significantly higher in patients with high MLR + high NLR, than those with either lower MLR or lower NLR, whereas the four groups had similar TVR ([table 2](#)).

Kaplan-Meier curves based on the cut-off values of MLR and NLR, are shown in [figure 2A](#) and [figure 2B](#), respectively. Significantly increased long-term MACE rates were observed in patients with high MLR (33.48% vs 13.71%, $p < 0.001$, [figure 2A](#)) and in patients with high NLR (31.52% vs 13.78%, $p < 0.001$, [figure 2B](#)). The Kaplan-Meier MACE-free curve based on the combined markers

is shown in [figure 2C](#). The MACE rates were significantly different among the four groups ($p < 0.001$) and patients in the high MLR + high NLR group had the highest MACE rate.

Independent predictors of long-term MACE

Univariate and multivariate COX regression analyses were used to determine the independent predictors of long-term MACE in patients with NSTEMI undergoing primary PCI. In univariate Cox analysis, leucocytes, neutrophils, lymphocytes, monocytes, MLR, NLR, LDL, hs-CRP, BNP and troponin I were found to be significantly associated with long-term MACE (see online supplementary table S1). After adjusting for covariates, both MLR (HR 2.128, 95% CI 1.458 to 3.105, $p < 0.001$) and NLR (HR 1.925, 95% CI 1.385 to 2.676, $p < 0.001$) were found to be significant predictors of long-term MACE in multivariate Cox regression. Moreover, the combination of MLR and NLR was found to be an independent predictor of long-term MACE (HR 4.055, 95% CI 2.550 to 6.448, $p < 0.001$ for patients with high MLR + high NLR vs patients with low MLR + low NLR). In addition to MLR and NLR, hs-CRP and BNP were also independent predictors of long-term MACE in patients with NSTEMI undergoing primary PCI ([table 3](#)). The details of multivariate Cox regression analyses are presented in online supplementary table S2.

Diagnostic efficacy of MLR in combination with NLR in differentiating MACE

ROC curves were used to evaluate and compare the predictive performance of MLR in combination with NLR with (1) MLR, NLR, hs-CRP and BNP alone. (2) All other pairwise combinations of the four biomarkers, for differentiating long-term MACE. [Figure 3A](#) shows that MLR in combination with NLR (AUC 0.715, 95% CI 0.679 to 0.748) achieved better performance in predicting long-term MACE, than MLR (AUC 0.683,

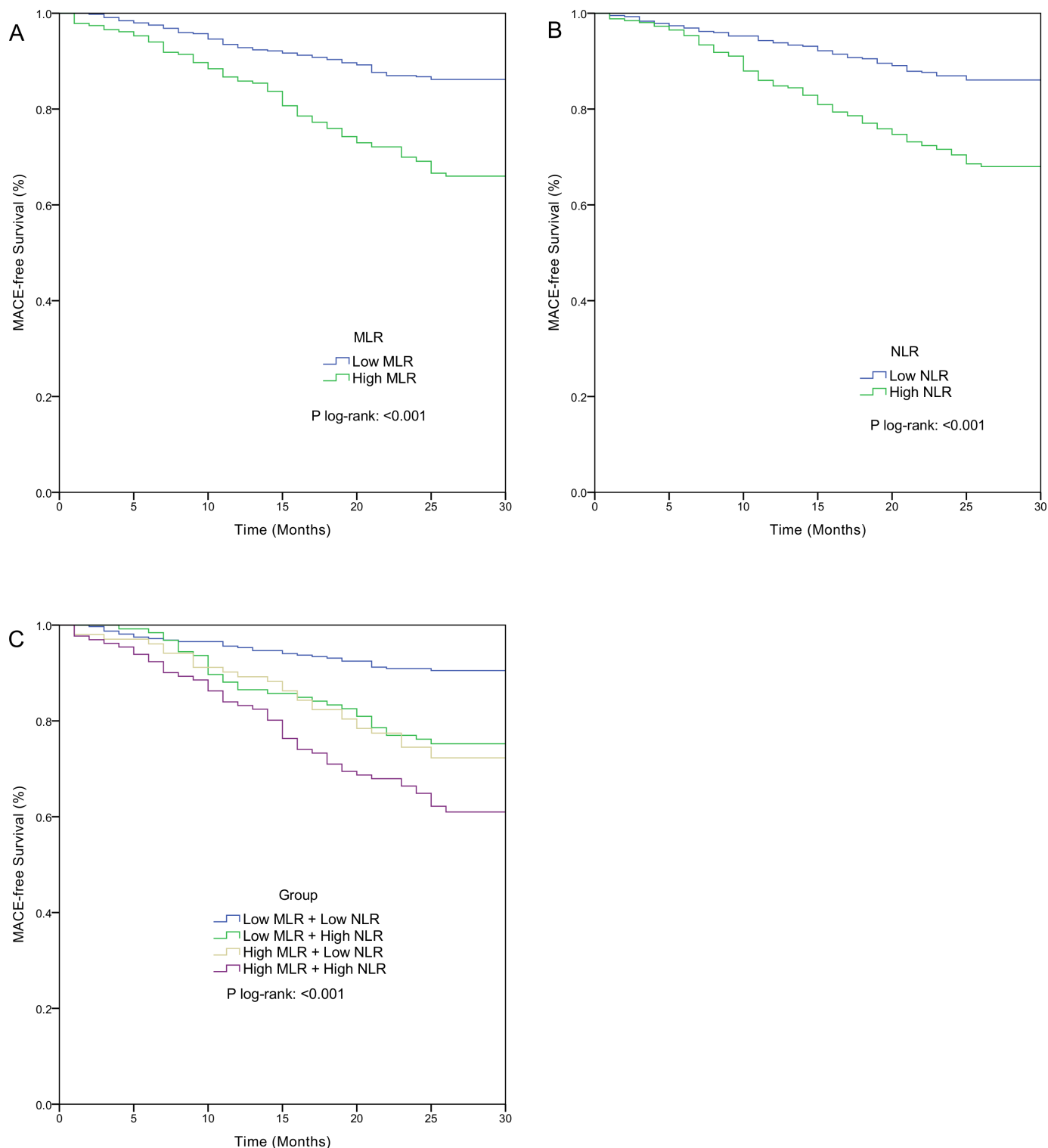


Figure 2 Kaplan-Meier cumulative MACE-free curves in patients with NSTEMI (A) according to the cut-off value of MLR; (B) according to the cut-off value of NLR; (C) according to MLR combined with NLR. MACE, major adverse cardiac events; MLR, monocyte-to-lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio; NSTEMI, non-ST elevated myocardial infarction.

95% CI 0.647 to 0.718), NLR (AUC 0.646, 95% CI 0.609 to 0.682), hs-CRP (AUC 0.642, 95% CI 0.593 to 0.691) and BNP alone (AUC 0.633, 95% CI 0.583 to 0.682) (all p values <0.05), whereas there was no statistically significant difference among the four individual biomarkers in AUC values. Additionally, MLR in combination with NLR

performed similarly to all other pairwise combinations of the four biomarkers (all p values ≥ 0.05 , figure 3B).

DISCUSSION

In this study, 139 of 678 patients (20.50%) presented with MACE during the follow-up period. The MACE rate in

Table 3 Independent predictors of long-term major adverse cardiac events in patients with non-ST elevated myocardial infarction (NSTEMI) by multivariate Cox regression analyses

Variable	HR	95% CI	P values
Model 1			
MLR			
Low MLR, MLR <0.36	Ref		
High MLR, MLR ≥0.36	2.128	1.458 to 3.105	<0.001
NLR			
Low NLR, NLR <2.15	Ref		
High NLR, NLR ≥2.15	1.925	1.385 to 2.676	<0.001
hs-CRP	1.747	1.173 to 2.601	0.006
BNP	1.950	1.156 to 3.290	0.012
Model 2			
Combination of MLR and NLR			
Low MLR + low NLR	Ref	-	-
Low MLR + high NLR	2.732	1.417 to 5.268	0.003
High MLR + low NLR	3.004	1.519 to 5.940	0.002
High MLR + high NLR	4.055	2.550 to 6.448	<0.001
hs-CRP	1.576	1.058 to 2.349	0.025
BNP	1.874	1.137 to 3.088	0.014

BNP, brain natriuretic peptide; hs-CRP, high-sensitivity C reactive protein; MLR, monocyte-to-lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio.

this study was comparable with that of the previous study (21.62%).¹⁷ The novel finding of the present study was that elevated MLR and NLR were independently associated with adverse clinical outcomes in patients with NSTEMI. Moreover, the study demonstrated for the first time that the combination of MLR with NLR has stronger predictive potential for long-term MACE in patients with NSTEMI undergoing primary PCI, compared with individual MLR or NLR.

Many compelling studies have clearly indicated that NLR can be a reliable prognostic factor for short-term and long-term adverse outcomes in patients with ACS undergoing PCI.^{18–19} Neutrophils, the most abundant leucocytes in the circulation, are actively involved in atherogenesis and plaque destabilisation.^{20–21} Several mechanisms can probably explain the pivotal role of neutrophils in atherosclerosis: (1) Neutrophils can infiltrate coronary atherosclerotic plaques and the infarcted myocardium, and mediate tissue damage by releasing matrix-degrading enzymes and reactive oxygen species. (2) Increases in neutrophil counts can aggravate endothelial dysfunction, modulate microvascular permeability and contribute to foam cell formation. (3) Neutrophils can promote endothelial erosion, weaken fibrous cap and accelerate neointima formation which contribute to plaque destabilisation.^{22–25} Lymphocytes are an integral part of the immune system, which participate in every phase of atherosclerosis. Lymphocytopenia, resulting from increased lymphocyte apoptosis, contributes to atherosclerotic plaque growth, lipid core development,

plaque destabilisation, postinfarct cardiac remodelling and progression.^{3,26} Lower lymphocyte count was reported to be an early marker of acute myocardial infarction, and was associated with worse cardiovascular outcomes.^{27–28} Obviously, it could be concluded that NLR, a composite marker of neutrophils and lymphocytes, can provide prognostic value in patients with ACS. In agreement with previous evidence, our study confirmed the prognostic role of increased NLR in patients with NSTEMI.

MLR, a novel haematological marker, has recently been reported to be a prognostic factor in many diseases, especially in various malignancies.^{29–30} To date, just a few studies have attempted to elucidate the impact of MLR on cardiovascular disease. In our previous studies, MLR had the potential to assess coronary lesion severity,⁹ and identify the vulnerable plaques in patients with stable angina.³¹ Siva *et al* showed that increased MLR level was associated with higher mortality in patients with acute heart failure.¹⁰ Kiris *et al* reported that elevated MLR level was independently associated with a higher risk of 6-month mortality in patients with STEMI undergoing primary PCI.¹¹ Gijsberts *et al* found that MLR significantly improved mortality prediction in patients with coronary angiography.¹² Thus, a high MLR was associated with adverse cardiac clinical outcomes, though fewer studies have been performed for MLR and cardiac prognosis, compared with those for NLR. Monocytes play an essential role in every stage of atherosclerosis³², which can recruit to the artery wall, differentiate into macrophages and stimulate activating the secretion of proinflammatory

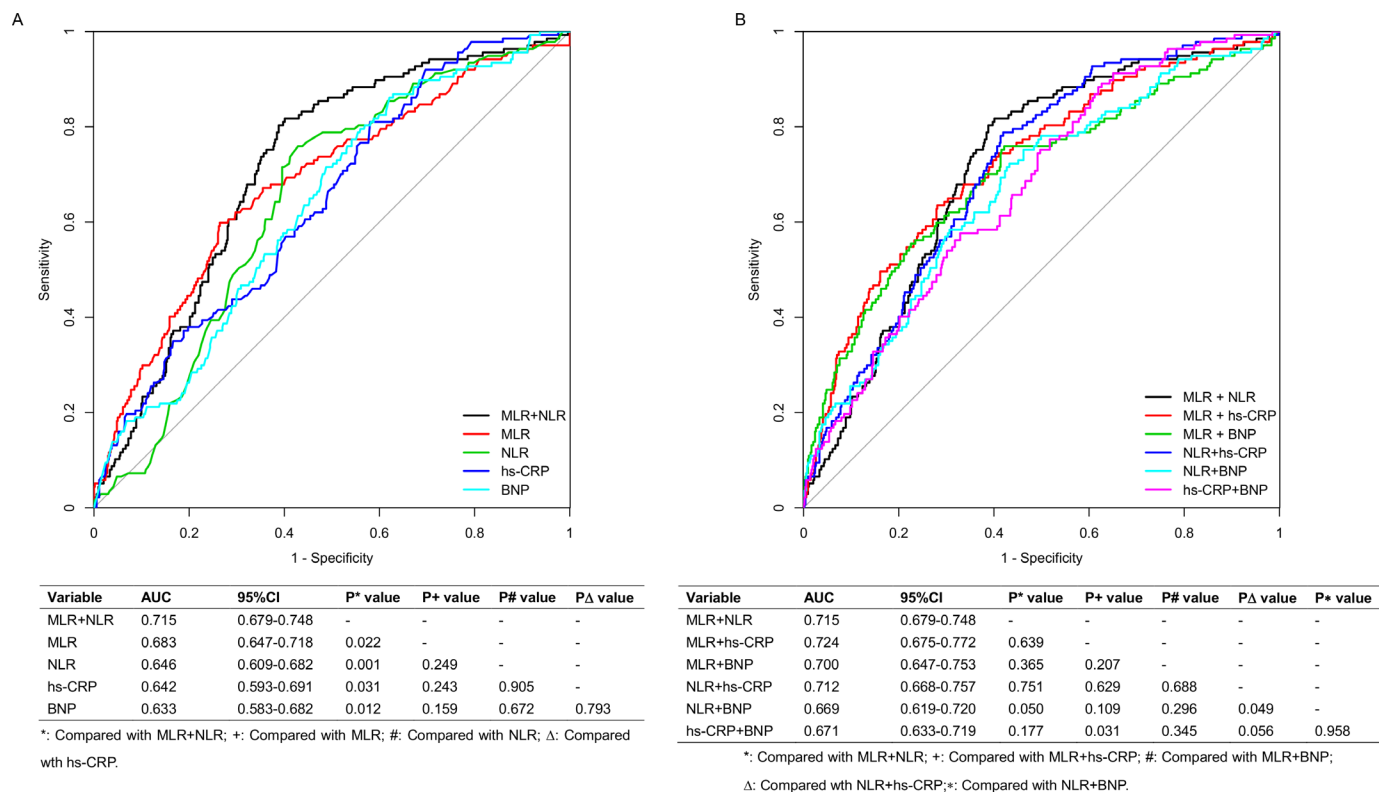


Figure 3 Receiver operating characteristic curves showing area under the curve (AUC) for (A) MLR in combination with NLR (MLR + NLR), MLR alone, NLR alone, hs-CRP alone and BNP alone; (B) MLR + NLR, MLR in combination with hs-CRP (MLR + hs-CRP), MLR in combination with BNP (MLR + BNP), NLR in combination with hs-CRP (NLR + hs-CRP), NLR in combination with BNP (NLR + BNP) and hs-CRP in combination with BNP (hs-CRP + BNP), for long-term MACE in patients with NSTEMI. BNP, brain natriuretic peptide; hs-CRP, high-sensitivity C reactive protein; MACE, major adverse cardiac events; MLR, monocyte-to-lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio; NSTEMI, non-ST elevated myocardial infarction.

cytokines.⁸ Compared with neutrophils, monocytes can produce higher levels of cytokines.³³ Recent pathological studies have found that monocytes can replace neutrophils and become the prominent infiltrating leucocytes within 48 hours of the onset of myocardial ischaemia.³⁴ On the other hand, MI may liberate haematopoietic stem and progenitor cells from bone marrow niches which could increase the availability of monocytes.³⁵ Therefore, MLR, being an integrated reflection of two important immune cells, could be a potential prognostic factor for ACS, and the present study confirmed this hypothesis. Our results revealed that MLR was an independent predictor of long-term MACE and had comparable diagnostic ability as NLR for long-term MACE in patients with NSTEMI undergoing primary PCI. Compared with STEMI, NSTEMI is much more common and tends to have increased mortality in the year following MI.³⁶ Furthermore, we evaluated the combined usefulness of MLR and NLR for predicting long-term MACE in patients with NSTEMI undergoing primary PCI. Our results showed that the combination of MLR with NLR was an independent predictor, more predictive than individual markers, in predicting long-term MACE in patients with NSTEMI.

In addition, our study found that hs-CRP and BNP were also independent predictors of long-term MACE in patients with NSTEMI undergoing primary PCI. hs-CRP

and BNP were classical biomarkers correlated with cardiovascular risk and prognosis. A recent meta-analysis of 14 studies concluded that elevated hs-CRP could predict the risk of cardiovascular mortality in the general population.³⁷ Cho KI *et al* showed that an increased hs-CRP level was a significant independent predictor of long-term adverse events in patients with NSTEMI/unstable angina.¹⁷ BNP has been established as a biomarker in vascular diseases used for monitoring disease progression. Porapakham *et al* performed a meta-analysis of eight randomised clinical trials and indicated that BNP could be used for guiding the treatment of chronic heart failure,³⁸ while Klok *et al* conducted a meta-analysis of 13 studies and revealed the prognostic value of BNP in patients with pulmonary embolism.³⁹ In patients with NSTEMI, Fukazawa *et al* showed that an increased concentration of BNP at admission was closely associated with poor prognosis.⁴⁰ Our study suggested that in addition to MLR and NLR, hs-CRP and BNP are also independent prognostic factors of long-term MACE in patients with NSTEMI undergoing primary PCI. Besides, compared with hs-CRP and BNP, the combined usefulness of MLR and NLR had a higher HR for predicting long-term MACE in patients with NSTEMI. ROC curves revealed that MLR in combination with NLR was superior to either MLR, NLR, hs-CRP or BNP alone in predicting long-term MACE, and it had

similar performance to all other pairwise combinations of the four biomarkers. Moreover, the measurement of MLR and NLR could be more cost-effective and easily accessible in clinical practice, which would possess practical clinical utility in the prediction of prognosis of NSTEMI.

This study had several limitations. First, this study comprised a modest sample size which may introduce selection bias. This single-centre study lacks external validation. Thus, these findings need further multi-institutional validation with larger samples. Second, we evaluated MLR and NLR on admission to the hospital, but didn't assess their dynamic changes during the follow-up period. Third, inflammatory biomarkers such as myeloperoxidase, interleukin 6 and tumour necrosis factor were not analysed in our patients. Finally, several scoring systems, for example, the HEART Score,⁴¹ have been developed to risk-stratify patients with ACS and have been to be associated with patients' prognosis. It would be of interest to investigate the additive value of MLR/NLR to the scoring systems, but this is beyond the scope of this study. Notwithstanding these limitations, this study first reported the prognostic value of the combination of MLR with NLR in patients with NSTEMI.

In conclusion, the combined usefulness of MLR with NLR gains a prognostic value in patients with NSTEMI, which could be used to identify the high-risk patients with poor outcomes and adjust their treatment accordingly. These findings provide a new perspective on the non-invasive, simple, economical and feasible biomarkers in predicting long-term MACE in patients with NSTEMI.

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