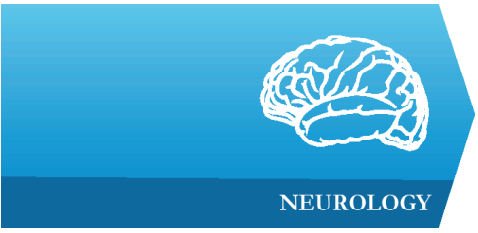
Original Research



Prognostic markers for ischemic stroke – are they truly reliable?

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Abstract

Background. Stroke is one of the leading causes of mortality and morbidity worldwide. Despite extensive research, to this date there is no panel of biomarkers for the prevention and prognosis of ischemic stroke and there is still much incomplete and insufficiently researched information.

Aim. We conducted a prospective, observational study between January and June 2020. The main objective of this study was to clarify the role of inflammation markers, i.e. neutrophil/ lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), high-sensitivity C – reactive protein (hsCRP) in ischemic stroke and whether there is or not a correlation between these markers and carotid stenosis.

Study design. In the study we included 150 subjects divided in two groups: study group – 100 subjects and control group – 50 subjects.

Methods. Data collected during the research (at the time of patient admission): 1) biological sample: 5 ml of peripheral blood were collected in a vial with clot activator and separating gel, from which the following laboratory tests were performed: hsCRP, neutrophils, lymphocytes, platelets. NLR and PLR were subsequently calculated as the ratio of neutrophils to lymphocytes, respectively platelets and lymphocytes), 2) paraclinical examinations: extracranial carotid Doppler ultrasound examination.

Results. The results were impressive: high-sensitivity C reactive protein (hsCRP), neutrophil/lymphocyte ratio (NLR) and platelet/lymphocyte ratio (PLR) were strongly, respectively moderately correlated with the severity of stroke (the severity being established with the NIHS (National Institute of Health Stroke) score. None of the inflammation markers included in the present study was correlated with carotid stenosis.

Conclusion. hsCRP, NLR and PLR may potentially be prognostic markers for ischemic stroke, being of major help in preventing its possible complications.

Keywords: stroke, inflammation, NLR, PLR, hsCRP, carotid stenosis, prognostic marker

DOI: 10.15386/mpr-2365

Manuscript received: 21.09.2021
Received in revised form: 16.03.2022
Accepted: 13.10.2022

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Introduction

Stroke is one of the main causes of mortality (21.64%) and morbidity (11.34%) in Romania, being a huge burden both for the caregivers and for the authorities (from a socio-economic point of view) [1,2]. For this reason, it was imperative to include in the research new biomarkers for the predictability and strict evaluation of stroke prognosis. Up to date, reliable markers for stroke prognosis are: imaging (computed tomography and MRI) and NIHSS (National Institute of Health Stroke Scale) score [3,4]. Numerous studies have questioned the role of inflammation in ischemic stroke, highlighting the role of biomarkers in the prognosis of patients with neurological vascular pathology. It was confirmed that inflammatory response aggravated ischemic brain damage and neurological dysfunction [4-6], being known that leukocytosis on admission can be associated with stroke severity and poor clinical outcomes in acute ischemic stroke (AIS) patients [7]. Neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR), as newfound and inexpensive biomarkers in systematic inflammation, have recently been proved to have diagnostic and predictive capabilities in multiple pathologies (including stroke) [8-10]. High neutrophil count and low lymphocyte count were regarded as correlation factors of unfavorable functional outcomes of acute cerebral infarction in many studies (Zhang et al., Xue et al.) [11-13]. As for platelets, information is not sufficiently clarified because the relativity between an increasing platelet count and clinical prognosis remains uncertain. Until now it is known only that platelets had a pivotal role in thrombogenesis and inflammation [14-16]. Therefore the role of inflammation in ischemic stroke is still insufficiently elucidated, which is why we need more studies that include different biomarkers physicians can rely on.

Methods

Study design

We conducted a prospective, observational, cross-sectional study during 2020, between January and June. The main objective of this study was to define the role of inflammation markers, i.e. neutrophil/ lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), high-sensitivity C – reactive protein (hsCRP) in ischemic stroke and whether there is a correlation of these markers with carotid stenosis. These would be helpful for physicians and for patients' relatives too, because it could predict disability and/or mortality in patients that suffered an ischemic stroke.

The study enrolled 150 patients, who were divided into two groups: a study group that included 100 patients (male and female) and a control group that included 50 patients (similar age and gender to the study group). The NIHSS Scale (National Institute of Health Stroke Scale) was used to assess the severity of the stroke.

The study was approved by the Ethics Committee of the "Lucian Blaga" University – Faculty of Medicine (code: PO-ULBS-PCD-301-CECSSUAE), the approved date is 2018.12.19, and was reapproved by the Ethics Committee of the Hospital.

Certain clear inclusion and exclusion criteria have been established in the study protocol, namely:

Inclusion criteria:

- adult, female or male subjects who have suffered an ischemic stroke
- brain imaging that highlights the lesion or its indirect signs
- onset of the disease <48 hours

Exclusion criteria for both groups:

- any condition that can influence inflammatory markers: infections, fever, solid neoplasms, autoimmune diseases, hematological diseases (lymphomas, multiple myeloma, etc.).
- patients who had recently (in the last 30 days) been treated with corticosteroids or immunosuppressants.
- patients with known severe cardiac pathology in the last 6 months: myocardial infarction, myocarditis.
- patients who at the time of the neurological examination sustained a traumatic brain injury.

Data collected retrospectively and at the time of inclusion in the study: age, gender, associated conditions, paraclinical examinations, brain imaging - computed tomography.

Data collected during the research (at the time of patient admission): 1) biological: 5 ml of peripheral blood were collected in a vial with clot activator and separating gel, from which the following laboratory tests were performed: hsCRP, neutrophils, lymphocytes, platelets. NLR and PLR were subsequently calculated as the ratio of neutrophils to lymphocytes, and platelets to lymphocytes and 2) paraclinical examinations: extracranial carotid Doppler ultrasound examination.

Both the neurological examination and the severity scale of the stroke (NIHSS) were performed by a neurologist at the time of hospitalization. Extracranial carotid Doppler ultrasound was performed during hospital admission in patients included in the study group. IMT was measured, cholesterol plaques were analyzed and possible carotid stenosis were quantified as a percentage (considering severe stenosis > 70%).

Statistical analysis

The database was created using Microsoft Office Excel 2010. SPSS 25.0 (SPSS Inc, Chicago, USA) was used for statistical analysis and data description. The normality of the quantitative data distribution was verified with the Shapiro-Wilk test (for small groups) or Kolmogorov - Smirnov. The significance level was α=0.05. The arithmetic mean ± standard deviation (DS) was used to describe the normally distributed continuous quantitative data. Qualitative data are expressed using absolute and

relative frequencies (%). The Student test (t-test) was used to compare the means of the corresponding quantitative variables of two independent groups. The Pearson correlation coefficient was calculated for the correlation analysis. Significance tests were used to estimate correlation coefficients (with α=0.05) and Colton's rules were used for empirical interpretation.

Results

To our knowledge, this is the first study in Romania that analyzes potential correlations between different inflammation markers, stroke severity and carotid stenosis.

Summary of results

1. Of the 150 subjects included in the research, in the study group the average age was 71.15±9.08 years, while in the control group the average age was 67.86±9.74 years, which is why we can conclude that age does not differ significantly between the two lots (Table I).

2. In both groups, the males were predominant, 60 male subjects were included in the study group and 28 in the control group. Gender distribution does not differ significantly between groups.

3. Women suffered an ischemic stroke at an older age (76 years) compared to men (68 years) - age is significantly higher in women (p<0.001), for the rest of the variables there are no significant differences in values in women compared to men (Table II).

4. In terms of risk factors, the most common one was smoking (in approximately 57% of subjects included in the study group), the second most frequent risk factor was obesity 43% (we mention that there were patients who are obese and smokers).

5. There is a significant difference between neu / lym ratio and plt/lym corresponding to the two groups.

6. In our study, hsCRP was strongly correlated with NIHS score (a strong and significant correlation - p<0.005), these patients having an unfavorable evolution of the stroke in most cases (Figure 1).

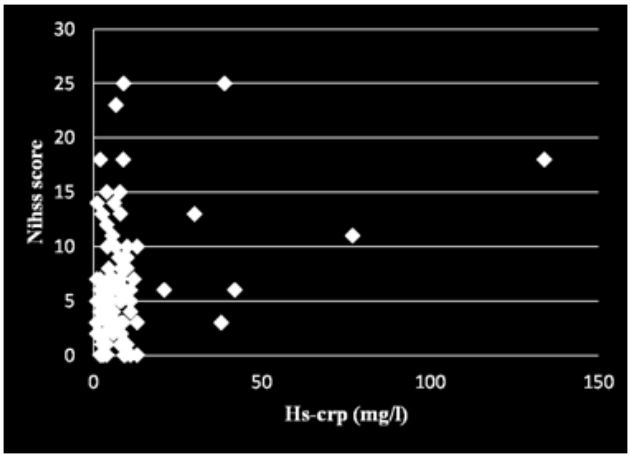

Figure 1. Strong correlation, directly proportional between, HsCRP and NIHSS (p < 0.005).

7. There was a strong, directly proportional correlation between plt / lym ratio and neu / lym ratio, with statistical significance (r=0.641, p<0.001).

8. NLR and PLR had a relatively linear correlation, with moderate strength directly proportional to NIHSS (p>0.005, index r in the range (0.25; 0.50)) (Figure 2, Figure 3).

Table I. Demographic, clinical and biochemical characteristics between the groups.

Variable	Study group	Control group	p-value
Age	71.15±9.08	67.86±9.74	0.051
NEU/LYM ratio	2.97±1.53	2.13±1.19	<0.001
PLT/LYM ratio	147.86±78.09	114.95±34.3	<0.001
PCR	9.48±16.1	2.13±2.42	<0.001
NIHSS at admission	6.61±5.35		
NIHSS at discharge	4.28±4.39		

Table II. – Comparisons of women vs. men in the study group.

Variable	Women	Men	p-value
Age	76.77±7.07	68.08±8.61	<0.001
Stenosis degree	68.51±25.49	68.59±26.67	0.989
NEU/LYM ratio	2.99±1.28	2.96±1.66	0.914
PLT/LYM ratio	156.73±73.84	143.02±80.47	0.406
PCR	8.54±13.48	9.99±17.45	0.670
NIHSS at admission	7.34±5.81	6.2±5.08	0.313

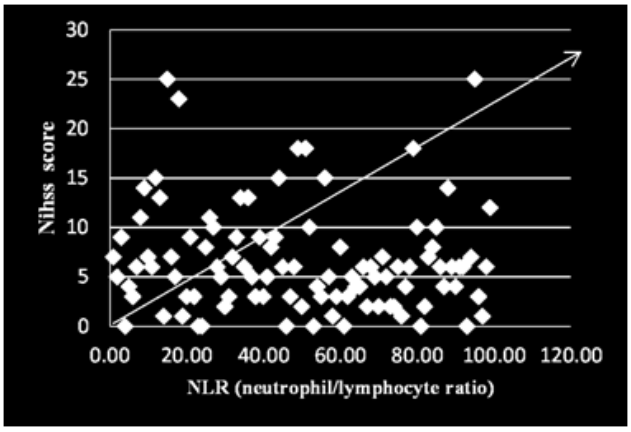

Figure 2. Moderate correlation, directly proportional, between NLR and NIHSS.

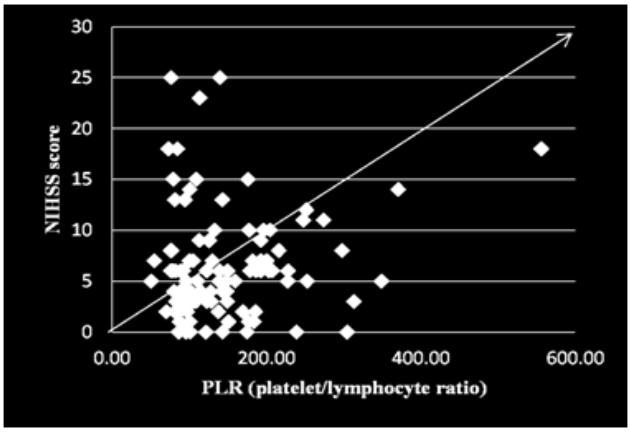

Figure 3. Moderate correlation, directly proportional, between PLR and NIHSS.

9. NLR and PLR were significantly higher in patients with a stroke caused by the occlusion of a large artery, located in the anterior territory (large vessel occlusion).

10. Brain imaging showed in approximately 72% of the subjects included in the study an ischemic stroke in the anterior territory, respectively 28% in the posterior territory.

11. Extracranial carotid Doppler ultrasound showed severe stenosis in approximately 59% of the subjects included in the study, most of them men and smokers.

12. Contrary to other studies published, the stenosis degree did not correlate with NLR and PLR reports, nor with hsCRP.

13. Of course, there were statistically significant differences for NIHSS at admission versus discharge for both women and men.

Discussion

Contrary to several studies in the literature (Rambe et al., 2013; Pranata et al., 2020) [3,4], in the present study, the prevalence of stroke was higher among male patients (64%), the severity being higher among female subjects included in the study (mean NIHSS at admission in female subjects: 7.34±5.81, male: 6.2±5.08). At the same time, as Boehme et al. (2016) pointed out, stroke is a pathology of the elderly, the risk increasing with each decade (it doubles every 10 years) [5].

The present study investigated the role of inflammation in cerebral ischemia, with the main purpose of developing a panel of biomarkers that can predict the evolution and prognosis of a patient who suffered an ischemic stroke. This would help health workers to provide as transparent, clear and relatively reliable information as possible, and caregivers, who could be informed from the onset of what to expect, without going through the uncertainty of the evolution of ischemic stroke.

Increased levels of HsCRP in acute cerebral ischemia indicate a clear inflammatory response as a result of tissue damage, which worsens the patient's condition (i.e. by activating chemotactic factors and opsonizing cells in close proximity of the brain injury) [6].

According to the latest studies in the literature (e.g. Reddy et al. 2020, Singh et al. 2019) [7,8], in the present study, HsCRP collected in the first 48 hours after onset, was strongly correlated ($p<0.005$) with NIHSS at admission and with large vessel occlusion (most often the occlusion of a large vessel in the anterior territory: the middle cerebral artery, respectively the anterior cerebral artery, correlates with a NIHSS that totals a large number of points). In support of this statement, we found several recent studies, including that of Ormstad et al. [9], which showed that a CRP>2.5 mg/l correlated with an infarct volume 10 times higher, compared to subjects who had a smaller CRP. Another study conducted at Teheran University by Ghabee et al. [10] showed that CRP and NIHSS have similar values in terms of both predictability and stroke prognosis as well as in patient mortality.

Different subtypes of white blood cells have different roles in ischemic stroke. Neutrophils accumulate in the blood vessel in the first hours after cerebral ischemia causing the extension of the infarction by preventing microvascular perfusion, while lymphocytes increase about 3-6 days after thrombosis, their role being insufficiently elucidated [11].

Recently NLR has been proposed as a predictive marker for coronary syndrome severity. Based on this, we considered extending the use of this ratio, along with the ratio between platelets and lymphocytes within the framework of cerebral ischemia. In the study we conducted, the two parameters (NLR and PLR) had a moderate, directly proportional correlation with the severity of the stroke ($p>0.005$, r index in the range (0.25; 0.50). There are multiple discussions in literature that support this moderate correlation, but which have implemented a cut off value of NLR.

Xue et al. [12] consider that a NLR>1.92 warns us of the patient's prognosis and confirms a high degree of stroke severity. There are studies, for example Őnder et

al. [13], which concluded that this cut-off value should be greater than 2.6, in order to have prognostic value, but the literature also presents studies (e.g. Lashin et al. [14]) which concluded that a NLR>1.34 is a reliable test for stroke predictability. We are in agreement with the literature, given that the average NLR in the group with neurological pathology included in the study conducted by us was 2.97, while in the control group the average was 2.19.

Circulating platelets have two main roles in ischemic stroke: first the well-known role in thrombosis and secondly the promoter of inflammation activators (chemokines, cytokines) that mediate other cells in the peripheral blood [15].

According to studies in recent literature (Du et al., 2020 [15]), platelet growth correlates with the risk of ischemic stroke, but without having any role in its predictability. Contrary to these studies, the authors claim that there is a moderate correlation in terms of PLR and stroke severity (NIHSS), in the study group the average value of PLR being 147.86, while in the control group 114.95. It should be noted that this biomarker is not as studied as NLR in the literature, but we believe that it has great potential in terms of prognosis of patients with stroke and should be expanded and studied in larger cohorts of patients.

Recently, it has been increasingly discussed that in studies that include patients with ischemic stroke and correlations with markers of inflammation and severity of stroke, they should be classified according to the affected vascular territory, more precisely the anterior or posterior territory. The reason behind this statement is that it is increasingly discussed that although the correlation of NLR with NIHSS in anterior and posterior stroke has been studied, there have recently been several studies that have shown that NLR is significantly better correlated with the volume of infarction in the anterior territory and also, it has no significant correlation in terms of infarction in the posterior territory (Kokaturk et al., 2019 [16]). We suspect that the reason that led to a moderate and not strong correlation of biomarkers (NLR and PLR) with stroke severity (NIHSS) was the fact that we did not classify patients according to the affected territory in our study. This can be explained by the fact that NIHSS helps us a lot in case of an anterior territory ischemia, having certain limitations in terms of posterior territory, which is why although patients may have an NIHSS that totals a small number of points, the condition can be very serious (for example, a small infarction in the posterior territory can be fatal, although it may have a relatively high NLR, but with a low NIHSS - bulbar infarction that may involve the respiratory centers, or minor hemorrhages with strong impact as in the case presented by Maier et al. [17] but which unfortunately it is not the subject of our study and warrants further thorough research).

More and more studies are discussing the fact that inflammation is closely linked to atherothrombosis, which has been partially demonstrated. To date, the literature certifies that the research is directed towards a correlation of inflammation markers (NLR, PLR, etc.) with carotid stenosis.

Koklu et al. 2016 [18] and Massiot et al. 2019 [19] found a strong correlation directly proportional between NLR and carotid stenosis in the patients included in the studies they conducted. Hyun et al. 2015 [20] correlated NLR with severe carotid stenosis in male patients, questioning the different hormonal involvement of the two genders. In our study, contrary to the current trend in the literature, and according to other contradictory studies published recently, NLR, PLR and hsCRP were not statistically significantly correlated with carotid stenosis. This was due to the fact that inflammation occurs in hypoechoic or ulcerated atheromatous plaques, which was not taken into account in the present study. At the same time, only the extracranial portion of the carotid arteries was analyzed. The study group included patients with predominantly fibrocalcalcic atheromatous plaques (no clear distinction was made in patients with hypo / hyperechoic plaques, respectively ulcerated), the reason for cerebral ischemia in these patients may be other than carotid stenosis / occlusion in the extracranial portion (for example paroxistic atrial fibrilation). Based on this finding, as Yuksel et al. 2016 [21] suggests, it can be argued that uncalcified plaques that produce intermediate stenosis of the internal carotid artery are associated with increased NLR (NLR limit value > 2.54, in our study NLR limit value > 2.61) may reflect a high risk of stroke and such cases should be closely monitored.

Limitations of the study

1. Relatively small study batch, conducted in a single center.

2. Follow-up of asymptomatic patients for more than one year in order to establish the risk of stroke over a long period of time was not taken into account.

3. The classification of patients according to the ischemic arterial territory was not performed and patients with fibrocalcic atheromatous plaques were not excluded.

4. Comparison of these markers with higher specificity markers such as: IL6, IL1β, TNF α may be considered in the future.

5. Serial blood collection could be considered for all biomarkers for comparison at a time interval (acute-subacute-chronic).

Conclusions

The results of our study hold the potential to have important clinical implications. The study showed remarkably increased levels of hsCRP, NLR and PLR during the few days after stroke onset and moderate to strong correlations with stroke severity. However, it failed to show significant association between carotid stenosis and any of the markers included in the study.

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