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Blood biomarkers in acute ischemic stroke: The prognostic value of neutrophil-to-lymphocyte ratio and mean platelet volume

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Abstract

Background. The neutrophil-to-lymphocyte ratio (NLR) and mean platelet volume (MPV) reflect systemic inflammation, which plays an important role in the process of treating ischemic strokes. Few studies have evaluated the association between blood biomarkers and clinical outcomes in ischemic strokes in intensive care units (ICUs).

Objectives. This retrospective study aims to explore the relationship between blood biomarkers and the clinical outcomes of acute ischemic stroke (AIS) patients.

Materials and methods. Basic descriptive statistics of the patients admitted to the ICU with the diagnosis of AIS according to sociodemographic, clinical and laboratory findings were collected. Receiver operating characteristic (ROC) curve analysis was used to determine the cutoff point for NLR and MPV variables based on the diagnosis in statistical analyses and crosstab analyses of variables. The χ^2 and Fisher's exact tests were used to assess the statistical relationship between categorical variables. In addition, the odds ratio (OR) was utilized to show the strength of the relationship between the categorical NLR, MPV and modified Rankin Scale (mRS) variables. Finally, the Mann–Whitney U test was used to compare the medians of 2 independent groups.

Results. A total of 1,379 records were identified in the database search. Eighty-seven patients who met the inclusion criteria and were hospitalized in the ICU were included in the study. The optimal cutoff point was determined to be 4.0 for NLR and 9.0 for the MPV. A statistically significant relationship was found between high medians of the NLR and the MPV and unfavorable functional outcomes using a 5% significance level ($p < 0.001$ and $p < 0.001$, respectively).

Conclusions. We showed that the NLR and MPV are associated with stroke severity, unfavorable functional outcomes and mortality in AIS. These findings provide new insights into the mechanisms and treatment strategies of AIS. The results show that these accessible values can be used as independent predictive biomarkers.

Key words: inflammation, prognosis, acute ischemic stroke, mean platelet volume, neutrophil-to-lymphocyte ratio

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Background

Our immune system carries out its defense function against harmful endogenous or exogenous factors with the functional cooperation of 2 main cell groups, collectively called leukocytes. Granulocytes and lymphocytes (agranulocytes) constitute the immune system's 2 cell groups. Granulocytes mainly include neutrophils, eosinophils, basophils, mast cells, dendritic cells, monocyte-macrophages, and phagocytes. Lymphocytes, on the other hand, consist of natural killer cells and some specialized cells in the T and B lymphocyte groups. Granulocytes directly inactivate pathogens. During these granulocyte functions, unlike lymphocytes, there is no need for mediator molecules or antigen presentation. Thanks to these features, they can actively display their functions from the first moments of life. Therefore, this innate immune response is called innate immunity. Lymphocytes, on the other hand, have acquired functional abilities, such as recognizing pathogenic structures and target molecules, keeping them in memory and synthesizing some specialized molecules to use when the time comes. Thus, they form the acquired immune system.[1] In situations that cause damage to our body, some cytokines, such as eicosanoids and leukotrienes, are secreted in the damaged areas, causing inflammation. This inflammation causes neutrophils to be directed to the area. Consequently, an inflammatory response develops in reaction to neutrophils, cytokines, dead pathogens, and cellular elements in the damaged tissue or site. In general, neutrophil counts do not increase in viral infections, while systemic infections or systemic inflammatory responses cause an increase in neutrophil counts in the blood.[2] Another inflammatory marker is the mean platelet volume (MPV), a measure of the mean platelet size that is used to determine the rate of platelet production and destruction in the bone marrow. Mean platelet volume is accepted as a marker of subclinical inflammation and inflammatory disease activity because platelets become active in the presence of inflammation and secrete pro-inflammatory and thrombotic factors. In the literature, many studies have shown a positive association between MPV, inflammation and coronary artery disease (CAD).[3,4]

Stroke is the 2nd leading cause of death and a major cause of disability worldwide.[5] Recovery from a stroke can take a very long time, and despite adequate treatment, this does not always result in a full recovery. Some studies have been conducted regarding the ability of various biomarkers to predict stroke prognosis. Fibrinogen levels have been associated with neurological deterioration in patients with acute ischemic stroke (AIS).[6,7] Interleukin-6 (IL-6) levels have been shown to be associated with stroke severity and functional outcomes during the first year of stroke.[8]

Post-stroke inflammation plays an important role in the pathogenesis of brain injury.[9] Inflammatory biomarkers have been associated with stroke severity and clinical outcomes. For example, elevated neutrophil counts have been associated with larger infarct volumes,[10] and elevated leukocyte counts have been associated with higher initial stroke severity and worse short- and long-term clinical outcomes.[11,12] In addition, increased serum concentrations of high-sensitivity C-reactive protein (hsCRP) have been found to be associated with the risk of stroke recurrence and worse functional outcomes.[13] A number of neural substrates play an essential role in the pathophysiology of stroke and its relationship with immune responses. An important consideration is the delicate balance between pro-inflammatory and anti-inflammatory responses during AIS. Understanding the interaction between these 2 types of responses and the complex mechanisms that regulate this balance can offer valuable insights into potential therapeutic interventions aimed at modulating immune responses to optimize stroke recovery. The synergistic relationships between various inflammatory markers such as hsCRP and interleukins are highly suggestive of their contribution to the overall inflammatory environment in these complex processes, and, hence, the impact of the immune response on the ischemic environment of the brain. In ischemic stroke patients with elevated hsCRP levels, there is a significant association between high platelet counts and unfavorable functional outcomes. In contrast, this was not the case in AIS patients with low hsCRP levels. Therefore, co-administration of antiplatelet and anti-inflammatory therapy in patients with AIS with high hsCRP levels may be a rational approach.[14]

An inflammatory condition plays a vital role at all stages of the ischemic cascade. The energy deficit caused by the loss of the neurons' ability to synthesize adenosine triphosphate (ATP) is the main mechanism of cell death in the region of cerebral ischemia. Disruption of ATP synthesis also causes a decrease in glutamate reuptake and an increase in its extracellular amount. Excessive activation of glutamate receptors causes excitotoxicity and accumulation of Ca^{2+} ions, leading to mitochondrial failure and apoptosis. The influx of Ca^{2+} ions activates catabolic enzymes by producing arachidonic acid and increasing the production of reactive oxygen species (ROS), mainly in neurons. The excitotoxicity and growth of ROS activate microglia and astrocytes that secrete cytokines, chemokines and matrix metalloproteinases (MMPs).[15,16] These inflammatory mediators induce the expression of endothelial cell adhesion molecules such as P-selectin, E-selectin, endothelial leukocyte adhesion molecule-1 (ELAM-1), and intercellular cell adhesion molecule-1 (ICAM-1), so that the neutrophils infiltrate into the ischemic areas of the brain.[17] As a result of cell damage in the hypoxic and/or necrotic brain, damage-associated molecular patterns (DAMPs) and high-mobility group box 1 (HMGB-1) are released into the environment, activating astrocytes and microglia. The DAMP–HMGB-1s stimulate pattern recognition receptors (PRRs) such as TLRs in the blood–brain barrier (BBB), leading to extravasation of leukocytes

(primarily neutrophils, macrophages and lymphocytes). The activation of TLR leads to inflammation, MMP activation, blood–brain barrier (BBB) breakdown, and leukocyte extravasation.[18] These mediators increase the expression of adhesion molecules on cerebral endothelial cells, which promotes adhesion and infiltration of the blood-derived leukocytes (neutrophils, macrophages and lymphocytes) into ischemic brain tissues. Activated M1 microglial cells release various pro-inflammatory mediators such as TNF-α, IL-1β and IL-6, contributing to the permeability of many immune cells and resulting in BBB disruption. Further reduced blood flow decreases ATP levels and increases the levels of Ca^{2+} and nitric oxide (NO), promoting free radical generation (ROS) and mitochondrial failure, which leads to cell death. Meanwhile, activated microglia/macrophages and infiltrated T cells also secrete some neuroprotective factors (e.g., IL-10 and TGF-β) that could suppress post-ischemic inflammation.[19] In this complex process, neutrophils infiltrating the ischemic/hypoxic tissue from the permeable BBB play a dominant role (Fig. 1).

The role of specific neuropharmacological adjuvants involved in neurochemical synaptic transmission and acting on brain plasticity processes in stroke may determine both our future therapeutic approach and the role of noninvasive brain stimulation techniques. One of the important questions in the coming years will be how modulation of neuronal substrates, such as the glutamatergic, noradrenergic and endocannabinoid systems, will have a curative effect on stroke in humans and, in particular, on the elimination of fear learning and anxiety disorders.[20] Understanding neurochemical synaptic transmission and brain plasticity processes, especially behavioral problems that may occur after stroke, may contribute to the correction of our abilities related to sensory perception inputs such as visuospatial attention, metacognitive abilities and objective performance gain. Regulation of neuronal substrates with neuromodulation techniques during attentional deployment is very important for understanding the effect of sensory input on objective performance and its atypical perceptual consequences.[21] The neurophysiological models developed regarding the shift of visual-spatial perception towards the right hemifield and deficiencies in perceiving left contralesional stimuli, especially in patients with stroke-related left hemispatial neglect. The regulation of neuronal substrates and their psychophysiological correlates were investigated using structural equation models calculated with discriminative electroencephalogram (EEG) measurements.[22]

Even with thrombolytic therapy in AIS, neuronal damage may not be prevented due to exaggerated inflammatory responses. Similar to peripheral molecular mimicry mechanisms, microglial activation may also be altered during ischemic stroke processes.[23] Increased astroglia and oligodendrocyte reactivity may reduce potential regenerative mechanisms of plasticity patterns, such as axonal growth, synaptic remodeling, remyelination, cortical remodeling, and reattachment of neural networks to healthy intact tissue. Attenuation of the pro-inflammatory effects and strengthening of the anti-inflammatory effects after stroke may guide our future treatment strategies. In particular, antagonists of cascades driven by pro-inflammatory chemokines and allosteric agonists of cascades driven by anti-inflammatory chemokines can alter neuronal plasticity by affecting GABAergic neurotransmission.[24] Concerning the C-X-C chemokine ligand 12 (CXCL12)/C-X-C chemokine receptor type 4 (CXCR4)/C-X-C chemokine receptor type 7 (CXCR7) chemokine pathway, rats subjected to transient middle cerebral artery occlusion (tMCAO) have been shown to improve functional outcomes after the administration of an experimental molecule, a CXCR4 receptor antagonist and allosteric CXCR7 agonist.[25] In addition, similar effects have been confirmed after the exogenous administration of C-X-C chemokine ligand 1 (CX3CL1)/C-X3-C chemokine receptor type 1 (CX3CR1) chemokines to wild-type mice.[26]

In summary, brain-resident immune cells such as microglia and astrocytes are activated to respond to injury. Subsequently, peripheral immune cells are activated and recruited to the brain to assist in the immune response. The constant influx of leukocytes leads to lymphocytopenia. This immunosuppression has an important contribution to increase the risk of post-stroke infections. The extent of these local and peripheral immune responses to a stroke is variable and plays an important role in determining patient outcomes and overall functional recovery in the acute and chronic phases after stroke.[27] Anxiety, depression and emotional disorders associated with stress have significant effects on both mental and physical health. The altered neurohormonal balance in AIS provides a controlled environment for examining the mechanisms underlying these disorders by identifying potential drug targets and treatment strategies within the framework of preclinical models. Therefore, understanding the biological mechanisms of neurobehavioral problems in both pre-ischemic and post-ischemic stroke may allow researchers to develop and test new therapeutic interventions.[28]

Another important research topic for AIS is reperfusion therapy. Reperfusion therapy aims to restore the blood flow of occluded blood vessels. However, successful recanalization is often associated with disruption of the BBB, leading to reperfusion injury. Delay in recanalization increases the risk of severe reperfusion injury, particularly cerebral edema and hemorrhage. With a better understanding of blood biomarkers and the biological mechanisms of stroke, the transient receptor potential melastatin-like subfamily member 4 (TRPM4) has emerged as an important drug target for the treatment of stroke and other autoimmune diseases. The TRPM4-blocking antibodies have been shown to ameliorate reperfusion injury and improve functional outcomes in animal models of early stroke reperfusion.[29]

Analyzing patient datasets and bioinformatic databases with artificial intelligence and machine learning will allow us to develop smart drugs on a molecular basis, to better

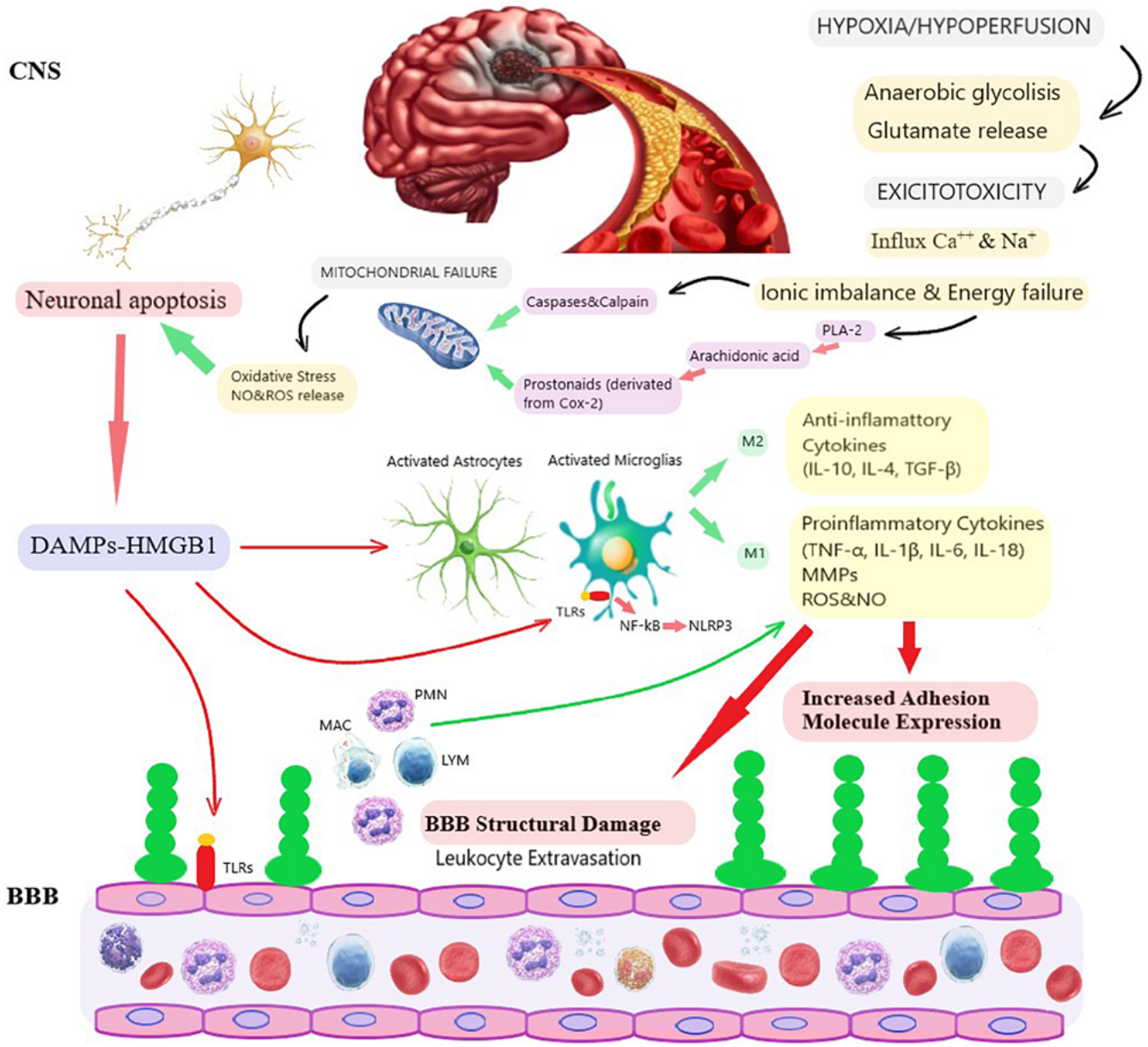

Fig. 1. Due to hypoxia, the deterioration in adenosine triphosphate (ATP) synthesis as a result of anaerobic glycolysis increases the reuptake of glutamate and its extracellular levels. With the overstimulation of glutamate receptors, excitotoxicity begins, and intracellular calcium (Ca^{2+}) influx develops. The increase of Ca^{2+} ions leads to ionic imbalance and energy failure. Thus, it leads to arachidonic acid from phospholipase A-2 (PLA-2) activity and, consequently, to an increase in the level of prostanoids derived from cyclooxygenase-2 (Cox-2). Through the simultaneous caspase and calpain enzymatic system, intracellular free radical generation or reactive oxygen species (ROS) production is increased, resulting in mitochondrial failure. Mitochondrial failure leads to oxidative stress, nitric oxide (NO) synthesis, and ROS release, resulting in cellular damage (neuronal apoptosis). As a result of cell damage, damage-associated molecular patterns (DAMPs) and high-mobility group box 1 (HMGB-1) are released into the environment, activating astrocytes and microglia. The DAMP–HMGB-1s stimulate pattern recognition receptors (PRRs) such as TLRs in the blood–brain barrier (BBB), leading to extravasation of leukocytes (primarily neutrophils, macrophages and lymphocytes). By stimulating M1 microglia again via TLRs, pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β, IL-6, and IL-18 are released from nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) via the nod-like receptor protein-3 (NLRP3) inflammasome, and the synthesis of matrix metalloproteases (MMPs), ROS, and NO is increased. These pro-inflammatory factors lead to structural damage and increase the expression of adhesion molecules such as P-selectin, E-selectin, endothelial leukocyte adhesion molecule (ELAM-1), and intercellular cell adhesion molecules-1 (ICAM-1) on cerebral endothelial cells causing the influx of blood-derived inflammatory cells such as neutrophils, macrophages and lymphocytes to the ischemic area. Extravasated PMLs from the peripheral circulation also trigger the pro-inflammatory process. T regulatory lymphocytes (Treg) and M2-activated microglia secrete anti-inflammatory cytokines such as IL-10, IL-4, and TGF-β

understand the pathogenesis and to discover new treatments for neuropsychiatric disorders. Interdisciplinary, synthesizable research models and their integration with biomechanics will be a milestone for our future treatment strategies.[30]

Stress and associated inflammation have been related to the activation of the metabolic system of tryptophan (Trp)–kynurenine (KYN), which visibly contributes to the development of pathological conditions, including neurological and psychiatric disorders. Mitochondria,

which are multifunctional organelles related to cellular energy, homeostasis, intracellular and intercellular signaling, and gene expression, and which are seen as the source of our life in a way, are structurally impaired in emotional disorders or biomechanical problems such as ischemia.[31] A number of gene polymorphisms have been found to contribute to laboratory aspirin resistance (AR), which is measured by platelet aggregation with arachidonic acid (AA) and adenosine diphosphate (ADP) in ischemic stroke patients. The effect of prostanoids, such as the AA pathway, in the pathogenesis of mitochondrial failure is well known.[32] The Trp–KYN metabolic system is closely linked to glutamate excitotoxicity, and blockade of this system can alleviate neuroinflammation by modifying microglia activation. As a result, there may be a therapeutic target for the treatment of neuroinflammatory conditions such as AIS, migraine and neuropathic pain.[33]

Rehabilitation of motor deficits after AIS is difficult. A series of impulses, such as mental practices and observation tasks, are believed to temporarily activate networks of neurons in the brain, known as mirror neurons and mentalization systems, to support healing. Understanding the effects of blood biomarkers on recovery, especially the change in rehabilitation level, may be the subject of further investigations.[34]

Experimental animal studies have also been conducted on the potential of xenografts, known as human umbilical mesenchymal stem cells (HUMSCs), in reducing inflammation and preventing tissue damage in chronic stroke patients, apart from treatment with rehabilitation or supportive interventions.[35]

The neutrophil-to-lymphocyte ratio (NLR) in whole blood analysis is accepted as a determinant of clinical/subclinical inflammation. In neurological diseases, studies on the prognostic value of NLR have been carried out, especially in diseases such as the spectrum of demyelinating diseases and ischemic stroke.[36,37] However, there is no consensus yet on what are the normal values of NLR, taking into account variables such as age, gender and ethnicity. A higher NLR has been associated with stroke severity and short-term mortality,[38] unfavorable functional outcomes, many post-stroke complications, including risk of intracerebral hemorrhage, and an increased risk of recurrent ischemic stroke.[39–41]

Until now, the studies have shown that NLR is associated with the severity of AIS and short-term functional outcomes; previous assessments of functional outcomes have been limited. In addition, there is no study in which easily measurable MPV values are compared with NLR and their prognostic value is analyzed. Determining the predictive value of these blood biomarkers, their interrelationships and their effects on the prognosis forms the basis of this study. Therefore, we conducted a single-center cross-sectional study to examine the relationship between blood biomarkers and stroke severity and to investigate the prognostic values of NLR and MPV in AIS patients.

Objectives

The neuroinflammatory response has been shown to play an important role in the pathophysiology of ischemic stroke. The NLR has recently been reported as a potential novel biomarker for baseline inflammatory processes. However, data on MPV are insufficient. Appropriate clinical decision-making tools and models are required to take advantage of the predictive value of NLR and MPV, which can be useful in identifying and monitoring high-risk patients to guide early treatment and achieve better outcomes. Our knowledge about the role of NLR and MPV in the immunopathogenesis of AIS and their impact on clinical practice is also insufficient. Biomarkers that may reliably predict post-stroke outcomes are necessary to plan appropriate interventions and treatment modalities. Given the limited availability, temporal constraints and problems associated with technical infrastructure standardization of advanced neuroimaging, simple, routinely collected blood-based biomarkers are of enormous clinical and translational importance. This article aimed to provide a comprehensive overview of the role of NLR and MPV in AIS patients admitted to the intensive care unit (ICU), to compare them with clinical tests such as the modified Rankin Scale (mRS) and the National Institutes of Health Stroke Scale (NIHSS), and to provide perspectives on future research areas in order to better understand the role of these blood biomarkers in the prognosis, treatment options and classification of patients.

Materials and methods

Patients

Patients who were admitted to the Medifema Hospital Level 3 Neurology ICU with the diagnosis of AIS between 2013 and 2020 and were hospitalized within the first 24 h from the onset of symptoms were included in the study. The inclusion criteria were as follows:

1. No active infection or fever at the time of application;
2. No history of major surgery or trauma in the last 3 months;
3. No history of stroke in the last 6 months;
4. No known autoimmune/inflammatory disease or malignancy;
5. Hemorrhage was excluded using computed tomography (CT) or magnetic resonance imaging (MRI);
6. Not under immunosuppressive therapy; and
7. Patients whose data could be accessed from the information file were included. The flowchart of the study is shown in Fig. 2.

Study data

In this retrospective study, sociodemographic and clinical characteristics used for database research were

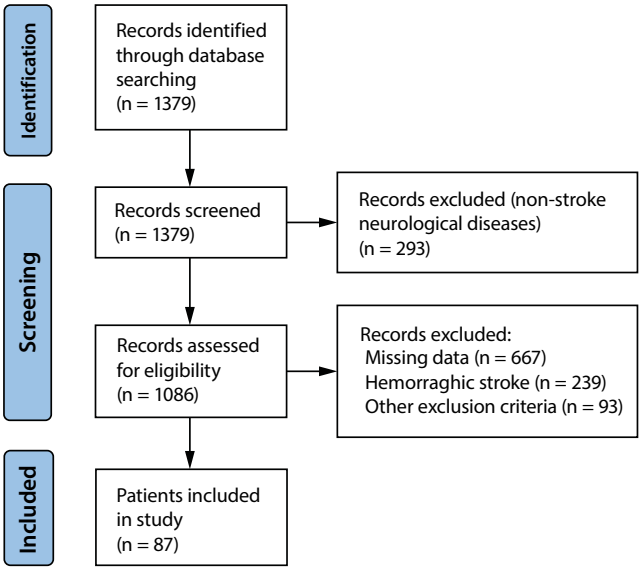

Fig. 2. Flowchart of the study

recorded using a data collection form. Data obtained from all patients at the time of admission and discharged from the ICU (or death of a patient) were taken into account. Demographic characteristics such as age, gender, medication history, medical history, comorbid diseases, and social habits (such as smoking and alcohol use) were recorded. The entrance hemogram and lipid profile of the patients in our hospital as well as the control hemogram values obtained at discharge were recorded as study data. The NLR and MPV values were reported according to hemogram results. Considering these characteristics, a total of 1,379 records were identified in the database search. Due to non-stroke diseases, 293 records were excluded. Additional 667 patients were excluded due to missing data, 239 patients due to hemorrhagic stroke, and 93 patients due to other exclusion criteria. Eighty-seven patients who met the inclusion criteria and were hospitalized in the ICU were included in the study.

Clinical tests

The neurological evaluations of the patients were evaluated with NIHSS, and their stroke-related disabilities were evaluated with mRS. All patients were evaluated by the same neurologist throughout the study. A mRS score of 0–2 was considered a favorable functional result and a score >3 an unfavorable functional result. Patients were categorized according to NIHSS severity in mild (1–4), moderate (5–15), moderate to severe (16–20), and severe stroke (21–42).

Classification of patients

The patients were classified into 3 subcategories according to the Bamford classification: anterior circulation, posterior circulation and lacunar infarcts.[42] Although the results of cerebral brain imaging were not required for classification, they were confirmed radiologically (with CT and MRI).

Statistical analyses

The sample size was calculated using G*Power v. 3.1 (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower). At 80% statistical power and an α-value of 0.05 (significance level), the smallest sample size required to determine a 0.25 Cohen's f effect size was calculated as a total of 85 participants with one-way analysis of variance (ANOVA).[43] We included 87 patients in the final sample. Alpha indicates a type 1 error, in which probability of finding a significant effect when there is no significant effect. A smaller level of significance increases the sample size. The study data were analyzed using IBM SPSS (Windows, v. 26.0) software (IBM Corp., Armonk, USA). Descriptive statistics (mean (M), standard deviation (SD), median) were used for continuous variables, and frequency and percentage values were used for categorical variables. It is more appropriate to use the median without the M when the data distribution is not normal and to use the M with SD when the data distribution is normal. Usually, normally distributed data are expressed as M ±SD. The receiver operating characteristic (ROC) curve analysis was used to determine the cutoff point for NLR and MPV variables, based on the diagnosis in statistical analyses and crosstab analyses of variables. With this analysis, continuous NLR and MPV variables were made categorically using statistical criteria such as sensitivity and specificity, and then used in relationship tests with other categorical variables. The χ^2 and Fisher's exact tests were used to find the statistical relationship between 2 categorical variables. In the R×C tables for the χ^2 test statistic, the expected frequencies should not be less than 1. The expected frequencies in 2×2 tables should be greater than 5. Otherwise, Fisher's exact test should be used. Another use of Fisher's exact test is when more than 20% of the expected frequencies are less than 5 in 2×2 tables. In this study, demographic, clinical and laboratory findings based on the diagnosis were obtained with the χ^2 relationship test. In addition, Fisher's exact and χ^2 tests were used to evaluate the relationship between risk factors of mortality and demographic characteristics. Under the assumption of normal distribution, the t-test is used to compare the means of 2 independent groups. If the assumption cannot be met, nonparametric tests are used to compare medians. In this study, the Mann–Whitney U test (M–W) was used to compare the medians of 2 independent groups. The odds ratio (OR) is a statistic that shows the strength of the relationship between 2 categorical variables. The OR value can range from 0 to infinity. If the OR is less than 1, this variable is interpreted as a protective factor, if it is greater than 1, this variable is a risk factor, and if it is equal to 1, it is interpreted as having no effect. In this

study, OR statistics were used to show the strength of the association of NLR and MPV risk factors on mRS variables. A p-value <0.05 was considered statistically significant.

Ethics

This study was conducted in accordance with the World Medical Association Declaration of Helsinki. The study was approved by the İzmir Bakırçay University Non-Interventional Transactions Ethics Committee (approval No. 860/840, January 25, 2023).

Results

In this study, the data of 87 patients who met the criteria and were hospitalized in the ICU were examined. Thirty (34.5%) female and 57 (65.5%) male patients were included, with a mean age of 77.59 ±11.82 (23–96). Eleven (12.6%) patients died during follow-up. The stroke type was anterior circulation in 54 (62.1%) patients, posterior circulation in 21 (24.1%) and lacunar stroke in 12 (13.8%). Clinical, demographic and medical history characteristics are summarized in Table 1. While mRS was evaluated in 2 classes as favorable (0–2) and unfavorable (3–6) functional outcomes, stroke severity was also evaluated using the NIHSS. The normality test of the continuous variables (Anderson–Darling) was performed within the scope of the study. According to this test, while low density lipoprotein (LDL) and high density lipoprotein (HDL) variables were normally distributed ($p > 0.05$), other demographic, clinical and laboratory findings were non-normally distributed ($p < 0.05$). Appropriate descriptive statistics (median or M ±SD) for these variables are summarized in Table 2. The NLR and MPV cutoff values for unfavorable functional outcomes (mRS: 3 and above) were determined separately with a ROC curve. The area under the ROC curve (AUC) provides an aggregated measure of performance across all possible classification thresholds. The AUC value ranges from 0 to 1. A model with 100% incorrect predictions has an AUC of 0, and a model with 100% correct predictions has an AUC of 1. The AUC for NLR was determined to be 94.7%. In addition, when the ROC curve was examined, it was decided that the optimal cutoff value was approx. 4, as the point where the highest sensitivity and specificity values were reached was between 3.33 and 4.37 on the ROC curve. For the value of 3.33, the sensitivity is 95% and the specificity is 89%, while for the value of 4.37, the sensitivity is 83% and the specificity is 99% (Fig. 3). With the selected value of 4, the sensitivity value will be between 83% and 95% and the specificity value will be between 89% and 99%. It is categorized as "0" for values below the cutoff point and "1" for values above the cut-off point for NLR to be used in crosstabs for statistical analysis. According to the results of the χ^2 test for the relationship between diagnosis and NLR, the relationship between these 2 variables was determined at a 5% significance level ($p < 0.05$). It was also observed that a NLR value above 4 was higher in those diagnosed with anterior ischemia (96.3%) and posterior ischemia (85.7%, Table 3).

In the ROC curve analysis performed for the cutoff value for MPV, the AUC was determined to be 84.9%. In addition, when the ROC curve was examined, it was decided that the optimal cutoff value was approx. 9, since the point where the highest sensitivity and specificity values were reached was between 8.80 and 9.05 on the ROC curve. For

Table 1. Frequency and percentage of patients according to clinical, demographic and medical history characteristics

Variable		Frequency (f)	Percentage (%)
Gender	female	30	34.5
	male	57	65.5
Comorbidity	stroke	25	28.7
	diabetes mellitus	44	50.6
	cardiovascular diseases	29	33.3
	hypertension	70	80.5
	hyperlipidemia	46	52.9
	chronic renal failure	12	13.8
	obesity	10	11.5
Bamford classification	anterior infarct	54	62.1
	posterior infarct	21	24.1
	lacunar infarct	12	13.8
mRS	2	9	10.3
	3	31	35.6
	4	25	28.7
	5	11	12.6
	6	11	12.6
Habitation	smoking (active smoker)	12	13.8
	smoking (ex-smoker)	54	62.1
	never smoked	21	24.1
	alcohol (actively consuming)	6	6.69
	alcohol (consumed in the past)	31	35.6
	never consume	50	57.5
Medication	warfarin	4	4.6
	new oral anticoagulant	21	24.1
	antiaggregant	42	48.3
	antihypertensive	59	67.8
	statin	32	36.8
	insulin/oral anti-diabetic	19	21.8
Carotid stenosis	<%50	35	40.2
	≥%50	52	59.8
Posterior circulation disorder		18	20.7
Exitus		11	12.6

mRS – modified Rankin Scale.

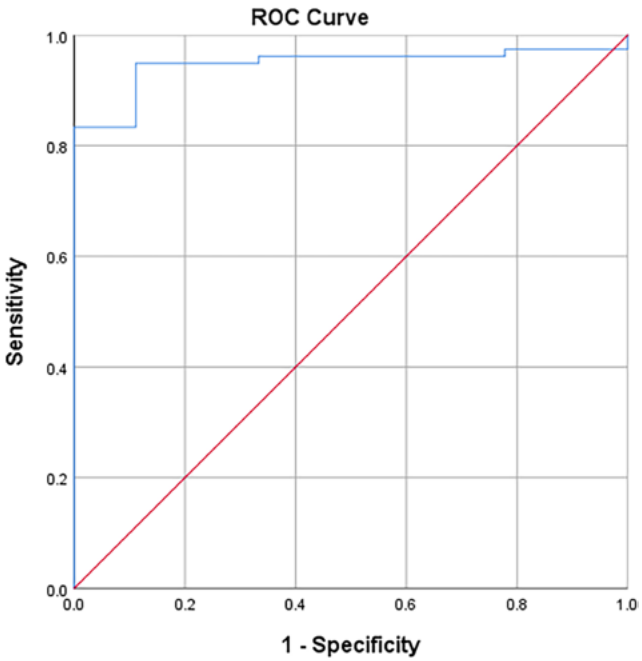

Fig. 3. Receiver operating characteristic (ROC) curve analysis for the neutrophil-to-lymphocyte ratio (NLR) to predict unfavorable functional outcomes (mRS ≥ 3) in acute ischemic stroke (AIS) patients. The area under the ROC curve (AUC) for NLR is 94.7. The ROC curve analysis in this study showed the optimal cutoff of 3.33 with a 95% sensitivity and 89% specificity, and the optimal cutoff 4.37 with a 83% sensitivity and 99% specificity

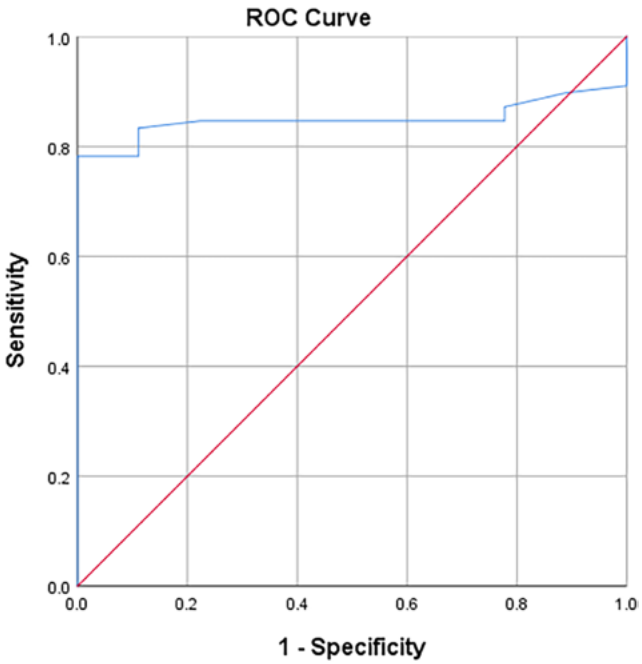

Fig. 4. Receiver operating characteristic (ROC) curve analysis for mean platelet volume (MPV) to predict unfavorable functional outcomes (mRS ≥ 3) in acute ischemic stroke (AIS) patients. The area under the ROC curve (AUC) for MPV is 84.9%. The ROC curve analysis in this study showed the optimal cutoff of 8.80 with a 83% sensitivity and 89% specificity and the optimal cutoff of 9.05 with a 78% sensitivity and 99% specificity

Table 2. Descriptive statistics of patients according to demographic, clinical and laboratory findings

Variable	Anderson–Darling p-value	Median or M ±SD	Min–max
Age	0.005*	78.5	23.00–96.00
NLR	0.048*	7.3	1.36–14.60
MPV	0.005*	11.6	5.90–17.90
LDL	0.877	145.28 ±29.55	75.00–224.00
Triglicerides	0.005*	162.5	95.00–480.00
HDL	0.543	39.40 ±8.99	16.00–63.00
NIHSS_Admission	0.005*	14.5	7.00–36.00
NIHSS_Exit	0.005*	8.5	3.00–40.00

* $p < 0.05$ (a statistically significant value, non-normal distribution); HDL – high density lipoprotein; LDL – low density lipoprotein; MPV – mean platelet volume; NIHSS – National Institute of Health Sciences Score; NLR – neutrophil-to-lymphocyte ratio; M ±SD – mean ± standard deviation.

the value of 8.80, the sensitivity is 83% and the specificity is 89%, while for the value of 9.05, the sensitivity is 78% and the specificity is 99% (Fig. 4). With the selected value of 9, the sensitivity value will be between 78% and 83%, and the specificity value will be between 89% and 99%. It is categorized as "0" for values below the cutoff point and "1" for values above the cutoff point for MPV to be used in crosstabs for statistical analysis. According to the results of the χ^2 test for the relationship between diagnosis and MPV, MPV value has a strong relationship with the diagnosis at a 5% significance level ($p < 0.05$). It was also observed that a MPV value above 9 was higher in those diagnosed with anterior ischemia (90.7%) and posterior ischemia (71.4%, Table 3). In addition, the frequency table and χ^2 relationship test results between diagnosis and other variables such as gender, mRS, comorbidity, habits, drugs, rate of carotid and vertebral artery stenosis, and exitus are given in Table 3.

In the mRS data, it was determined that 78 patients (89.7%) had a severe stroke (mRS ≥ 3) compared to unfavorable functional outcomes, and this rate was also very high. At the same time, since the NLR and MPV variables were not normally distributed, the M–W test was used to assess the statistical difference between the medians of these variables in the mRS classes. Considering the small number of observations in the mRS class (mRS < 3), it was decided that the distributions of these variables were approximately similar based on the histograms and central tendency measures (mode < median = M). According to this approach, a statistically significant difference was found between the NLR and MPV medians of the mRS groups with a 5% significance level ($p = 0.000$ and $p = 0.001$, respectively). Based on these data, a statistically significant association was established between high medians of NLR and MPV and unfavorable functional outcomes (Table 4).

Table 3. The χ^2 relationship test results between diagnosis and demographic, clinical and laboratory findings

Diagnosis frequency									
Demographic, clinical and laboratory findings		anterior infarct		posterior infarct		lacunar infarct		χ^2	p-value
		f	(%)	f	(%)	f	(%)		
Gender	female	21	38.9	6	28.6	3	34.5	1.266	0.531
	male	33	61.1	15	71.4	9	75.0		
NLR	0	2	3.7	3	14.3	7	58.3	24.648	<0.001
	1	52	96.3	18	85.7	5	41.7		
MPV	0	5	9.3	6	28.6	10	83.3	29.717	<0.001
	1	49	90.7	15	71.4	2	16.7		
MRS	3+	52	96.3	20	95.2	6	50.0	23.620	<0.001
	1–2	2	3.7	1	4.8	6	50.0		
Stroke	absence	30	55.6	20	95.2	12	100	17.240	<0.001
	presence	24	44.4	1	4.8	0	0		
Hypertension	absence	4	7.4	11	52.4	2	16.7	19.525	<0.001
	presence	50	92.6	10	47.6	10	83.3		
Diabetes mellitus	absence	24	44.4	8	38.1	11	91.7	10.180	0.006*
	presence	30	55.6	13	61.9	1	8.3		
Hyperlipidemia	absence	15	27.8	17	81.0	9	75.0	21.498	<0.001
	presence	39	72.2	4	19.0	3	25.0		
Obesity	absence	46	85.2	19	90.5	12	100	2.224	0.329
	presence	8	14.8	2	9.5	0	0		
CRF	absence	43	79.6	20	95.2	12	100	5.325	0.070
	presence	11	20.4	1	4.8	0	0		
Cardiovascular disease	absence	31	57.4	16	76.2	11	91.7	6.315	0.043*
	presence	23	42.6	5	23.8	1	8.3		
Carotid stenosis	absence	32	59.3	3	14.3	0	0	22.088	<0.001
	presence	22	40.7	18	85.7	12	100		
Vertebral artery stenosis	absence	37	68.5	20	95.2	12	100	10.210	0.006*
	presence	17	31.5	1	4.8	0	0		
Exitus	no	43	79.6	21	100	12	100	7.695	0.021*
	yes	11	20.4	0	0	0	0		
Smoking	active smoker	2	3.7	2	9.5	8	66.7	35.225	<0.001
	ex-smoker	36	66.7	16	76.2	2	16.7		
	never smoked	16	29.6	3	14.3	2	16.7		
Insulin	absence	40	74.1	16	76.2	12	100	3.929	0.140
	presence	14	25.9	5	23.8	0	0		
Antiaggregant	absence	24	44.4	14	66.7	7	58.3	3.234	0.199
	presence	30	55.6	7	33.3	5	41.7		
Antihypertensive	absence	11	20.4	14	66.7	3	25.0	15.177	<0.001
	presence	43	79.6	7	33.3	9	75.0		
Statin	absence	26	48.1	19	90.5	10	83.3	14.072	<0.001
	presence	28	51.9	2	9.5	2	16.7		
NOAC	absence	37	68.5	17	81.0	12	100	5.706	0.058
	presence	17	31.5	4	19.0	0	0		

* $p < 0.05$ (a statistically significant value, there is a relationship between variables); CRF – chronic renal failure; MPV – mean platelet volume; NLR – neutrophil-to-lymphocyte ratio; NOAC – new oral anticoagulant; f – frequency.

Table 4. Mann–Whitney U test results for the neutrophil-to-lymphocyte ratio (NLR) and mean platelet volume (MPV) variables according to the modified Rankin Scale (mRS) for a cutoff point of 3

Variable	mRS ≥ 3 (f = 78)		mRS < 3 (f = 9)		M–W	p-value
	median	mean rank	median	mean rank		
NLR	7.45	48.03	2.77	9.11	37.0	<0.001*
MPV	12.30	47.14	8.35	16.78	106.0	<0.001*

* p < 0.05 (a statistically significant value, different medians); mRS ≥ 3 (an unfavorable functional outcome); mRS < 3 (a favorable functional outcome); M–W – Mann–Whitney U test.

Table 5. Fisher's exact test results for the relationship between unfavorable functional outcome and neutrophil-to-lymphocyte ratio (NLR) and mean platelet volume (MPV)

Variable		mRS ≥ 3	mRS < 3	OR (95% CI)	p-value
NLR	0	4 (5.1)	8 (88.9)	reference	
	1 (≥4)	74 (94.9)	1 (11.1)	148 (14.70–1490.31)	<0.001*
MPV	0	13 (16.7)	8 (88.9)	reference	
	1 (≥9)	65 (83.3)	1 (11.1)	40 (4.60–347.70)	<0.001*

* p < 0.05 (a statistically significant value, different medians); mRS ≥ 3 (an unfavorable functional outcome); mRS < 3 (a favorable functional outcome); mRS – modified Rankin Scale; OR – odds ratio; 95% CI – 95% confidence interval.

Table 6. Mann–Whitney U test results of neutrophil-to-lymphocyte ratio (NLR) and mean platelet volume (MPV) variables according to the modified Rankin Scale (mRS) for a cutoff point of 6

Variable	mRS ≥ 6 (f = 11)		mRS < 6 (f = 76)		MW	p-value
	median	mean rank	median	mean rank		
NLR	9.6	74.77	6.45	39.55	79.5	<0.001*
MPV	15.35	73.91	11.20	39.67	89.0	<0.001*

* p < 0.05 (a statistically significant value, different medians); M–W – Mann–Whitney U test.

The categorical values for NLR and MPV were cross-tabulated. The categorical values separated as mRS ≥ 3 (an unfavorable functional outcome) and mRS < 3 (a favorable functional outcome) were evaluated according to the cut-points of NLR and MPV, and a statistically significant relationship was found with a 5% significance level according to the Fisher's exact test (p = 0.000). According to this result, it can be determined that there is a significant relationship between unfavorable functional outcomes and high NLR and MPV values. The categorical relationship between unfavorable functional outcomes and cut-points of NLR and MPV values is summarized in Table 5.

Considering the small number of observations in the mRS class (mRS = 6), it was decided by looking at the histograms and central tendency measures that the distributions of these variables were approximately similar (mode < median = M). According to this approach, when the relationship between the median NLR and MPV values of patients who died (mRS = 6) and did not die (mRS < 6) during intensive care follow-up was examined, a statistically significant difference was found between the NLR medians of the mRS and the MPV medians with 95% confidence intervals (95% CIs) compared to the M–W test (p < 0.000 and p = 0.001, respectively). Based on these data, a relationship between stroke-related mortality and high NLR and MPV values can be confirmed (Table 6).

To summarize the results, both NLR and MPV variables were separately associated with unfavorable functional outcomes and mortality. In addition, the severity of stroke and clinical worsening increased as the levels of NLR and MPV increased.

Discussion

The ischemic stroke injury is caused by a complex neuroinflammatory process. Detailed analysis and correct understanding of this process are very important for the formation of current and reliable data on the natural history, severity, progression, and, ultimately, the treatment of the disease. In addition to determining the prognosis of the disease, the detection of easy and accessible blood biomarkers will enable the development of future-focused therapeutic strategies.

In this study, we particularly focused on the early and relatively easily detectable responses of this neuroinflammation. In particular, extravasation of neutrophils by the stimulation of PRRs by DAMPs, which occurs with BBB disruption and glial damage, was evaluated as an early neuroinflammatory response. However, since confirming this situation with another blood biomarker will provide a better understanding of the process, it was planned to evaluate the increased platelet volume in the circulation

simultaneously with the increase in neutrophils, to understand their effects on the prognosis, and as a result, to develop future-focused treatment strategies for this cascade.

Ischemic stroke is a life-threatening disease with high mortality and morbidity rates. There are several studies on the role of neuroinflammation in ischemic stroke.[44] Early determination of stroke severity is critical for disease management, follow-up and prognostic evaluation of patients. Studies have shown that parameters such as NLR, MPV, neutrophil, leukocyte, and platelet counts are significantly associated with survival.[45] The NLR and MPV have been reported as novel biomarkers, especially showing the initial inflammatory response, and are considered to be potential predictors of prognosis in patients with ischemic stroke.[39] The NLR is an easy, available, inexpensive, and practical indicator that can be calculated using complete blood count data. Studies have been conducted to show that the NLR can be used as a predictive marker not only for ischemic stroke but also for CAD,[46] colorectal cancer[47] and multiple sclerosis.[48]

In the pathogenesis of ischemic stroke, the first response to damaged tissue is the migration of neutrophils to the site. In this inflammatory reaction, which results in liquefactive necrosis, the released cytokines, eicosanoids and adhesion molecules regulate the migration of leukocytes. As a result of neutrophil activity in this region, it is thought that several proteolytic enzymes, such as acid phosphatase, cause damage not only in the core area of the infarct but also in the penumbra. Therefore, it is assumed that there is a correlation between neutrophil density and the severity of the injury. Although there are opinions to the contrary, several cytokines and growth factors secreted by T lymphocytes are suggested to be involved in the repair of inflamed tissue by modulating microglial activation.[49] Therefore, an increase in the NLR rate can be linked to a poor prognosis. It has been shown that the NLR hemogram values taken within the first 24 h in AIS patients with a mRS ≥ 3 or NIHSS ≥ 15, which are considered unfavorable functional outcomes, are in line with the literature. The NLR has also been shown to be an independent risk factor for mortality, similar to unfavorable functional outcomes. It was thought that both the decrease in neutrophils and the increase in lymphocytes were effective in causing the relative decrease in the NLR values obtained from the hemograms of patients during the first week.

While some studies report the NLR cutoff values for unfavorable functional outcomes in AIS as 4.0,[50] 3.3[40] and 3.51,[51] some studies propose cutoff values such as 4.1 for the estimation of mortality in stroke patients.[52]

In our study, the NLR and MPV cutoff values for unfavorable functional outcomes were found to be 4.0 and 9.0, respectively, with the ROC curve. The MPV is an accurate measurement of volume-based platelet sizes calculated using hematology analyzers during routine hemogram testing. Significantly developed or abnormal thrombocytopoiesis, the result of activating factors on platelets, can lead to changes in the ratios between MPV and platelet count (PLT). Therefore, possible applications of these parameters in the diagnosis of certain diseases have been proposed. In addition, MPV is associated with platelet activation and is therefore considered a marker of platelet activity.[53] High MPV values are observed in patients with acute cerebrovascular ischemia, as well as in some inflammatory diseases such as ischemic heart disease,[54] some respiratory diseases and rheumatoid arthritis.[55] Individuals with high MPV values are at greater risk of acute stroke than those with normal MPV values. Studies have shown a decrease in platelet values along with an increase in MPV values.[56] Although no significant correlation was observed between 6-month survival and the evaluation of prognosis, higher mortality rates were reported in patients with high MPV values. Therefore, the use of MPV as a prognostic marker has been suggested.[57] In our study, it was observed that MPV values were high in patients with unfavorable functional outcomes (mRS ≥ 3). This risk was found to be more pronounced in patients with an MPV value ≥9. It was found that high MPV was associated with unfavorable functional outcomes and mortality.

In our study, a statistically significant relationship was found between the diagnosis (anterior, posterior and lacunar) and risk factors such as stroke, hypertension, diabetes mellitus, hyperlipidemia, chronic renal failure (CRF), carotid stenosis, vertebral artery stenosis, exitus, smoking, antihypertensive therapy, and statin therapy. In addition, a statistical relationship was found between mRS and NLR and between mRS and MPV variables.

In addition to the use of existing stroke risk factors, NLR and MPV have the potential to be used as an easy, accessible and low-cost prognostic scale. While clinical assessment scales such as the NIHSS and mRS are of great prognostic importance, simple laboratory markers can guide the course and severity of the disease. In ICUs, where prognostic assessment is of greater importance, there may be some confusing factors that determine the prognosis of the disease, including nosocomial infections, electrolyte disorders and organ failures.[58] However, when these conditions are excluded, independent clinical laboratory tests that can be clinically correlated with stroke severity and an unfavorable functional outcome can guide clinicians in establishing a global decision-making framework.

Limitations

The fact that this study was conducted in stroke patients admitted to the ICU increased the likelihood of certain infections that could affect neutrophil and lymphocyte counts. However, other clinical and laboratory parameters related to infection were strictly controlled for in all patients, and these processes were excluded. However, it should be kept in mind that the length of stay in the ICU may be prolonged, and therefore the risk of infections may increase. The limitations of our study include its

retrospective nature, very strict exclusion criteria for the reliability of the file data, the selection of participants from only one institution, and the relatively small sample size. With larger sample sizes, prospective follow-up studies and multicenter measurements, possible estimations can be made more powerful.

Conclusions

The results demonstrate the importance of the inflammatory response in the pathophysiology of both mortality and stroke severity. Increasing evidence has shown that the inflammatory response can provoke cell death following ischemic cerebral injury, as well as play a beneficial role by serving a complex function in the pathophysiologic process. As the molecular-based pathogenesis of ischemic stroke is understood, our future-focused treatment strategies will be updated. Therefore, it is necessary to investigate the experimental drugs that support the anti-inflammatory process and suppress the pro-inflammatory response.

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