ORIGINAL ARTICLE

CONTENTS

Diagnostic value of lipoprotein-associated phospholipase A2 in patients in the early recovery period of atherothrombotic stroke

Olga J. Mykhaloiko, Viktoriya A. Gryb, Ivanna-Romana M. Fabryka, Oleksandra I. Hotsaniuk, Uliana P. Shalamai, Tetiana I. Salyzhyn, Igor J. Mykhaloiko
IVANO-FRANKIVSK NATIONAL MEDICAL UNIVERSITY, IVANO-FRANKIVSK, UKRAINE

ABSTRACT

Aim: The purpose of the study is to study changes in Lp-PLA2 in the blood of patients in the early recovery period of atherothrombotic stroke depending on the degree of stenosis and type of atherosclerotic plaque.

Materials and Methods: A clinical and laboratory analysis of 130 patients in the early recovery period of atherothrombotic stroke was conducted. Among those examined were 69 men and 61 women aged (60.42±7.4) years. The control group consisted of 30 practically healthy persons (16 men and 14 women) without a history of severe somatic pathology, aged (58.7±6.3) years. Ultrasound scanning of the vessels of the neck was performed on a Siemens Acuson X 300 device. The amount of lipoprotein-associated phospholipase A2 (Lp-PLA2) was determined by the immunoenzymatic method. STATISTICA 8 software was used for statistical processing.

Results: In all examined patients, an increase in the content of Lp-PLA2 in the blood up to 260 (220.4; 295.7) ng/ml was found compared to individuals of the control group ($p<0.05$). Increased levels of Lp-PLA2 in stroke patients were associated with an increased degree of atherosclerotic stenosis and depended on the type of atherosclerotic plaque, reflecting its instability.

Conclusions: Increased concentrations of Lp-PLA2 in patients with cerebral atherosclerosis can be considered as circulating biomarkers of atherosclerotic plaque vulnerability. Patients in the early recovery period of an ischemic stroke with a soft atherosclerotic plaque according to ultrasound data in combination with an increase in Lp-PLA2 indicators in the blood belong to the group of high risk of developing a repeated stroke.

KEY WORDS: lipoprotein-associated phospholipase A2, atherothrombotic stroke, atherosclerotic plaque, early recovery period of ischemic stroke

Wiad Lek. 2025;78(6):1119-1124. doi: 10.36740/WLek/204797 DOI

INTRODUCTION

Despite significant advances in modern medicine, cardiovascular diseases and their complications, including strokes, remain the main cause of mortality worldwide. 30-40% of all ischemic strokes are the atherothrombotic subtype of stroke, the pathogenetic mechanism of which is arterio-arterial embolism from thrombotic deposits on the surface of atherosclerotic plaque, which lead to occlusion of intracranial arteries. According to modern ideas, the basis of the development and progression of atherosclerosis is endothelial dysfunction, which occurs against the background of chronic inflammation [1-2]. The initial phase of the inflammatory process in the area of atherosclerotic artery damage is asymptomatic. Clinical manifestations are present only in the case of hemodynamically significant atherosclerotic stenosis of the artery, which is manifested by a lack of blood supply to the affected area, or due to the development of thrombogenic complications arising from the destabilization and disintegration of the atherosclerotic plaque [3-4].

The traditional definition of atherosclerotic lesions of cerebral vessels is an ultrasound examination (US) with measurement of the lumen of the stenosis and the characteristics of the atherosclerotic plaque. However, despite the informativeness of ultrasound of atherosclerotic vascular damage, predicting the vulnerability of plaques remains difficult. In search of new opportunities to detect the stage of inflammation in atherosclerotic plaque, vascular markers of inflammation and their relationship with the risk of developing complications have been widely studied in recent years [2, 5]. Determination of potentially new biomarkers of the progression of atherothrosclerosis in comparison with clinical and ultrasound parameters would allow to characterize the activity of the atherosclerotic process, predict the risk of its progression and determine the probability of embolic complications.

One of the most studied markers of inflammation in recent years is lipoprotein-associated phospholipase A2 (Lp-PLA2). The role of lipoprotein A as a risk factor for cardiovascular diseases has been studied for many years [5-6]. Features of Lp-PLA2 that attract the attention of researchers are the specificity for vascular inflammation.

European recommendations for the treatment of dyslipidemias, issued by the European Society of Cardiology together with the European Society of Atherosclerosis, recommend measuring Lp-PLA2 in people with early development of cardiovascular diseases, in people with familial hypercholesterolemia, in people with a family history of early cardiovascular diseases (CVD) and in individuals with recurrent CVD episodes despite optimal lipid-lowering therapy, who have a 10-year SCORE risk ≥5% [6-7]. On the other hand, the American Lipid Association does not include Lp-PLA2 screening as one of the indicators of the standard lipid panel, which is partly due to the difficulty of standardizing methods for its determination [2, 8].

Lp-PLA2 belongs to the phospholipase A2 family and is produced by monocytes, mast cells, Kupffer cells, and T lymphocytes.

In plasma, 80% of Lp-PLA2 is bound to LDL-C, the remaining 20% is bound to HDL-C and VLDL. The main properties of Lp-PLA2 include reflecting the degree of intravascular inflammation and instability of atherosclerotic plaque. That is, Lp-PLA2 has specificity in relation to vascular inflammation, while other biomarkers, such as CRP, reflect the presence of systemic inflammation. Lp-PLA2 takes a direct part in atherogenesis, causing modification of lipids by hydrolysis of phosphatidylcholine with the formation of lyso-phosphatidylcholine and oxidation of free fatty acids, which stimulate the development of atherosclerosis. An increase in Lp-PLA2 in the blood directly reflects an increase in the risk of cardiovascular events [3, 8-9].

According to the results of one of the European studies, when observing patients with stable coronary heart disease, the level of Lp-PLA2 from 30 to 50 mg/dL was associated with a threefold increase in the risk of amputation of the lower limbs, while in patients with Lp-PLA2 >50 mg/dL - with twentyfold [4, 10-11].

The MONICA (The Monitoring Trends and Determinants in Cardiovascular Diseases) study examined the relationship between Lp-PLA2 levels and the risk of coronary events in 934 healthy men aged 45–64 years. Observations were carried out for 14 years. During the follow-up period, 97 men experienced acute coronary events and had significantly higher baseline Lp-PLA2 levels compared to controls.

In the Atherosclerosis Risk in Communities (ARIC) study of 12819 middle-aged men and women followed for 6 years, the combination of high Lp-PLA2 and high CRP was superior to either marker taken alone. by the ability to predict the risk of developing coronary events [2, 10].

The categories of patients who need to determine the concentration of Lp-PLA2, as well as the cut-off level, are actively discussed by experts. A value of ≥200 ng/ml was taken as the clinical threshold of the Lp-PLA2 level, which gives the right to make a decision on reclassification of risks. The researchers approved this threshold, which is based on the results of a study that showed a significant increase in the risk of cardiovascular events in patients who exceeded this threshold. Interpretation of Lp-PLA2 levels in blood serum is carried out as follows: low degree of cardiovascular risk - (< 200 ng/ml); average degree of risk - (200 - 235 ng/ml); high degree of risk - (> 250 ng/ml). The level of Lp-PLA2 more than 250 ng/ml has a high correlation with endothelial dysfunction, which in turn is closely related to the process of progression of atherosclerosis [2, 6, 12].

The understanding that Lp-PLA2 is actively synthesized in the places of atherosclerotic lesions and causes multiple proatherogenic and prothrombotic effects determines the relevance of determining this marker for diagnosis and assessment of the severity of the atherosclerotic process.

AIM

The aim of the study is to study changes in Lp-PLA2 in the blood of patients in the early recovery period of atherothrombotic stroke depending on the degree of stenosis and type of atherosclerotic plaque.

MATERIALS AND METHODS

A clinical and laboratory analysis of 130 patients in the early recovery period of atherothrombotic stroke (ERPAS) was performed. Among the examined were 69 men and 61 women aged (60.42±7.4) years.

The control group consisted of 30 practically healthy persons (16 men and 14 women) without a history of severe somatic pathology and disorders of cerebral blood circulation, aged (58.7±6.3) years.

Ultrasound duplex scanning of the vessels of the neck with the determination of the degree of atherosclerotic stenosis and the nature of the atherosclerotic plaque was performed on a Siemens Acuson X 300 device with a linear multifrequency sensor from 4–10 mHz) according to standard methods.

The classification of stenotic-occlusive lesions of cerebral arteries was carried out according to the classification of B. V. Gaidar et al.. According to these

Table 1. The level of lipoprotein-associated phospholipase A2 in the blood of patients in the early recovery period of atherothrombotic stroke depending on the degree of atherosclerotic stenosis

Lp-PLA2; ng/ml Me (q_1 ; q_3)	control group (n=30)	The degree of atherosclerotic damage			
		group A: <40% (n=26)	group B: 40– 60% (n=48)	group C: 60-75 (n=42)	group D: 75-95% (n=14)
	200.75 (185.1; 202,2)	255.9 (234.5;263.3) p<0,05	272.9 (268.5;280.3) p<0,05 p_1<0,05	280.9 (275.5;285.3) p<0,001 p_1<0,05 p_2>0,05	290.9 (284.5;294.3) p < 0,001, p_1 < 0.05, p_2<0,05, p_3>0,05.

Notes:
p – the reliability of the differences in indicators compared to the control group;
p1 – the reliability of the differences in indicators of groups B, C, D in comparison with group A;
p2 – the reliability of the differences in indicators of groups C, D in comparison with group B;
p3 – the reliability of the differences in the indicators of groups D in comparison with group C.

criteria, stenosis of the main artery of the 1st degree was diagnosed when the vessel narrowed to 40% of the diameter, 2nd degree – 40–59%, 3rd degree – 60–74%, 4th degree – 75–90%, 5th degree – more than 90%, hemodynamically significant considered stenosis IV–V degree.

The nature of atherosclerotic plaques was divided into five types according to the Nicolaidese and Geroulaka classification: I type: only echonegative ("soft" homogeneous plaque); II type: mostly echonegative with more than 50% hypoechoic areas (heterogeneous hypoechoic plaque); Type III: mostly echo-positive with more than 50% hyperechoic areas (heterogeneous hyperechoic plaque); IV type: only echo-positive ("dense" homogeneous plaque); V type: pronounced calcinosis, which gives an acoustic shadow.

The amount of Lp-PLA2 was determined using an enzyme-linked immunosorbent assay (ELISA) kit (Diazyme Laboratories Inc., Poway, California, USA). Plasma was added to the microplate wells with anti-Lp-PLA2 monoclonal antibodies (2C10) and incubated for 10 min at room temperature. Then the second monoclonal antibodies (4B4), labeled with horseradish peroxidase enzyme, were added and incubated for 180 min. Wells were washed and tetramethylbenzidine substrate was added. After 20 min of incubation, absorbance at 450 nm was measured, which is directly proportional to the concentration of Lp-PLA2 in the plasma. The concentration of Lp-PLA2 is expressed in units of ng/ml.

The study was approved by the Bioethics Commission of Ivano-Frankivsk National Medical University (protocol No 21 dated 09.27.2022). Patients gave written informed consent for the above diagnostic procedures and participation in this research project.

STATISTICA 8 software was used for statistical processing. The frequency of qualitative indicators was presented in absolute (n) and relative (%) frequencies. When analyzing quantitative data, the nature of the distribution of indicator values was determined using the Shapiro-Wilk's test method. For quantitative data with a normal distribution, the results were presented in the form of «M (σ)» (where M is the average value, and σ is the mean square deviation), and for quantitative data with a non-normal distribution - in the form of «Me (Q1; Q3)» (where Me is the median, and Q1, Q3 are quartiles). Quantitative indicators with non-normal distribution of values in 2 independent groups were compared by the Mann–Whitney method. Comparison of 2 independent groups according to the qualitative indicator was carried out according to Fisher's exact test.

RESULTS

According to a duplex scan of the vessels of the head and neck, all patients in the early recovery period of atherothrombotic stroke had atherosclerotic stenoses of varying degrees. The localization of the atherosclerotic plaque corresponded to the side of the affected cerebral hemisphere. The largest number of patients had atherosclerotic layers, causing stenosis by 40-75%. There were 14 patients with hemodynamically significant stenoses, 4 of whom had more than 90% stenosis, all of whom were recommended carotid endarterectomy.

An increase in the content of Lp-PLA2 in blood up to 260 (220.4; 295.7) ng/ml (p<0.05) was found in all examined patients with ERPAS compared to 200.75 (185.1; 202.2) in healthy individuals. Depending on the severity of the degree of atherosclerotic stenosis, an increase in the level of Lp-PLA2 was noted. The highest values of Lp-PLA2 were recorded in the group of patients with hemodynamically significant atherosclerotic stenoses (Table 1).

Table 2. The level of lipoprotein-associated phospholipase A2 in the blood of patients in the early recovery period of atherothrombotic stroke depending on the type of atherosclerotic plaques

	Types of atherosclerotic plaques					control group, (n=30)
	I (n=18)	II (n=44)	III (n=34)	IV (n=48)	V (n=16)	
Lp-PLA2; ng/ml Me (q_1; q_3)	292.2 (280.7;293.3) p<0,001	283.9 (274.5;285.5) p<0,05 p_1>0,05	275.9 (268.5;281..5) 280.3) p<0,05 p_1<0,05 p_2>0,05	267.9 (268.5;272.3) p<0,05 p_1<0,05 p_2<0,05 p_3>0,05	258.9 (244.5;262.3) p<0,05 p_1<0,05 p_2<0,05 p_3<0,05 p_4<0,05	200.75 (185.1; 202,2)

Notes:
p– the reliability of the differences in indicators compared to the control group;
p_1 – reliability of differences in indicators from II, III, IV, V in comparison with group I;
p_2 – the reliability of the differences in indicators of groups III, IV, V in comparison with group II;
p_3 – the reliability of the differences in indicators of groups IV, V in comparison with group III;
p_4 – the reliability of the differences in indicators of groups V in comparison with group IV.

All patients were taking antiplatelet agents, statins, and hypoglycemic drugs (in the presence of diabetes) for the purpose of secondary prevention of stroke. It should be noted that statin therapy causes biochemical remodeling of the atherosclerotic plaque with a predominant effect on the lipid components than on the total plaque size. Despite this fact, atherosclerotic plaques of type I (echo-negative homogeneous plaques) and type II (echo-negative with hypoechoic inclusions more than 50%) were noted in 18 patients, which have a high degree of embologenicity and, accordingly, can be the cause of repeated atherothrombotic stroke.

Unstable atherosclerotic plaques, for the most part, had a heterogeneous structure with hypoechoic inclusions, an uneven surface, and existing layers. Stable plaques were homogeneous, hyperechoic. Concentration changes of Lp-PLA2 also depended on the structure of the atherosclerotic plaque. The highest levels of Lp-PLA2 were recorded in patients with I, II and III types of atherosclerotic plaques. Accordingly, in the group with stable, calcified atherosclerotic plaques, the level of Lp-PLA2 was the lowest among the subjects, however, it was higher than in the control group (Table 2).

When analyzing the obtained data, it was established that in patients with the same parameters of the level of stenosis, but different morphological density of atherosclerotic layers, significantly higher values of Lp-PLA2 were recorded in patients with soft, unstable atherosclerotic plaques (285.9 (265.5; 295.3)) compared to stable (250.2 (245.9; 267.3)). That is, the increase in the concentration of Lp-PLA2 depended more on the structure of the atherosclerotic plaque than on its size. Based on the results obtained by us, patients with a soft atherosclerotic plaque according to ultrasound data in combination with an increase in Lp-PLA2 indicators in the blood belong to the group of high risk of recurrent stroke.

DISCUSSION

Atherosclerosis is a chronic inflammatory disease that causes dysfunction of endothelial cells, influx of inflammatory leukocytes into the subendothelial space and oxidation of LDL. As plaque inflammation develops, it destabilizes and becomes prone to rupture. Plaque rupture is responsible for acute manifestations of atherosclerosis, including myocardial infarction, unstable angina, stroke, and death. Identification of markers of plaque destabilization at the preclinical stage is a justified direction in the search for predictors of cardiovascular catastrophes. Among all known systemic markers of inflammation and endothelial dysfunction, Lp-PLA2 has specificity for vascular inflammation, which is why it is of interest. [1, 4, 7].

The results of our study, which indicate the diagnostic significance of determining Lp-PLA2 in patients at high risk of recurrent stroke, echoed the results of other studies. The JUPITER study showed that patients with high Lp-PLA2 activity (fourth quartile) had a more than two-fold increased relative risk (HR 2.15, 95% CI: 1.13-4.08) of cardiovascular events compared to patients with low activity (first quartile). The Bruneck study showed that the population in the third tertile of Lp-PLA2 activity had a higher relative risk of cardiovascular events (HR 2.2, 95% CI: 1.1-4.8) compared with those in the first tertile [2, 11]. Similar results were obtained in the LIPID trial that entailed subjects with history of acute coronary syndrome in whom Lp-PLA2 activity was

associated to a higher risk of cardiovascular mortality (HR 1.32, 95%CI: 1.00-1.75) [12].

Lipoprotein-associated phospholipase A2 (Lp-PLA2) is a promising marker of atherosclerotic plaque destabilization, which plays a key role in the metabolism of pro-inflammatory phospholipids and in the generation of proatherogenic metabolites. Since there is much evidence for the key role of inflammation in atherothrombosis, and the main product Lp-PLA2 is a potent pro-inflammatory molecule, it may be not only a predictor of cardiovascular disease risk, but also a target for therapeutic intervention.

CONCLUSIONS

The level of Lp-PLA2 in patients in the ERPAS was significantly higher compared to the control group.

Increased levels of Lp-PLA2 in stroke patients were associated with an increased degree of atherosclerotic stenosis and depended on the type of atherosclerotic plaque, reflecting its instability.

Patients in the early recovery period of an ischemic stroke with a soft atherosclerotic plaque according to ultrasound data in combination with an increase in Lp-PLA2 indicators in the blood belong to the group of high risk of developing a repeated stroke.

REFERENCES

1. Benjamin EJ, Virani SS, Callaway CW et al. Heart Disease and Stroke Statistics 2018 update: a report from the American Heart Association. Circulation. 2018;137(12):67-492. doi: 10.1161/CIR.0000000000000558. DOI
2. Garg PK, McClelland RL, Jenny NS et al. Lipoprotein-associated phospholipase A2 and risk of incident cardiovascular disease in a multi-ethnic cohort: The multi ethnic study of atherosclerosis. Atherosclerosis, 2015;241(1):176-182. doi: 10.1016/j.atherosclerosis.2015.05.006. DOI
3. Banerjee C, Chimowitz M. Stroke caused by atherosclerosis of the major intracranial arteries. Circulation Research. 2017;120(3):502–513. doi: 10.1161/CIRCRESAHA.116.308441. DOI
4. Ezhov M, Safarova M, Afanasieva O. Lipoprotein(a) level and apolipoprotein(a) phenotype as predictors of long-term cardiovascular outcomes after coronary artery bypass grafting. Atherosclerosis. 2014;235:477-82. doi: 10.1016/j.atherosclerosis.2014.05.944. DOI
5. Han L, Zhong C, Bu X. Prognostic value of lipoproteinassociated phospholipase A2 mass for all-cause mortality and vascular events within one year after acute ischemic stroke. Atherosclerosis. 2017;266:1–7. doi: 10.1016/j.atherosclerosis.2017.09.013. DOI
6. Marcovina S, Albers J. Lipoprotein(a) measurements for clinical application. J Lipid Res. 2016;(57):526-37. doi: 10.1194/jlr.R061648. DOI
7. Mykhalojko OY, Mykhalojko IY. Potential circulating biomarkers of atherosclerotic plaque vulnerability in patients in the early recovery period of atherothrombotic stroke. Ukrainian Biochemical Journal. 2023;(95)1:58-63. doi: 10.15407/ubj95.01.058. DOI
8. Liu H, Yao Y, Wang Y et al. Association between high-sensitivity C-reactive protein, lipoprotein-associated phospholipase A2 and carotid atherosclerosis: A cross-sectional study. J Cell Mol Med. 2018;22(10):5145-5150. doi: 10.1111/jcmm.13803. DOI
9. Huang F, Wang K, Shen J. Lipoprotein-associated phospholipase A2: the story continues. Medicinal Research Reviews. 2020;40(1):79–134. doi: 10.1002/med.21597. DOI
10. Wong N, Zhao Y, Sung J, Browne A. Relation of first and total recurrent atherosclerotic cardiovascular disease events to increased lipoprotein(a) levels among statin treated adults with cardiovascular disease. Am J Cardiol. 2021;(145):12-17. doi: 10.1016/j.amjcard.2020.12.075. DOI
11. Maiolino G, Bisogni V, Rossitto G, Rossi GP. Lipoprotein-associated phospholipase A2 prognostic role in atherosclerotic complications. World J Cardiol. 2015;7(10): 609-620. doi: 10.4330/wjc.v7.i10.609. DOI
12. Karakas M, Koenig W. Lp-PLA2 Inhibition – The Atherosclerosis Panacea? Pharmaceuticals. 2010;3(5):1360-1373. doi: 10.3390/ph3051360. DOI

CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Olga J. Mykhaloiko
Ivano-Frankivsk National Medical University
2 Halytska St., 760008 Ivano-Frankivsk, Ukraine
e-mail: myhalojko@i.ua

ORCID AND CONTRIBUTIONSHIP
Olga J. Mykhaloiko: 0000-0003-0618-4501 A B C D E F
Viktoriya A. Gryb: 0000-0001-6111-7921 F
Ivanna-Romana M. Fabryka: 0000-0002-1154-3241 A B
Oleksandra I. Hotsaniuk: 0000-0001-9691-7419 B
Uliana P. Shalamai: 0000-0001-7044-3585 B C
Tetiana I. Salyzhyn: 0000-0003-4206-622X B
Igor J. Mykhaloiko: 0000-0002-6288-1806 A B C E F

A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval of the article

RECEIVED: 07.11.2024
ACCEPTED: 07.05.2025