High Neutrophil-Lymphocyte Ratio and Low Lymphocyte-Monocyte Ratio Combination After Thrombolysis is a Potential Predictor of Poor Functional Outcome of Acute Ischemic Stroke

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Research Article

Keywords: stroke, neutrophil-lymphocyte ratio, lymphocyte-monocyte ratio, acute ischemic stroke

Posted Date: December 7th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1123187/v1

Abstract

Ischemic stroke is one of the leading causes of death and disability. An inflammatory response is observed in multiple stages of cerebral ischemia, particularly in the acute phase. Recent publications revealed that neutrophil-lymphocyte ratio (NLR) and lymphocyte-monocyte ratio (LMR) may be used to predict long term prognosis in acute ischemic stroke (AIS) after thrombolysis. To test whether there is a relationship between the combination of these parameters and long-term prognosis, we analyzed NLR-LMR combination in AIS patients treated with intravenous recombinant tissue plasminogen activator (rtPA). The study included 285 adults with diagnosis of AIS and rtPA treatment within 4.5h time window. Blood samples were obtained at admission and 24h after thrombolysis to calculate pre- and post-thrombolysis NLR and LMR. Clinical data including NIHSS was registered on admission and day 1. Long-term outcome was defined 90 days post-event by the modified Rankin Scale (mRS). Therapy-associated intracranial hemorrhage (ICH) was classified according to ECASS II. Receiver operating characteristic curve (ROC) analysis was performed to determine optimal cutoffs of NLR and LMR as predictors of therapy outcomes. Patients were stratified by cutoffs of 5.73 for NLR and 2.08 for LMR. Multivariate logistic regression model including all possible confounders displayed no significant association of NLR or LMR with 3-months functional prognosis. The combination of high NLR-low LMR in patients vs. low NRL-high LMR in patients as obtained 24h after thrombolysis was found to be an independent predictor of poor 3-months functional outcome (mRS ≥2; OR 3.407, 95% CI 1.449 to 8.011, p = 0.005). The proportion of patients between low NLR-high LMR and high NLR-low LMR groups from admission to day 1 showed no significant change in the good outcome group. On the other hand, in the poor outcome group (mRS ≥2), low NLR-high LMR and high NLR-low LMR groups displayed a significant shift of patient proportions from 67% and 21% at admission (p = 0.001) to 36% and 49% at 24h after thrombolysis (p < 0.001), respectively. Our study demonstrated for the first time that a high NLR-low LMR combination as observed at 24h after thrombolysis can serve as an independent predictor of 3-months poor outcome in AIS patients. This simple and readily available data may help clinicians to improve the prognostic estimation of patients and may provide guidance in selecting patients for intensified care post-thrombolysis.

Introduction

Despite recent advances in the treatment of acute ischemic stroke (AIS), the disease puts a heavy burden on individuals, families, and on the health care system. Although mechanical thrombectomy has revolutionized stroke care in the past decade [1], intravenous (i.v.) thrombolysis by recombinant tissue plasminogen activator (rt-PA) remains the most commonly used pharmacological therapy of AIS. With the extended time-window of 4.5 hours [2], about half of the patients receiving thrombolysis attain total or nearly total neurological recovery at 3 months [3]. On the other hand, a large proportion of patients will not benefit from thrombolysis, moreover, 6-8% of treated patients will develop potentially fatal intracerebral haemorrhage. Identification of patients in whom thrombolysis will not be effective or would cause potential side-effects could be a key approach to personalize acute stroke care, to improve long term

quality of life, and reduce the global burden of AIS. To achieve this, a rapid, cheap, simple, easily accessible and reliable prognostic marker of thrombolysis outcome is needed. Neutrophil-lymphocyte ratio (NLR) and lymphocyte-monocyte ratio (LMR) have been shown to be associated with various pathological conditions [4–6]. NLR and LMR are potential novel biomarkers of inflammation and immune response [7]. They simply drive from complete blood count and in this way, they are rapidly and readily available markers for clinical use before administering the thrombolytic agent. Recently, traces of evidence indicated a causal link between the prognosis of AIS and NLR and LMR levels [8,9]. Here we analyzed the potential relationship between NLR and LMR levels and the outcome of AIS after intravenous rtPA administration.

Methods

Study Population. Consecutive AIS patients admitted to the Department of Neurology, University of Debrecen, Hungary between September 2016 and April 2018 with AIS were enrolled in the study. Inclusion and exclusion criteria of patients were identical to the standard criteria of intravenous rtPA administration of the 2008 ESO guideline [10]. The diagnosis of ischemic stroke was confirmed by using non-contrast computerized tomography (NCCT) scan, and computed tomography angiography (CTA). Clinical data including the National Institutes of Health Stroke Scale (NIHSS) was registered on admission and day 1. Thrombolysis was performed within the 4.5h time window from the onset of symptoms using intravenous rtPA according to standard protocols [10]. Patients receiving mechanical thrombectomy in addition to thrombolysis were not included in the study. A control NCCT was performed on day 1 and early ischemic changes based on admission NCCT and day 1 NCCT were calculated using the Alberta Stroke Program Early CT Score (ASPECTS) as assessed by four independent radiologists [11]. Stroke etiology was defined by Trial of ORG 10172 in Acute Stroke Treatment (TOAST) criteria [12]. The presence of intracerebral hemorrhage (ICH) was tested on day 1 using NCCT, and patients with hemorrhage were divided into two groups: symptomatic (SICH) or asymptomatic (aSICH) based on the European Cooperative Acute Stroke Study (ECASS) II criteria [2]. Short-term outcome evaluation was performed on day 1 after thrombolysis. Favorable short-term outcome was defined as a decrease in the NIHSS score by at least 4 points or to 0, while a poor short-term outcome was defined as an increase in NIHSS score by at least 4 points [13]. The modified Rankin Scale (mRS) was registered to define long term outcome at 90 days. Unfavorable outcome was defined as mRS greater than 1 (mRS≥2).

The study was approved by the Ethics Committee of the University of Debrecen, Hungary and the Ethics Board of the Medical Research Council of the Hungarian Ministry of Human Capacities, Hungary. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. All patients or their relatives provided written informed consent.

Blood sampling and laboratory measurements. Peripheral blood samples were drawn before the initiation of rt-PA infusion, and 24 hours after thrombolysis. From the blood samples taken on admission, routine

laboratory examinations were performed (ions, glucose levels, renal and liver function tests, high-sensitivity C-reactive protein (hsCRP)) by standard methods (Roche Diagnostics, Mannheim, Germany).

Complete blood counts were assessed from blood samples obtained before and 24 h after thrombolysis using an automated analyzer (XE 2100, Sysmex Europe GmbH, Hamburg, Germany). Hematological parameters were determined immediately after blood collection. The absolute neutrophil-to-lymphocyte count (NLR), and the absolute lymphocyte-to-monocyte count (LMR) were calculated from blood samples obtained at both occasions.

Statistical methods. Continuous variables were expressed either as mean ± SD or median and interquartile range (IQR) as appropriate. Categorical data were expressed as numbers and percentages. Multiple groups of continuous data were compared using one-way analysis of variance (ANOVA) using Bonferroni post-hoc test or Kruskal-Wallis analysis with Dunn-Bonferroni post-hoc test. Categorical data were compared using the χ^2 of Fisher's exact tests where appropriate. Receiver operating characteristic (ROC) curves were built by plotting sensitivity vs. 1-specificity and calculating the area under the curve (AUC). Optimal threshold values were calculated based on Youden's J statistics. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated using contingency tables and $\chi 2$ test or Fisher's exact at statistically optimal threshold values. Multivariable logistic regression models were used to test the independent effect of NLR, LMR, their combination, or each leukocyte subtype count on outcome measures before and after adjustment for major baseline characteristics. The p-value cut-off point of 0.25 in univariate analysis was used to select candidate variables for inclusion in multivariable analyses [14].

Statistical analyses were performed using SPSS 18.0 (Chicago, IL, USA) and MedCalc 14.8.1 software (Mariakerke, Belgium).

Results

Baseline characteristics of enrolled patients. During the study period, a total of 285 consecutive AIS patients undergoing i.v. thrombolysis treatment were enrolled in the study. Baseline characteristics of included patients are listed in Table 1. Mean age of the patients was 66 ±12.9 years, with 44.2% being female. Median baseline NIHSS score was 6 (IQR [5, 9.1]) and median 90-day mRS was 1.0. Patients with poor outcome (mRS ≥2) at 90 days after stroke were significantly older, had higher blood pressure and atrial fibrillation more frequently, and more severe neurologic deficit on admission as compared to those with a good outcome. Moreover, patients with poor outcome showed significantly higher NLR and significantly lower LMR as compared to those with good outcome (Table 1).

White blood cell counts, NLR and LMR during thrombolysis. In the total cohort, the median neutrophil count, monocyte count and NLR increased, whereas median LMR decreased 24 h after thrombolysis when compared with admission results (Table 2). An inverse but modest correlation was found between neutrophil count and lymphocyte count at admission (r = -0.166, p = 0.002), and at day 1 (r = -0.200, p =

0.001). Significant but modest positive correlation was found between lymphocyte count and monocyte count at admission ($r = 0.261$, $p < 0.001$), but not at day 1. Neutrophil count also correlated with monocyte count at admission ($r=0.381$, $p < 0.001$) and at day 1 ($r = 0.598$, $p < 0.001$).

None of the leukocyte indices showed association with stroke etiology, stroke severity at admission or with hemorrhagic transformation at admission (Table 3 and Supplementary Supplementary Table S1). On the other hand, neutrophil count, monocyte count and NLR significantly increased, while lymphocyte count and LMR significantly decreased 24 h post-rtPA

Table 1

Baseline characteristics of enrolled patients according to long term outcomes (modified Rankin Scale at 90 days post event). Results are depicted as mean ± SD or median (interquartile range). *ACE* angiotensin converting enzyme, *ASPECTS* Alberta Stroke Program Early CT Score, *BA* basilar artery, *BMI* body mass index, *DM* diabetes mellitus, *hsCRP* high sensitivity C reactive protein measurement, *ICA* internal carotid artery, *LMR* lymphocyte-monocyte ratio, *mRS* modified Rankin Scale, *NIHSS* National Institutes of Health Stroke Scale, *NLR* neutrophil-lymphocyte ratio, *PAD* peripheral artery disease, *SICH* symptomatic intracerebral hemorrhage, *aSICH* asymptomatic intracerebral hemorrhage, *TIA* transient ischemic attack, *TOAST* Trial of ORG 10172 in Acute Stroke Treatment, *WBC* white blood cell count.

	All Patients *n* = 285	**Good outcome (mRS=0-1)** *n* = 190	**Poor outcome (mRS=2-6)** *n* = 95	**p value**
Demographic characteristics				
Age (year)	66 ± 12.9	62.8 ± 12.9	72.0 ± 10.2	< 0.001
Gender, male (%)	159 (55.8)	107 (56.3)	52 (54.7)	0.802
BMI (kg/m^2)	28.5 ± 5.9	28.5 ± 5.6	28.4 ± 6.5	0.900
Baseline laboratory results				
High sensitivity C-reactive protein (g/L)	2.8 (1.4-6.0)	2.5 (1.3-5.2)	3.5 (1.7-7.7)	0.060
White blood cell count (G/L)	8.1 (6.5-9.9)	8.04 (6.45-9.59)	8.15 (6.48-10.33)	0.455
Neutrophil count (G/L)	5.2 (4.0-7.1)	5.12 (3.99-6.86)	5.62 (4.17-7.55)	0.157
Lymphocyte count (G/L)	1.7 (1.2-2.3)	1.77 (1.31-2.3)	1.61 (1.15-2.24)	0.053
Monocyte count (G/L)	0.56 (0.44-0.69)	0.54 (0.43-0.69)	0.58 (0.47-0.71)	0.164
NLR	2.9 (1.94-4.82)	2.72 (1.86-4.66)	3.18 (2.17-5.94)	0.036
LMR	3.22 (2.42-4.29)	3.41 (2.51-4.55)	2.97 (1.87-3.92)	0.005
Vascular risk factors				
Smoking, No. (%)				
Non-smoker	204 (71.6)	131 (68.8)	73 (76.8)	0.152
Previous smoker	2 (0.7)	2 (1.1)	0	
Current smoker	79 (27.7)	57 (30.2)	22 (23.2)	

	All Patients *n* = 285	Good outcome (mRS=0-1) *n* = 190	Poor outcome (mRS=2-6) *n* = 95	p value
Previous stroke/TIA, No. (%)	67 (23.5)	38 (20)	29 (30.5)	0.055
Atrial fibrillation, No. (%)	29 (10.2)	14 (7.4)	15 (15.8)	0.026
PAD, No. (%)	9 (3.2)	6 (3.2)	3 (3.2)	1.000
Hyperlipidemia, No. (%)	181 (63.5)	123 (64.7)	58 (61.0)	0.602
DM, No. (%)	71 (24.9)	41 (21.6)	30 (31.6)	0.081
Hypertension, No. (%)	246 (86.3)	158 (83.2)	88 (92.6)	0.029
Therapy at stroke onset, No. (%)				
ACE inhibitor	148 (51.9)	92 (48.4)	56 (58.9)	0.103
Diuretic	118 (41.4)	71 (37.4)	48 (50.5)	0.056
Beta blocker	97 (34)	62 (32.6)	35 (36.8)	0.509
Calcium channel blocker	69 (24.2)	46 (24.2)	23 (24.2)	1.000
Alfa blocker	23 (8.1)	14 (7.4)	9 (9.5)	0.645
Hypertension therapy	189 (66.3)	121 (63.7)	68 (71.6)	0.231
Acetylsalicylic acid	86 (30.2)	52 (27.4)	34 (35.8)	0.171
Clopidogrel	23 (8.1)	16 (8.4)	7 (7.4)	0.822
Anticoagulant therapy, No. (%)				
Vitamin K antagonist	9 (3.2)	5 (2.6)	4 (4.2)	
Direct thrombin inhibitor	1 (0.4)	1 (0.5)	0	
Direct factor Xa inhibitor	0	0	0	
Low molecular weight heparin	3 (1.1)	2 (1.1)	1 (1.1)	
Lipid lowering therapy, No. (%)	78 (25)	44 (23.3)	27 (28.4)	0.384
Antidiabetic therapy, No. (%)	52 (17)	27 (14.2)	21 (22.1)	0.094
Stroke severity, No. (%)				
NIHSS at day 1				
0-5	110 (38.7)	93 (48.9)	17 (18.1)	< 0.001

	All Patients *n* = 285	Good outcome (mRS=0-1) *n* = 190	Poor outcome (mRS=2-6) *n* = 95	p value
6-10	98 (34.5)	65 (34.2)	33 (35.1)	
11-15	50 (17.6)	24 (12.6)	26 (27.7)	
>15	26 (9.2)	8 (4.2)	18 (19.1)	
NIHSS at day 7				
0-5	129 (46.9)	109 (57.7)	20 (23.3)	< 0.001
6-10	113 (41.1)	77 (40.7)	36 (41.9)	
11-15	24 (8.7)	3 (1.6)	21 (24.4)	
>15	9 (3.3)	0	9 (10.5)	
Hemorrhagic transformation				
aSICH, No. (%)	13 (4.6)	4 (2.1)	9 (9.5)	0.110
SICH, No. (%)	7 (2.5)	0	7 (7.4)	
Stroke localization				
ICA, No. (%)	193 (67.7)	112 (58.9)	81 (85.3)	< 0.001
VB, No. (%)	92 (32.3)	78 (41.1)	14 (14.7)	
Stroke etiology (TOAST)				
Large-artery atherosclerosis, No. (%)	62 (21.8)	55 (28.9)	7 (7.4)	< 0.001
Small-vessel occlusion, No. (%)	103 (36.1)	59 (31.1)	44 (46.3)	
Cardioembolic, No. (%)	23 (8.1)	17 (8.9)	6 (6.3)	
Other/undetermined, No. (%)	97 (34)	59 (31.1)	38 (40)	

Table 2

Leukocyte counts and ratios in acute ischemic stroke patients before and 24h after thrombolysis. *NLR* neutrophil-lymphocyte ratio, *LMR* lymphocyte-monocyte ratio, statistics: Wilcoxon Signed Rank test.

	Before thrombolysis	**24h after thrombolysis**	**p value**
Neutrophil (G/L)	5.24 (4.04-7.14)	6.26 (4.7-8.3)	< 0.001
Lymphocyte (G/L)	1.74 (1.25-2.3)	1.69 (1.28-2.15)	0.061
Monocyte (G/L)	0.56 (0.44-0.69)	0.66 (0.53-0.83)	< 0.001
NLR	2.9 (1.94-4.82)	3.58 (2.48-5.6)	< 0.001
LMR	3.22 (2.42-4.29)	2.58 (1.74-3.56)	< 0.001

Table 3

Leukocyte counts and ratios at admission and 24h after thrombolysis according to stroke severity at admission and thrombolysis safety. Data depicted as median (inter-quartile range). *NLR* neutrophil-lymphocyte ratio, *LMR* lymphocyte- monocyte ratio, *aSICH* asymptomatic intracerebral hemorrhage, *SICH* symptomatic intracerebral hemorrhage, *ECASS II* European Co-operative Acute Stroke Study-II, *NIHSS* National Institutes of Health Stroke Scale. Statistics: Kruskal-Wallis.

	Time of blood sampling	**Neutrophil (G/L)**	**Lymphocyte (G/L)**	**Monocyte (G/L)**	**NLR**	**LMR**
Hemorrhagic transformation according to ECASS II (24h post-lysis)	At admission					
No hemorrhage (n = 264)		5.2 (4.1-7.1)	1.7 (1.2-2.3)	0.56 (0.44-0.70)	2.88 (1.93-4.82)	3.22 (2.42-4.30)
aSICH (n = 13)		5.3 (3.8-7.2)	1.7 (1.3-1.9)	0.45 (0.39-0.58)	3.07 (2.32-6.50)	3.82 (2.68-5.10)
SICH (n = 7)		6.2 (3.6-8.0)	1.8 (1.4-2.2)	0.60 (0.53-0.68)	3.41 (1.96-4.54)	2.97 (2.56-3.91)
p value		0.987	0.688	0.152	0.805	0.551
Hemorrhagic transformation according to ECASS II (24 h post-lysis)	24h after thrombolysis					
No hemorrhage (n = 264)		6.1 (4.6-8.2)	1.7 (1.3-2.2)	0.66 (0.52-0.83)	3.44 (2.45-5.20)	2.63 (1.75-3.59)
aSICH (n = 13)		8.2 (6.6-9.1)	1.3 (1.1-1.9)	0.69 (0.62-0.87)	5.63 (3.31-8.58)	2.07 (1.2-2.59)
SICH (n = 7)		9.7 (7.3-15.4)	1.3 (0.8-2.2)	0.91 (0.80-1.17)	7.12 (4.15-19.74)	1.51 (0.8-2.04)
p value		0.002	0.091	0.030	0.002	0.005
Stroke severity at admission	At admission					
NIHSS 0-5 (n = 110)		5.1 (4.0-7.0)	1.8 (1.4-2.4)	0.57 (0.44-0.71)	2.75 (1.81-3.98)	3.45 (2.51-4.51)
NIHSS 6-10 (n = 97)		5.5 (4.4-6.8)	1.7 (1.2-2.3)	0.57 (0.45-0.70)	2.78 (2.00-4.95)	3.01 (2.33-4.34)

	Time of blood sampling	Neutrophil (G/L)	Lymphocyte (G/L)	Monocyte (G/L)	NLR	LMR
NIHSS 11-16 (n = 50)		5.1 (4.0-7.5)	1.6 (1.2-2.1)	0.53 (0.42-0.66)	2.99 (2.08-6.56)	3.11 (2.41-4.13)
NIHSS >16 (n = 25)		5.3 (3.6-6.9)	1.6 (1.1-1.9)	0.55 (0.44-0.63)	3.27 (2.10-5.73)	3.04 (2.36-4.06)
p value		0.782	0.067	0.581	0.330	0.441
Stroke severity at admission	24h after thrombolysis					
NIHSS 0-5 (n = 110)		5.4 (4.3-7.5)	1.8 (1.4-2.4)	0.61 (0.49-0.79)	3.08 (2.10-4.47)	2.95 (2.27-3.92)
NIHSS 6-10 (n = 97)		6.4 (4.7-8.0)	1.7 (1.4-2.2)	0.66 (0.56-0.82)	3.30 (2.48-5.17)	2.54 (1.85-3.59)
NIHSS 11-16 (n = 50)		7.7 (5.0-9.7)	1.4 (1.2-2.0)	0.67 (0.56-0.85)	4.66 (3.04-6.85)	2.26 (1.67-2.87)
NIHSS >16 (n = 25)		9.7 (7.2-13.4)	1.2 (0.9-1.7)	0.83 (0.68-1.08)	8.42 (4.05-12.98)	1.34 (1.04-1.87)
p value		< 0.001	< 0.001	0.004	< 0.001	< 0.001

in patients with more severe stroke. Similar pattern was seen in those who suffered therapy-associated intracerebral hemorrhage (Table 3).

Neutrophil count and NLR were significantly higher in ASPECTS 10-8 group compared with ASPECTS ≤7 group at day 1 (Supplementary Table S2). Univariate logistic regression proved a significant protective effect of higher LMR at admission against functional dependence at 3 months post-event (OR = 0.755, 95%CI [0.631, 0.903], p = 0.002) (Supplementary Table S3). Similar analysis showed no association between NLR and long-term functional outcome. Besides LMR, age, lymphocyte count at admission, hypertension and stroke characteristics (NIHSS, hemorrhagic transformation, stroke localization and etiology) showed association with long-term outcome of therapy, but in the multivariate model only age, NIHSS, hemorrhagic transformation and stroke localization remained as significant variables (Supplementary Table S3). Although in the univariate model, white blood cell counts, NLR and LMR measured at 24h after rtPA therapy showed highly significant association with functional outcomes at 3 months post-event, a multivariate logistic regression model including all possible confounders displayed no significant association of these parameters with 3-months functional outcome (Table 4).

Table 4

Univariable and multivariable logistic regression analyses depicting the associations of day 1 NLR, LMR and baseline characteristics with functional independence at 3 months post-event (mRS ≥ 2). *ACE* angiotensin converting enzyme, *ASPECTS* Alberta Stroke Program Early CT Score, *BA* basilar artery, *BMI* body mass index, *DM* diabetes mellitus, *hsCRP* high sensitivity C reactive protein measurement, *ICA* internal carotid artery, *LMR* lymphocyte-monocyte ratio, *mRS* modified Rankin Scale, *NIHSS* National Institutes of Health Stroke Scale, *NLR* neutrophil-lymphocyte ratio, *PAD* peripheral artery disease, *SICH* symptomatic intracerebral hemorrhage, *aSICH* asymptomatic intracerebral hemorrhage, *TIA* transient ischemic attack, *TOAST* Trial of ORG 10172 in Acute Stroke Treatment, *WBC* white blood cell count.

Parameters	**Univariable Logistic Regression Analysis**		**Multivariable Logistic Regression Analysis**	
	OR (95%CI)	p value	OR (95%CI)	p value
Age (year)	1.076 (1.048-1.105)	< 0.001	1.056 (1.011-1.111)	0.014
Gender, male (%)	0.938 (0.572-1.539)	0.800		
BMI (kg/1.72m2)	0.997 (0.956-1.040)	0.894		
hsCRP (g/L)	1.011 (1.001-1.022)	0.038	1.005 (0.979-1.031)	0.722
WBC (G/L)	1.283 (1.173-1.402)	< 0.001	1.127 (0.796-1.595)	0.502
Neutrophil (G/L)	1.348 (1.221-1.489)	< 0.001	0.807 (0.527-1.237)	0.325
Lymphocyte (G/L)	0.403 (0.260-0.625)	< 0.001	8.299 (1.489-67.141)	0.050
Monocyte (G/L)	7.470 (2.818-19.802)	< 0.001	0.229 (0.094-2.426)	0.117
NLR	1.417 (1.266-1.585)	< 0.001	1.416 (0.963-2.083)	0.077
LMR	0.453 (0.347-0.591)	< 0.001	0.311 (0.103-1.413)	0.056
Smoking, No. (%)	0.654 (0.377-1.135)	0.131	0.961 (0.732-4.296)	0.204
Previous stroke/TIA, No. (%)	1.758 (1.001-3.086)	0.050	1.774 (0.732-4.296)	0.204
Atrial fibrillation, No. (%)	1.656 (1.086-2.527)	0.019	0.347 (0.191-0.981)	0.033
PAD, No. (%)	1.000 (0.245-4.089)	1.000		
Hyperlipidemia, No. (%)	0.854 (0.513-1.420)	0.543		
DM, No. (%)	1.677 (0.964-2.918)	0.067	1.148 (0.219-6.008)	0.870
Hypertension, No. (%)	2.546 (1.079-6.007)	0.033	0.541 (0.117-2.493)	0.430

Parameters	Univariable Logistic Regression Analysis		Multivariable Logistic Regression Analysis	
Angiotensin converting enzyme inhibitor therapy	0.654 (0.397-1.076)	0.094	1.823 (0.570-5.829)	0.311
Beta blocker therapy	0.830 (0.496-1.390)	0.480		
Diuretic therapy	0.609 (0.370-1.003)	0.051	1.216 (0.459-3.217)	0.694
Calcium channel blocker therapy	1000 (0.563-1.777)	1.000		
Alfa blocker therapy	0.760 (0.316-1.826)	0.539		
Hypertension therapy	0.696 (0.480-1.189)	0.185	1.028 (0.248-4.268)	0.969
Acetylsalicylic acid therapy	0.676 (0.399-1.145)	0.145	0.593 (0.245-1.431)	0.245
Clopidogrel therapy	1.156 (0.459-2.914)	0.759		
Anticoagulant therapy, No. (%)	1.025 (0.601-1.748)	0.927		
Lipid lowering therapy, No. (%)	0.759 (0.434-1.327)	0.334		
Antidiabetic therapy, No. (%)	0.548 (0.310-1.099)	0.096	0.954 (0.156-5.821)	0.595
NIHSS at day 1	2.333 (1.757-3.098)	< 0.001	0.925 (1.420-3.948)	0.177
NIHSS at day 7	4.613 (2.949-7.215)	< 0.001	1.537 (1.335-1.769)	< 0.001
Hemorrhagic transformation	6.874 (2.441-19.357)	< 0.001	4.102 (0.589-28.579)	0.154
Stroke localization	0.248 (0.131-0.469)	< 0.001	0.393 (0.137-1.131)	0.083
Stroke etiology (TOAST)	1.313 (1.060-1.626)	0.013	1.104 (0.758-1.608)	0.606

At baseline, the mean values of NLR and LMR for the study population were 2.9 (IQR [1.94, 4.82]) and 3.22 (IQR [2.42, 4.29]), respectively. The optimal threshold values for the prediction of poor functional outcome at 3 months post-event based on the best Youden index by ROC analysis were 5.73 for NLR and 2.08 for LMR (Fig. 1). According to the optimal cut-off values of NLR and LMR at admission, patients were classified into four groups: low NLR-high LMR, high NLR-high LMR, low NLR-low LMR, high NLR-low LMR.

Out of 190 patients with favorable outcome, 77% of patients fell in the category of low NLR-high LMR combinations, while the high NLR-low LMR group only 6.8% of patients at admission. The proportion of patients with favorable outcome as stratified according to low NLR-high LMR and high NLR-low LMR

were 76% and 7.8% at day 1, respectively. Out of 95 patients in the poor outcome group, 67% of patients were stratified as low NLR-High LMR while only 21% as high NLR-low LMR before the administration of thrombolysis (p = 0.001) (Fig. 2). At 24h after thrombolysis, the proportion of patients with poor outcome displayed a significant shift in the above groups as 36% of patients could be stratified as having low NLR-high LMR while the high NLR-low LMR group included 49% (p < 0.001) of patients (Fig. 2).

The combination of NRL and LMR as determined at 24h after thrombolysis was found to be an independent predictor of poor functional outcome at 3 months post-event (OR = 3.407, 95% CI [1.449, 8.011], *p* = 0.005 for high NLR-low LMR patient group vs. low NRL-high LMR patient group) after controlling for all potential confounders (Table 5).

Table 5
Association of NLR-LMR combinations at admission and 24h after thrombolysis with poor functional outcome (mRS ≥2) at 3 months post-event. *CI* confidence interval, *OR* odds ratio, *NLR* neutrophil-lymphocyte ratio, *LMR* monocyte-lymphocyte ratio. [a] Controlled for: age, sex, atrial fibrillation, hypertension, NIHSS at day 1, hemorrhagic transformation, stroke localization, stroke etiology (TOAST).

Characteristics	Univariate Analysis, OR (95% CI)	p value	Multivariate Analysis, OR (95% CI)[a]	p value
At admission				
Low NLR-High LMR (n=211)	Ref	-	Ref	-
High NLR-High LMR n=22)	0.766 (0.310-1.891)	p = 0.563	0.338 (0.075-1.530)	p = 0.159
Low NLR-Low LMR (n=19)	0.993 (0.516-1.914)	p = 0.507	1.486 (0.462-4.779)	p = 0.507
High NLR-Low LMR (n=33)	5.496 (3.236-9.336)	p < 0.001	3.049 (1.205-7.714)	p = 0.019
At day 1				
Low NLR-High LMR (n=178)	Ref	-	Ref	-
High NLR-High LMR n=10)	1.412 (0.555-3.591)	p = 0.469	4.860 (0.816-28.944)	p = 0.082
Low NLR-Low LMR (n=35)	1.831 (0.914-3.671)	p = 0.088	1.168 (0.439-3.107)	p = 0.755
High NLR-Low LMR (n=62)	10.134 (5.685-18.066)	p < 0.001	6.353 (2.774-14.548)	p < 0.001

Discussion

The present study, to the best of our knowledge, is the first to provide and discuss the evidence in support of a combination of NLR and LMR as measured at 24 h post-thrombolysis as an independent prognostic

factor that possesses clinical significance and feasibility to identify 3-months poor outcome of AIS patients treated with intravenous thrombolysis. Confirmed by a multivariate analysis, in addition to gender, NIHSS at day 1, and TOAST classification, high NLR-low LMR remained an independent prognostic factor for 3-months poor outcome for AIS patients after thrombolysis.

Inflammation has a very important role in ischemic brain injury. Ischemia following stroke activates microglia and consequently circulating monocytes, neutrophils and lymphocytes are recruited to injury site [15]. The resident microglia cell activation occurs immediately after brain injury and blood-derived immune cells infiltration into the brain tissue follows within hours to a few days [16]. Immune cells release different agents which exacerbate tissue damage [17]. Experimental stroke models have shown increased hematopoiesis and greater output of neutrophils from the bone marrow, via increased stimulation of the autonomic nervous system post-stroke [18]. Post thrombolysis NLR as a marker of increased risk for poor outcome within 3 months after stroke onset has been also found by others [19]. Others analyzed the ability of admission NLR for 90-day stroke outcome prediction after endovascular stroke therapy [20]. They found NLR (cut-off point at ≥5.9) a valuable prognostic marker for poor outcome. Pektezel et al conducted a retrospective evaluation of acute stroke patients treated with rtPA by comparing favorable outcome (mRS≤3) with excellent outcome (mRS 0 or 1) at admission and after 24h [21]. This study showed that significant elevation of NLR from admission to 24h post-event, and NLR ≤3.6 after 24h revealed favorable prognosis. They conclude that elevated NLR during the first 24 hours is an epiphenomenon of poor prognosis. On the contrary, Shi et al found no significant difference in NRL at admission in patients with good versus poor outcome [19]. We experienced similar cut-off point for NLR (at ≥5.73) and NRL did not show significant difference at admission between poor and good outcome groups. Others found, in a small group of patients, no significant relation between NRL or LMR and long-term outcome in AIS patients treated by thrombolysis [22]. In the acute phase of ischemic stroke, different circulating immune cells infiltrate into the brain [15]. Among them, monocytes have been shown to play a particularly important role. In the ischemic brain, the monocyte-derived macrophages (MDMs) differentiate from monocytes [23]. MDMs are potent phagocytic cells and involve in the long-term spontaneous functional recovery of brain after ischemia [24]. LMR at admission also was evaluated for 3-months prognosis in patient with stroke with thrombolysis therapy [25]. They demonstrated that higher LMR value (cut-off point at 3.48) was an independent factor to predict the clinical outcome of stroke before rtPA administration.

The relation between leukocyte profile and stroke outcome after mechanical thrombectomy has also been investigated [26,27]. In one study, higher counts of neutrophils and NLR at admission, and at day 1, as well as lower lymphocyte counts at day 1 were associated with poor prognosis (mRS>2) [26]. In another study, higher NLR and lower LMR at 24-h after mechanical thrombectomy but not at admission were significant predictors of mRS at 3 months functional outcome [27]. They found optimal cut-off values of 5.5 for NLR and 2.0 for LMR after thrombectomy.

Our study is the first to discuss the value of the combined post-thrombolysis high NLR-low LMR ratio in evaluating the prognosis of AIS patients at 3 months post-event. Our findings corroborated with the

observations of previous studies which had revealed the lack of reliability of the pre-thrombolysis prognostic value of the NLR measurement [19]. Our results show that the combination of NLR-LMR as obtained 24 h post-lysis categorized patients according to 3-months outcome more precisely and with better diagnostic accuracy.

Conclusion

In conclusion, by combining NLR and LMR results of AIS patients as obtained 24h after thrombolysis, estimation of patient outcomes can be significantly improved. The combination of high NLR-low LMR was found to be an independent risk factor for poor outcome at 3 months post event (OR = 3.407, 95% CI [1.449, 8.011], p = 0.005).

Declarations

Author contributions

AHS conceived the study. KSZ, LC, EB and ZB oversaw the statistical analysis plan. AHS, KSZ, FS and FS conducted statistical analysis. FS, FS, IS, EGS, NV, and RK contributed to data acquisition. FS, FS and IS contributed to data quality assurance and data quality analysis. AHS, KSZ and ZB contributed to data interpretation. AHS, ZB, FS and FS drafted the initial manuscript and all remaining authors critically revised the manuscript. All authors gave final approval for publication.

Competing interestsStatement

The authors declare no competing interests.

Funding

Supported by grants from the National Research, Development and Innovation Fund (K120042, FK128582), by GINOP-2.3.2-15-2016-00048 and the Eötvös Loránd Kutatási Hálózat (ELKH-DE Cerebrovascular and Neurodegenerative Research Group).

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Figures

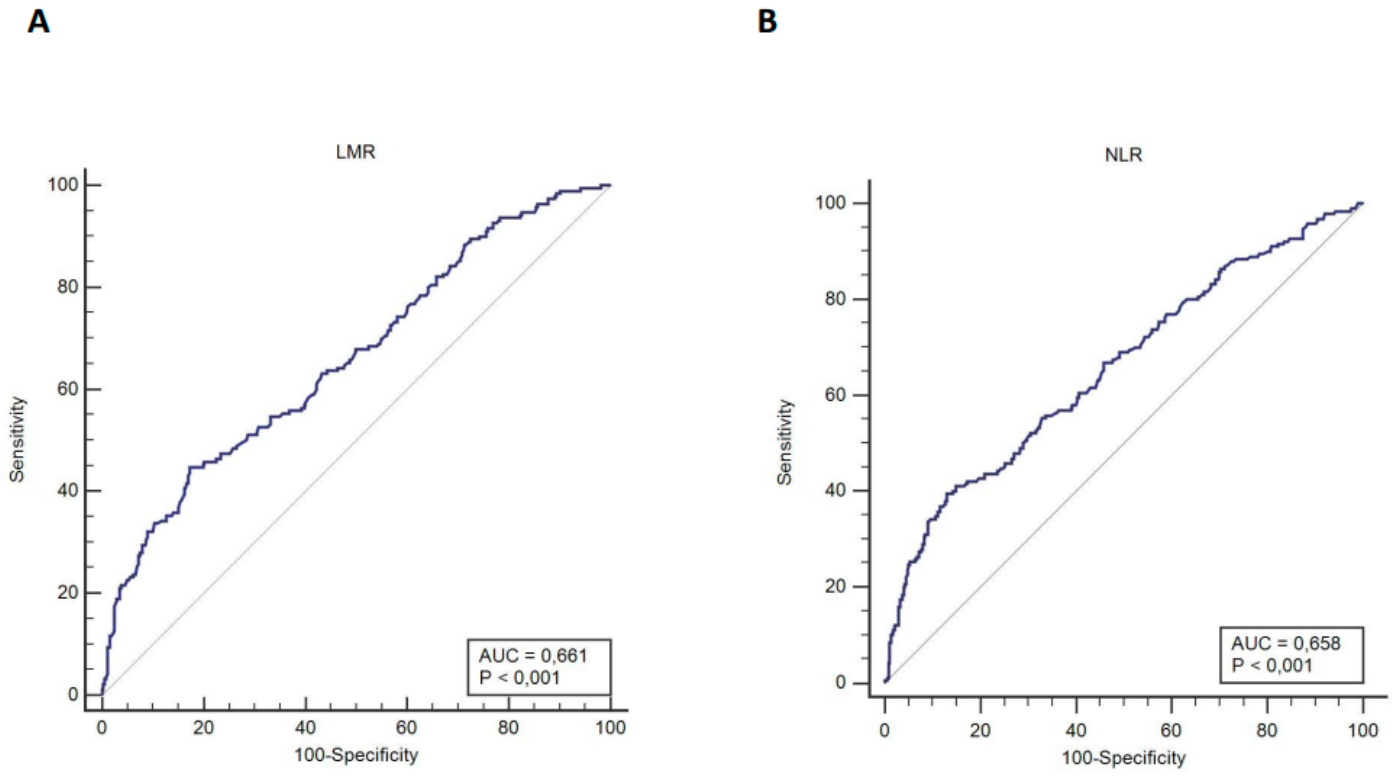

Figure 1

Receiver operating characteristics (ROC) curve analysis of admission neutrophil-to-lymphocyte ratio (NLR) (A) and lymphocyte-to-monocyte ratio (LMR) (B) values predictingfunctional dependence (mRS2) at 3 months post-event.

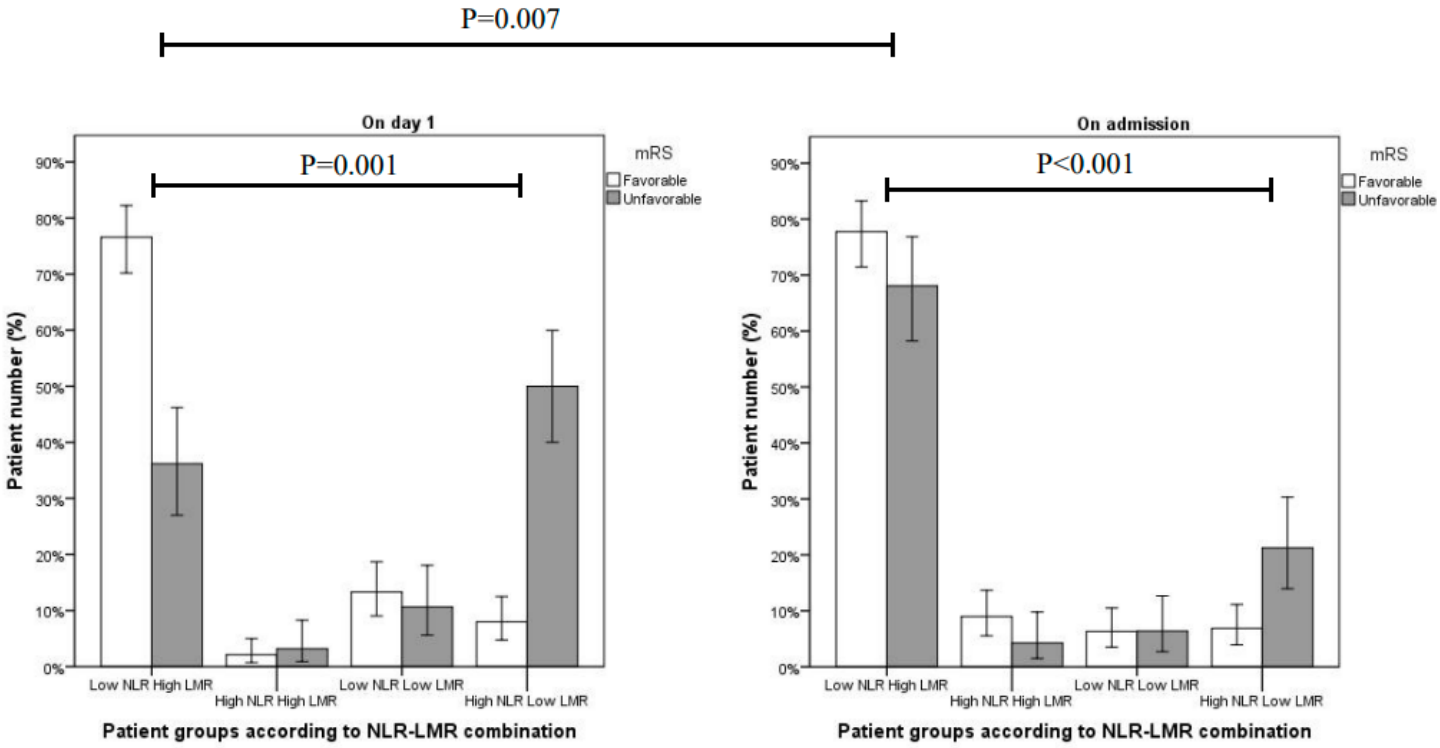

Figure 2

Proportion of patients in different NLR-LMR combination groups at admission and at day 1.

Supplementary Files

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- SupplementaryTables.pdf