

DISSERTATION FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

HEMATOLOGIC INDICES IN ACUTE ISCHEMIC STROKE

By Dr. Farzaneh Sadeghi

Supervisor:

Dr. Amir-Houshang Shemirani, PhD



UNIVERSITY OF DEBRECEN

Kálmán Laki Doctoral School

Debrecen, 2024

HEMATOLOGIC INDICES IN ACUTE ISCHEMIC STROKE

By Dr. Farzaneh Sadeghi

Supervisor: Dr. Shemirani Amir Houshang, PhD

Doctoral School of Kálmán Laki, University of Debrecen

Head of the Defense Committee: Prof. Dr. József Balla, PhD, DSc, MHAS

Reviewers: Prof. István Takács, PhD

Dr. Miklós Fagyas, PhD

Members of the Defense Committee: Prof. Dr. Barna Vásárhelyi, PhD, DSc

Dr. Kitti Bernadett Kovács, PhD

The PhD Defense takes place at the Lecture Hall of Bldg. A Department of Internal Medicine, Faculty of Medicine, University of Debrecen

13:00, 8th October 2024

1. Introduction

Stroke is one of the predominant causes of mortality and morbidity in the world, with incidence rates that vary by region. Platelets have been identified as crucial participants in the pathophysiology of atherothrombosis and ischemic stroke, marker such as, Mean Platelet Volume (MPV) can assess platelet turnover. Elevated MPV, which is generally associated with more immature and bigger platelets, is associated with an amplified risk of thrombosis.

Meanwhile, despite significant advances in treatment, acute ischemic stroke (AIS) still poses a substantial burden, with recombinant tissue plasminogen activator (rt-PA) being a frequently used therapeutic method. Personalizing AIS care is critical because thrombolysis does not assist all patients and others may experience severe effects, including intracerebral hemorrhage. The Neutrophil-Lymphocyte (NLR) and Lymphocyte-Monocyte Ratio (LMR), which are generated from standard complete blood count tests, have emerged as possible prognostic markers for AIS outcomes, providing quick and easy insights.

Given the diversity in findings across prior research, this study intends to shed light on the association between MPV, Platelet Count (PC), and atherosclerosis. In addition, we look into the possible relationship between NLR and LMR and prognosis of patients with AIS after thrombolysis.

2. Background of ischemic stroke

Stroke can be divided into two main general types: ischemic stroke and hemorrhagic stroke, which latter group comprises subarachnoid hemorrhage and intracerebral hemorrhage. It is a conspicuous cause of mortality and disability worldwide. According to American Heart association Ischemic stroke (IS), account for 87% of all stroke subtypes globally. Ischemic stroke is characterized by infarction of the central nervous system: brain, spinal cord, or retina. Because of advancements in technology, specifically in radiological imaging, the description of ischemic stroke has drifted from a predominantly clinical assessment to a

classification based on tissue characteristics. Many cases with transient neurological symptoms with full clinical recovery in the presence of parenchymal damage on brain MRI are classified as stroke. When blood flow is temporarily interrupted, a transient ischemic attack (TIA) occurs, where both blood flow as well as appeared symptoms get resolved before causing any permanent damage.

The pathogenesis of TIA is indistinguishable to that of ischemic stroke, as well as the inquiries into the root cause and strategies for secondary prevention.

Very early studies in 1868 and another pivotal research conducted in the 1970s unveiled a groundbreaking revelation: a substantial portion of the initial clinical impairments observed in stroke patients can be attributed to insufficient blood perfusion in the region surrounding the ischemic core known as the ischemic penumbra, which lacks electrical activity. If perfusion is not resorted, this penumbral area undergoes a transformation into irreparably damaged tissue, just like the ischemic core. The pace of this progression, however, exhibits significant variability among individuals.

This penumbral brain area, however, has the potential for rescue and the restoration of its normal function through swift reperfusion. Ever since the publication of the inaugural successful trial of thrombolysis in stroke patients in 1995, this pivotal revelation has underpinned the development of treatments. Consequently, there have been a revolution in the outcomes for individuals afflicted by stroke.

Patients with ischemic stroke are presently best managed geologically in the specific stroke division. These centers are facilitated by an experienced interdisciplinary health care team and clinicians follow the best-evidence stroke guidelines. Thrombolytics given intravenously can reduce disabilities when treatment is administered within time frame of 270 minutes from beginning of stroke. In the certain cases, a favorable brain perfusion CT/MRI examination

might enable intravenous thrombolysis (IVT) up to 540 minutes from stroke onset, including wake-up stroke cases.

Mechanical clot retrieval via catheter angiography, also known as Endovascular thrombectomy, results in a more than twofold decrease in disabilities for a wide range of patients with large blood vessel occlusion. This holds true if the procedure is conducted within 360 minutes from the beginning of onset of symptoms, which even could be extend up to 24 hours post-stroke for patients selected using brain perfusion imaging. Nevertheless, it is essential to acknowledge that both intravenous thrombolysis and endovascular thrombectomy share a common constraint, which is time. Addressing this time-related challenge is of paramount importance in order to optimize the advantages of these therapies within healthcare systems and expedite treatment.

2.1. Epidemiology of ischemic stroke

Across the globe roughly 14 million people are affected by stroke each year. It is the secondary leading contributor to mortality, accounting for approximately five million deaths per year. According to estimations 25% of adult population will experience episode of stroke in their lifetime, more over there are more than 80 million stroke survivors worldwide.

Secondary preventive measures are centered on high-risk demographic of stroke survivors. Ischemic stroke prevalence and incidence have changed throughout time. There were 9.5 million ischemic stroke incidents worldwide in 2016. 2.7 million People died in 2017 as a result of ischemic stroke. Over the 1990–2013 time-frame, ischemic stroke's prevalence, mortality, and disability-adjusted life years all dropped globally. In years 1990 to 2005 there was an increase, then fell from 2005 to 2013, and finally there was a small statistically not significant rise in the worldwide stroke incidence from 1990 to 2013.

A more than twofold decrease in fatalities due to stroke can be attributable to remarkably improved preventive measures. It is interesting to point out that between years 1990 and

2010, the epidemiology of ischemic stroke altered depending on the economy of a nation. For instance, nations with higher income had declines in prevalence of death rate, disabilities and lower mortality-to-incidence ratio. On the other hand, in case of low- and middle-income nations there was no appreciable variations in this period of time. These discrepancies may result from variations in the population's age distribution, life expectancy, health state, and standards of healthcare delivery.

4.2. Risk factors of ischemic stroke

Age, gender, and inherited factors are amongst the ischemic stroke risk factors that cannot be changed. The consequence of advancing age as a risk factor for ischemic stroke varies, depending on a country's level of advances. In developed countries incidence of stroke had a greater rise after age 49 whereas in developing countries the prevalence of stroke was seen after age 39. The prevalence of ischemic stroke has increased approximately by 37% in related disability-adjusted life years in those aged 20 to 64 worldwide between 1990 and 2013. According to a survey from 2013, men had a greater incidence of ischemic stroke than women.

Most incidences of ischemic stroke are sporadic, despite the fact that some monogenic causes have been found. Using genome-wide complex trait analysis, the inheritance of ischemic stroke is predicted to be 37.9%. There are several modifiable ischemic stroke risk factors. The INTERSTROKE study found out that globally 10 variables were constantly concomitant with ischemic stroke across geographic provinces, gender, and age categories. These variables are responsible for approximately ninety-one percent of the population risk attribution for ischemic stroke. History of hypertension (HT), inconsistent physical exercises, an elevated apolipoprotein B -to- apolipoprotein A1 ratio, unhealthy diet, an excessive waist/hip ratio, psychological stress, smoking, cardiac conditions (atrial fibrillation and prior myocardial

infarction), excessive consumption of alcohol, and diabetes mellitus (DM) were among the risk factors.

Hypertension was associated with the highest risk (OR 3.14, 99% CI 2.67-3.71) and a population-attributable risk of 45.2% (99% CI 40.3-50.0%). Sleep apnea, none acute inflammation, gum disease, and chronic renal illness are all potential risk factors. Furthermore, in a few studies exposure to air pollution have found links between temporary increases in stroke prevalence.

4.3. Mechanism/patho-physiology of ischemic stroke

The vast majority of ischemic strokes are due to formation of thromboembolism, either by formation of atherosclerosis in the large arteries or/and cardiac conditions. Regarding the cardiac source of embolism, atrial fibrillation chiefly serves as its usual source. Disease in the small arteries (more common in Asia) which is connected to HT and DM, Vasculitis, dissection of arteries, paradoxical embolism through patent foramen ovale (PFO), and hematological diseases are considered as another causes of ischemic stroke. The identification of the origin of an ischemic stroke is of great importance due to the fact that it could direct physician to different therapeutic approaches in order to avoid further strokes.

5. Atherosclerosis as an arterial cause of stroke

In the presence of atherosclerosis within the cerebral vasculature, there is a possible scenario where the lipid core within atherosclerotic plaques might be exposed to the circulation, thereby creating a predisposition for the development of thrombi. This exposure is frequently incited by inflammatory mechanisms and the resultant in formation of ulcer within the enveloping fibrous cap. Vessels which are narrowed by atherosclerotic plaques, might be occluded by thrombi or embolize distally in the main vessels associated with stroke. The internal carotid artery (ICA) is where atherosclerotic plaques are more frequently found in western populations.

Internal lining of vessels known as intima is thought that, thickens and reduced nitric oxide release. This is linked to low shear stress and causes vulnerability to cholesterol plaque development. Despite the fact that cerebral atherosclerosis is infrequently seen in patients in western countries, it is still far more frequent in Asia and is typically found in heavy smokers and people with DM. In fact, compared to white patients who have 5-10% of strokes, Asian patients experience 30-50% of ischemic strokes due to cerebral atherosclerosis. Atherosclerosis makes routine thrombectomy difficult because it is linked to higher re-occlusion rates following thrombectomy. It also necessitates more stenting with a higher risk of complications, especially hemorrhage linked to the use of medications such as antiplatelet therapies to maintain stent patency.

6. Cardiac causes of stroke

6.1. Atrial fibrillation

Blood can become stagnant during fast electrical discharge patterns of atrial fibrillation or flutter especially in the left atrial appendage which increases the chances of thrombosis. Cardioembolic ischemic stroke risk is increased by both persistent and intermittent atrial fibrillation. Atrial fibrillation is becoming more common in older and obese population. Chronic HT, ischemic cardiac disease, valvular disease, DM, hyperthyroidism, binge drinking, and obstructive sleep apnea are additional risk factors for atrial fibrillation. The CHA₂DS₂-VASc score, which considers congestive heart failure (CHF) history, age, stroke/TIA history, gender, DM, HT, and vascular disease ((VD), including prior MI, PAD or aortic plaque)) can be utilized for determination of stroke risk for patients with arterial fibrillation requiring anticoagulation. This score system can serve as a means to assess the likelihood of ischemic stroke in patients diagnosed with atrial fibrillation. Patients with score of 2 or higher are recommended to take oral anticoagulant.

6.2. Patent foramen ovale

Foramen ovale in the heart during pregnancy permits oxygenated placental blood to bypass pulmonary circulation. There is an increase in left atrial pressure after birth, which in turn results in the closure of the foramen ovale. However, in 25% of the population, foramen ovale exists as a residual patency (a PFO). Paradoxical embolism and ischemic stroke may result because of this patency. There was a considerable drop in the risk of subsequent ischemic stroke with endovascular PFO closure which has attracted attention to its implication in young stroke patient.

6.3. Hematological Causes

Ischemic stroke has a few important but uncommon causes, including hematological diseases. Three of the more prevalent hematological diseases that enhance the risk of thrombus formation are essential thrombocytosis, polycythemia vera, and antiphospholipid syndrome. The presenting symptom of these illnesses can be a stroke. Moreover, sickle cell anemia can result in stroke which is a significant factor in pediatric stroke in people of African heritage.

7. Diagnosis, screening and prevention of ischemic stroke

7.1. Non-contrasted Computed Tomography

Sensitivity of native computed Tomography (CT) examination of the brain is near-perfect for detecting extra parenchymal and parenchymal hemorrhage. Traditionally in a patient with signs of AIS, thrombolysis treatment decisions have been based on this method of radiological examination, by which hemorrhage is excluded. Positive diagnosis of stroke in some individuals can be achieved based on early ischemic changes such as indistinction between white and grey matter, hypoattenuation of deep nuclei, and cortical hypodensity with parenchymal swelling. However, these symptoms may go undetected in the initial hours following the onset of a stroke due to the difficulties in distinguishing between the loss of grey matter and white matter.

7.2. CT angiography and CT perfusion

CT perfusion enables the assessment of static acquisition and a time-resolved series of cerebral vasculature following the infusion of an iodinated contrast agent. If arterial atherosclerosis or artery dissection is identified, CT angiography can diagnose ischemic stroke, providing valuable insights into the stroke's pathogenesis due to its accuracy in detecting arterial stenosis and occlusion. All patients diagnosed with ischemic stroke should routinely have CT angiography from arch of aorta to the cerebral vertex in order to determine suitability for endovascular thrombectomy. The degree of collateral flow can also be evaluated by CT angiography which adds more prognostic data about the expected severity of tissue damage. Functional scan of brain is characterized by CT perfusion. CT perfusion is more frequently implemented as a diagnostic tool to identify the ischemic penumbra due to the availability of many automatic software methods.

7.3. Magnetic Resonance Imaging

Perfusion and diffusion Magnetic Resonance Imaging (MRI), along with T2-based sequences, are just a few examples of the numerous MRI sequences available. These can be used to evaluate a wide range of functional and structural features of brain parenchyma. The utmost sensitive imaging technique for the identification of acute ischemia is diffusion MRI. This method measures the mobility of random molecules of water. Diffusion is constrained in areas with cytotoxic oedema, where the water distribution has shifts from extracellular to intracellular compartments. Within minutes of onset of an ischemic stroke, diffusion MRI appears abnormal, and regions with diffusion restriction rarely regain their original appearance. Ionic and vasogenic oedema develop during the subsequent days (18-96 hours) as a result of more blood-brain barrier damage, and it can be seen on both native CT as well as some MRI sequences.

8. Management of acute ischemic stroke

8.1. Antiplatelet therapy

Use of aspirin within the first 48 hours of symptoms lowers the prevalence of further strokes and improves treatment results. Although the benefit is less compared to other reperfusion treatments, aspirin is commonly used, and it is also reasonably priced.

Aspirin, when used in conjunction with dipyridamole, or clopidogrel used without aspirin, is somewhat more effective but also more expensive. In patients at high risk, application of aspirin together with clopidogrel within 12 hours of a mild stroke or TIA and continued for around 21 days, decreased the occurrence of recurring stroke.

8.2. Reperfusion therapies

8.2.1. Intravenous thrombolysis

Alteplase and Tenecteplase are the two foremost medications which are available for intravenous thrombolysis. A recombinant version of tissue plasminogen activator (rt-TPA), known as alteplase, breaks down plasminogen into plasmin. Then, plasmin breaks down fibrin and removes the thrombus. This treatment is approved globally as a standard thrombolytic therapy after ischemic stroke. Several experiments have assessed the ideal time frame for alteplase delivery. For instance, part A and B studies of NINDS tPA demonstrated a clinical advantage once alteplase was administered within three hours of the start of a stroke. In subsequent experiments, administration of alteplase up to 6 hours after stroke onset showed different degree of benefits. Individual patient data meta-analysis has revealed that the treatment of stroke by alteplase up to 270 minutes following the appearance of symptoms has a substantial therapeutic benefit (a considerable decrease in disability and mortality). The number of patients that must be treated to successfully treat one more patient over the course of 4.5 hours grows from 4.5 patients within 90 minutes to 15 patients between 3 and 4.5 hours. This result is due to the diminishing amount of recoverable penumbral tissue over

time. Moreover, the thrombus' susceptibility to lysis may weaken with time, decreasing the effectiveness of treatment with delayed administration.

The most important complication of thrombolysis is hemorrhage, distinctively transformation of ischemic stroke into hemorrhagic stroke. This transformation in extreme situations could exacerbate cerebral injury and enhance the mass effect. In order to be eligible for intravenous thrombolysis beside time frame and radiological imaging proof for salvageable brain, it is important to eliminate any conditions which could increase risk of cerebral or systemic hemorrhage. Patients may be disqualified from receiving thrombolytic therapy for a variety of reasons, including previous cerebral hemorrhage, recent operation, trauma, or systemic bleeding, usage of anticoagulation medication, coagulopathy, and unrestrained hypertension. Depending on the definition used, there are different risk factors for symptomatic intracerebral hemorrhage.

8.3. Beyond Traditional Approaches

According to recent data, rates of morbidity and mortality are significantly reduced when treatment is initiated within an hour of the onset of symptoms. The Golden Hour is a term used frequently to describe this remarkable 60-minute window following the onset of symptoms, currently rt-TPA stands as the benchmark treatment. About 50% of the patients receiving rt-TPA will either partially or fully recover after three months given the extended time frame of 270 minutes. However, thrombolysis will not be beneficial for a significant portion of patients, and 6-8% of those who receive it will experience an intracerebral hemorrhage that could be fatal. Personalizing acute stroke care, enhance quality of life over lifespan of time, and globally lessen the burden of AIS, it may be important to identify patients in who would benefit from rt-TPA treatment. To accomplish this, it is necessary to develop a prognostic marker for the outcome of thrombolysis, which is quick, affordable, easily accessible, and accurate.

It has been established that thrombocytes play a significant part in the development of atherothrombosis and ischemic stroke. Platelet turnover is measured by mean platelet volume.

Platelets with more granules tend to be more immature and thus larger, emitting a greater quantity of chemical messengers, which promote further platelet aggregation and stimulation. The risk of thrombosis increases when the MPV and PC are both elevated. It has been discovered that patients with pulmonary embolism have isolated elevated PC and substantially increased MPV. It has been suggested that altered platelet functions in patients with either type of stroke result in a hypercoagulable state. Both PC and MPV, in primary intracerebral hemorrhage were linked to poor outcome. According to some studies patients with AS have substantially higher MPV or PC than controls, while other studies reported a lower MPV or PC values in patients with AS. Nevertheless, some investigations found no correlation between these parameters and AS.

9. Thrombocytes

The smallest and most reactive of all the blood cells are thrombocytes. These cells are primarily involved in maintenance of normal hemostasis and fibrosis. Their multifunctionality has been sufficiently supported by recent investigations. When chemicals like ADP, TXA₂, PAF, and inflammatory cytokines (e.g., IL-1, IL-6, TNF alpha) activate platelets, they initially accumulate at the injury site. This leads to changes in shape, pseudopodia formation, release of cytoplasmic granular content, and subsequent aggregation, causing fibrosis and inflammation. As per the extensive literature, MPV serves as a highly informative parameter, offering crucial insights into the progression and prognosis of a broad spectrum of pathological conditions. These conditions span cardiovascular diseases, respiratory ailments, Crohn's disease, rheumatoid arthritis, juvenile systemic lupus erythematosus, DM, and most of malignant neoplasms.

During thrombocytopoiesis, platelets are produced as non-nucleated, disc-shaped fragments of megakaryocyte cytoplasm. This complex process is intricately connected with the presence of thrombopoietin (Tpo), ultimately leading to the formation of blood platelets. Megakaryocytes undergo cell proliferation, intracellular platelet protein production, and a loss of proliferative potential when their c-MPL receptor for Tpo is activated. Following that, the repeated processes of endomitosis result in the construction of the polypoidal nucleus of the megakaryocyte, which enhances cell metabolism and results in the formation of the system of membranes, cell organelles, and specialized granules required for normal platelet formation. The ploidy level of the megakaryocytes determines the quantity and size of the generating platelets. The more cytoplasm and distinct platelet structure a megakaryocyte nucleus contains, the higher its ploidy. Proplatelets are formed by an activated cell's cytoplasmic processes that resemble pseudopodia and serve as an intermediary form between megakaryocytes and thrombocytes. Proplatelets are lengthy megakaryocyte cytoplasmic processes that include organelles which are characteristic of thrombocytes but lack definite boundary zones. Because mature proplatelets are smaller than stem cells, they can move to peripheral arteries after being released into bone marrow vessels. Proplatelets may even be hundred times bigger than blood platelets, indicating that their cytoplasm has already begun to fragment in order to give rise to platelet.

Tpo is primarily responsible for activating megakaryocytes and increasing thrombocyte release. It has been shown that some inflammatory cytokines can stimulate blood platelet precursor cells in inflammatory conditions. Megakaryocytes are directly affected by IL-6's action via the membranous receptor IL-6R and are also more likely enhance production of Tpo in the liver as a result of this action. This implies that a significant increase in blood PC may occur in an inflammatory condition. The variables that directly influence the ploidy of megakaryocytes, the maturity of progenitor cells, and the activation and wear of blood

platelets during coagulation and pro-inflammatory processes determine any variations in the count, total platelet mass, morphology, and function. The lifespan of the Platelets in the blood is 8 to 12 days. About 70% of the total mass of platelets are typically in the circulatory pool in healthy individuals, with the remaining 30% of thrombocytes being stored in the spleen.

9.1. Platelets and Inflammatory Response

Leukocytes are drawn to the site of injury during an inflammatory reaction due to the release of cytokines and chemokines by blood platelets. Platelet-leukocyte aggregates are the result of interactions between blood platelets and leukocytes during the inflammatory process.

While pro-inflammatory cytokines stimulate megakaryocytes and cause a significant rise in the synthesis and release of thrombocytes, coagulation may cause the count to drop.

9.2. Platelet Morphological Parameters

During a standard blood morphology test, fundamental platelet characteristics are evaluated, providing important data on PC, MPV, platelet distribution width (PDW), plateletcrit (PCT), and the percentage of large platelets with MPV > 15 fL (P-LCR). The most recent studies have demonstrated that platelet parameters can help diagnose a patient's overall health and have prognostic significance in particular illnesses.

9.3. Mean Platelet Volume and ischemic stroke

Hematological analysts use volume distribution from a standard blood morphology test to calculate MPV, which is a precise measurement of platelet size. While the percentage of large platelets should be between 0.2 and 5.0% of the total platelet population, MPV ranges between 7.5 and 12.0 fL. In physiological settings, MPV is negatively correlated with platelet count, which is connected to maintaining both hemostasis and consistent platelet mass. This physiological proportion is distorted in several illnesses.

Changes in the ratios of MPV and PC could result from abnormally or noticeably accelerated thrombocytopoiesis, increased wear, or the effect of activating agents on blood platelets. As a

result, it has been suggested that these criteria might be used in diagnosis of some disorders. Additionally, MPV is considered as a sign of platelet activity because it corresponds with platelet activity. Those with greater MPV (>15 fL) tend to be younger and have higher levels of reactivity than people with MPV in the reference range. They are produced as a result of megakaryocytes being activated by cytokines, which increases their ploidy and encourages the release of larger platelets. Moreover, it is hypothesized that big thrombocytes have more cell granules, express adhesion molecules more strongly, and activate more quickly, leading to platelet hyperactivity and a higher risk of clot formation. Increased platelet aggregation, TXA2 production, and release are all correlated with elevated MPV.

9.4. MPV in Inflammation

In healthy individuals, the elevated PC induces the liver to significantly reduce the production of Tpo through a feedback mechanism, which in turn triggers platelet release by megakaryocytes to maintain continual platelet mass. Nevertheless, the rising levels of proinflammatory cytokines, particularly IL-6, in patients with persistent inflammation can trigger platelet release. This is connected to how IL-6 directly affects megakaryocytes and how it stimulates the production of Tpo. Megakaryocytic nuclei's ploidy and cytoplasm volume both increase as a result of IL-6 and as a consequent result in an abundance of blood platelets. The advance of an inflammatory condition is also linked with an increase in the percentage of large platelets, which is most likely due to intracellular synthesis of procoagulatory and proinflammatory factors, granule degranulation, and stimulation of the platelet pool stored in the spleen.

9.5. Factors Influencing PC and MPV Values

According to a number of experts, MPV and platelet count should always be evaluated simultaneously because PC and MPV have a nonlinear inverse relation. Other factors, such as ethnicity, gender race, age, and lifestyle may also have a significant impact on MPV and PC.

Due to genetic variations, PC and MPV have a high heritability of 84% and 75%, respectively.

10. Neutrophils

Neutrophils are one of the primary cells to respond to an AIS. They also participate in clot formation, atherosclerosis, and other pathogenesis-related ischemic stroke processes. Many disorders, including IS, diabetic retinopathy, MI, sickle cell disease, renal micro-vasculopathy, transfusion-related acute lung injury and, acute respiratory distress syndrome, have worsened outcomes when associated to neutrophils. They have numerous functions intended to destroy pathogens; however, they also have an effect on several processes which may cause or facilitate cerebral infarction. Reactive oxygen species (ROS) including superoxide and hypochlorous acid are produced by neutrophils during the phagocytosis process via NADPH oxidase and myeloperoxidase (MPO), respectively. These ROS can directly damage the blood brain barrier (BBB) and possibly brain cells.

Elastase, cathepsin G, Proteinase 3, MMPs elastase, and cathepsin G are some of the proteases that neutrophils produce during the pathogen-killing process of degranulation, which might harm the blood brain barrier (BBB) and brain cells by breaking down extracellular matrix.

Proinflammatory cytokines including IL-1, IL-6, IL-8, and TNF- α , as well as chemokines like MCP-1, CCL2, MIP-1 α , and CCL5 that may exacerbate ischemic brain injury, can also be released by or reacted to by neutrophils. Several neutrophil receptors engage in interactions with damaged endothelium as well as platelets to encourage clotting/thrombosis and atherosclerosis.

Following ischemia, neutrophils arrive within 6-12 hours' time frame to the ischemic brain territory. These leukocytes promote ischemic cerebral injuries in various ways including the release of proinflammatory mediators. Reflow phenomenon happens when flow of

erythrocytes is impaired due to adhesion of white blood cell to the endothelium, disturbing the microvasculature leading to further injury.

Activated lymphocytes promote secondly production of ROS, proteases, gelatinases and collagenase by stimulating the endothelium and possibly salvage brain tissues and vessels. Thirdly, creation of biologically active substances may cause vasoconstriction and surge in platelet aggregation. Finally, as result of leukocyte infiltrating into penumbra adjacent to infarct core, further neuronal injury is achieved by the release of proinflammatory cytokines and other immune molecules.

Several animal studies with lymphocytes have demonstrated a protective effect on nerves, increased lymphocytes repressed inflammatory cytokines including IL-6 or TNF, at the same time, stimulated anti-inflammatory regulation of interleukin 10. According to Cep Juli et al. poor neurological outcome on NIHSS scale was linked to low lymphocyte level. This outcome is explained through activation of renin angiotensin system together with increase in cortisol level post stroke, resulting in apoptosis of lymphocytes.

At least two different populations of monocytes have been described based on their functions and surface markers. IL-10, acting as an anti-inflammatory cytokine, is produced by classic monocytes, while TNF is produced by "pro-inflammatory" monocytes.

Post ischemic stroke pro-inflammatory monocytes rapidly migrate to site of injury. These cells there then differentiate into macrophages and Dendritic cells.

NLR and LMR have shown links with various pathological conditions, positioning them as potential emerging indicators for assessing inflammation and immune response. Recent investigations have revealed initial signs suggesting a fundamental association between the prognosis of AIS and the levels of NLR and LMR. Significantly, these laboratory parameters can be conveniently extracted from a complete blood count, making them promptly

accessible and valuable markers for clinical use, particularly prior to administration of thrombolytic agents.

11. Meta-analysis in relation to ischemic stroke, PC and MPV

11.1. Protocol and Registration

According to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines we conducted our systemic review and meta-analysis in addition to this we verified our study according to the MOOSE (Meta-analysis of Observational Studies in Epidemiology). The systematic review and meta-analysis protocol was also registered with the PROSPERO database at:

https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=67864.

11.2. Search Strategy and Study Selection

The suitability criteria conformed to the inclusion standards of the Patient Population or Problem, Intervention, Comparator, Outcomes, and Setting (PICOS). We made alterations to the PICOS by omitting the 'I' which pertains to Interventions, given that no interventional studies were integrated. Suitable studies were those that met the following requirements: (1) centered on the adult population, (2) encompassed a control group, (3) investigated the diagnosis or assessment of acute stroke, (4) provided comprehensive data on platelet indices in both the patient and control groups, (5) constituted either cross-sectional or case-control studies. There were no restrictions concerning geographic location, language or publication date. Excluded from consideration were abstracts, editorials comments, reviews, and conference proceedings.

Our analysis was founded on the thorough examination of pertinent research published up to July 2017 in, Google Scholar, EBSCOhost, ScienceDirect, Scopus, Web of Science and Medline, In August 2019 we reconducted our search, and incorporated new published data.

Google Scholar was explored through Harzing's Publish or Perish software (Harzing, A.W., 2007, Publish or Perish, accessible at <https://harzing.com/resources/publish-or-perish>).

11.3. Data Extraction

Two individuals conducted data extraction using a standard form then cross-validated the information. Any disagreements were resolved through consensus or by engaging in discussions with the tertiary reviewer. We also referenced the bibliographies of the retrieved articles to identify other pertinent studies. All the data was systematically collected. Initially, following the first search, we retained reviews in the list to gather suitable references. In the subsequent phase, we excluded all reviews from the list of accepted publications. The quality of the eligible studies was assessed using the Newcastle-Ottawa Scale (NOS). The NOS encompasses three domains: the study population's selection, the comparability of groups, and the outcome ascertainment. Articles garnering NOS scores of 6–9 were classified as high quality, while scores of 0–5 indicated poorer quality. In cases where studies only provided median values with a range or interquartile range, we calculated the mean and standard deviation in accordance with Wen et al.

11.4. Data Synthesis and Statistical Analysis

This study was conducted in accordance with the guidelines outlined by Viechtbauer and the work of Huzsvai and Balogh. Our meta-analysis encompassed variations between studies and also included covariates to address potential publication biases. We calculated the Standardized Mean Differences (SMDs) for MP and PC within each individual study. Additionally, we carried out subgroup analysis via a random-effect (RE) meta-analysis to evaluate heterogeneity, considering the type of anticoagulant (EDTA or citrate), the type of analyzer (Sysmex or Coulter), and the type of infarction [Ischemic Stroke (IS)]. Then, a mixed-effect (ME) meta-regression was performed using the type of infarction and type of analytes as the moderator variables. We also conducted an outlier detection for MPV

concerning storage time and type of analyte. Across this study we quantified heterogeneity using the I square (I^2) statistics.

12. Study of Neutrophil lymphocytes ratio and lymphocytes monocytes ratio in relation to stroke

12.1. Study Population

This research enrolled a consecutive group of AIS patients admitted to the Department of Neurology at the University of Debrecen, Hungary, spanning from September 2016 to April 2018. Patient inclusion and exclusion criteria were in accordance with the established standards for intravenous rtPA administration as outlined in the 2008 ESO guideline. The diagnosis of ischemic stroke was definitively confirmed via non-contrast computerized tomography (NCCT) scans and computed tomography angiography CTA. Clinical data, which encompassed the assessment of the National Institutes of Health Stroke Scale (NIHSS) scores, were precisely recorded upon admission and on the first day of hospitalization. Thrombolysis, following the established protocols, was executed within the 270 minutes time window commencing from the onset of symptoms through intravenous rtPA application. Patients who underwent mechanical thrombectomy in conjunction with thrombolysis were not within the scope of this study.

On the first day, a control non-contrast CT (NCCT) scan was conducted, and early ischemic changes were determined through the assessment of Alberta Stroke Program Early CT Score (ASPECTS) using evaluations from four independent radiologists. Stroke etiology was classified in accordance with the Trial of ORG 10,172 based on the Acute Stroke Treatment (TOAST) criteria. The presence of intracerebral hemorrhage (ICH) was examined on the first day via NCCT, and patients with hemorrhage were categorized into two groups: symptomatic (SICH) or asymptomatic (aSICH), following the criteria from the European Cooperative Acute Stroke Study (ECASS) II.

Assessment of short-term outcomes occurred one day after thrombolysis. A favorable short-term outcome was characterized by a reduction in the NIHSS score by at least four points or a score reaching zero, while an unfavorable short-term outcome was indicated by an increase in the NIHSS score of at least four points. To determine long-term outcomes at ninety days, the modified Rankin Scale (mRS) was used. An adverse outcome was defined as an mRS greater than one ($\text{mRS} > 1$). Our study received approval from 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 protocol adhered to the ethical principles of the 1975 Declaration of Helsinki. All patients or their family members provided written consent.

12.2. Blood Sampling and Laboratory Measurements

Peripheral blood samples were obtained prior to the commencement of rt-PA infusion and again 24 hours post thrombolysis. Upon admission, standard laboratory assessments, including ion levels, glucose levels, liver enzymes and renal function test, and high-sensitivity C-reactive protein (hsCRP), were conducted using established techniques (Roche Diagnostics, Mannheim, Germany).

Complete blood counts were analyzed using an automated analyzer (XE 2100, Sysmex Europe GmbH, Hamburg, Germany) from blood samples collected both before and 24 hours after thrombolysis. Hematological parameters were promptly determined following blood collection, and the LMR and NLR were computed from blood samples collected on both occasions.

12.3. Statistical Methods

For continuous parameters, we presented the outcomes either as the mean combined with standard deviation (SD) or as the median accompanied by the interquartile range (IQR) when suitable. Categorical data were conveyed in terms of counts and the associated percentages.

To evaluate multiple groups of continuous data, we utilized one-way analysis of variance (ANOVA) and applied either the Bonferroni post-hoc test or employed Kruskal-Wallis analysis, complemented by the Dunn-Bonferroni post-hoc examination when required.

Categorical data were analyzed through χ^2 or Fisher's exact tests where appropriate. To construct Receiver Operating Characteristic (ROC) curves, we plotted sensitivity against 1-specificity and determined the area under the curve (AUC). Threshold values were derived based on Youden's J statistics. We employed multivariable logistic regression models to evaluate the independent impact of NLR, LMR, their combination, or each leukocyte subtype count on outcome measures, both before and after adjusting for major baseline characteristics. Significance was defined as a p-value less than 0.05. Statistical analyses were conducted using SPSS 18.0 (Chicago, IL, USA) and MedCalc 14.8.1 software (Mariakerke, Belgium).

14. Results of our meta-analysis

Initially we identified 6812 studies. Subsequently, 2746 duplicates (40.3%) were removed, and 3810 articles (55.9%) were excluded following a review of their titles and/or abstracts. The residual 256 articles underwent eligibility assessment by screening their abstracts and titles, then. thirty-nine studies were chosen for qualitative assessment. We attempted to obtain data from the authors of five studies; however, the data was unavailable. Ultimately, we included thirty-four articles in this meta-analysis.

Of the studies conducted, three were situated in the United States, while twelve took place in Europe, 10 in Asia, 1 in Africa, and 8 studies had an international focus. The patient populations varied, ranging from 12 to 384 individuals. A significant proportion of the included studies primarily centered on AIS (82%). In 13 studies (38%), no information regarding the type of analyte was reported, and a nearly equivalent number of studies (41%) succeeded in providing details about the type of hematological automated analyzer used. For

approximately 20.5% of the studies, the elapsed time between blood collection and measurement exceeded 2 hours. Furthermore, a considerable number of studies omitted reporting stroke-related comorbidities.

To estimate ME and RE models for PC and MPV, we utilized the Dersimonian-Laird estimator. The funnel plot highlighted the presence of publication biases in the RE model, particularly regarding MPV. However, the ME model for both variables displayed no evidence of publication biases. We applied a weighted regression model featuring multiplicative dispersion to calculate t-values. Notably, Egger's test detected publication biases in the RE model, specifically for MPV ($p < 0.001$). In the case of PC, t-statistics suggested a more robust symmetry for the mixed-effects model. Notably, our outlier detection procedure did not reveal any falsely elevated MPV values. To enhance the reliability of both RE and ME models and prevent overfitting due to a limited number of studies, we employed bootstrap sampling.

14.1. Results regarding PC

Overall, 6107 participants were drawn from 24 publications, with 2492 allocated to the patient group and 3615 to the control group. Three studies indicated that patients with AS exhibited higher PC than controls. In contrast, eleven studies revealed lower PC in the patient group, while eleven other studies found no significant difference in PC between patients and controls. One study did not provide PC results. The average PC across all publications was 234 G/L (95% CI: 224–244) for patients and 248 G/L (95% CI 238–259) for controls. The ME model indicated a substantial residual heterogeneity for PC [$\chi^2 = 230.2$; $df = 27$; $I^2 = 88.3\%$ (95% CI: 80.0–93.1); $p < .001$]. Regarding ischemic stroke, the estimated pooled mean difference in PC was 0.48 G/L [$Z = 1.61$; $p = 0.107$] for the ME model. For the RE model without moderating effects, it was -0.30 ($Z = -3.15$, 95% CI: -0.49 – -0.11 , $p = 0.002$).

14.2. Results regarding MPV

Out of the twenty-seven studies, seventeen reported that patients with stroke demonstrated greater MPV compared to controls. In contrast, ten studies found no significant difference in MPV between patients and controls, interestingly two studies indicated a higher MPV in the control group.

The average MPV across all published studies was 9.8 fL (95% CI 9.4–10.1) for patients and 9.2 fL (95% CI, 8.8–9.6) for controls. Considerable residual heterogeneity was observed in the ME model for MPV [$\chi^2 = 333.5$; $df = 20$; $I^2 = 94\%$ (95%CI: 89–97); $p < 0.001$]. Regarding the effect of analyte (EDTA compared to Citrate), the estimated pooled mean difference in MPV was 0.86 ($Z = 5.509$; $p < .001$) for the ME model. In absence of moderating effects, the RE model indicated a mean difference of 0.52 fL ($Z = 4.29$, $p < 0.001$).

14.3. Results of subgroup Analysis

We also conducted subgroup and sensitivity analyses to explore the relationship between these parameters, based on the analyte, analyzer, and type stroke for both MPV and PC. In total, twelve studies performed complete blood count measurements in 120 minutes or less, while seven studies assessed blood samples after 120 minutes, and we analyzed these in distinct subgroups. Among the thirty-four studies included in this meta-analysis, three studies had further subdivided their analyses into various subgroups. In the case of AIS, involving twenty-three studies, 2007 cases exhibited significantly lower PC compared to 3090 control individuals (SMD = -0.18 ; 95% CI: -0.35 to -0.01 , $p < 0.001$).

In the context of PICH, six studies presented more significant findings involving 485 patients compared to 525 controls (SMD = -0.94 ; 95% CI: -1.62 to -0.25 , $p < 0.001$). For PC measurements with citrated samples, the results were significantly higher in 100 patients in

comparison to 173 controls (SMD = -0.36 ; 95% CI: -0.68 to -0.04 , $p < 0.05$). MPV was notably higher among 2444 patients who experienced acute ischemic stroke when compared to 3405 controls (SMD = 0.57 ; 95% CI: 0.31 – 0.83 , $p < 0.001$). Conversely, in the case of acute hemorrhagic stroke, MPV was lower in patients compared to controls, but this difference failed to reach statistical significance (SMD = 0.07 ; 95% CI: -0.10 – 0.25 , $p > 0.05$). MPV measured from EDTA anticoagulated samples was evaluated in seventeen studies, and it was significantly higher among 1974 cases in contrast to 3209 control individuals (SMD = 0.86 ; 95% CI: 0.55 – 1.17 , $p < 0.001$). Furthermore, citrated samples from 208 cases exhibited lower MPV compared to 186 controls (SMD = -0.13 ; 95% CI: -0.69 – 0.42 , $p < 0.001$).

Across 14 studies utilized Sysmex hematology analyzers, encompassing 2727 controls, notably elevated MPV values were observed in 1557 patients (SMD = 0.64 ; 95% CI: 0.28 – 1.01 , $p < 0.001$). In parallel, within six studies featuring Coulter hematology analyzers, 432 patients exhibited markedly increased MPV when contrasted with 347 controls (SMD = 0.58 ; 95% CI: 0.08 – 1.08 , $p < 0.001$). Additionally, we explored MPV outcomes in two subcategories based on the time elapsed between venipuncture and analysis, distinguishing between durations exceeding 120 minutes and those less than or equal to 120 minutes. Intriguingly, in relation to the control group, MPV displayed significant elevation within both the ≤ 120 -minute and > 120 -minute groups (SMD = 0.72 ; 95% CI: 0.36 – 1.08 , $p < 0.001$) and (SMD = 0.70 ; 95% CI: 0.08 – 1.32 , $p < 0.001$), respectively.

15. Results of NLR-LMR study

15.1. Baseline Characteristics of Enrolled Patient

Throughout duration of our study, a total of 285 consecutive patients experiencing AIS and receiving intravenous thrombolysis were included in the research. The baseline characteristics of the enrolled patients. The average age of these patients was 66 years with a standard deviation of 12.9 years, and 44.2% of them were female. Their baseline NIHSS score had a

median value of 6, with an interquartile range (IQR) between 5 and 9.1. The median mRS score at 90 days was 1.0.

Patients who had a poor outcome ($\text{mRS} \geq 2$) at 90 days post-stroke tended to be older, had higher blood pressure levels, and had a higher incidence of atrial fibrillation. They also presented with more severe neurological deficits at admission compared to those who achieved a favorable outcome. Additionally, patients with poor outcomes exhibited significantly higher NLR and considerably lower LMR in comparison to those with favorable outcomes.

15.2. White Blood Cell Counts, NLR and LMR during Thrombolysis

Within the entire cohort, it was observed that 24 hours after thrombolysis, the median counts of neutrophils and monocytes, as well as the NLR, had all increased, while the median LMR had decreased when compared to the admission results. We identified a modest inverse correlation between neutrophil count and lymphocyte count at admission ($r = -0.166$, $p = 0.002$) and on day 1 ($r = -0.200$, $p = 0.001$). Moreover, a significant yet modest positive correlation was identified between lymphocyte counts and monocyte counts at admission ($r = 0.261$, $p < 0.001$), though this correlation was not evident on day 1. Furthermore, neutrophil counts exhibited a correlation with monocyte counts both at admission ($r = 0.381$, $p < 0.001$) and on day 1 ($r = 0.598$, $p < 0.001$).

The results are presented as either mean with standard deviation (SD) or median along with the interquartile range. None of the leukocyte indices demonstrated an association with stroke etiology, stroke severity upon admission, or hemorrhagic transformation at admission. Conversely, the monocyte, neutrophil count, and NLR exhibited significant increases, whereas the LMR and lymphocyte count showed notable decreases 24 hours post-rtPA treatment in patients with more severe stroke.

A comparable trend was noted in individuals who encountered intracerebral hemorrhage associated with therapy.

In contrast, the ASPECTS 10-8 group exhibited notably lower neutrophil counts and NLR values when compared to the ASPECTS 7 group on day 1. Univariate logistic regression analysis unveiled a considerable protective effect of elevated LMR at admission in relation to functional dependence at the 3-month mark following the event (OR = 0.755, 95% CI: 0.631, 0.903, $p = 0.002$). However, this analysis did not reveal any discernible link between NLR and long-term functional outcomes. Furthermore, alongside LMR, elements such as age, hypertension, atrial fibrillation, and various stroke

characteristics (including NIHSS, hemorrhagic transformation, and stroke localization) exhibited associations with the long-term outcome of therapy. Yet, within the multivariate model, only age, NIHSS, hemorrhagic transformation, and stroke localization emerged as the significant contributing factors.

Despite the univariate model highlighting a robust link between NLR and LMR, assessed 24 hours after rtPA therapy, and functional outcomes at the 3-month mark following the event, the multivariate logistic regression model, which considered all potential confounding factors, showed no noteworthy correlation between these parameters and functional outcomes at 3 months.

Initially, the study population exhibited median values at baseline, with NLR at 2.9 (interquartile range, IQR: 1.94, 4.82) and LMR at 3.22 (IQR: 2.42, 4.29). The optimal thresholds for predicting unfavorable functional outcomes at the 3-month post-event mark, determined through ROC analysis by maximizing the Youden index, were set at 5.73 for NLR and 2.08 for LMR. These optimal cutoff values at admission led to the classification of patients into four distinct groups: low NLR–high LMR, high NLR–high LMR, low NLR–low LMR, and high NLR–low LMR.

Among the 190 patients who experienced favorable outcomes, a remarkable 77% found themselves in the low NLR–high LMR category at admission. In contrast, only 6.8% of patients were situated in the high NLR–low LMR group. These proportions saw little change by day 1, with 76% in the low NLR–high LMR category and 7.8% in the high NLR–low LMR category. On the other hand, within the 95-patient group with unfavorable outcomes, 67% belonged to the low NLR–high LMR category at admission, while a mere 21% were classified as high NLR–low LMR ($p = 0.001$). At the 24-hour mark following thrombolysis, the distribution among these groups underwent a significant transformation. Approximately 36% of patients were categorized as having low NLR–high LMR, whereas the high NLR–low LMR group accounted for 49% ($p < 0.001$) of patients.

The joint evaluation of NLR and LMR at the 24-hour mark post-thrombolysis has been identified as identified predictor of adverse functional outcomes at 3 months post-event (OR = 3.407, 95% CI [1.449, 8.011], $p = 0.005$) for individuals categorized in the high NLR–low LMR group as opposed to those in the low NLR–high LMR group. This finding remains robust even when adjusting for all conceivable confounding variables

16. Discussion

We undertook a comprehensive systematic review and meta-analysis of the existing literature to investigate PC and MPV in individuals with AS. Larger platelets, characterized by elevated MPV, are acknowledged to exhibit heightened metabolic and functional activity. Platelets with a high MPV possess greater stores of glycogen, ADP, ATP, and are more responsive to activating agents . Defect in platelet activity has been associated with the occurrence of PICH. Furthermore, platelets play a crucial role in thrombus formation, which can serve as the initiating events leading to stroke symptoms.

Recent meta-analyses have provided insights into elevated MPV in various pathological conditions. Conditions such as HT, DM, dyslipidemia, CAD, and smoking habits are

established as important medical predispositions and comorbidities in the context of stroke. A growing body of evidence from existing literature has indicated a direct link between MPV and these conventional risk factors for cerebrovascular events. For instance, among individuals with coronary artery disease, MPV was notably elevated (0.65 fL), and individuals with higher MPV displayed a 17% increased risk of experiencing cardiovascular events compared to those with lower MPV. Furthermore, another meta-analysis revealed a significant elevation in MPV among individuals with acute MI compared to those without. In our study, we found a substantial increase in MPV among acute ischemic stroke patients, along with a significant decrease in PC in both acute ischemic and hemorrhagic stroke cases. Various authors have explored the potential role of MPV assessments as a promising biomarker for AS or a predictive tool for post-stroke patient outcomes. The association between MPV and stroke may be attributed to the heightened platelet activity, aggregation propensity, and rapid turnover observed in individuals with elevated MPV. Studies have indicated that increased MPV correlates with the presence of more hemostatically active platelets with a heightened tendency to aggregate. Furthermore, due to accelerated turnover of platelets bone marrow releases younger, more active, and larger platelets. Our comprehensive analysis uncovered a statistically significant mean difference of 0.51 fL, signifying substantially higher MPV values in AS patients compared to controls. Furthermore, even after meticulous adjustments for the type of analyte or analyzer, MPV remained notably elevated in patients compared to controls. It's important to note that while these differences are statistically significant, they represent relatively minor changes in absolute terms. These findings likely reflect distinct characteristics of the platelet population and the prevalence of more responsive platelets within this patient group's bloodstream. As platelets typically have a lifespan ranging from 8 to 10 days, the influence of heightened MPV on prothrombotic tendencies and the increased risk of stroke likely predates the onset of

a stroke. Notably, stroke patients exhibited elevated platelet activation, as evidenced by increased CD62P (P-selectin) levels and the presence of circulating monocyte-platelet complexes. Conversely, the levels of reticulated platelets, indicative of platelet production, mirrored those of controls among stroke patients. These findings suggest an ongoing stimulation of platelet activation without excessive platelet production following a stroke. Continuous monitoring of stroke patients revealed persistent elevations in MPV even three to 6 months post-stroke. A limited number of studies concerning acute hemorrhagic stroke and hyporeactive platelets, characterized by significantly reduced platelet aggregation rates induced by ADP, may partially account for the sustained MPV levels in acute hemorrhagic stroke cases.

The decline in platelet count (PC) during the acute phase of ischemic stroke can be ascribed to heightened platelet consumption. However, comprehending the underlying reasons for the reduced PC levels in patients with acute hemorrhagic stroke is a more intricate matter, in some cases, low platelet count before stroke might cause hemorrhagic stroke. It is improbable that non-traumatic acute hemorrhagic strokes involve hemorrhages extensive enough to cause a significant depletion of platelets. Alternative factors, such as medications like antiepileptics or antibiotics, may have contributed to the observed decrease in PC. It becomes evident that modifying PC as an acute-phase marker requires a timeframe that exceeds the duration of acute stroke. Moreover, it is imperative to acknowledge that pre-analytical conditions play a pivotal role in the assessment of mean MPV. To regard MPV as a potential risk factor for acute stroke, standardizing its measurement emerges as an essential consideration. This necessity extends not only to laboratory protocols but also holds paramount significance for the clinical community in terms of accurate interpretation and clinical decision-making.

The selection of anticoagulants for blood samples exerts a substantial impact on the outcomes of platelet parameters when assessed through traditional hematology analyzers. Existing

evidence has substantiated that the introduction of EDTA as an anticoagulant within the sample may give rise to artificial platelet swelling. This phenomenon is, in part, attributable to alterations in the platelet cell wall, modifications to the membranes lining the canalicular system, and shifts in the phosphorylation patterns of platelet proteins, all induced by exposure to EDTA. Intriguingly, among the studies encompassed in this meta-analysis, 61% opted for EDTA as the anticoagulant, a choice that correlated with markedly higher mean platelet volume (MPV) values for the patient cohort. It is worth noting that nearly half of the studies omitted any specification regarding the type of analyte employed in their investigations. Moreover, the type of anticoagulant utilized for blood samples can profoundly influence platelet count (PC) outcomes. Notably, the use of citrate anticoagulant may incite the formation of minuscule platelet micro-aggregates. Our findings reveal that, when considering the patient cohort in its entirety versus the control group, the calculated pooled mean difference for PC was -0.30 G/L. Furthermore, it is paramount to acknowledge that hematology analyzers encompass varying methodologies for the measurement of platelet parameters. These methodologies predominantly hinge on impedance or optical techniques, which, however, do not account for the morphological changes in platelets.

Consequently, these methodologies exhibit incongruities, and another pivotal parameter influencing MPV is the duration of sample storage. Despite the fact that not all investigations documented the time-frame for sample preservation, it was ascertained that MPV demonstrated a significant elevation in comparison to the control group, whether the samples were stored for ≤ 120 minutes or >120 minutes. In order to enhance the uniformity of MPV reporting, it is recommended to standardize the interval between venipuncture and MPV assessment.

It is important to acknowledge the limitations of this study. The heterogeneity observed in the literature regarding the correlation between MPV and stroke is multifactorial. The meta-

analysis revealed substantial heterogeneity, primarily arising from disparities in preanalytical factors and the demographic attributes of the study cohorts. Regrettably, these essential factors were often unreported, representing a potential wellspring of heterogeneity. Furthermore, only a restricted number of studies factored in the potential confounding influence of traditional cerebrovascular risk factors, and these studies displayed profound variations in preanalytical factors. As such, the results must be interpreted judiciously in light of the significant heterogeneity evident in certain studies. The variations in the timeframe between the onset of stroke and blood sampling, along with the lack of specificity in reporting the type of analyte or analyzer employed, may have contributed to the divergent findings. Additionally, the protracted contact between blood samples and anticoagulants can yield spurious results. The heterogeneity among the studies may also be partially attributed to the diversity in the analyte utilized for anticoagulation within the samples or the assay methods. Consequently, it is highly advisable that forthcoming research endeavors adhere to a standardized set of covariates and preanalytical factors. This standardization can be achieved by establishing a specific window for the measurement of sample results, specifying the type of anticoagulant utilized in the sample tubes, and delineating the method of measurement. The absence of comprehensive documentation concerning patient-associated preanalytical factors has markedly contributed to the augmented variability among the studies.

In summary, the assessment of MPV as an integral facet of standard complete blood counts offers a readily accessible and potentially informative marker of platelet turnover. The findings from this investigation underscore the pivotal role of platelets in the pathogenesis of stroke. Both PC and MPV demonstrated statistically significant deviations in comparison to control populations. Remarkably, individuals afflicted with acute ischemic stroke displayed a substantial elevation in MPV. In contrast, patients with both acute ischemic and hemorrhagic strokes exhibited notably diminished PC counts. These distinctive attributes may bear

significance in the etiology of stroke and its underlying pathophysiology. Crucially, this study stands as a pioneering endeavor, being the first to present and deliberate upon the evidence bolstering the combination of NLR and LMR, appraised at the 24-hour mark post-thrombolysis, as an autonomous prognostic factor bearing clinical import. This innovative approach not only demonstrates practical feasibility in identifying patients with acute ischemic stroke who face an unfavorable outcome at the 3-month juncture following intravenous thrombolysis but also holds its own as an independent prognostic factor. This standing was corroborated via a comprehensive multivariate analysis, further cementing the autonomy of high NLR–low LMR in prognosticating suboptimal outcomes at the 3-month threshold in patients with acute ischemic stroke post- rt-PA therapy. The predictive prowess of this model extends its reach to encompass other determinants, including gender, NIHSS scores, and TOAST classification.

Inflammation plays a pivotal role in the pathogenesis of ischemic brain injury. Following a stroke, ischemia triggers microglia activation, subsequently leading to the infiltration of blood-derived immune cells into the brain tissue within hours to a few days. Neutrophils swiftly reach the infarcted area in the initial hours following brain infarction, monocytes arrive within the first 24 hours, and lymphocytes make their entrance between 24 and 48 hours. This influx of immune cells results in the release of reactive oxygen species and a plethora of inflammatory mediators, adhesion molecules, cytokines, chemokines, and proteases, which collectively exacerbate tissue damage. Experimental stroke models have revealed increased hematopoiesis and an augmented release of neutrophils from the bone marrow due to heightened stimulation of the autonomic nervous system post-stroke.

Studies have indicated that post-thrombolysis NLR serves as a marker for an elevated risk of poor outcomes within three months following the onset of a stroke, and similar findings have been reported by other research groups. Our results align with these studies, demonstrating no

independent association between infarct size and NLR or LMR twenty-four hours post-stroke. Pektezel et al. conducted a retrospective analysis of acute stroke patients treated with rtPA by comparing those with favorable outcomes (mRS score of 3) to those with excellent outcomes (mRS score of 0 or 1) at admission and after 24 hours. Their study revealed a significant elevation in NLR from admission to 24 hours post-event, with an NLR value of 3.6 after 24 hours indicative of a favorable prognosis. They concluded that the elevated NLR during the first 24 hours is a secondary phenomenon associated with a poor prognosis. In contrast, a recent meta-analysis could not amass sufficient data to substantiate this assertion. Shi et al. conducted a study in which they did not identify any statistically significant differences in NLR upon admission between patients who later exhibited favorable versus poor outcomes. Our investigation aligns with these findings, as we determined a similar NLR threshold (established at 5.73) and observed no significant disparities in NLR at the time of admission between individuals who experienced unfavorable and favorable outcomes. Additionally, other researchers, although working with a relatively small patient cohort, did not discern any significant associations between NLR or the LMR and the long-term outcomes of patients with AIS treated with thrombolysis.

Beyond these considerations, it is noteworthy that monocytes assume a pivotal role in the inflammatory processes occurring within the cerebral infarct area resulting from cerebral ischemia and hypoxia. Monocytes release a spectrum of inflammatory mediators, encompassing chemokines, intercellular adhesion molecule-1, interleukin-1, IL-6, IL-8, and tumor necrosis factor, thereby actively contributing to the progression of inflammation. Furthermore, monocytes have a role in the promotion of thrombosis and vascular occlusion by forming aggregates in conjunction with platelets, thus amplifying the extent of ischemic injury.

Within the ischemic cerebral environment, monocyte-derived macrophages (MDMs) undergo differentiation from their precursor monocytes, as delineated in previous research. These MDMs exhibit considerable phagocytic potency and play a pivotal role in the protracted and spontaneous functional recuperation of the brain following an ischemic insult.

Furthermore, the Lymphocyte-to-Monocyte Ratio (LMR) at the time of patient admission has also been a subject of investigation for its potential to prognosticate the clinical outcomes at the 3-month mark in stroke patients receiving thrombolysis therapy. This analysis unveiled a crucial finding: a higher LMR value, with a defined cutoff point at 3.48, emerged as an independent determinant for forecasting the clinical outcomes of stroke patients, even prior to the administration of rt-PA.

Additionally, an examination into the temporal variations in the levels of peripherally circulating leukocytes revealed a striking phenomenon: an immediate post-ischemic stroke event is marked by an exponential decrease in the lymphocyte count. This noteworthy observation provides valuable insights into the dynamics of immune response following ischemic stroke.

Reduced lymphocyte counts, signifying severe brain damage, have shown predictive value for unfavorable neurological recovery and poor functional outcomes post-stroke. Our analysis aligns with this trend, as it revealed a significant rise in the number of patients experiencing poor outcomes within the high NLR–low LMR group in the first 24 hours post-thrombolysis. This observation underscores the relative association between diminished lymphocyte counts and unfavorable outcomes.

One potential mechanistic explanation is that regulatory lymphocytes both B and T cells, characterized as shielding cells that curtail disease progression, promote immune homeostasis, and generate anti-inflammatory agents, witness an upsurge in brain tissue following ischemic injury. Notably, this subdivision of WBCs regulates expression of

sympathomimetic and parasympathetic mediated receptors on their cell surface. Lymphocytes display countless cholinergic receptors on their surfaces meanwhile lower number of receptors of sympathetic mediators, while granulocytes exhibit a reverse relation with receptors. This dynamic interplay among leukocyte subsets may underlie the observed trends. Our research, in line with previous findings, demonstrates that there is no substantial association between SICH, severity of stroke, and leukocyte indices measured at time of patient is admitted to hospital. The association between WBC profiles and stroke outcomes after mechanical thrombectomy is the subject of some investigations. In a study, higher counts of neutrophils and NLR at admission and on day 1, as well as lower lymphocyte counts on day 1, were linked to poor prognosis ($mRS > 2$). In another study, higher NLR and lower LMR 24 hours after mechanical thrombectomy (but not at admission) were significant predictors of mRS at the 3-month functional outcome. They established optimal cut-off values of 5.5 for NLR and 2.0 for LMR post-thrombectomy.

Our study is the first to delve into the value of the combined post-thrombolysis high NLR–low LMR ratio for assessing the prognosis of AIS patients at 3 months post-event. Our findings are supported by previous research, which indicated the limited reliability of the pre-thrombolysis prognostic value of NLR measurements. Our results reveal that the NLR–LMR index, as determined 24 hours post-lysis, more accurately categorizes patients according to their 3-month outcomes, providing superior diagnostic accuracy. In summary, our investigation has unveiled that an elevated NLR-low LMR composite, evaluated 24 hours post-thrombolysis, stands as an autonomous prognosticator of unfavorable functional outcomes at the 3-month juncture subsequent to an ischemic episode. The remarkable increase in the representation of patients within the high NLR-low LMR category among those experiencing unfavorable outcomes conveys the potential of this combination to signify

a less optimistic prognosis. However, when we isolate the assessment of NLR and LMR, no substantial correlations with extended-term outcomes manifest.

Our findings underscore the importance of incorporating MPV, PC, NLR, and LMR measurements, both individually and in combination, to enhance the precision of prognostic predictions in AIS patients. These parameters can be easily obtained through routine blood test, offering a easily accessible and cost-effective tool for healthcare providers. The identification of individuals at a higher risk of adverse outcomes can facilitate the customization and optimization of post-thrombolysis therapy, potentially leading to improved longstanding quality of life as well as a reduced liability of acute ischemic stroke. Further research is warranted to validate these findings and explore the underlying mechanisms that link NLR, LMR, platelet indices, and stroke.



Nyilvántartási szám: DEENK/475/2023.PL
Tárgy: PhD Publikációs Lista

Jelölt: Sadeghi, Farzaneh
Doktori Iskola: Laki Kálmán Doktori Iskola

A PhD értekezés alapjául szolgáló közlemények

1. **Sadeghi, F., Sarkady, F., Zsóri, K. S., Szegedi, I., Orbán-Kálmándi, R. A., Székely, E. G., Molnárné Vasas, N., Berényi, E., Csiba, L., Bagoly, Z., Shemirani, A. H.:** High Neutrophil-Lymphocyte Ratio and Low Lymphocyte-Monocyte Ratio Combination after Thrombolysis Is a Potential Predictor of Poor Functional Outcome of Acute Ischemic Stroke.
J. Pers. Med. 12 (8), 1-12, 2022.
DOI: <http://dx.doi.org/10.3390/jpm12081221>
IF: 3.4
2. **Sadeghi, F., Kovács, S., Zsóri, K. S., Csiki, Z., Bereczky, Z., Shemirani, A. H.:** Platelet count and mean volume in acute stroke: a systematic review and meta-analysis.
Platelets. 31 (6), 731-739, 2020.
DOI: <http://dx.doi.org/10.1080/09537104.2019.1680826>
IF: 3.862





További közlemények

3. Hurják, B., Kovács, Z., Dönczö, B., Katona, É., Haramura, G., Erdélyi, F., Shemirani, A. H.,
Sadeghi, F., Muszbek, L., Guttman, A.: N-glycosylation of blood coagulation factor XIII
subunit B and its functional consequence.
J. Thromb. Haemost. 18 (6), 1302-1309, 2020.
DOI: <http://dx.doi.org/10.1111/jth.14792>
IF: 5.824

A közlő folyóiratok összesített impakt faktora: 13,086

**A közlő folyóiratok összesített impakt faktora (az értekezés alapjául szolgáló közleményekre):
7,262**

A DEENK a Jelölt által az iDEa Tudóstérbe feltöltött adatok bibliográfiai és tudománymetriai
ellenőrzését a tudományos adatbázisok és a Journal Citation Reports Impact Factor lista alapján
elvégezte.

Debrecen, 2023.10.24.

