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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 was 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). High MLR + high NLR group had lower MACE-free survival rate than the other three groups (P log-rank < 0.001). Both MLR (hazard ratio (HR): 2.144, 95%CI: 1.510-3.045) and NLR (HR: 2.059, 95%CI: 1.446-2.933) were independent predictors of long-term MACE. Moreover, the patients in high-MLR + high-NLR group had an HR of 4.660 (95%CI: 2.957-7.342) for long-term MACE, compared with the patients in low-MLR + low-NLR group. And ROC curves revealed that the combination of MLR with NLR (areas under curve

(AUC): 0.715) had better performance in differentiating long-term MACE, compared with individual MLR (AUC: 0.683) and NLR (AUC: 0.646) biomarkers (P = 0.022, and P = 0.001, respectively).

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.

Keywords

Monocyte-to-lymphocyte ratio; Neutrophil-to-lymphocyte ratio; Major adverse cardiac events; Non-ST elevated myocardial infarction

Strengths and limitations of this study

- This is the first study considering the incorporate use of monocyte-to-lymphocyte ratio (MLR) and neutrophil-to-lymphocyte ratio (NLR) for predicting long-term major adverse cardiac events (MACE) in patients with non-ST elevated myocardial infarction (NSTEMI) undergoing primary percutaneous coronary intervention (PCI).
- Elevated MLR and NLR were independently associated with long-term MACE in patients with NSTEMI, while 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.
- MLR and NLR, non-invasive, simple, economical and feasible biomarkers, possess practical clinical utility in the prognosis prediction of NSTEMI.
- These findings need further multi-institutional with larger samples validation.

INTRODUCTION

Previous studies have verified that inflammatory response plays a vital role in the development of atherosclerosis and cardiovascular diseases.[1 2] White blood cell and its subtypes including neutrophils, monocytes and lymphocytes are important immune cells involved in the initiation, formation and destabilization of atherosclerosis.[3] 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] And 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 pro-inflammatory cytokines which play a crucial role at every level of the atherosclerotic process.[8] Monocyte-to-lymphocyte ratio (MLR) has emerged as a novel systematic inflammatory marker related with increased cardiovascular risk.[9] Recently, MLR is reported to be associated with adverse clinical outcomes in patients with ST-elevation myocardial infarction (STEMI) and acute heart failure.[10 11] However, the prognostic value of the combined usefulness of MLR and NLR in 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 Civil Aviation General Hospital, Beijing, China. A total of 818 consecutive NSTEMI patients who underwent primary PCI at the institution from July 2010 to July 2015 were considered for participation in this study. The NSTEMI was defined by typical ischemia symptoms, elevation level of cardiac troponin-I or creatine kinase-MB and no evidence of ST segment elevation in electrocardiography. We excluded patients who had serious heart failure (NYHA Class III or IV), rheumatic heart disease, valvar heart disease, congenital heart disease, pulmonary heart disease, active or chronic inflammatory conditions, acute infection, hemodynamic disorders, malignancies, severe renal (estimated glomerular filtration rate [eGFR] < 30 ml/min/1.73m^2) or hepatic (alanine-aminotransferase > 40 U/l) disease, steroid therapy in the preceding 3 months, history of cerebrovascular vascular events or incomplete blood cell count or medical records. Hypertension was defined as current use of an antihypertensive medication or, a systolic blood pressure ≥ 140 mmHg and/or a diastolic blood pressure ≥ 90 mmHg. Diabetes mellitus was defined as active use of an antidiabetic agent, or fasting plasma glucose level ≥ 7.0 mmol/L or casual plasma glucose level ≥ 11.1 mmol/L.

This research was approved by the Ethical Committee of Civil Aviation General Hospital, and informed consent was obtained from all patients.

Study procedures and laboratory analysis

Complete blood counts and biochemical indicators were measured at the time of admission by the core laboratory of Civil Aviation General Hospital. Complete blood counts included hemoglobin, white blood cells, neutrophils, monocytes, lymphocytes, and platelets. The biochemical indicators included total cholesterol, triglycerides, creatinine, low density lipoprotein (LDL), high density lipoprotein (HDL), creatinine, high-sensitivity C-reactive protein (hs-CRP) and brain natriuretic peptide (BNP) . eGFR was calculated using the Chronic Kidney Disease Epidemiology (CKD EPI) creatinine equation. The 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 to 100 U/kg) to maintain an activated clotting time 250-300 seconds 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 (TIMI) II or III flow in the treated artery. Angiographic characteristics were collected for all the patients.

Clinical outcomes

The main outcomes were MACE 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 revascularization (TLR) and target vessel revascularization (TVR) according to the Academic Research Consortium definition.[12] Cardiac death was defined as death resulting from any cardiac-related causes (eg, MI, heart failure, lethally cardiac arrhythmia). Nonfatal MI was defined based on the European Society of Cardiology, American Heart Association, American College of Cardiology, and World Heart Federation definitions.[13] TLR was defined as a repeat revascularization caused by a stenosis ≥ 50% within the stent or within a 5-mm proximal or distal to the stent. TVR was defined as a 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

Continuous data were expressed as mean ± standard deviation (SD) or median (interquartile range). The Kolmogorov-Smirnov test was used to analyze the normal distribution of continuous data. Student's t test or the Mann-Whitney U test, or one-way ANOVA model was used for the comparisons of continuous variables. Categorical data were expressed as numbers and percentages and compared using Chi-square tests. ROC curves were utilized to determine the ability of MLR and NLR to differentiate MACE. The optimal cut-off values were derived from Youden's index. 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 Log-rank test. Univariate and multivariate COX regression analyses were carried out to identify the independent predictors of MACE. The

statistical significance was considered as a 2-tailed $P < 0.05$. Statistical analyses were performed using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

818 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 the 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** depicted the clinical layout of the study cohort.

Baseline clinical characteristics

A MLR cut-off value of 0.36 had 54.74% sensitivity and 73.57% specificity and a NLR cut-off value of 2.35 had 77.37% sensitivity and 55.08% specificity for differentiating long-term MACE, via ROC analysis. According to the optimal cut-off values of MLR 0.36 and NLR 2.35, participants were classified into four groups: low-MLR + low-NLR group (MLR < 0.36, NLR < 2.35, n=319), low-MLR + high-NLR group (MLR < 0.36, NLR ≥ 2.35, n=126), high-MLR + low-NLR group (MLR ≥ 0.36, NLR < 2.35, n=102) and high-MLR + high-NLR group (MLR ≥ 0.36, NLR ≥ 2.35, n=131). The clinical characteristics were summarized in **Table 1**. The distribution of cardiovascular conventional risk factors, prior medications and angiographic findings were similar between the four groups. However, patients in 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 and BNP, whereas they had lower lymphocytes.

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-value
Age /(year)	61.1±14.38	63.63±15.05	64.05±15.15	65.23±16.34*	0.036
Male, n (%)	209(65.5%)	71(56.3%)	65(63.7%)	90(68.7%)	0.188
Family history, n (%)	33(10.3%)	17(13.5%)	11(10.8%)	20(15.3%)	0.463
Hypertension, n(%)	221(69.3%)	86(68.3%)	73(71.6%)	105(80.2%)	0.100
Diabetes Mellitus, n(%)	125(39.2%)	56(44.4%)	39(38.2%)	59(45.0%)	0.522
Dyslipidemia, n (%)	114(35.7%)	51(40.5%)	35(34.3%)	49(37.4%)	0.758
Current Smoker, n (%)	108(33.9%)	45(35.7%)	33(32.4%)	51(38.9%)	0.702
Killip class (> I)	192(60.2%)	86(68.3%)	69(67.6%)	99(75.6%)*	0.015
Laboratory parameters					
White blood cell, $\times 10^9$	6.67±1.72	6.77±2.03	7.06±1.98*	7.25±2.11*	0.027
Neutrophil, $\times 10^9$	4.26±1.07	4.69±1.19*	4.56±1.15*	5.08±1.27*+#	<0.001
Lymphocyte, $\times 10^9$	2.31±0.53	1.98±0.51*	2.37±0.67+	2.02±0.64*#	<0.001
Monocyte, $\times 10^9$	0.18±0.09	0.22±0.11*	0.49±0.19*+	0.46±0.13*+	<0.001
Hemoglobin, g/L	136.7±30.38	129.6±27.57*	133.7±39.76	127.4±36.01*	0.039
Total cholesterol /(mmol/L)	5.7±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	3.1±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/1.73m^2	85.7±29.1	81.9±28.7	78.9±26.3	81.3±27.1	0.102
BNP/(pg/ml)	265.1±82.74	245.6±79.38*	269.7±87.57*+	298.7±97.15*+#	<0.001
Medical Treatment					
Aspirin, n(%)	309(96.9%)	119(94.4%)	96(94.1%)	129(98.5%)	0.202
Anticoagulant, n(%)	305(95.6%)	117(92.9%)	93(91.2%)	124(94.7%)	0.340
Statin, n(%)	292(91.5%)	112(88.9%)	90(88.2%)	121(92.4%)	0.589
ACEI or ARB, n(%)	186(58.3%)	71(56.3%)	61(59.8%)	84(64.1%)	0.602

Beta-blocker, n(%)	259(81.2%)	98(77.8%)	86(84.3%)	105(80.2%)	0.654
Calcium-channel blockers, n(%)	67(21%)	30(23.8%)	26(25.5%)	28(21.4%)	0.767
Nitrate drugs, n(%)	257(80.6%)	96(76.2%)	75(73.5%)	106(80.9%)	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-vessel, n(%)	76(23.82%)	39(30.95%)	32(31.37%)	42(32.06%)	
three-vessel/ 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(8.5%)	16(12.7%)	11(10.8%)	14(10.7%)	0.575
Moderate or severe calcification, n(%)	29(9.1%)	14(11.1%)	13(12.7%)	15(11.5%)	0.706

*: compared with low-MLR and low-NLR group, $p<0.05$;

+: compared with low-MLR and high-NLR group, $p<0.05$;

#: compared with high-MLR and high-NLR group, $p<0.05$.

ACEI, angiotensin-converting enzyme 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.

Clinical outcomes

During the median follow-up period of 26 months, long-term MACE were observed in 139 (20.50%) patients. 10 (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 to 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**).

The Kaplan-Meier curves based on the cut-off value of MLR and NLR, were presented in **Figure 2A** and **Figure 2B**, respectively. Significantly increased long-term MACE rates were observed in patients with high MLR (33.5% vs. 13.7%, $P < 0.001$, **Figure 2A**) and in patients with high NLR (31.5% vs. 13.8%, $P < 0.001$, **Figure 2B**). The Kaplan-Meier MACE-free curve based on the combined markers was shown in **Figure 2C**. The MACE rates were significantly different among the four groups ($P < 0.001$) and patients in high-MLR + high-NLR group had the highest MACE rate.

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-value
Follow-up 2 years	30(9.4%)	31(24.6%)*	28(27.5%)*	50(38.2%)*+#	<0.001
All- cause death	2(0.6%)	1(0.8%)	1(1.0%)	6(4.6%)*	0.010
Cardiac death	1(0.3%)	1(0.8%)	1(1.0%)	5(3.8%)*	0.018
Nonfatal MI	9(2.8%)	8(6.3%)	9(8.8%)*	14(10.7%)*	0.006
Stroke	4(1.3%)	6(4.8%)*	5(4.9%)*	9(6.9%)*	0.017
TLR	14(4.4%)	16(12.7%)*	12(11.8%)*	19(14.5%)*	0.001
TVR	1(0.3%)	0(0%)	1(1.0%)	2(1.5%)	0.335

*: compared with low-MLR and low-NLR group, p<0.05;

+: compared with low-MLR and high-NLR group, p<0.05;

#: compared with high-MLR and high-NLR group, p<0.05.

MI, myocardial infarction; TLR: target lesion revascularization; TVR: target vessel revascularization.

Independent predictors of long-term MACE

Multivariate COX regression analyses were utilized to determine the independent predictors

of long-term MACE in patients with NSTEMI undergoing primary PCI. On multivariate COX analysis, both MLR (HR 2.144, 95% CI 1.510-3.045, $P < 0.001$) and NLR (HR 2.059, 95% CI 1.446-2.933, $P < 0.001$) were found to be significant predictors of long-term MACE. And the combination of MLR and NLR was found to be an independent predictor of long-term MACE (HR 4.660, 95% CI: 2.957-7.342, $P < 0.001$ 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 independent predictors of long-term MACE in patients with NSTEMI undergoing primary PCI (**Table 3**).

Table 3 Independent predictors of long-term MACE in Patients with NSTEMI by COX regression analyses

Variable	Hazard Ratio	95% Confidence Interval	P value
Model 1			
MLR			
Low MLR, MLR<0.36	Ref	-	-
High MLR, MLR≥0.36	2.144	1.510-3.045	<0.001
NLR			
Low NLR, NLR<2.15	Ref	-	-
High NLR, NLR≥2.15	2.059	1.446-2.933	<0.001
BNP	1.791	1.134-2.829	0.004
hs-CRP	2.054	1.173-3.597	0.012
Model 2			
Combination of MLR and NLR			
Low-MLR + Low-NLR	Ref	-	-
Low-MLR + High-NLR	2.807	1.699-4.637	<0.001
High-MLR + Low-NLR	3.002	1.785-5.049	<0.001
High-MLR + High-NLR	4.660	2.957-7.342	<0.001
BNP	1.976	1.200-3.254	0.007
hs-CRP	2.034	1.207-3.428	0.008

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

Diagnostic efficacy of MLR and NLR in differentiating MACE

ROC curves were to test the diagnostic efficacy of MLR, NLR and the combination of MLR with NLR, for differentiating long-term MACE. MLR (AUC: 0.683, 95%CI: 0.647-0.718) had a similar AUC value as NLR (AUC: 0.646, 95%CI: 0.609-0.682). Additionally, the combination of MLR with NLR has the largest AUC area (AUC: 0.715, 95%CI: 0.679-0.748). Pairwise comparisons of ROC curves revealed that the combination of MLR with NLR had the highest discriminative potential for the detection of long-term MACE (P = 0.022, for MLR+NLR vs. MLR; P = 0.001, for MLR+NLR vs. NLR), whereas MLR had the comparable ability with NLR for differentiating long-term MACE (P = 0.247) (**Figure 3**).

DISCUSSION

In this study, there were 139 of 678 patients (20.5%) presented with MACE, during the follow-up period. The MACE rate was comparable between our study and the previous study (21.6%).[14] [14]. 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 and long-term adverse outcomes in patients with acute coronary syndrome

undergoing PCI.[15 16] Neutrophils, the most abundant white blood cells in the circulation, are actively involved in the atherogenesis and plaque destabilization.[17 18]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 destabilization.[19-22] Lymphocytes are an integral part of the immune system, which participate in every phase of atherosclerosis. Lymphocytopenia, resulting from increased lymphocytes apoptosis, contributes to post-infarct cardiac remodeling and progression.[3 23] Lower lymphocyte count was reported to be associated with worse cardiovascular outcomes.[24] Obviously, it could be concluded that NLR, a composite marker of neutrophils and lymphocytes, can provide prognostic value in ACS patients. In agreement with previous evidence, our study confirmed the prognostic role of increased NLR in patients with NSTEMI.

MLR, a novel composite marker, has been recently reported as a prognostic factor in many diseases, especially various malignancies.[25 26] 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 in assessing coronary lesion severity,[9] and identifying the vulnerable plaques in patients with stable angina[27]. Siva et al. showed that increased MLR level was associated with higher morality in patients with acute heart failure.[10] Kiris et al. reported that

elevated MLR level was independently associated with a higher risk of six-month mortality in patients with STEMI undergoing primary PCI.[11] Thus, a high MLR was associated with adverse cardiac clinical outcomes, though fewer studies have been performed for MLR and cardiac prognosis, compared to those for NLR. Monocytes also play an essential role in every stage of atherosclerosis, though numbers of monocytes are lower than that of neutrophils.[28 29] They can recruit to the artery wall, differentiate into macrophages and stimulate activating the secretion of pro-inflammatory cytokines.[8] And compared with neutrophils, monocytes can produce higher levels of cytokines.[30] Moreover, recent pathological studies have found that monocytes can replace neutrophils and become the prominent infiltrating leukocytes within 48 h after the onset of myocardial ischemia.[31] Therefore, MLR could be a potentially prognostic factor for acute coronary syndrome, and the present study confirmed the 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 an increased mortality in the year following the myocardial infarction.[32] 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, superior to individual markers, in predicting the long-term MACE in patients with NSTEMI. Thus, it could be concluded that 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, the combined usefulness of MLR and NLR, in predicting long-term adverse outcomes 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.[33] 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.[14] BNP has been established as a biomarker in vascular diseases used for monitoring disease progression. Porapakkham et al. performed a meta-analysis of eight randomized clinical trials and indicated that BNP could be used for guiding the treatment of chronic heart failure [34], while Klok et al. conducted a meta-analysis of 13 studies and revealed the prognostic value of BNP in patients with pulmonary embolism.[35] In patients with NSTEMI, Fukazawa et al. showed that an increased concentration of BNP at admission was closely associated with poor prognosis.[36] In our study, multivariate COX regression analyses suggested that hs-CRP and BNP are independent prognostic factors of long-term MACE in patients with NSTEMI undergoing primary PCI. However, compared with hs-CRP and BNP, the combined usefulness of MLR and NLR was a significant prognostic factor with higher HR. And the measurement of MLR and NLR could be more cost-effectiveness and flexible in clinical practice, which possess practical clinical utility in the prognosis prediction of NSTEMI.

This study had several limitations. Firstly, this study comprised of a modest sample size

which may introduce selection bias. And the single center study was lack of external validation. Thus, these findings need further multi-institutional with larger samples validation. Secondly, we evaluated the MLR and NLR on admission of the hospital, but didn't assess their dynamic changes during the follow-up period. Finally, inflammatory biomarkers such as myeloperoxidase, interleukin-6 and tumor necrosis factor were not analyzed in our patients. Notwithstanding these limitations, this study firstly reported the prognostic value of the combination of MLR with NLR in patients with NSTEMI.

In conclusion, MLR and NLR, and especially their combination, were all independent predictors of long-term MACE in patients with NSTEMI who underwent primary PCI. Moreover, the combined usefulness of MLR with NLR performed better than individual MLR or NLR in predicting long-term MACE in patients with NSTEMI who underwent primary PCI.

Contributors ZF made the major contribution to the conception and design of the study, and drafted the manuscript. YL and HJ contributed to the data acquisition and performed the statistical analyses. XJ revised the manuscript. XJ is the guarantor of the study.

All authors read and approved the final manuscript prior to the submission.

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Competing interests None declared.

Ethics approval This research was approved by the ethics committee of the Ethical Committee of Civil Aviation General Hospital.

Data Sharing Statement Raw data can be obtained by contacting the corresponding author.

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Figure Legend

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.

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.

Figure 3. Receiver-operating characteristic curves identifying the discrimination thresholds of MLR, NLR, and the combination of MLR and NLR (MLR+NLR) for the prediction of long-term MACE.

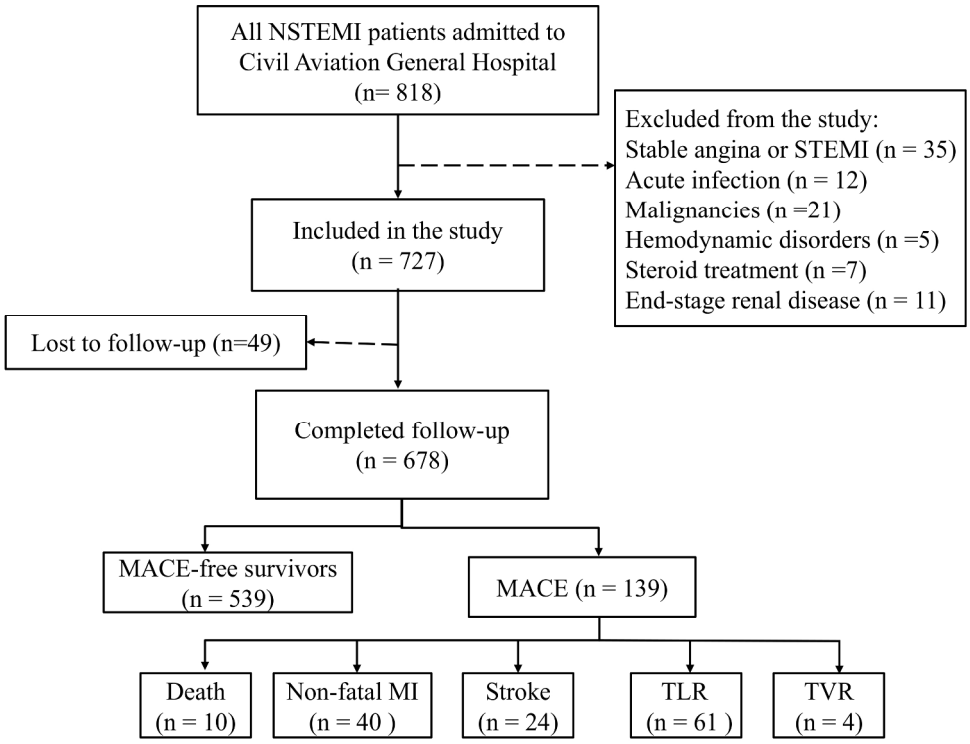

Figure 1. Flow Chart of the study cohort. The flow chart presents the selection criteria of the study and the clinical layout of the study population.

254x190mm (300 x 300 DPI)

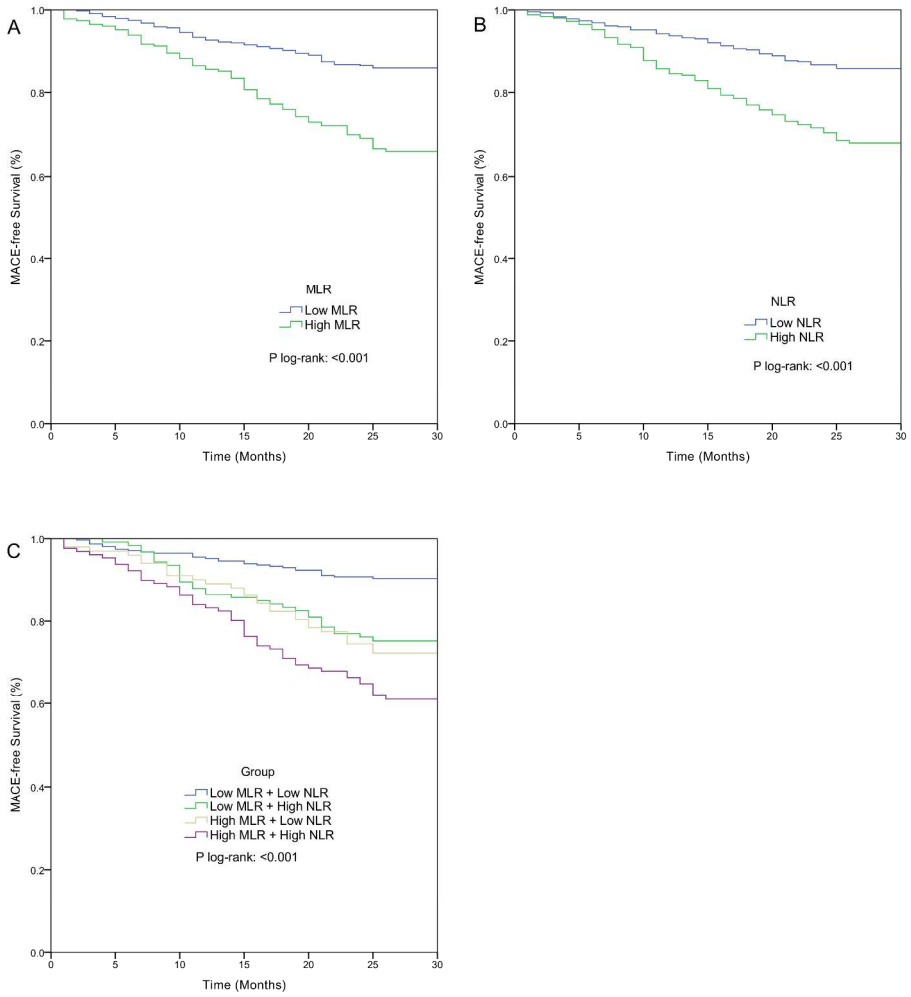

Figure 2. Kaplan-Meier cumulative MACE-free curves in patients with NSTEMI (A) according to the cut-off value of MLR; (B) according to the cut-off value of NLR; (C) according to MLR combined with NLR.

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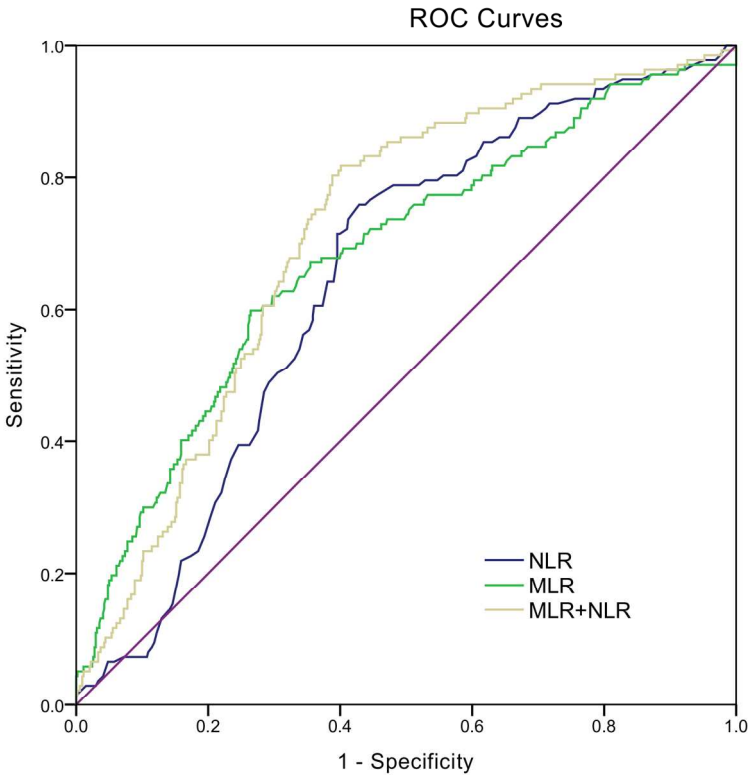

Variable	AUC	SE [a]	95% CI [b]	P value
MLR	0.683	0.0264	0.647 - 0.718	<0.001
NLR	0.646	0.0242	0.609 - 0.682	<0.001
MLR+NLR	0.715	0.0229	0.679 - 0.748	<0.001

[a] DeLong et al., 1988

[b] Binormia exact

Figure 3. Receiver-operating characteristic curves identifying the discrimination thresholds of MLR, NLR, and the combination of MLR and NLR (MLR+NLR) for the prediction of long-term MACE.

152x194mm (300 x 300 DPI)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1,2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6-7
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	Not applicable
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-7
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	*6*
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	7
		(*c*) Explain how missing data were addressed	7
		(*d*) If applicable, explain how loss to follow-up was addressed	7
		(*e*) Describe any sensitivity analyses	7
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	8
		(b) Give reasons for non-participation at each stage	8
		(c) Consider use of a flow diagram	8, Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8-10
		(b) Indicate number of participants with missing data for each variable of interest	8
		(c) Summarise follow-up time (eg, average and total amount)	8
Outcome data	15*	Report numbers of outcome events or summary measures over time	10-11
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	11-12
		(*b*) Report category boundaries when continuous variables were categorized	8
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	11-12
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	13
Discussion			
Key results	18	Summarise key results with reference to study objectives	13
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	16-17
Generalisability	21	Discuss the generalisability (external validity) of the study results	17
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	17

*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.

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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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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). High MLR + high NLR group had lower MACE-free survival rate than the other three groups (P log-rank < 0.001). Both MLR [hazard ratio (HR): 2.128, 95% CI: 1.458-3.105] and NLR (HR: 1.925, 95% CI: 1.385-2.676) were independent predictors of long-term MACE. Moreover, the patients in high-MLR + high-NLR group had a HR of 4.055 (95% CI: 2.550-6.448) for long-term MACE, with low-MLR + low-NLR group as reference. Comparisons of ROC curves revealed that the combination of MLR with NLR achieved

better performance in differentiating long-term MACE, compared with MLR, NLR, hs-CRP and BNP 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.

Keywords

Monocyte-to-lymphocyte ratio; Neutrophil-to-lymphocyte ratio; Major adverse cardiac events; Non-ST elevated myocardial infarction

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 long-term major adverse cardiac events (MACE) in patients with non-ST elevated myocardial infarction (NSTEMI) undergoing primary percutaneous coronary intervention (PCI).
- MLR in combination with NLR, has good performance in prognosis prediction in patients with NSTEMI undergoing primary PCI, through a comparison with (i) MLR, NLR, hs-CRP and BNP alone, and (ii) all other pairwise combinations of the four biomarkers.
- MLR and NLR, non-invasive, simple, economical and feasible biomarkers, possess practical clinical utility in the prognosis prediction of NSTEMI.
- These findings need further multi-institutional with larger samples validation.

INTRODUCTION

Previous studies have verified that inflammatory response plays a vital role in the development of atherosclerosis and cardiovascular diseases.[1 2] White blood cell and its subtypes including neutrophils, monocytes and lymphocytes are important immune cells involved in the initiation, formation and destabilization of atherosclerosis.[3] 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 pro-inflammatory cytokines which play a crucial role at every level of the atherosclerotic process.[8] Monocyte-to-lymphocyte ratio (MLR) has emerged as a novel systematic inflammatory marker related with 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 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 Civil Aviation General Hospital,

Beijing, China. A total of 818 consecutive NSTEMI patients who presented to the emergency department and underwent primary PCI from July 2010 to July 2015 were considered for participation in this study. The NSTEMI was defined by typical ischemia symptoms, elevation level of cardiac troponin-I or creatine kinase-MB and no evidence of ST segment elevation in electrocardiography. We excluded patients who had serious heart failure (NYHA Class III or IV), rheumatic heart disease, valvar heart disease, congenital heart disease, pulmonary heart disease, active or chronic inflammatory conditions, acute infection, hemodynamic disorders, malignancies, severe renal [estimated glomerular filtration rate (eGFR) < 30 ml/min/1.73m^2] or hepatic (alanine-aminotransferase > 40 U/l) disease, steroid therapy in the preceding 3 months, history of cerebrovascular vascular events or incomplete blood cell count or medical records. Hypertension was defined as current use of an antihypertensive medication or, a systolic blood pressure ≥ 140 mmHg and/or a diastolic blood pressure ≥ 90 mmHg. Diabetes mellitus was defined as active use of an antidiabetic agent, or fasting plasma glucose level ≥ 7.0 mmol/L or casual plasma glucose level ≥ 11.1 mmol/L.

This research was approved by the Ethical Committee of Civil Aviation General Hospital (CAGH-EC100201), and informed consent was obtained from all patients. The study was designed and performed in accordance with the Declaration of Helsinki.

Study procedures and laboratory analysis

At the time of admission, venous blood samples were collected from each patient. All hematological 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 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 hemoglobin, white blood cells, neutrophils, monocytes, lymphocytes, and platelets. The biochemical indicators were determined using a Hitachi7600 automatic biochemistry analyzer (Hitachi, Tokyo, Japan).The biochemical indicators included 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. eGFR was calculated using the Chronic Kidney Disease Epidemiology (CKD EPI) creatinine equation. The 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 to 100 U/kg) to maintain an activated clotting time 250-300 seconds 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 (TIMI) II or III flow in the treated artery. Angiographic characteristics were collected for all the patients.

Clinical outcomes

The main outcomes were MACE 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 revascularization (TLR) and target vessel revascularization (TVR) according to the Academic Research Consortium definition.[13] Cardiac death was defined as death resulting from any cardiac-related causes (eg, MI, heart failure, lethally cardiac arrhythmia). Nonfatal MI was defined based on the European Society of Cardiology, American Heart Association, American College of Cardiology, and World Heart Federation definitions.[14] TLR was defined as a repeat revascularization caused by a stenosis ≥ 50% within the stent or within a 5-mm proximal or distal to the stent. TVR was defined as a 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

Kolmogorov-Smirnov test was employed to test the normality of the continuous variables in each group. Continuous variables distributed normally were expressed as mean ± standard deviation (SD), while categorical data were expressed as numbers and percentage. We initially used ROC curves to determine the ability of MLR and NLR to differentiate MACE. Subsequently optimal cut-off values, specificity and sensitivity were derived. Based on the optimal cut-off values, participants were assigned into 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 ANOVA followed by Tukey's post hoc tests, while categorical data were compared

by Chi square 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 Log-rank 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 hazard ratio (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 group = 3]. The effect sizes were expressed as HR and their 95% confidence intervals (CI). Afterwards, we used ROC curves to evaluate the diagnostic performance of individual biomarkers and their pairwise combinations in predicting long-term MACE. The AUCs were compared by Delong's tests. The statistical significance was considered as a 2-tailed $P < 0.05$. Statistical analyses were performed using SPSS version 22.0 (SPSS Inc., Chicago, IL, 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

818 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 the 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** depicted 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 a NLR cut-off value of 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 + low-NLR group (MLR < 0.36, NLR < 2.35, n=319), low-MLR + high-NLR group (MLR < 0.36, NLR ≥ 2.35, n=126), high-MLR + low-NLR group (MLR ≥ 0.36, NLR < 2.35, n=102) and high-MLR + high-NLR group (MLR ≥ 0.36, NLR ≥ 2.35, n=131). The clinical characteristics were summarized in **Table 1**. The distribution of prior medications and angiographic findings were similar between the four groups. However, patients in 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 hemoglobin.

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-value
Age /(year)	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
Dyslipidemia, 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					
White blood cell, $\times10^9$	6.67±1.72	6.77±2.03	7.06±1.98*	7.25±2.11*	0.027
Neutrophil, $\times10^9$	4.26±1.07	4.69±1.19*	4.56±1.15*	5.08±1.27*+#	<0.001
Lymphocyte, $\times10^9$	2.31±0.53	1.98±0.51*	2.37±0.67+	2.02±0.64*#	<0.001
Monocyte, $\times10^9$	0.18±0.09	0.22±0.11*	0.49±0.19*+	0.46±0.13*+	<0.001
Hemoglobin/(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/1.73m^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-vessel, n(%)	76(23.82)	39(30.95)	32(31.37)	42(32.06)	
three-vessel/ 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 low-MLR + low-NLR group, p<0.05;

+: compared with low-MLR+ high-NLR group, p<0.05;

#: compared with high-MLR+ low-NLR group, p<0.05.

ACEI, angiotensin-converting enzyme 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.

Clinical outcomes

During the median follow-up period of 26 months, long-term MACE were observed in 139 (20.50%) patients. 10 (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 to 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**).

The Kaplan-Meier curves based on the cut-off value of MLR and NLR, were presented

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 was shown in **Figure 2C**. The MACE rates were significantly different among the four groups ($P < 0.001$) and patients in high-MLR + high-NLR group had the highest MACE rate.

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-value
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
Nonfatal 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 low-MLR + low-NLR group, p<0.05;

+: compared with low-MLR + high-NLR group, p<0.05;

#: compared with high-MLR + low-NLR group, p<0.05.

MI, myocardial infarction; TLR, target lesion revascularization; TVR, target vessel revascularization.

Independent predictors of long-term MACE

Univariate and multivariate COX regression analyses were utilized to determine the independent predictors of long-term MACE in patients with NSTEMI undergoing primary PCI. In univariate COX analysis, white blood cells, 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: 1.458-3.105, $P < 0.001$) and NLR (HR 1.925, 95% CI: 1.385-2.676, $P < 0.001$) 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: 2.550-6.448, $P < 0.001$ 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 were presented in online supplementary **Table S2**.

Table 3 Independent predictors of long-term MACE in patients with NSTEMI by multivariate COX regression analyses

Variable	Hazard Ratio	95% Confidence Interval	P value
Model 1			
MLR			
Low MLR, MLR<0.36	Ref		
High MLR, MLR≥0.36	2.128	1.458-3.105	<0.001
NLR			
Low NLR, NLR<2.15	Ref		
High NLR, NLR≥2.15	1.925	1.385-2.676	<0.001
hs-CRP	1.747	1.173-2.601	0.006
BNP	1.950	1.156-3.290	0.012
Model 2			
Combination of MLR and NLR			
Low-MLR + Low-NLR	Ref	-	-
Low-MLR + High-NLR	2.732	1.417-5.268	0.003
High-MLR + Low-NLR	3.004	1.519-5.940	0.002

High-MLR + High-NLR	4.055	2.550-6.448	<0.001
hs-CRP	1.576	1.058-2.349	0.025
BNP	1.874	1.137-3.088	0.014

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

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 (i) MLR, NLR, hs-CRP and BNP alone, and (ii) all other pairwise combinations of the four biomarkers, for differentiating long-term MACE. **Figure 3A** showed that MLR in combination with NLR (AUC: 0.715, 95%CI: 0.679-0.748) achieved better performance in predicting long-term MACE, than MLR (AUC: 0.683, 95%CI: 0.647-0.718), NLR (AUC: 0.646, 95%CI: 0.609-0.682), hs-CRP (AUC: 0.642, 95%CI: 0.593-0.691) and BNP alone (AUC: 0.633, 95%CI: 0.583-0.682) (All P values < 0.05), whereas there was no statistical difference among the four individual biomarkers in AUC values. Additionally, MLR in combination with NLR showed similar performance to all other pairwise combinations of the four biomarkers (All *P* values ≥ 0.05, **Figure 3B**).

DISCUSSION

In this study, there were 139 of 678 patients (20.50%) presented with MACE, during the follow-up period. The MACE rate was comparable between our study and the previous study (21.62%)[15]. 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 and long-term adverse outcomes in patients with acute coronary syndrome undergoing PCI.[16 17] Neutrophils, the most abundant white blood cells in the circulation, are actively involved in the atherogenesis and plaque destabilization.[18 19]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 destabilization.[20-23] Lymphocytes are an integral part of the immune system, which participate in every phase of atherosclerosis. Lymphocytopenia, resulting from increased lymphocytes apoptosis, contributes to atherosclerotic plaque growth, lipid core development, plaque destabilization, post-infarct cardiac remodeling and progression.[3 24] Lower lymphocyte count was reported to be not only an early marker of acute myocardial infraction, but also associated with worse cardiovascular outcomes.[25 26] Obviously, it could be concluded that NLR, a composite marker of neutrophils and lymphocytes, can provide prognostic value in ACS patients. In agreement with previous evidence, our study confirmed the prognostic role of increased NLR in patients with NSTEMI.

MLR, a novel hematological marker, has been recently reported as a prognostic factor in many diseases, especially various malignancies.[27 28] 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 in assessing coronary lesion severity,[9] and identifying the vulnerable plaques in patients with stable angina.[29] 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 six-month mortality in patients with STEMI undergoing primary PCI.[11] Gijsberts et al. found that MLR significantly improved mortality prediction in coronary angiography patients.[12] Thus, a high MLR was associated with adverse cardiac clinical outcomes, though fewer studies have been performed for MLR and cardiac prognosis, compared to those for NLR. Monocytes play an essential role in every stage of atherosclerosis.[30] They can recruit to the artery wall, differentiate into macrophages and stimulate activating the secretion of pro-inflammatory cytokines.[8] Compared with neutrophils, monocytes can produce higher levels of cytokines.[31] Recent pathological studies have found that monocytes can replace neutrophils and become the prominent infiltrating leukocytes within 48 h after the onset of myocardial ischemia.[32] On the other hand, myocardial infarction may liberate hematopoietic stem and progenitor cells from bone marrow niches which could increase the availability of monocytes.[33] Therefore, MLR, being an integrated reflection of two important immune cells, could be a potentially prognostic factor for acute coronary syndrome, and the present study confirmed the 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 an increased mortality in the year following the myocardial infarction.[34]

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 the 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.[35] 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.[15] BNP has been established as a biomarker in vascular diseases used for monitoring disease progression. Porapakkham et al. performed a meta-analysis of eight randomized clinical trials and indicated that BNP could be used for guiding the treatment of chronic heart failure,[36] while Klok et al. conducted a meta-analysis of 13 studies and revealed the prognostic value of BNP in patients with pulmonary embolism.[37] In patients with NSTEMI, Fukazawa et al. showed that an increased concentration of BNP at admission was closely associated with poor prognosis.[38] 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 was with higher HR. 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-effectiveness and easily accessible in clinical practice, which would possess practical clinical utility in the prognosis prediction of NSTEMI.

This study had several limitations. Firstly, this study comprised of a modest sample size which may introduce selection bias. The single center study was lack of external validation. Thus, these findings need further multi-institutional with larger samples validation. Secondly, we evaluated the MLR and NLR on admission of the hospital, but didn't assess their dynamic changes during the follow-up period. Thirdly, inflammatory biomarkers such as myeloperoxidase, interleukin-6 and tumor necrosis factor were not analyzed in our patients. Finally, several scoring systems, for example, the HEART Score, have been developed to risk stratify patients with ACS and reported 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 firstly 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.

Contributors ZF made the major contribution to the conception and design of the study, and

drafted the manuscript. YL and HJ contributed to the data acquisition and performed the statistical analyses. XJ revised the manuscript. XJ is the guarantor of the study. All authors read and approved the final manuscript prior to the submission.

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Competing interests None declared.

Ethics approval This research was approved by the ethics committee of the Ethical Committee of Civil Aviation General Hospital (CAGH-EC100201). The study was designed and performed in accordance with the Declaration of Helsinki.

Data Sharing Statement Raw data can be obtained by contacting the corresponding author.

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Figure Legend

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.

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.

Figure 3. Receiver-operating characteristic curves showing area under the curve 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 NSTEMI patients.

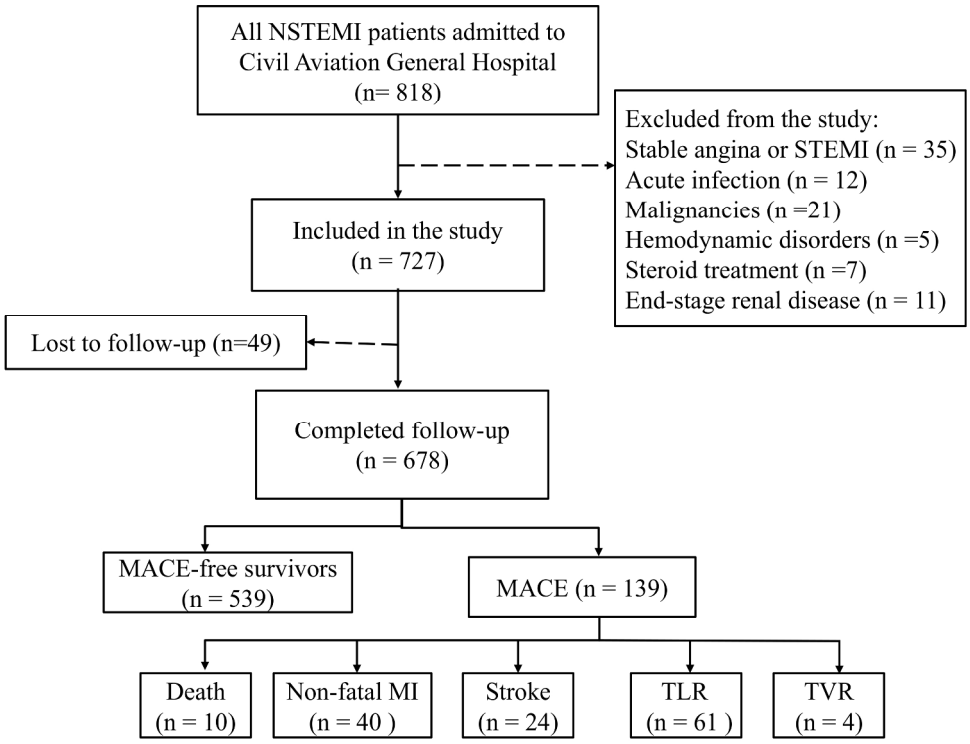

Figure 1. Flow Chart of the study cohort. The flow chart presents the selection criteria of the study and the clinical layout of the study population.

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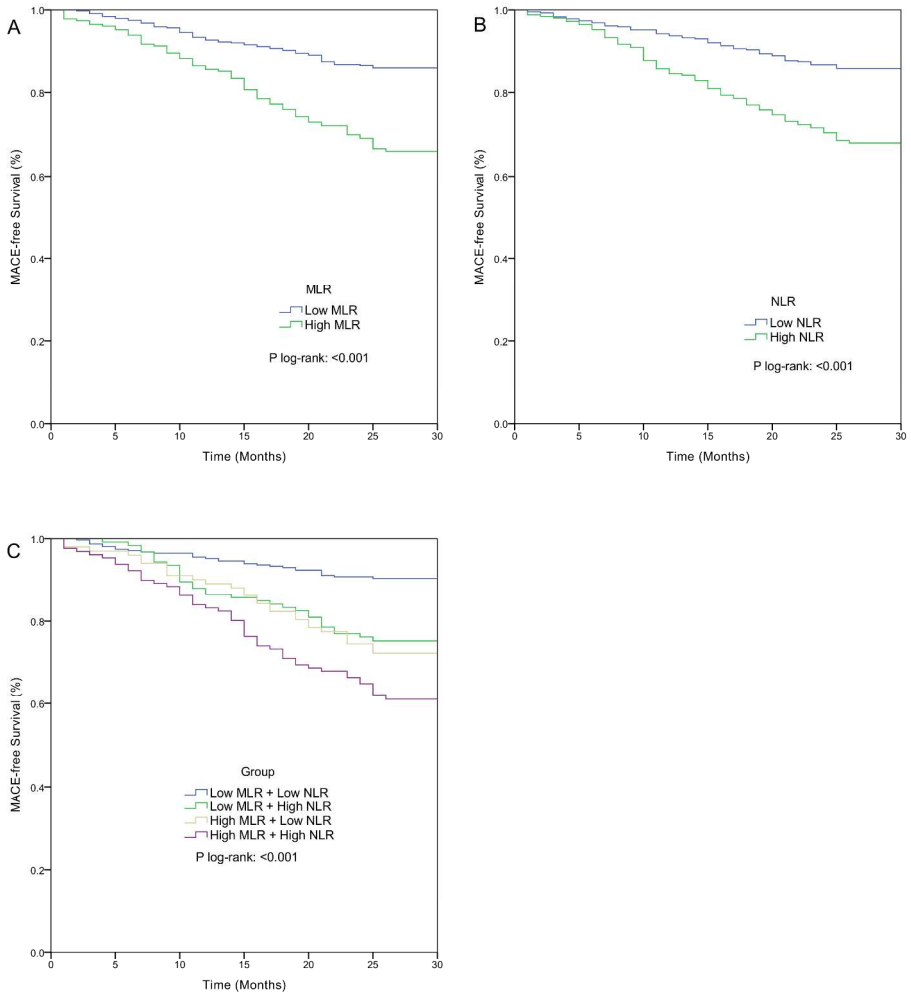
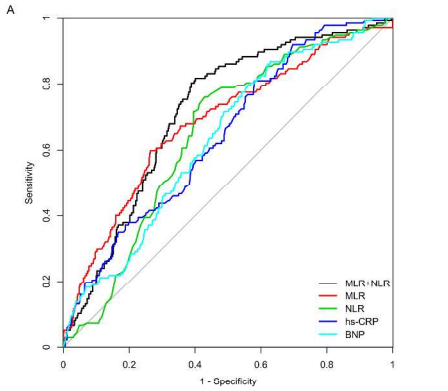


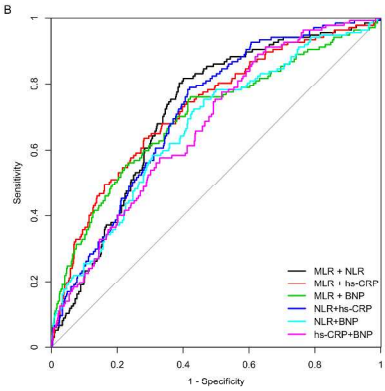
Figure 2. Kaplan-Meier cumulative MACE-free curves in patients with NSTEMI (A) according to the cut-off value of MLR; (B) according to the cut-off value of NLR; (C) according to MLR combined with NLR.

282x289mm (300 x 300 DPI)



Variable	AUC	95%CI	P* value	P+ value	P# value	PΔ value
MLR+NLR	0.715	0.679-0.748	-	-	-	-
MLR	0.683	0.647-0.718	0.022	-	-	-
NLR	0.646	0.609-0.682	0.001	0.249	-	-
hs-CRP	0.642	0.593-0.691	0.031	0.243	0.905	-
BNP	0.633	0.583-0.682	0.012	0.159	0.672	0.793

*: Compared with MLR+NLR; +: Compared with MLR; #: Compared with NLR; Δ: Compared with hs-CRP.



Variable	AUC	95%CI	P* value	P+ value	P# value	PΔ value	P⋆ value
MLR+NLR	0.715	0.679-0.748	-	-	-	-	-
MLR+hs-CRP	0.724	0.675-0.772	0.639	-	-	-	-
MLR+BNP	0.700	0.647-0.753	0.365	0.207	-	-	-
NLR+hs-CRP	0.712	0.668-0.757	0.751	0.629	0.688	-	-
NLR+BNP	0.669	0.619-0.720	0.050	0.109	0.296	0.049	-
hs-CRP+BNP	0.671	0.633-0.719	0.177	0.031	0.345	0.056	0.958

*: Compared with MLR+NLR; +: Compared with MLR+hs-CRP; #: Compared with MLR+BNP; Δ: Compared with NLR+hs-CRP;⋆: Compared with NLR+BNP.

Figure 3. Receiver-operating characteristic curves showing area under the curve 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 NSTEMI patients.

359x211mm (300 x 300 DPI)

Table S1 Associated factors of long-term MACE in patients with NSTEMI by univariate COX regression analyses (n=678)

Variable	non-MACE (n=539)	MACE (n=139)	Hazard Ratio	95%CI	P Value
Age /(year)	62.18±15.54	65.26±13.78	1.127	0.056-1.127	0.033
Male, n (%)	345(64.01)	90(64.75)	0.968	0.656-1.430	0.871
Family history, n (%)	67(12.43)	14(10.07)	1.267	0.689-2.330	0.445
Hypertension, n(%)	379(70.32)	106(76.26)	0.737	0.479-1.136	0.167
Diabetes Mellitus, n(%)	215(39.89)	64(46.04)	0.778	0.534-1.132	0.189
Dyslipidemia, n (%)	194(35.99)	55(39.57)	0.859	0.586-1.259	0.436
Current Smoker, n (%)	191(35.44)	46(33.09)	1.110	0.748-1.647	0.606
Killip class (> I)	348(64.56)	98(70.50)	0.762	0.508-1.143	0.189
Ejection fraction	65.59±10.25	64.37±10.06	1.68	0.414-1.68	0.210
Laboratory parameters					
White blood cell, $\times 10^9$	6.75±2.09	7.28±2.59	1.138	1.029-1.258	0.012
Neutrophil, $\times 10^9$	4.42±1.16	5.02±1.18	1.198	1.121-1.280	<0.001
Lymphocyte, $\times 10^9$	2.29±0.65	1.85±0.61	0.859	0.824-0.896	<0.001
Monocyte, $\times 10^9$	0.27±0.13	0.36±0.15	2.606	1.987-3.419	<0.001
High MLR, n(%)	141(26.16)	92(66.19)	5.525	3.702-8.245	<0.001
High NLR, n(%)	172(31.91)	85(61.15)	3.359	2.283-4.942	<0.001
Hemoglobin /(g/L)	132.87±36.22	134.26±32.85	1.146	0.598-2.195	0.681
Total cholesterol /(mmol/L)	5.69±1.41	5.82±1.38	1.162	0.859-1.571	0.331
Triglycerides /(mmol/L)	1.67±0.52	1.65±0.54	1.113	0.659-1.879	0.688
LDL/(mmol/L)	2.91±1.09	3.40±1.10	2.773	1.809-4.252	<0.001
HDL/(mmol/L)	1.38±0.56	1.31±0.58	1.142	0.935-1.394	0.193
hs-CRP/(mg/dL)	2.21±0.65	2.68±0.77	2.601	2.003-3.379	<0.001
Creatinine /(umol/L)	109.58±35.12	113.67±34.78	1.384	0.823-2.327	0.220
eGFR/(ml/min/1.73m^2)	83.94±26.98	79.94±25.32	1.672	0.882-3.169	0.115
BNP/(pg/ml)	264.78±80.25	283.77±81.78	1.296	1.055-1.591	0.013
Troponin I (ng/mL)	7.05±3.18	8.11±3.32	1.732	1.268-2.364	0.001
Medical Treatment					
Aspirin, n(%)	516(95.73)	137(98.56)	0.328	0.076-1.406	0.133
Anticoagulant, n(%)	510(94.62)	129(92.81)	1.363	0.648-2.870	0.414
Statin, n(%)	486(90.17)	129(92.81)	0.711	0.352-1.436	0.341
ACEI or ARB, n(%)	315(58.44)	87(62.59)	0.841	0.573-1.234	0.375

Beta-blocker, n(%)	429(79.59)	119(85.61)	0.655	0.391-1.100	0.110
Calcium-channel blockers, n(%)	116(21.52)	35(25.18)	0.815	0.528-1.258	0.358
Nitrate drugs, n(%)	418(77.55)	116(83.45)	0.685	0.419-1.119	0.131

ACEI, angiotensin-converting enzyme 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 S2 Independent predictors of long-term MACE in patients with NSTEMI by multivariate COX regression analyses

Variable	Hazard Ratio	95% Confidence Interval	P value
Model 1			
White blood cell	1.142	0.969-1.347	0.113
Neutrophil	1.152	0.954-1.393	0.142
Lymphocyte	2.130	0.904-5.020	0.084
Monocyte	1.208	0.935-1.561	0.149
MLR			
Low MLR, MLR<0.36	Ref		
High MLR, MLR≥0.36	2.128	1.458-3.105	<0.001
NLR			
Low NLR, NLR<2.15	Ref		
High NLR, NLR≥2.15	1.925	1.385-2.676	<0.001
LDL	1.173	0.953-1.443	0.132
hs-CRP	1.747	1.173-2.601	0.006
BNP	1.950	1.156-3.290	0.012
Troponin I	1.179	0.961-1.446	0.115
Model 2			
White blood cell	1.140	0.957-1.358	0.142
Neutrophil	1.145	0.971-1.349	0.108
Lymphocyte	0.903	0.758-1.076	0.255
Monocyte	2.125	0.904-5.010	0.084
Combination of MLR and NLR			
Low-MLR + Low-NLR	Ref	-	-
Low-MLR + High-NLR	2.732	1.417-5.268	0.003

High-MLR + Low-NLR	3.004	1.519-5.940	0.002
High-MLR + High-NLR	4.055	2.550-6.448	<0.001
LDL	1.173	0.953-1.443	0.132
hs-CRP	1.576	1.058-2.349	0.025
BNP	1.874	1.137-3.088	0.014
Troponin I	1.170	0.966-1.417	0.108

Effect sizes are expressed as hazard ratios with 95% confidence intervals. BNP, brain natriuretic peptide; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; MLR, monocyte-to-lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio.

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1,2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2-3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6-7
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	Not applicable
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-7
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	*6*
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7-8
		(*b*) Describe any methods used to examine subgroups and interactions	7-8
		(*c*) Explain how missing data were addressed	7-8
		(*d*) If applicable, explain how loss to follow-up was addressed	7-8
		(*e*) Describe any sensitivity analyses	7-8
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	8-9
		(b) Give reasons for non-participation at each stage	8-9
		(c) Consider use of a flow diagram	8, Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9-11
		(b) Indicate number of participants with missing data for each variable of interest	9
		(c) Summarise follow-up time (eg, average and total amount)	9
Outcome data	15*	Report numbers of outcome events or summary measures over time	11-12
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	12-14
		(*b*) Report category boundaries when continuous variables were categorized	9
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	12-14
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	14
Discussion			
Key results	18	Summarise key results with reference to study objectives	14
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	18
Generalisability	21	Discuss the generalisability (external validity) of the study results	18
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	19

*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.

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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Keywords:	Monocyte-to-lymphocyte ratio, Neutrophil-to-lymphocyte ratio, Major adverse cardiac events, Non-ST elevated myocardial infarction

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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WORD COUNT 3105

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). High MLR + high NLR group had lower MACE-free survival rate than the other three groups (P log-rank < 0.001). Both MLR [hazard ratio (HR): 2.128, 95% CI: 1.458-3.105] and NLR (HR: 1.925, 95% CI: 1.385-2.676) were independent predictors of long-term MACE. Moreover, the patients in high-MLR + high-NLR group had a HR of 4.055 (95% CI: 2.550-6.448) for long-term MACE, with low-MLR + low-NLR group as reference. Comparisons of ROC curves revealed that the combination of MLR with NLR achieved

better performance in differentiating long-term MACE, compared with MLR, NLR, hs-CRP and BNP 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.

Keywords

Monocyte-to-lymphocyte ratio; Neutrophil-to-lymphocyte ratio; Major adverse cardiac events; Non-ST elevated myocardial infarction

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 (PCI).
- ROC curves were performed to extensively explore and compare the diagnostic efficacies of MLR alone, NLR alone, high-sensitivity C-reactive protein (hs-CRP) alone, brain natriuretic peptide (BNP) alone and their pairwise combinations in differentiating MACE in NSTEMI patients.
- MLR and NLR, non-invasive, simple, economical and feasible biomarkers, possess practical clinical utility in the prognosis prediction of NSTEMI.
- These findings need further multi-institutional with larger samples validation.

INTRODUCTION

Previous studies have verified that inflammatory response plays a vital role in the development of atherosclerosis and cardiovascular diseases.[1 2] White blood cell and its subtypes including neutrophils, monocytes and lymphocytes are important immune cells involved in the initiation, formation and destabilization of atherosclerosis.[3] 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 pro-inflammatory cytokines which play a crucial role at every level of the atherosclerotic process.[8] Monocyte-to-lymphocyte ratio (MLR) has emerged as a novel systematic inflammatory marker related with 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 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 Civil Aviation General Hospital,

Beijing, China. A total of 818 consecutive NSTEMI patients who presented to the emergency department and underwent primary PCI from July 2010 to July 2015 were considered for participation in this study. The NSTEMI was defined by typical ischemia symptoms, elevation level of cardiac troponin-I or creatine kinase-MB and no evidence of ST segment elevation in electrocardiography. We excluded patients who had serious heart failure (NYHA Class III or IV), rheumatic heart disease, valvar heart disease, congenital heart disease, pulmonary heart disease, active or chronic inflammatory conditions, acute infection, hemodynamic disorders, malignancies, severe renal [estimated glomerular filtration rate (eGFR) < 30 ml/min/1.73m^2] or hepatic (alanine-aminotransferase > 40 U/l) disease, steroid therapy in the preceding 3 months, history of cerebrovascular vascular events or incomplete blood cell count or medical records. Hypertension was defined as current use of an antihypertensive medication or, a systolic blood pressure ≥ 140 mmHg and/or a diastolic blood pressure ≥ 90 mmHg. Diabetes mellitus was defined as active use of an antidiabetic agent, or fasting plasma glucose level ≥ 7.0 mmol/L or casual plasma glucose level ≥ 11.1 mmol/L.

This research was approved by the Ethical Committee of Civil Aviation General Hospital (CAGH-EC100201), and informed consent was obtained from all patients. The study was designed and performed in accordance with the Declaration of Helsinki.

Study procedures and laboratory analysis

At the time of admission, venous blood samples were collected from each patient. All hematological 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 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 hemoglobin, white blood cells, neutrophils, monocytes, lymphocytes, and platelets. The biochemical indicators were determined using a Hitachi7600 automatic biochemistry analyzer (Hitachi, Tokyo, Japan).The biochemical indicators included 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. eGFR was calculated using the Chronic Kidney Disease Epidemiology (CKD EPI) creatinine equation. The 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 to 100 U/kg) to maintain an activated clotting time 250-300 seconds 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 (TIMI) II or III flow in the treated artery. Angiographic characteristics were collected for all the patients.

Clinical outcomes

The main outcomes were MACE 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 revascularization (TLR) and target vessel revascularization (TVR) according to the Academic Research Consortium definition.[13] Cardiac death was defined as death resulting from any cardiac-related causes (eg, MI, heart failure, lethally cardiac arrhythmia). Nonfatal MI was defined based on the European Society of Cardiology, American Heart Association, American College of Cardiology, and World Heart Federation definitions.[14] TLR was defined as a repeat revascularization caused by a stenosis ≥ 50% within the stent or within a 5-mm proximal or distal to the stent. TVR was defined as a 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

Kolmogorov-Smirnov test was employed to test the normality of the continuous variables in each group. Continuous variables distributed normally were expressed as mean ± standard deviation (SD), while categorical data were expressed as numbers and percentage. We initially used ROC curves to determine the ability of MLR and NLR to differentiate MACE. Subsequently optimal cut-off values, specificity and sensitivity were derived. Based on the optimal cut-off values, participants were assigned into 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 ANOVA followed by Tukey's post hoc tests, while categorical data were compared

by Chi square 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 Log-rank 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 hazard ratio (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 group = 3]. The effect sizes were expressed as HR and their 95% confidence intervals (CI). Afterwards, we used ROC curves to evaluate the diagnostic performance of individual biomarkers and their pairwise combinations in predicting long-term MACE. The AUCs were compared by Delong's tests. The statistical significance was considered as a 2-tailed $P < 0.05$. Statistical analyses were performed using SPSS version 22.0 (SPSS Inc., Chicago, IL, 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

818 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 the 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** depicted 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 a NLR cut-off value of 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 + low-NLR group (MLR < 0.36, NLR < 2.35, n=319), low-MLR + high-NLR group (MLR < 0.36, NLR ≥ 2.35, n=126), high-MLR + low-NLR group (MLR ≥ 0.36, NLR < 2.35, n=102) and high-MLR + high-NLR group (MLR ≥ 0.36, NLR ≥ 2.35, n=131). The clinical characteristics were summarized in **Table 1**. The distribution of prior medications and angiographic findings were similar between the four groups. However, patients in 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 hemoglobin.

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-value
Age /(year)	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
Dyslipidemia, 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					
White blood cell, $\times10^9$	6.67±1.72	6.77±2.03	7.06±1.98*	7.25±2.11*	0.027
Neutrophil, $\times10^9$	4.26±1.07	4.69±1.19*	4.56±1.15*	5.08±1.27*+#	<0.001
Lymphocyte, $\times10^9$	2.31±0.53	1.98±0.51*	2.37±0.67+	2.02±0.64*#	<0.001
Monocyte, $\times10^9$	0.18±0.09	0.22±0.11*	0.49±0.19*+	0.46±0.13*+	<0.001
Hemoglobin/(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/1.73m^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-vessel, n(%)	76(23.82)	39(30.95)	32(31.37)	42(32.06)	
three-vessel/ 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 low-MLR + low-NLR group, p<0.05;

+: compared with low-MLR+ high-NLR group, p<0.05;

#: compared with high-MLR+ low-NLR group, p<0.05.

ACEI, angiotensin-converting enzyme 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.

Clinical outcomes

During the median follow-up period of 26 months, long-term MACE were observed in 139 (20.50%) patients. 10 (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 to 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**).

The Kaplan-Meier curves based on the cut-off value of MLR and NLR, were presented

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 was shown in **Figure 2C**. The MACE rates were significantly different among the four groups ($P < 0.001$) and patients in high-MLR + high-NLR group had the highest MACE rate.

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-value
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
Nonfatal 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 low-MLR + low-NLR group, p<0.05;

+: compared with low-MLR + high-NLR group, p<0.05;

#: compared with high-MLR + low-NLR group, p<0.05.

MI, myocardial infarction; TLR, target lesion revascularization; TVR, target vessel revascularization.

Independent predictors of long-term MACE

Univariate and multivariate COX regression analyses were utilized to determine the independent predictors of long-term MACE in patients with NSTEMI undergoing primary PCI. In univariate COX analysis, white blood cells, 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: 1.458-3.105, $P < 0.001$) and NLR (HR 1.925, 95% CI: 1.385-2.676, $P < 0.001$) 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: 2.550-6.448, $P < 0.001$ 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 were presented in online supplementary **Table S2**.

Table 3 Independent predictors of long-term MACE in patients with NSTEMI by multivariate COX regression analyses

Variable	Hazard Ratio	95% Confidence Interval	P value
Model 1			
MLR			
Low MLR, MLR<0.36	Ref		
High MLR, MLR≥0.36	2.128	1.458-3.105	<0.001
NLR			
Low NLR, NLR<2.15	Ref		
High NLR, NLR≥2.15	1.925	1.385-2.676	<0.001
hs-CRP	1.747	1.173-2.601	0.006
BNP	1.950	1.156-3.290	0.012
Model 2			
Combination of MLR and NLR			
Low-MLR + Low-NLR	Ref	-	-
Low-MLR + High-NLR	2.732	1.417-5.268	0.003
High-MLR + Low-NLR	3.004	1.519-5.940	0.002

High-MLR + High-NLR	4.055	2.550-6.448	<0.001
hs-CRP	1.576	1.058-2.349	0.025
BNP	1.874	1.137-3.088	0.014

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

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 (i) MLR, NLR, hs-CRP and BNP alone, and (ii) all other pairwise combinations of the four biomarkers, for differentiating long-term MACE. **Figure 3A** showed that MLR in combination with NLR (AUC: 0.715, 95%CI: 0.679-0.748) achieved better performance in predicting long-term MACE, than MLR (AUC: 0.683, 95%CI: 0.647-0.718), NLR (AUC: 0.646, 95%CI: 0.609-0.682), hs-CRP (AUC: 0.642, 95%CI: 0.593-0.691) and BNP alone (AUC: 0.633, 95%CI: 0.583-0.682) (All P values < 0.05), whereas there was no statistical difference among the four individual biomarkers in AUC values. Additionally, MLR in combination with NLR showed similar performance to all other pairwise combinations of the four biomarkers (All *P* values ≥ 0.05, **Figure 3B**).

DISCUSSION

In this study, there were 139 of 678 patients (20.50%) presented with MACE, during the follow-up period. The MACE rate was comparable between our study and the previous study (21.62%)[15]. 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 and long-term adverse outcomes in patients with acute coronary syndrome undergoing PCI.[16 17] Neutrophils, the most abundant white blood cells in the circulation, are actively involved in the atherogenesis and plaque destabilization.[18 19]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 destabilization.[20-23] Lymphocytes are an integral part of the immune system, which participate in every phase of atherosclerosis. Lymphocytopenia, resulting from increased lymphocytes apoptosis, contributes to atherosclerotic plaque growth, lipid core development, plaque destabilization, post-infarct cardiac remodeling and progression.[3 24] Lower lymphocyte count was reported to be not only an early marker of acute myocardial infraction, but also associated with worse cardiovascular outcomes.[25 26] Obviously, it could be concluded that NLR, a composite marker of neutrophils and lymphocytes, can provide prognostic value in ACS patients. In agreement with previous evidence, our study confirmed the prognostic role of increased NLR in patients with NSTEMI.

MLR, a novel hematological marker, has been recently reported as a prognostic factor in many diseases, especially various malignancies.[27 28] 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 in assessing coronary lesion severity,[9] and identifying the vulnerable plaques in patients with stable angina.[29] 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 six-month mortality in patients with STEMI undergoing primary PCI.[11] Gijsberts et al. found that MLR significantly improved mortality prediction in coronary angiography patients.[12] Thus, a high MLR was associated with adverse cardiac clinical outcomes, though fewer studies have been performed for MLR and cardiac prognosis, compared to those for NLR. Monocytes play an essential role in every stage of atherosclerosis.[30] They can recruit to the artery wall, differentiate into macrophages and stimulate activating the secretion of pro-inflammatory cytokines.[8] Compared with neutrophils, monocytes can produce higher levels of cytokines.[31] Recent pathological studies have found that monocytes can replace neutrophils and become the prominent infiltrating leukocytes within 48 h after the onset of myocardial ischemia.[32] On the other hand, myocardial infarction may liberate hematopoietic stem and progenitor cells from bone marrow niches which could increase the availability of monocytes.[33] Therefore, MLR, being an integrated reflection of two important immune cells, could be a potentially prognostic factor for acute coronary syndrome, and the present study confirmed the 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 an increased mortality in the year following the myocardial infarction.[34]

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 the 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.[35] 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.[15] BNP has been established as a biomarker in vascular diseases used for monitoring disease progression. Porapakkham et al. performed a meta-analysis of eight randomized clinical trials and indicated that BNP could be used for guiding the treatment of chronic heart failure,[36] while Klok et al. conducted a meta-analysis of 13 studies and revealed the prognostic value of BNP in patients with pulmonary embolism.[37] In patients with NSTEMI, Fukazawa et al. showed that an increased concentration of BNP at admission was closely associated with poor prognosis.[38] 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 was with higher HR. 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-effectiveness and easily accessible in clinical practice, which would possess practical clinical utility in the prognosis prediction of NSTEMI.

This study had several limitations. Firstly, this study comprised of a modest sample size which may introduce selection bias. The single center study was lack of external validation. Thus, these findings need further multi-institutional with larger samples validation. Secondly, we evaluated the MLR and NLR on admission of the hospital, but didn't assess their dynamic changes during the follow-up period. Thirdly, inflammatory biomarkers such as myeloperoxidase, interleukin-6 and tumor necrosis factor were not analyzed in our patients. Finally, several scoring systems, for example, the HEART Score, have been developed to risk stratify patients with ACS and reported 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 firstly 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.

Contributors ZF made the major contribution to the conception and design of the study, and

drafted the manuscript. YL and HJ contributed to the data acquisition and performed the statistical analyses. XJ revised the manuscript. XJ is the guarantor of the study. All authors read and approved the final manuscript prior to the submission.

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Competing interests None declared.

Ethics approval This research was approved by the ethics committee of the Ethical Committee of Civil Aviation General Hospital (CAGH-EC100201). The study was designed and performed in accordance with the Declaration of Helsinki.

Data Sharing Statement Raw data can be obtained by contacting the corresponding author.

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Figure Legend

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.

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.

Figure 3. Receiver-operating characteristic curves showing area under the curve 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 NSTEMI patients.

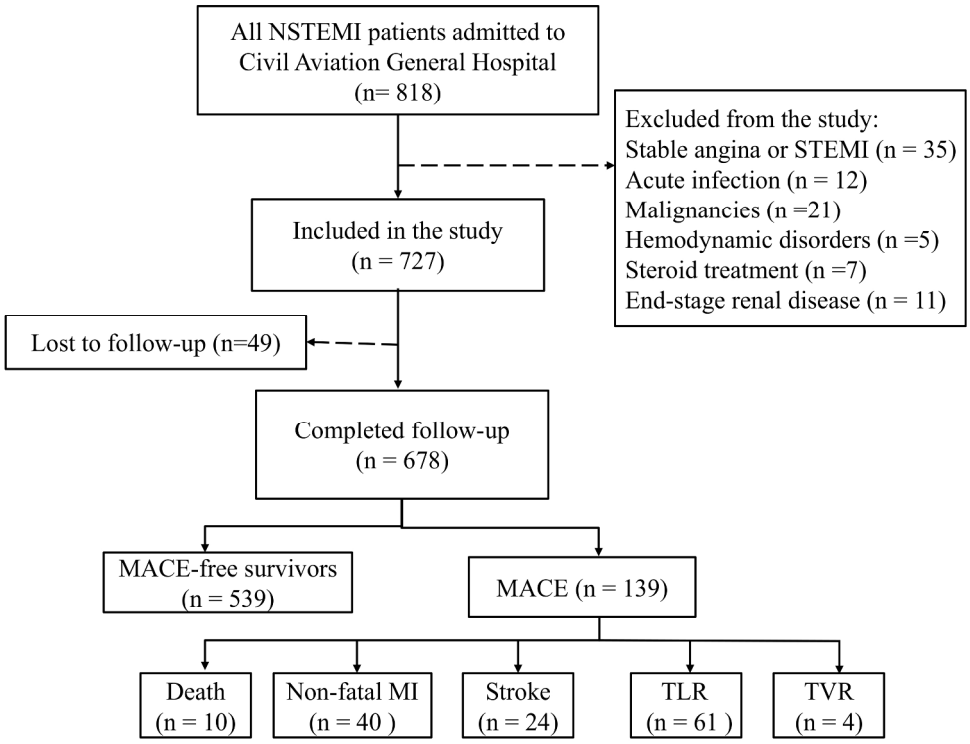


Figure 1. Flow Chart of the study cohort. The flow chart presents the selection criteria of the study and the clinical layout of the study population.

254x190mm (300 x 300 DPI)

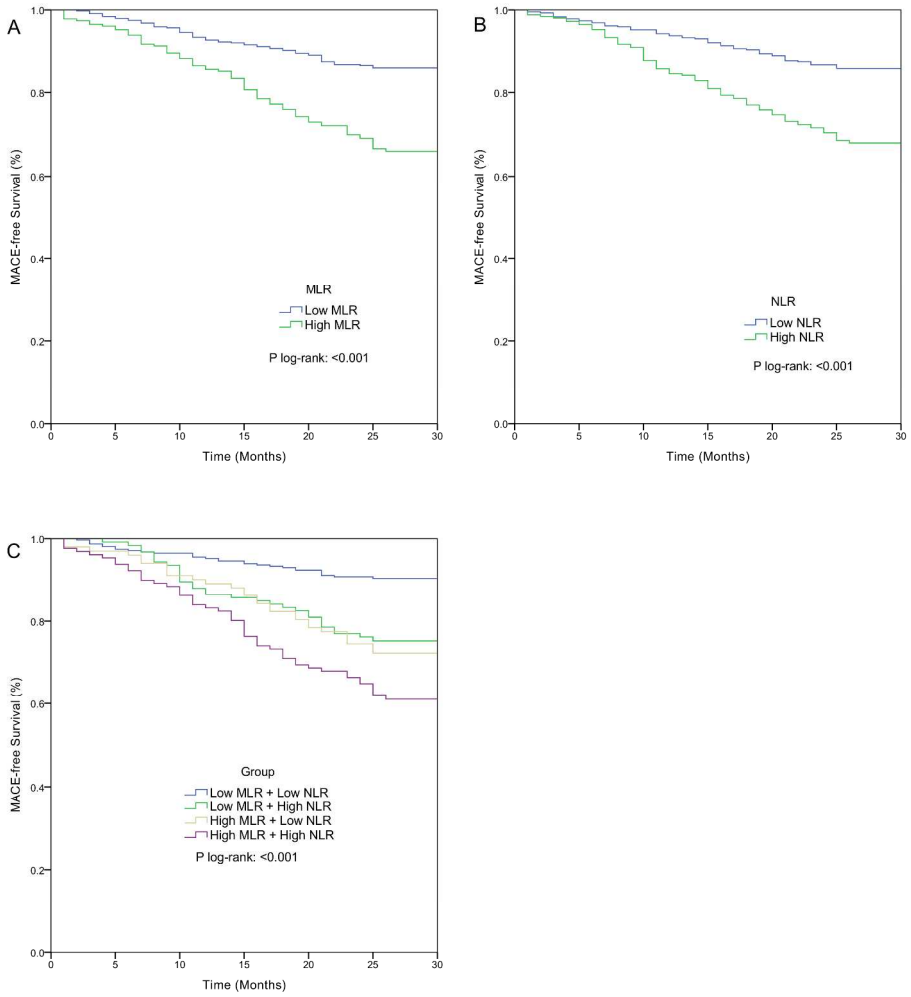
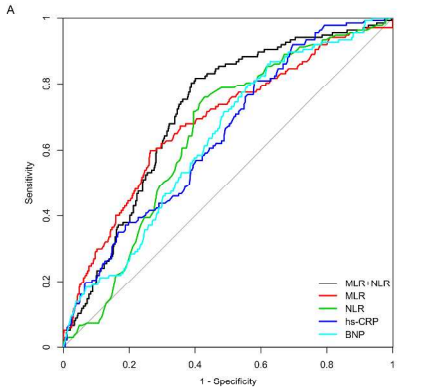


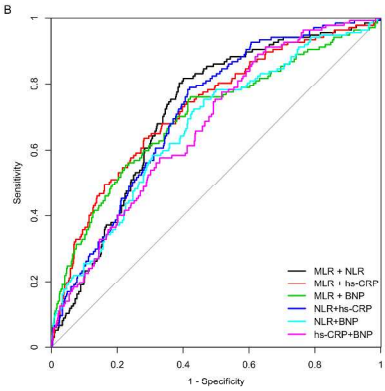
Figure 2. Kaplan-Meier cumulative MACE-free curves in patients with NSTEMI (A) according to the cut-off value of MLR; (B) according to the cut-off value of NLR; (C) according to MLR combined with NLR.

282x289mm (300 x 300 DPI)



Variable	AUC	95%CI	P* value	P+ value	P# value	PΔ value
MLR+NLR	0.715	0.679-0.748	-	-	-	-
MLR	0.683	0.647-0.718	0.022	-	-	-
NLR	0.646	0.609-0.682	0.001	0.249	-	-
hs-CRP	0.642	0.593-0.691	0.031	0.243	0.905	-
BNP	0.633	0.583-0.682	0.012	0.159	0.672	0.793

*: Compared with MLR+NLR; +: Compared with MLR; #: Compared with NLR; Δ: Compared with hs-CRP.



Variable	AUC	95%CI	P* value	P+ value	P# value	PΔ value	P⋆ value
MLR+NLR	0.715	0.679-0.748	-	-	-	-	-
MLR+hs-CRP	0.724	0.675-0.772	0.639	-	-	-	-
MLR+BNP	0.700	0.647-0.753	0.365	0.207	-	-	-
NLR+hs-CRP	0.712	0.668-0.757	0.751	0.629	0.688	-	-
NLR+BNP	0.669	0.619-0.720	0.050	0.109	0.296	0.049	-
hs-CRP+BNP	0.671	0.633-0.719	0.177	0.031	0.345	0.056	0.958

*: Compared with MLR+NLR; +: Compared with MLR+hs-CRP; #: Compared with MLR+BNP; Δ: Compared with NLR+hs-CRP;⋆: Compared with NLR+BNP.

Figure 3. Receiver-operating characteristic curves showing area under the curve 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 NSTEMI patients.

359x211mm (300 x 300 DPI)

Table S1 Associated factors of long-term MACE in patients with NSTEMI by univariate COX regression analyses (n=678)

Variable	non-MACE (n=539)	MACE (n=139)	Hazard Ratio	95%CI	P Value
Age /(year)	62.18±15.54	65.26±13.78	1.127	0.056-1.127	0.033
Male, n (%)	345(64.01)	90(64.75)	0.968	0.656-1.430	0.871
Family history, n (%)	67(12.43)	14(10.07)	1.267	0.689-2.330	0.445
Hypertension, n(%)	379(70.32)	106(76.26)	0.737	0.479-1.136	0.167
Diabetes Mellitus, n(%)	215(39.89)	64(46.04)	0.778	0.534-1.132	0.189
Dyslipidemia, n (%)	194(35.99)	55(39.57)	0.859	0.586-1.259	0.436
Current Smoker, n (%)	191(35.44)	46(33.09)	1.110	0.748-1.647	0.606
Killip class (> I)	348(64.56)	98(70.50)	0.762	0.508-1.143	0.189
Ejection fraction	65.59±10.25	64.37±10.06	1.68	0.414-1.68	0.210
Laboratory parameters					
White blood cell, $\times 10^9$	6.75±2.09	7.28±2.59	1.138	1.029-1.258	0.012
Neutrophil, $\times 10^9$	4.42±1.16	5.02±1.18	1.198	1.121-1.280	<0.001
Lymphocyte, $\times 10^9$	2.29±0.65	1.85±0.61	0.859	0.824-0.896	<0.001
Monocyte, $\times 10^9$	0.27±0.13	0.36±0.15	2.606	1.987-3.419	<0.001
High MLR, n(%)	141(26.16)	92(66.19)	5.525	3.702-8.245	<0.001
High NLR, n(%)	172(31.91)	85(61.15)	3.359	2.283-4.942	<0.001
Hemoglobin /(g/L)	132.87±36.22	134.26±32.85	1.146	0.598-2.195	0.681
Total cholesterol /(mmol/L)	5.69±1.41	5.82±1.38	1.162	0.859-1.571	0.331
Triglycerides /(mmol/L)	1.67±0.52	1.65±0.54	1.113	0.659-1.879	0.688
LDL/(mmol/L)	2.91±1.09	3.40±1.10	2.773	1.809-4.252	<0.001
HDL/(mmol/L)	1.38±0.56	1.31±0.58	1.142	0.935-1.394	0.193
hs-CRP/(mg/dL)	2.21±0.65	2.68±0.77	2.601	2.003-3.379	<0.001
Creatinine /(umol/L)	109.58±35.12	113.67±34.78	1.384	0.823-2.327	0.220
eGFR/(ml/min/1.73m^2)	83.94±26.98	79.94±25.32	1.672	0.882-3.169	0.115
BNP/(pg/ml)	264.78±80.25	283.77±81.78	1.296	1.055-1.591	0.013
Troponin I (ng/mL)	7.05±3.18	8.11±3.32	1.732	1.268-2.364	0.001
Medical Treatment					
Aspirin, n(%)	516(95.73)	137(98.56)	0.328	0.076-1.406	0.133
Anticoagulant, n(%)	510(94.62)	129(92.81)	1.363	0.648-2.870	0.414
Statin, n(%)	486(90.17)	129(92.81)	0.711	0.352-1.436	0.341
ACEI or ARB, n(%)	315(58.44)	87(62.59)	0.841	0.573-1.234	0.375

Beta-blocker, n(%)	429(79.59)	119(85.61)	0.655	0.391-1.100	0.110
Calcium-channel blockers, n(%)	116(21.52)	35(25.18)	0.815	0.528-1.258	0.358
Nitrate drugs, n(%)	418(77.55)	116(83.45)	0.685	0.419-1.119	0.131

ACEI, angiotensin-converting enzyme 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 S2 Independent predictors of long-term MACE in patients with NSTEMI by multivariate COX regression analyses

Variable	Hazard Ratio	95% Confidence Interval	P value
Model 1			
White blood cell	1.142	0.969-1.347	0.113
Neutrophil	1.152	0.954-1.393	0.142
Lymphocyte	2.130	0.904-5.020	0.084
Monocyte	1.208	0.935-1.561	0.149
MLR			
Low MLR, MLR<0.36	Ref		
High MLR, MLR≥0.36	2.128	1.458-3.105	<0.001
NLR			
Low NLR, NLR<2.15	Ref		
High NLR, NLR≥2.15	1.925	1.385-2.676	<0.001
LDL	1.173	0.953-1.443	0.132
hs-CRP	1.747	1.173-2.601	0.006
BNP	1.950	1.156-3.290	0.012
Troponin I	1.179	0.961-1.446	0.115
Model 2			
White blood cell	1.140	0.957-1.358	0.142
Neutrophil	1.145	0.971-1.349	0.108
Lymphocyte	0.903	0.758-1.076	0.255
Monocyte	2.125	0.904-5.010	0.084
Combination of MLR and NLR			
Low-MLR + Low-NLR	Ref	-	-
Low-MLR + High-NLR	2.732	1.417-5.268	0.003

High-MLR + Low-NLR	3.004	1.519-5.940	0.002
High-MLR + High-NLR	4.055	2.550-6.448	<0.001
LDL	1.173	0.953-1.443	0.132
hs-CRP	1.576	1.058-2.349	0.025
BNP	1.874	1.137-3.088	0.014
Troponin I	1.170	0.966-1.417	0.108

Effect sizes are expressed as hazard ratios with 95% confidence intervals. BNP, brain natriuretic peptide; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; MLR, monocyte-to-lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio.

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1,2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2-3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6-7
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	Not applicable
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-7
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	*6*
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7-8
		(*b*) Describe any methods used to examine subgroups and interactions	7-8
		(*c*) Explain how missing data were addressed	7-8
		(*d*) If applicable, explain how loss to follow-up was addressed	7-8
		(*e*) Describe any sensitivity analyses	7-8
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	8-9
		(b) Give reasons for non-participation at each stage	8-9
		(c) Consider use of a flow diagram	8, Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9-11
		(b) Indicate number of participants with missing data for each variable of interest	9
		(c) Summarise follow-up time (eg, average and total amount)	9
Outcome data	15*	Report numbers of outcome events or summary measures over time	11-12
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	12-14
		(*b*) Report category boundaries when continuous variables were categorized	9
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	12-14
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	14
Discussion			
Key results	18	Summarise key results with reference to study objectives	14
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	18
Generalisability	21	Discuss the generalisability (external validity) of the study results	18
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	19

*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.