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Padi4 In Predicting The Severity And Recovery Of Early Stroke

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Abstract: A comparison of the results of the study with data on the role of PADI4 in transport and immunomodulation shows that this protein is important for cell regeneration and the prevention of irreversible consequences through the catalysis of posttranslational modifications. The level of PADI4 below 80.1 u/ml is associated with an increased risk of mortality, while its excess improves the chances of survival and correlates with favorable functional outcomes. Thus, measuring the level of PADI4 on the first day after an ischemic stroke can serve as an indicator of prognosis and a basis for correcting metabolic therapy.
Key words: PADI4, ischemic stroke, PLA2G2E, DGLA

Introduction. Today, ischemic stroke is a serious medical and social problem due to its prevalence and serious consequences for the health and quality of life of patients [1].

Just as with other pathological conditions, the body, through certain markers, gives signals about the degree of the state of the processes occurring in it. One of them is presumably the level of PADI4 (peptidylargine deiminase) in blood plasma. The significance of this signaling protein has not been found and confirmed in some studies [2].

The brain has a limited ability to regenerate after injury. The mechanisms of self-healing embedded in the brain itself are still poorly understood. A study by Nakamura Akari et al. (2023) found that secreted phospholipase PLA2G2E, produced by neurons near the lesion, creates digomo-γ-linolenic acid (DGLA), which plays a key role in activating cerebral self-healing after ischemic stroke. A decrease in Pla2g2e levels leads to a decrease in the expression of peptidyl arginine deiminase 4 (Padi4), an important transcription regulator in peri-infarct neurons. Single-cell RNA sequencing (scRNA-seq) and epigenetic analysis have shown that neural PADI4 is able to activate genes involved in recovery from ischemic stroke through histone citrullination. Among the DGLA metabolites, 15-hydroxy-eicosatrienoic acid (15-HETrE) was identified as a substance that stimulates PADI4 activity in neurons near the lesion site. The introduction of 15-HETrE into the body improved functional recovery after ischemic stroke. These results indicate the prospects of using brain self-healing activated by specific lipids to stimulate regeneration processes after traumatic brain injuries. The main functions of PADI4 are the catalysis of posttranslational protein modifications (PTMs) providing new functional groups that can be used to regulate protein function. In particular, it prevents the hydrolytic deamination of arginine protein to form citrulline, which plays an important role in protein homeostasis, which may be useful for reducing the risk of sarcopenia.

Since this substance can be metabolized in the body to arginine, the amino acid can promote the formation of nitric oxide and increase the production of this compound in the human body. Nitric oxide, in turn, can cause vasodilation of blood vessels, which leads to increased blood flow and increased efficiency of oxygen and nutrient transport to tissues and organs. Vasodilation caused by increased nitric oxide production can also lead to a decrease in blood pressure [3].

PAD4 was initially discovered as a protein whose expression increases during terminal differentiation of HL-60 cells, a line of leukemic cells, into granulocytes and monocytes [4], suggesting a possible role for this isoenzyme in cellular differentiation. Although this role has not yet been established [4], it is clear that PAD4 is expressed in the nucleus and cytoplasmic granules of differentiated neutrophils and plays a role in the formation of PTM. In addition to differentiation, PAD4 also affects gene expression through its ability to deiminate histones H2A, H3, and H4 [5]. This observation was very significant because it is known that

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histone modifications (e.g., methylation, phosphorylation, and acetylation) regulate gene expression, DNA repair, and replication by changing the local, and in some cases global, architecture of chromatin [6].
Currently, the main areas of PADI4 study are rheumatoid arthritis, multiple sclerosis, cancer, spondylitis, osteoarthritis, Alzheimer's disease, and HIV/AIDS. [3]. Given the biological role of PADI4, it can be assumed that it may be involved not only in the development of inflammatory autoimmune diseases, but also in other pathological processes unrelated to others, for example, in ischemic stroke, when extensive cell damage occurs. Currently, there is very little data in the scientific literature on the study of the role of PAD4 in patients with ischemic stroke, which served as the basis for conducting and defining the goals of this study.
Materials. The study examined 138 patients admitted to the basic hospital of the Department of Neurology of the Tashkent Medical Academy and the 4th City Clinical Hospital of Tashkent. The conditions for participation in the study were: diagnosed ischemic stroke, confirmed by neuroimaging methods, the age of patients aged 18 years and above, admission to the clinic in the first 24 hours after the onset of the disease, the absence of exacerbations of chronic or acute diseases of internal organs, as well as oncological diagnoses.
A total of 138 patients participated in the clinical trial. The average age of the patients was 64.7±0.92 years. Patients were divided into 2 groups, with low (GLL PADI4) and high (GHL PADI4) levels of PADI4 (Table 1).

Table 1.

Characteristics of the clinical research object

	Characteristics of the groups		Women		Men	
			n	Age M_{cp}	n	Age M_{cp}
1	GHL PADI4	n = 70	23	64,1±1,95	47	64,5±1,42
2	GLL PADI4	n = 68	26	63,4÷2,08	42	65,2±1,83

When considering the possibility of thrombolysis, additional criteria for selecting patients were applied, corresponding to the current recommendations for thrombolysis [7].
The patients were prescribed standardized basic treatment, and if necessary, thrombolytic and antithrombotic drugs were used [7].
In these groups, both clinical neurological and anamnestic examinations were performed in accordance with the standards of neurological examination [8,9]. The diagnosis of AI was established according to the criteria of ICD 10, the classification of O.S. Levin (2006), after collecting anamnesis, a thorough clinical and neurological examination, a study of the functions of the cognitive sphere, and magnetic resonance imaging (MRI). Nonparametric methods were used to process the results of laboratory tests and obtain clinical statistics. The degree of axiomaticity of P was 0.05. A clinical and neuropsychological assessment of patients was performed using special Rankin scales, Rivermead, MoCA (The Montreal Cognitive Assessment), NIHHS (National Institutes of Health Stroke Scale), Barthel index (Table 2). Neuroimaging research methods (MRI) were performed.

Scale indicators in the examined patients at the beginning of treatment (score) (M±m)

Groups	NIHSS	Rankin	Rivermead	MOCA	Barthel
GHL PADI4 (n=70)	9,70±0,32	3,59±0,09	5,11±0,45	21,1±0,31	64,8±0,81
GLL PADI4 (n=68)	9,91±0,44	3,72±0,07	4,42±0,51	19,4±0,22	51,9±0,92
3-rd group (healthy) (n=30)	1,12±0,18	0,52±0,05	14,8±0,11	26,8±0,18	99,8±0,34

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PADI4 (peptidylargine deiminase) was determined using a Sigma-Aldrich human recombinant kit expressed in E. coli, aqueous solution. The ABAP peptidylarginine deiminase Activity assay (antibody-based assay for peptidylarginine deiminase activity) for determining PAD activity was developed in 2007 [10] and is based on the detection of citrullinated peptides by an antibody that reacts with these peptides in a citrulline-dependent manner. Citrulline is a non-protein amino acid, this compound can stimulate protein synthesis and, thus, can play an important role in protein homeostasis, which may be useful for reducing the risk of sarcopenia. Since this substance can be metabolized in the body to arginine, the amino acid can contribute to the formation of nitric oxide and increase the production of this compound in the human body. Nitric oxide, in turn, can cause vasodilation of blood vessels, which leads to increased blood flow and increased efficiency of oxygen and nutrient transport to tissues and organs. Vasodilation caused by increased nitric oxide production can also lead to a decrease in blood pressure.

Peptides containing arginine, for example, a peptide corresponding to the citrullinated filaggrin epitope recognized by RA autoantibodies, are immobilized on a 96-well microtiter plate and incubated with PAD-containing samples. The conversion of peptidylarginine to peptidyl citrulline can subsequently be detected using an antibody that specifically reacts with the citrullinated peptide, for example, a single-stranded variable fragment of RA3 [11] or antibodies from the serum of patients with RA positive for ACPA [10]. Finally, detection and visualization of bound antibodies are performed using classical ELISA procedures. It was shown that the ABAP analysis is relatively sensitive, since the threshold for the detection of citrulline was about 5 pmol, which is between the colorimetric analysis in microtiter plates and the fluorimetric HPLC method. In addition, the presence of endogenous uranium-containing compounds does not affect the analysis, which makes it very suitable for determining PADI4 activity in complex biological samples. In healthy patients, PADI4 activity levels were 80-200 u/ml.

The data obtained during the clinical trial was analyzed using the STATISTICA program for Windows 6.0.

Results and discussion. When patients with ischemic stroke were monitored for a month, death was observed in 16.6% of cases (23 people), which was most often recorded on the tenth day of the disease (Q 4.9-16), mainly due to cerebral edema. Fatal outcomes were observed in the group with low PADI4 values. Patients with a good outcome on the Rankine, MOSS, NIHSS scale and the Bartel index after a month of rehabilitation could be noted in the GBU PADI4 group, with a weak presence of a good outcome in the GBU PADI4 group. According to the results of the Cox regression analysis conducted in stages, statistically significant negative effects of low PADI4 levels on the probability of death were observed (B = - 0.328). The odds ratio (OR) was 0.71.

According to the results of the study, a decrease in the level of PADI4 by one unit was accompanied by an increase in the probability of death by 0.71 times. Kaplan—Meyer curves demonstrated a higher risk of death at low levels of PADI4 compared to the probability of survival. The Kaplan—Meyer analysis revealed a threshold level of PADI4 associated with an increased mortality risk of 80.1±5.7 (93%) units/ml, which is confirmed by the coordinates on the characteristic curve.

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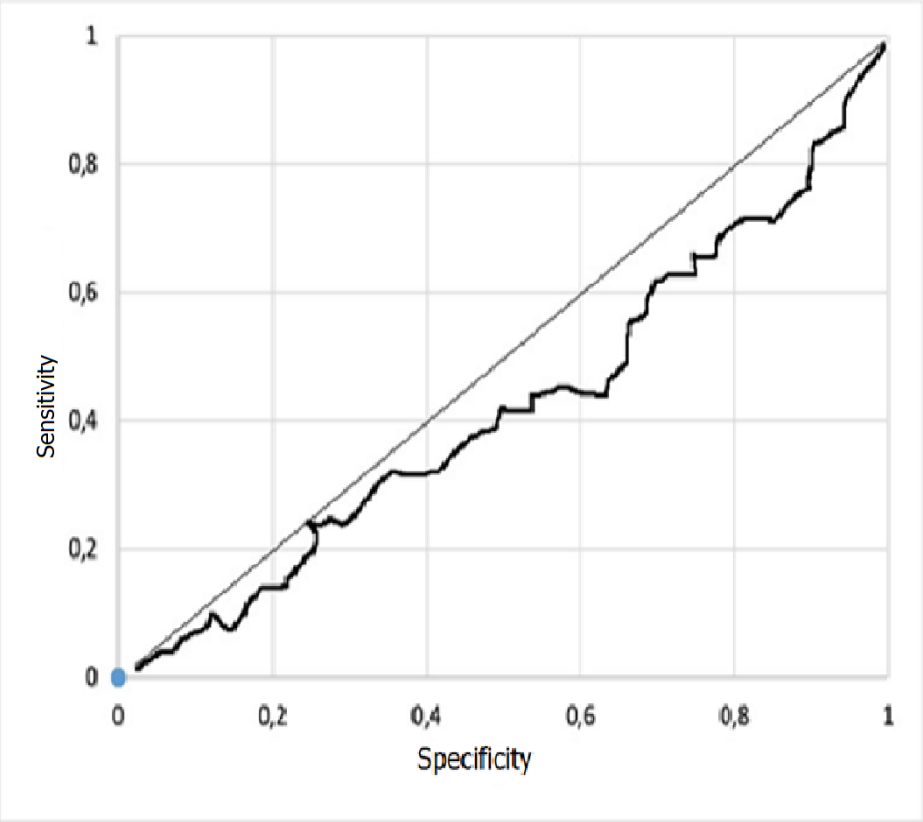

Figure 1 Relationship between the level of PADI4 and the probability of death

The accuracy of the PADI4 test at levels above 80.1 u/ml for predicting death was 47.1% (95% confidence interval: 36.6—50.7%), and at levels below 75.4% (95% confidence interval: 66.8—82.3%). An analysis of the operating curve reflecting the probability of death showed that PADI4 was statistically significantly associated not with the death outcome, but with the opposite - with survival, since the curve was located on the same diagonal line (see Fig.1). Therefore, an increase in PADI4 concentration above 80.1 u/ml correlates with an increased chance of survival in patients who have suffered an ischemic stroke.

To study the effect of PADI4 on improving the functional state measured on the Rankin scale, a sequential logistic regression analysis was performed. The odds ratio (OR) was 1.52, which indicates that an increase in the concentration of PADI4 per unit is associated with an increase in the probability of a favorable result on the Rankine scale by 1.52 times. The analysis based on the Kaplan-Meyer method revealed the threshold level of PADI4, which is important for predicting functional improvement on the Rankine scale, which was confirmed by the analysis of curve points – 80.1u/ml (95%). The prognostic value of exceeding this threshold (PADI4>80.1u/ml) for a favorable outcome was 68.2%, while for an unfavorable outcome it was 51.5%. The curve reflecting the prognosis of the severity of the condition confirmed the statistical significance of PADI4 in predicting an improvement in functional status on the Rankine scale. The area under the curve was 0.711.

Data analysis performed using logistic regression, the Kaplan-Meyer method, and graphs reflecting the dependencies of the Barthel index and the MOSS scale demonstrated similarity with the results obtained using the Rankine scale to evaluate PADI4. In this regard, the same threshold level of PADI4 as for the Rankin scale was used to predict a favorable functional outcome according to the Barthel index and the MOSS scale: 80.1u/ml. This threshold proved to be more informative when assessing the probability of good functional recovery using the Barthel index and the MOSS scale than when using the Rankine scale. The relative risk (OR) was 90.7, which means that an increase in the PADI4 level by one is associated with a 1.5-fold increase in the probability of achieving a good functional result according to the Barthel index and the MOSS scale. The prognostic value of the positive test (PADI4 > 80.1u/ml) for functional recovery according to the Barthel index and the MOSS scale was 79.7%, and the negative test was 53.5%.

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Conclusion. To summarize, a comparison of the results of the study with the existing data on the transport and immunomodulatory role of PADI4 allows us to conclude that this protein reflects the adequacy of cell regeneration, which is crucial for the restoration of affected tissues and the prevention of irreversible consequences by catalyzing posttranslational modifications of proteins (PTMs) providing new functional groups that can use it to regulate the function of proteins. A PADI4 value below 80.1 u/ml is associated with an increased risk of mortality. The level of PADI4 exceeding the specified limit significantly improves the chances of survival, and its further increase, above 80.1 u/ml, correlates with the subsequent favorable functional outcome, estimated by the Rankine and Bartel scales. Therefore, measuring PADI4 levels on the first day after the onset of ischemic stroke can serve as an indicator of disease prognosis and a basis for correcting metabolic therapy in order to improve survival and functional recovery.

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