“Stroke-Stop” Formula: A Tool for Risk Index Determination in Development of Acute Cerebrovascular Disease in Patients with Asymptomatic Internal Carotid Artery Stenosis

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Abstract

Background: Extracranial carotid artery disease is considered a risk factor for developing acute cerebrovascular diseases. The paper presents the “Stroke-Stop” formula proposed for the determination of the risk of developing stroke in patients with asymptomatic internal carotid artery (ICA) stenosis. The formula is based on a mathematical calculation of the major risk factors for stroke: the degree of ICA stenosis, the morphological structure of the atherosclerotic plaque and the level of lipoprotein-associated phospholipase A2 (Lp-PLA2) concentration.

Methods: The study included 70 patients with atherosclerotic ICA stenosis. Among vascular inflammatory markers, Lp-PLA2 was determined with concentration 252.7-328.6 mg/l. The obtained results were evaluated using descriptive statistics (the frequency, percentage ratio) as well as the one-way analysis of variance (ANOVA) and chi-square test.

Results: The risk of stroke development is eminently increasing with the progression of ICA stenosis and elevation of Lp-PLA2 levels. In patients with soft atherosclerotic plaque, the risk of stroke development was significantly higher in correlation with patients with hard atherosclerotic plaque. Based on calculations using “Stroke-Stop” formula, three main groups were generated: low (<70 points), medium (70 – 100 points) and high (>100 points) risk of stroke development.

Conclusions: The “Stroke-Stop” formula may serve as an additional criterion for individual selection of patients with asymptomatic ICA stenosis for carotid endarterectomy. This model could be used as a diagnostic and prognostic tool to identify patients with potentially high risk development of ischemic stroke in clinical praxis.

1 Background

Extracranial carotid artery disease is considered a risk factor for developing acute cerebrovascular diseases [1]. Despite a large number of scientific researches dedicated to prevention, diagnosis and treatment of stroke, the problem is still relevant and remains unresolved [2].

The ratio of ischemic: hemorrhagic stroke incidence is (80–85%):(15–20%), respectively and the leading cause of cerebral ischemia (approximately 40–45%) is atherosclerotic internal carotid artery (ICA) stenosis [3].

In patients with symptomatic ICA stenosis > 50%, the average annual risk of recurrent stroke is 37–40%, while in patients with asymptomatic ICA stenosis > 70%, the risk of developing stroke is 3–6%. At the same time, the risk of developing postoperative complications is from 2 to 5% [4, 5]. It indicates the fact that performing carotid endarterectomy in patients with symptomatic ICA stenosis allows reducing the risk of stroke development by approximately 9–10 times, while in patients with asymptomatic ICA stenosis – by 1.5-2 times only [6]. Therefore, the criteria for selecting patients for surgical prevention of acute cerebrovascular disease among patients with asymptomatic ICA stenosis are broadly discussed

nowadays [7, 8]. There have been several studies, where in addition to atherosclerotic ICA stenosis, the morphological structure of the atherosclerotic plaque was taken into consideration [9–11]. In recent years, researchers have widely investigated vascular inflammatory markers as well as the relationship between their concentration and the risk of developing atherosclerotic complications [12]. Specific biomarkers are crucial tool since enabling the early prevention of non-symptomatic stages of diseases and also support prognostic/predicted and patient- centered treatment. The useful biomarker has to correlate with clinical parameters, such as specific symptoms, clinical signs and validated diagnostic tests [13]. One of the inflammatory markers correlating to atherosclerosis complications with high specificity is the lipoprotein-associated phospholipase A2 (Lp-PLA2) [14, 15].

Duplex ultrasonography is one of the main screening methods for detection of atherosclerotic ICA stenosis [16, 17]. It allows detecting the degree of ICA stenosis as well as evaluating the structure of the atherosclerotic plaque [18]. It is the degree of stenosis that serves as the main indication for performing carotid endarterectomy [5]. However, according to recent reports, approximately 20–25% of symptomatic patients have ICA stenosis < 70% [19]. This means that one criterion alone, namely the degree of ICA stenosis is not enough to determine the risk of stroke development in asymptomatic patients since this category of patients (20–25%) is not under active supervision by physicians.

Together, these data confirm that the problem of early detection of patients at the highest risk of developing stroke remains relevant.

2 Methods

2.1 Patient selection

This study has been cleared by the Ethics Committee of the East Slovak Institute of Cardiovascular Diseases (07/12/2015) for human studies and that patients have signed an informed consent.

The study included 70 patients with atherosclerotic ICA stenosis (symptomatic stenosis ICA > 50%, and asymptomatic stenosis ICA > 70%), where 44 (63%) patients were males, and 26 (37%) patients were females who were hospitalized and subjected to carotid endarterectomy at the Clinic of Vascular Surgery of the Eastern Slovak Institute of Cardiovascular Diseases and the Faculty of Medicine of Pavol Jozef Safarik University, Kosice.

Symptomatic patients were defined as patients who had suffered an ischemic stroke or transient ischemic attack (TIA) within last 6 months, while asymptomatic patients were defined as those who did not suffer any stroke or TIA within last 6 months.

The average patients' age was 69 ± 7.5 years. Depending on the clinical course, the patients were divided into two groups:

Group I included 30 (43% of all 70 tested) patients with symptomatic ICA stenosis; among them, 20 (66.6% of Group I) patients had a history of ischemic stroke, and 10 (33.3% of Group I) patients had a history of transient ischemic attack (TIA);

Group II included 40 (57% of all 70 tested) patients with asymptomatic ICA stenosis.

There was no statistically significant difference in age and sex between the groups.

The control group included 20 (10 men and 10 women) individuals; without any statistically significant difference in age and sex. The average age was 38 ± 5.2 years. The study characteristics of the current participants (excluding negative controls) are presented in the Table 1.

Table 1
Characteristics of the study groups

	Total	Group I (sympt. n = 30)	Statistical importance	P-value	Group I (asympt. n = 40)	Statistical importance	P-value
Male	43 (61.4%)	22 (73.3%)	N/I	0.6	21 (26.7%)	N/I	0.6
Female	27 (38.6%)	16 (53.3%)	N/I	0.4	11 (46.7%)	N/I	0.4
Current smoking	29 (41.4%)	14 (46.7%)	N/I	0.8	15 (37.5%)	N/I	0.8
Ischemic heart disease	48 (68.5%)	22 (73.3%)	N/I	0.7	26 (65.0%)	N/I	0.9
Hypertension	65 (92.9%)	28 (93.3%)	N/I	1	37 (92.5%)	N/I	1
Diabetes mellitus	17 (24.3%)	8 (26.7%)	N/I	0.8	9 (22.5%)	N/I	0.9
Peripheral artery disease	3 (4.3%)	2 (6.7%)	N/I	0.6	1 (2.5%)	N/I	0.6
N/I – not important; sympt. - patients with symptomatic ICA stenosis; asympt. - patients with asymptomatic ICA stenosis							

Exclusion criteria were: severe neurologic impairment (hemiplegia, coma), recent heart failure, uncontrolled hypertension, atrial fibrillation, critical limb ischemia, acute and chronic liver disease, acute and chronic renal failure, decompensated metabolic disease, acute and chronic infection, patients with history of cancer and autoimmune disease.

2.2 Preoperative ultrasound imaging of the atherosclerotic plaque

The degree of ICA stenosis, as well as the morphological structure of the atherosclerotic plaque, was determined in the preoperative period using ultrasonography. Duplex scanning of the carotid arteries was performed using the Philips HD11XE ultrasound machine with an 8 MHz linear transducer. The degree of stenosis was evaluated according to the “Consensus Panel Gray-Scale and Doppler US Criteria for Diagnosis of ICA Stenosis” through the determination of the peak systolic velocity (PSV) and the end diastolic velocity (EDV) within the ICA [20].

The structure and composition of the atherosclerotic plaque was assessed using echogenicity: hypoechogenic plaque – soft plaque, heterogenic plaque – mixed plaque, hyperechogenic plaque – hard plaque.

Hypoechogenic and heterogenic plaques were assessed as “unstable” plaques; hyperechogenicplaques were assessed as “stable” plaques [17].

2.3 Marker of inflammation in patients with carotid artery stenosis

Blood samples of all patients were taken one day before the surgery, and the concentration of specific markers, namely, Hpx, Lp-PLA2, and IL-4, were determined. The levels of these parameters were determined using the ELISA method (human lipoprotein associated phospholipase A2, Cussabio, USA; Human Hpx, Abcam, UK), and the results were evaluated with the Tukey test. The plasma level of IL-4 was measured using an ELISA kit Human IL-4 Platinum ELISA (eBioscience, San Diego, USA). Blood samples for the measurement of specific markers were centrifuged after collection at 2.500 rpm for 10 min. Serum samples were then frozen at -80 °C until analysis. Samples were processed using Synergy H4 multiplate reader (BioTek Vermont, USA).

2.4 Histopathology

In the postoperative period, histological assessment of the atherosclerotic plaque removed during carotid endarterectomy was performed. The selected atherosclerotic plaque was fixed in a 4% solution of neutral formalin; then, decalcification was carried out, and the material was poured in paraffin blocks 1 cm in size. 5-μm-thick sections of paraffin blocks cut by a microtome were studied histologically. The blocks were examined in the longitudinal section, in the most stenotic area. The hematoxylin and eosin staining were used. Histopathological characteristics of the retrieved carotid plaques were reported according to the updated American Heart Association classification of advanced atherosclerosis and a previously well-validated scoring system published by Lovett et al. (11). For each plaque, the following features were recorded: the rupture of the fibrous cap, lipid core size, nodular calcification, neovascularisation, inflammatory infiltrate, infiltration of the fibrous cap, proportions of fibrous tissue, intraplaque hemorrhage, presence of foam cells, and surface thrombus. Based on the presence of these features in each plaque, an overall stability rating was given by the histopathologist as “unstable” or “stable.” Unstable plaques demonstrated many or all features, and “stable” plaques demonstrated none of them.

2.5 Statistical methods

The obtained results were evaluated using descriptive statistics (the frequency, percentage ratio) as well as the one-way analysis of variance (ANOVA) (Minitab Inc. version 11.24, Coventry, UK) and chi-square test (Preacher, K. J. (2001, April). Calculation for the chi-square test: An interactive calculation tool for chi-square tests of goodness of fit and independence). The relationship between two variables – the group of symptomatic patients and the group of asymptomatic ones – was evaluated using the one-way analysis of variance (ANOVA) (Minitab Inc. version 11.24, Coventry, UK). The correlative relationship was evaluated using Pearson's correlation coefficient (Pearson correlation test, MINITAB Inc., Coventry, United Kingdom). The relationship between more than two variables (symptomatic patients, asymptomatic patients, and the control group) was evaluated using the Tukey's HSD test (Minitab Inc. version 11.24, Coventry, UK); the data were considered statistically significant at $p < 0.05$.

3 Theory

- This study is aimed to search for new criteria for assessing the risk of stroke in patients with atherosclerotic ICA stenosis, based on the principles of personalized medicine. Proposed "Stroke-Stop" formula (based on a mathematical calculation of the degree of ICA stenosis, the morphological structure of the atherosclerotic plaque and the level of lipoprotein-associated phospholipase A2 (Lp-PLA2) concentration) determine the risk of stroke development.

4 Results

The assessment of the atherosclerotic plaque echogenicity according to the ultrasonographic data has revealed that soft atherosclerotic plaque was observed in 19 (27.1%) patients, a mixed atherosclerotic plaque was detected in 29 (41.4%) patients and a hard atherosclerotic plaque was found in 22 (31.5%) patients. The ratio of the atherosclerotic plaque echogenicity between Group I and Group II is presented in Table 2.

Table 2
Morphology of the atherosclerotic plaque in Group I and Group II

	Group I (sympt. n = 30)			**Group II (asympt. n = 40)**		
Stenosis severity	Soft	Mixed	Hard	Soft	Mixed	Hard
< 60%	3 (10%)	4 (13.3%)	1 (3.3%)	-	-	-
60 ≥ x < 80%	6 (20%)	5 (16.7%)	3 (10.0%)	2 (5%)	5 (12.5%)	7 (17.5%)
x ≥ 80%	1 (3.3%)	3 (10%)	4 (13.3%)	6 (15%)	9 (22,5%)	11 (27.5%)
Correlation of stenosis severity of soft plaque between Group I and Group II showed statistical significance with p-value 0.025, while statistical analysis of hard plaque showed none significance (p-value 0.352).						
Sympt - patients with symptomatic ICA stenosis; asympt - patients with asymptomatic ICA stenosis						

Histological assessment was performed to test the statistical significance of the quality of atherosclerotic plaque density interpretation using ultrasound. Ultrasound detected unstable plaques

(patients with soft and mixed atherosclerotic plaque) in 62.9% of patients, while histological assessment detected unstable plaques in 64.3% of patients. It indicated the fact that ultrasound interpretation of the atherosclerotic plaque structure is highly informative and could be considered in assessing the risk of developing atherosclerotic complications.

Among vascular inflammatory markers, lipoprotein-associated phospholipase A2 (Lp-PLA2) showed a statistically significant difference ($P < 0.01$) in both the structure of atherosclerotic plaque and its clinical manifestations. In the patients studied, the concentration of Lp-PLA2was increased at 252.7-328.6 ng/ml in correlation with negative controls 205–240 ng/ml.

When studying vascular inflammatory markers, the ratio of Lp-PLA2 concentration was compared between patients of Group I and those of Group II (symptomatic and asymptomatic ICA stenosis). The symptomatic group had a higher mean plasma level of Lp-PLA2 (285.30 ± 2.05 μg/l) than the asymptomatic group (274.35 ± 3.38 μg/l). The difference in the Lp-PLA2 levels between the symptomatic and asymptomatic groups (Fig. 1a) was significant ($p < 0.05$). No significant differences were noted in the plasma levels of Lp-PLA2 between men and women within the two groups.

On the other hand, the level of Lp-PLA2in 15% of asymptomatic patients with soft atherosclerotic plaque was higher than that in symptomatic patients with hard atherosclerotic plaque.

In addition, the dependence of Lp-PLA2 concentration from the structure of the atherosclerotic plaque was evaluated. While assessing the obtained results, a statistical significance ($p < 0.05$) between Lp-PLA2 concentration in patients with soft atherosclerotic plaque, mixed atherosclerotic plaque and patients with hard atherosclerotic plaque was detected (Fig. 1b).

When assessing the results a statistical significance was found between the increase in Lp-PLA2 concentration and the morphological structure of the atherosclerotic plaque ($p < 0.001$).

Histograms in Fig. 2 indicate a number of patients with a different type of hardness based on Lp-PLA2 concentration. Noteworthy, the peaks and tails of histograms overlap. That means that we cannot separate or identify patients using only Lp-PLA2 concentration level. One could notice from Fig. 1a symptomatic patients have Lp-PLA2 > 250 mg/L. In the current paper, we propose to take into account additional parameters such as Systolic to Diastolic Velocities Ratio (SDVR) of IVA and the type of plaque.

In Fig. 3 Lp-PLA2 concentration is represented as a function of SDVR for each type of plaque. The red dots denote the patients with symptomatic stenosis of ICA inside the corresponding types of plaque. For the soft atherosclerotic plaque, red dots are spread almost homogenously. However, for the mixed and hard atherosclerotic plaque, the red dots are located close to the edge of the point locations. The latter fact can be easily explained by the multiplication of two parameters Lp-LPA2 and SDVR. The geometrical

outcome of the multiplication is the area of the corresponding rectangle. In Fig. 3, three rectangles are shown for one point belonging to each type of atherosclerotic plaque. So the higher the value of the area, the higher the probability for a patient having symptomatic stenosis of ICA. We propose the area, i.e. **Lp-PLA2 * SDVR**, as a modified parameter to predict symptomatic stenosis of ICA. Moreover, one can introduce the scaling factor (SF) for each type of plaque defining the high risk at 100. To satisfy the latter assumption, one must have SF = 5 for the soft atherosclerotic plaque, and SF = 10 and SF = 15 for mixed and hard atherosclerotic plaques, correspondingly. The risk factor consequence can be calculated as follows:

RF = area / SF

We need to provide a more efficient way to determine patient status. Using an empiric equation, we are introducing the Risk index as the universal parameter to identify patient status.

From Fig. 4, one can see the separate peaks for symptomatic and asymptomatic patients with probabilities 37% (asymptomatic) and 26% (symptomatic) which correspond to risk indices 60 and 90, respectively. Even though, the tails are overlapping more than the half asymptomatic patients have risk index < 70 while the majority of the symptomatic patients, 80% have risk index > 70. One can underline that the risk index is independent of gender, age of patients and depends only on well-defined and measured values such as Lp-LPA2 concentration and velocities ratio.

To study the effect of the atherosclerotic plaque structure, the degree of ICA stenosis, and the concentration of inflammatory markers increasing the risk of development of stroke were analyzed. 3D visualization technology revealed that the risk of stroke development is increased in case of the progression of ICA stenosis and elevation of Lp-PLA2 levels (Fig. 5). In patients with soft atherosclerotic plaque, the risk of stroke development was significantly higher in contrast to the patients with hard atherosclerotic plaque.

To assess a dominant risk factor for stroke development, all the patients were analyzed according to the structure of the atherosclerotic plaque.

According to the given empirical scale, all the patients with soft atherosclerotic plaque (both symptomatic and asymptomatic ones), ICA stenosis greater than 70% and Lp-PLA2concentration of more than 285 mg/l had the risk of stroke development of > 100 points (Fig. 6a).

Among patients with mixed atherosclerotic plaque, the risk of stroke development with > 100 points was observed in patients with hemodynamically significant ICA stenosis (> 80%) and Lp-PLA2concentration of more than 285 mg/l. (Fig. 6b).

Patients with hard atherosclerotic plaque had low or medium risk of stroke development (Fig. 6c).

Based on the results obtained, a mathematical calculation of the major risk factors (the degree of stenosis, the morphological structure of the atherosclerotic plaque and the level of inflammatory marker concentration) has been proposed to calculate the risk index for developing of stroke in patients with asymptomatic ICA stenosis.

The principle of mathematical calculation using the formula “Stroke-Stop” is the following: in patients with asymptomatic atherosclerotic disease of the carotid arteries, the concentration of Lp-PLA_2 is determined using ELISA; both diastolic velocity and systolic velocity of blood flow within the ICA are measured using ultrasound thereby determining the degree of ICA stenosis;the structure of the atherosclerotic plaque is evaluated; and finally, the risk index for development of ischemic stroke using the proposed formula “Stroke-Stop” is calculated.

$$\text{Stroke} - \text{Stop}" = \frac{\frac{\text{systolic velocity of the ICA}}{\text{diastolic velocity of the ICA}} * \text{level of (Lp} - \text{PLA2)}}{\text{coefficient of atherosclerotic plaque density}}$$

The numerator represents the ratio of ICA systolic velocity to ICA diastolic velocity multiplied by the indicator of Lp-PLA2 concentration, and the denominator represents the density coefficient of “5” in soft hypoechogenic atherosclerotic plaque, the density coefficient of “10” in mixed atherosclerotic plaque and the density coefficient of “15” in hard hyperechogenic atherosclerotic plaque. If the indicator is 50–70 points, the index of stroke risk is low, if the indicator is from 70 to 100 points, the index of stroke risk is medium, and if the indicator is more than 100 points – the index of stroke risk is high.

The use of the coefficient does not affect changes in the results since it is used as an identifier of the atherosclerotic plaque structure in all the patients. The results of calculating the risk of developing ischemic stroke by the formula “Stroke-Stop” are presented in Table 3.

Table 3
Calculation of the risk of stroke development by the “Stroke-Stop” formula

№	Index Stroke –Stop	Lp-PLA_2	Plaque	Stenosis	Sympt Patients	Asympt patients
Minimal Risk						
1	58	252.9	Hard	70		+
2	59	256.1	Hard	70		+
3	62	265.9	Hard	70		+
4	64	261.1	Hard	75		+
5	68	268.2	Hard	80		+
6	69	279.8	Hard	75		+
Medium Risk						
7	73	257.3	Hard	80		+
8	78	261.3	Hard	80		+
9	79	253.8	Hard	85		+
10	79	266.5	Hard	85		+
11	82	253.6	Hard	85		+
12	83	263.5	Hard	85		+
13	83	252.8	Hard	85		+
14	86	253.9	Hard	89		+
15	86	254.7	Hard	87		+
16	87	256.6	Hard	90		+
17	89	263.8	Hard	87		+
18	89	254.7	Mixed	70		+
19	91	252.7	Hard	90		+
20	92	253.4	Hard	90		+
21	93	267.1	Hard	90		+

Dividing of the studied samples into groups with minimum, medium and high risk of stroke development after interpretation of “Stroke-Stop” formula

Sympt - patients with symptomatic ICA stenosis; asympt - patients with asymptomatic ICA stenosis

№	Index Stroke –Stop	Lp-PLA_2	Plaque	Stenosis	Sympt Patients	Asympt patients
22	98	264.9	Mixed	75		+
High Risk						
23	102	267.1	Hard	70	+	
24	102	276.1	Mixed	65	+	
25	107	274.1	Mixed	50	+	
26	107	285.9	Mixed	60	+	
27	111	264.7	Mixed	85		+
28	112	266.7	Mixed	80		+
29	112	288.9	Mixed	70	+	
30	115	256.8	Mixed	85		+
31	115	285.3	Soft	55	+	
32	117	285.1	Mixed	80	+	
33	117	286.1	Mixed	50	+	
34	118	263.5	Mixed	90		+
35	118	263.5	Mixed	85	+	
36	119	266.5	Mixed	90		+
37	119	253.9	Mixed	90	+	
38	123	290.1	Soft	75	+	
39	125	284.7	Mixed	95		+
40	126	315.3	Mixed	70		+
41	127	286.3	Soft	70	+	
42	127	285.7	Mixed	90	+	
43	129	296.9	Mixed	70		+
44	132	275.7	Mixed	90	+	

Dividing of the studied samples into groups with minimum, medium and high risk of stroke development after interpretation of “Stroke-Stop” formula

Sympt - patients with symptomatic ICA stenosis; asympt - patients with asymptomatic ICA stenosis

№	Index Stroke –Stop	Lp-PLA_2	Plaque	Stenosis	Sympt Patients	Asympt patients
45	136	285.7	Mixed	90	+	
46	137	274.2	Mixed	85	+	
47	137	285.6	Mixed	95	+	
48	139	286.3	Mixed	90	+	
49	139	285.9	Mixed	90	+	
50	142	284.9	Mixed	95		+
51	143	285.2	Mixed	95	+	
52	148	295.7	Mixed	80	+	
53	157	295.5	Mixed	95		+
54	164	300.1	Soft	60	+	
55	210	300.9	Soft	85	+	
56	216	299.9	Soft	90		+
57	219	297.8	Soft	75		+
58	229	328.6	Soft	75		+
59	238	287.1	Soft	85	+	
60	239	299.9	Soft	80	+	
61	259	287.9	Soft	85	+	
62	260	289.5	Soft	90	+	
63	268	298.9	Soft	80		+
64	270	300.7	Soft	85	+	
65	278	286.2	Soft	95	+	
66	279	301.2	Soft	90		+
67	284	298.4	Soft	90		+
68	285	304.7	Soft	90		+

Dividing of the studied samples into groups with minimum, medium and high risk of stroke development after interpretation of “Stroke-Stop” formula

Sympt - patients with symptomatic ICA stenosis; asympt - patients with asymptomatic ICA stenosis

№	Index Stroke –Stop	Lp-PLA_2	Plaque	Stenosis	Sympt Patients	Asympt patients
69	292	304.4	Soft	95	+	
70	293	301.6	Soft	90		+
Dividing of the studied samples into groups with minimum, medium and high risk of stroke development after interpretation of “Stroke-Stop” formula						
Sympt - patients with symptomatic ICA stenosis; asympt - patients with asymptomatic ICA stenosis						

The results of calculating the risk of developing ischemic stroke by the formula “Stroke-Stop” presented in Table 2 clearly show the dependence and the influence of three risk factors (ICA stenosis, atherosclerotic plaque structure and Lp-PLA2concentration) on stroke development.

An alternative to the mathematical calculation of the risk of stroke development by the formula “Stroke-Stop” is its graphic display in the form of three diagrams, where each represents the structure of the atherosclerotic plaque (Fig. 7).

Red color points represent a high risk of stroke development; yellow color is used to indicate a medium risk of stroke development; blue color indicates a low risk of stroke development.

The diagrams (Fig. 7a-c) show how the risk of stroke development in patients with ICA stenosis of 80% and Lp-PLA_2 concentration at the level of 285 mg/l depends on the structure of the atherosclerotic plaque; concretely Fig. 7a presents a high risk (soft atherosclerotic plaque), Fig. 7b presents a medium risk (mixed atherosclerotic plaque); Fig. 7c presents a low risk (hard atherosclerotic plaque). The use of the diagram simplifies the process of determining the “Stroke-Stop” index as there is no need for mathematical calculations and the risk of stroke development is determined by the zone of contact between two indicators (ICA stenosis and Lp-PLA2 concentration) on the graphical chart where stroke risk threshold depends on the dominant risk factor, namely atherosclerotic plaque structure.

5 Discussion

It was established that the carotid endarterectomy is the effective treatment to reduce the risk of subsequent stroke in symptomatic patients with carotid stenosis [6]. Moreover, the risk-benefit ratio prefers surgery for about 70% symptomatic stenosis. In correlation with symptomatic stenosis, it is the unusual behavior for the asymptomatic lesions [7]. Therefore, the stenosis degree stand-alone might not be sufficient to predict the risk of a stroke. Clearly, additional markers are required to characterize more precisely the patients who would benefit the most from a surgery.

The development of new diagnostic technologies is contributing to the transition to a multi-parameter systematic model that allows the formation of a personalized approach to the diagnosis and prevention of stroke.

The main criteria, evaluated in our study, were the degree of ICA stenosis, the morphological structure of the atherosclerotic plaque and the level of Lp-PLA2 concentration.

We propose to further study unique biomarker Lp-PLA2 – an enzyme produced by inflammatory cells and hydrolyzes oxidized phospholipids in LDL, which in connection with individual patient plague stability may lead to earlier detection of atherosclerosis progression / manifestation. Lipoprotein-associated phospholipase A2 is also known as platelet-activating factor acetylhydrolase (PAF-AH). In the blood it is mainly connected with low density lypoprotein (LDL, near 80%) and only less than 20% of this enzyme is associated with high density lypoprotein (HDL) [14].

The combination of three factors – stenosis, ulceration of the atherosclerotic plaque and the inflammatory process within it – is one of the leading mechanisms of embologenicity as well as the development of stroke [21, 22]. This mechanism is not taken into consideration by the classical approach to determining the indications for carotid endarterectomy where the main criterion is the degree of ICA stenosis (50% in symptomatic patients and 70% in asymptomatic patients).

Alongside with the degree of stenosis, plaque morphology can provide crucial information to predict the stroke risk. The recent ultrasound studies showed a higher risk of cerebrovascular events for hypo- or anechogenic plaques compared to echogenic ones [23].

Among symptomatic patients, unstable carotid plaques were found in 76.6% of cases, while stable plaques were detected in 23.4% of cases only. The difference was statistically significant ($p < 0.0001$).

Ultrasonographic assessment of tissue characteristics is performed according to the overall distribution of grey tones (overall brightness). There are anechogenic (dark) and hyperechogenic (bright) plaques [22].

To provide more objective description, there were developed more detailed classifications; however, an interobserver agreement was weak and there was observed a low correlation with the histopathological findings [24, 25].

Several classifications of plaque echogenicity have been reported in the literature. However, echogenicity on plaque character should be standardized against three reference structures: flowing blood for anechogenic, sternocleidomastoid muscle for isoechogenic, and the adjacent transverse apophysis of the cervical vertebrae for hyperechogenicity [26, 27].

Unstable carotid plaques are associated with increased risk of stroke not only in symptomatic but also in asymptomatic patients. A meta-analysis of eight prospective studies, with a total of 7,557 patients, observing patients with asymptomatic carotid stenosis found that patients with unstable, echolucent

plaques had a 2.31-fold increased risk of stroke compared to patients with stable plaque based on ultrasound assessment [22, 28].

Although, according to the study ACST-1, carotid plaque echolucency assessment offered no predictive value for stroke risk [29].

In our research, 25% of patients with asymptomatic ICA stenosis had unstable atherosclerotic plaque.

Experimental studies have shown a key role of inflammation in destabilization and rupture of the atherosclerotic plaque [9, 11]. Specific vascular markers may serve as one of the criteria for assessing inflammation and destabilization of atherosclerotic plaque. In scientific journals, many articles on the comparison of different vascular markers were published [12, 13]. However, Lp-PLA2 is currently considered an independent biomarker for stroke, as well as coronary artery disease and peripheral arterial occlusive disease [15].

The JUPITER trial confirmed that patients with high Lp-PLA2 activity had more than twofold higher risk of developing cardiovascular events compared to those with low Lp-PLA2 activity [30].

Our study showed that patients with symptomatic stenosis of the internal carotid artery had significantly higher plasma levels of Lp-PLA2 compared to patients with asymptomatic stenosis.

When assessing the results, there was found a statistical significance between the increase in Lp-PLA2 concentration and the morphological structure of the atherosclerotic plaque.

Our results strongly confirm the role of Lp-PLA2 in the pathophysiology and clinical presentation of an unstable carotid plaque. Similar to our findings, some studies have reported the association of increased plasma levels of Lp-PLA2 in patients with unstable atherosclerotic plaque [31, 32].

The obtained results indicated the fact that in patients with soft atherosclerotic plaque, the level of Lp-PLA2 concentration was statistically higher compared to patients with hard atherosclerotic plaque. In addition, in patients with soft atherosclerotic plaque, the level of Lp-PLA2 increased to the level of Lp-PLA2 concentration observed in symptomatic patients.

Therefore, we can state that soft atherosclerotic plaque, as well as an increased concentration of Lp-PLA2, is a risk factor for developing stroke.

The innovative approach proposed, clinically verified individually detected formula for determining the risk of stroke development. It considers three main risk factors: the degree of ICA stenosis, atherosclerotic plaque structure and Lp-PLA2 concentration.

According to the results of our study, the consideration of several factors increases the accuracy of calculating the risk of stroke development for particular individuals, which is the basis for risk stratification algorithms.

The use of the proposed method for mathematical calculation of the risk index for stroke development using the formula “Stroke-Stop” may serve as an auxiliary criterion at the stage of determining and selecting treatment tactics for patients with ICA stenosis greater than 70%, and finally to apply predictive and prognostic patient-specific treatment of atherosclerosis supporting the shift from reactive medicine to predictive, preventive, and personalized medicine.

In addition, thus proposed formula accompanied and accomplished with lipid individual profile can be applied alternatively to typical post symptomatic treatment of atheroslerosis. HDL-associated Lp-PLA2 may substantially contribute to the HDL antiatherogenic activity, and could be additionally applicable for the prediction of the efficacy of prescribed medication. Correspondingly, our Stop-Stroke formula is recommended for implementation for personalized clinical application of therapies. It is anticipated that, ultimately, this change in diagnosis and therapy will help in the future design and development of new, more selective and effective therapies for each individual patient.

6 Conclusions

- The “Stroke-Stop” formula for the determination of the risk of stroke development is proposed for patients with asymptomatic internal carotid artery (ICA) stenosis
- This formula is based on a mathematical calculation of the major risk factors for stroke: the degree of ICA stenosis, the morphological structure of the atherosclerotic plaque and the level of lipoprotein-associated phospholipase A2 (Lp-PLA2) concentration
- Based on calculations using “Stroke-Stop” formula, three main groups were generated: low (< 70 points), medium (70–100 points) and high (> 100 points) risk of stroke development
- This model could be used as a diagnostic and prognostic tool to identify patients with potentially high risky development of ischemic stroke in clinical praxis

Declarations

7 Ethics approval and consent to participate

The study was approved by the Ethics Committee of the East Slovak Institute of Cardiovascular Diseases (07/12/2015). All the patients, including negative controls, were informed and gave written consent to participate in the study.

8 Consent for publication

All the patients, including negative controls, were informed and gave written consent to participate in the study.

9 Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

10 Competing interests

The authors declare that they have no competing interests.

11 Funding

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12 Authors' contributions

IK – Writing - Original Draft, Methodology, Conceptualization, Investigation, Project administration, final approval of the version to be submitted

PB – Methodology, Validation, Resources, final approval of the version to be submitted

PS – Methodology, Investigation, Visualization, Writing - Original Draft, final approval of the version to be submitted

DL – Conceptualization, Software, Visualization, final approval of the version to be submitted

RM - Formal analysis, Funding acquisition, Writing - Review & Editing, final approval of the version to be submitted

ZH – Investigation, Data Curation, final approval of the version to be submitted

ST – Methodology, Visualization, final approval of the version to be submitted

NB – Supervision, Funding acquisition, final approval of the version to be submitted

VS – Methodology, Validation, final approval of the version to be submitted

13 Acknowledgements

Not applicable

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Figures

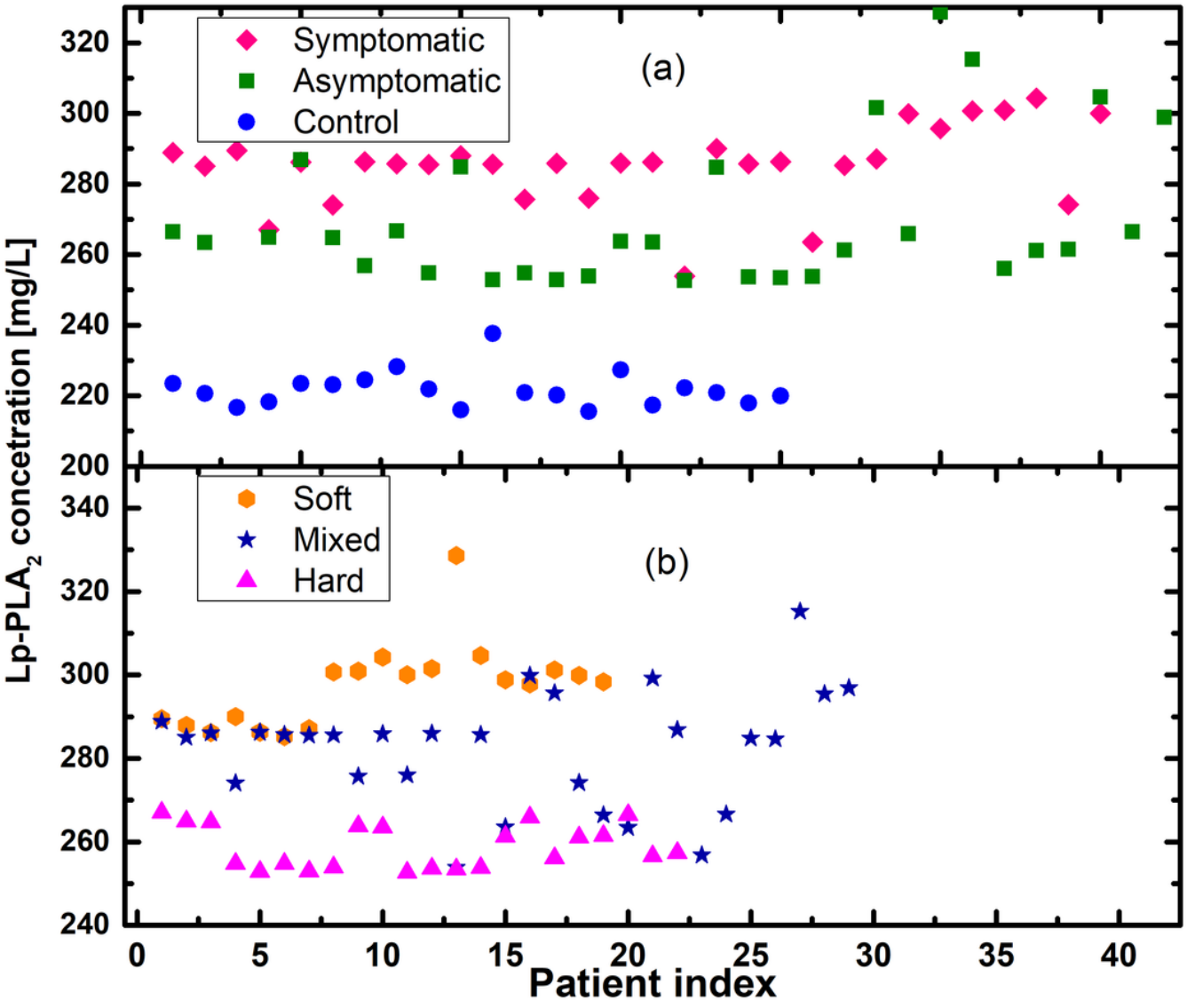

Figure 1

Changes in concentration of Lp-PLA2 according to symptomatic manifestations and plaque stability (a) Concentration of Lp-PLA2 in symptomatic, asymptomatic patients and control group Symptomatic = patients with symptomatic stenosis of ICA; Asymptomatic = patients with asymptomatic stenosis of ICA; Control = control group; (b) Concentration of Lp-PLA2 in patients with soft atherosclerotic plaque, mixed

atherosclerotic plaque and patients with hard atherosclerotic plaque; Soft = soft atherosclerotic plaque, Mixed = mixed atherosclerotic plaque, Hard = hard atherosclerotic plaque

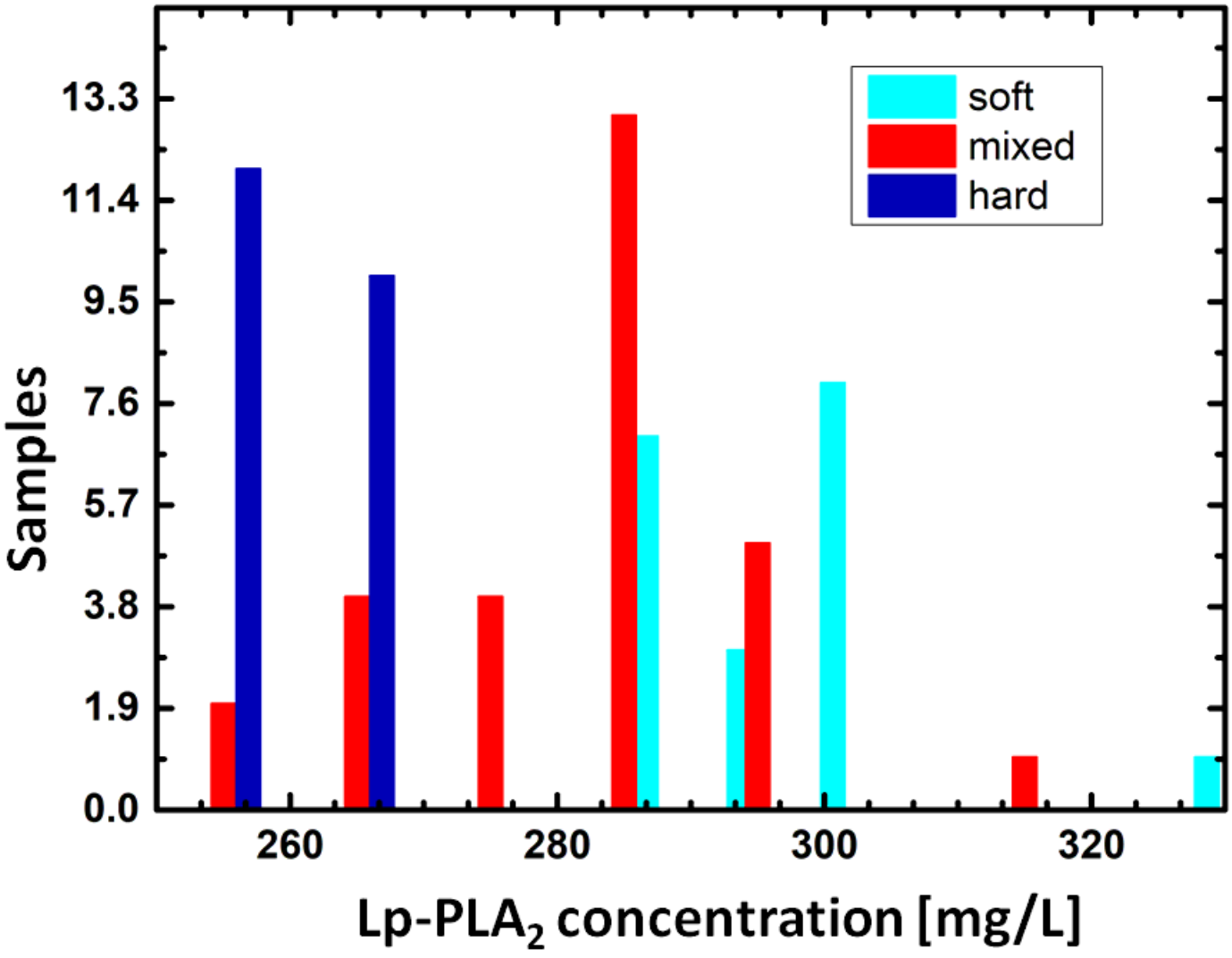

Figure 2

Histograms for soft (cyan), mixed (red), and hard (blue) The results are statistically significant at p□0.001 (the Pearson correlation coefficient, MINITAB Inc., Coventry, United Kingdom)

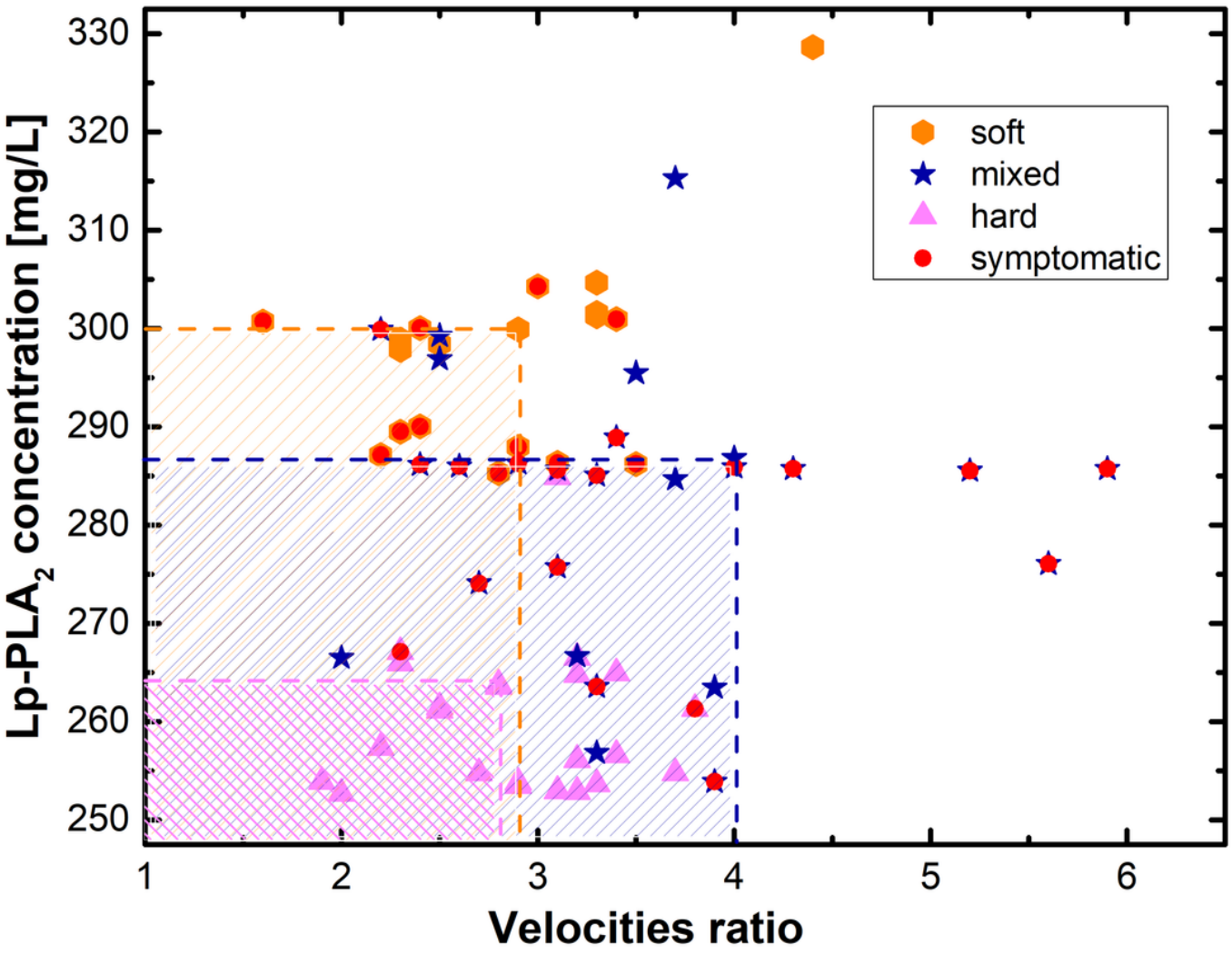

Figure 3

Lp-PLA2 concentration as a function of SDVR for each type of plaque Concentration is represented as a function of SDVR for each type of plaque

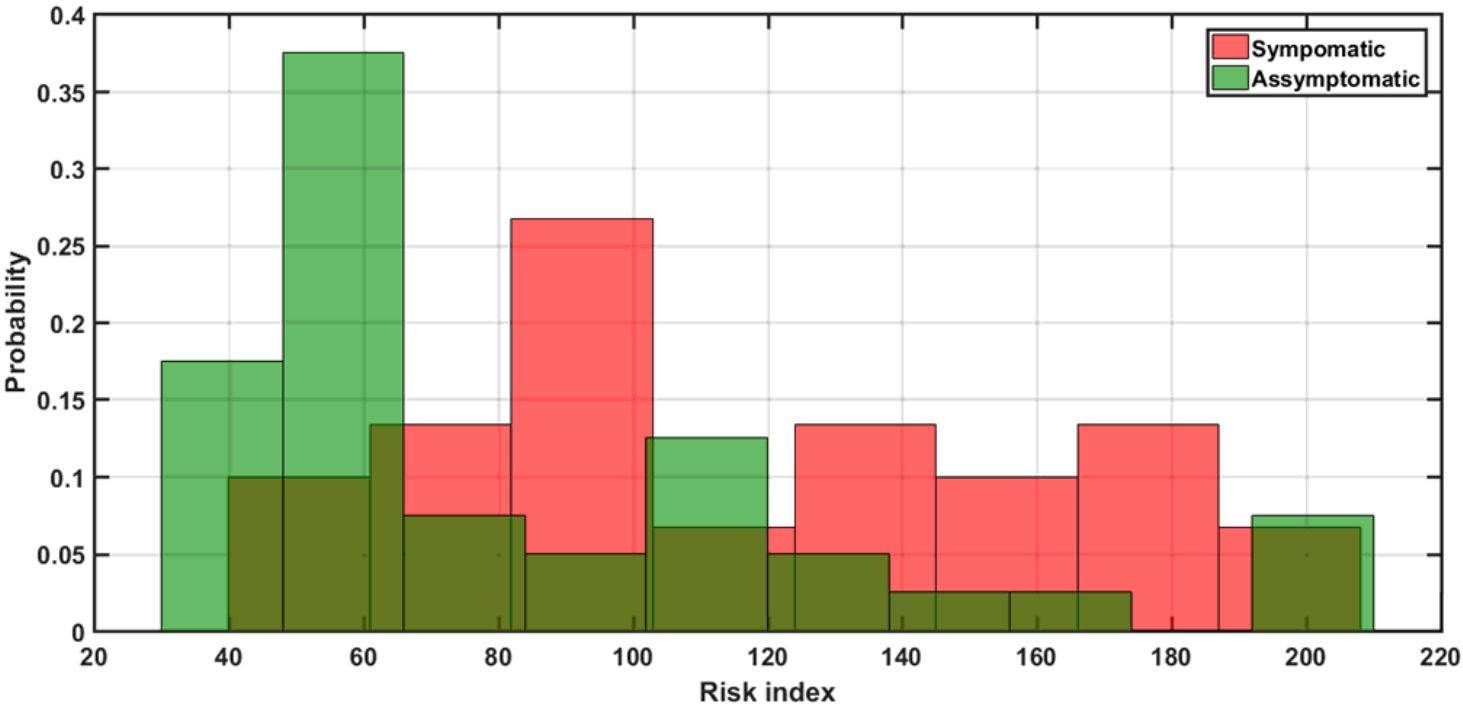

Figure 4

Determination of the risk of stroke development in symptomatic and asymptomatic patients The probability of histograms based on the risk index

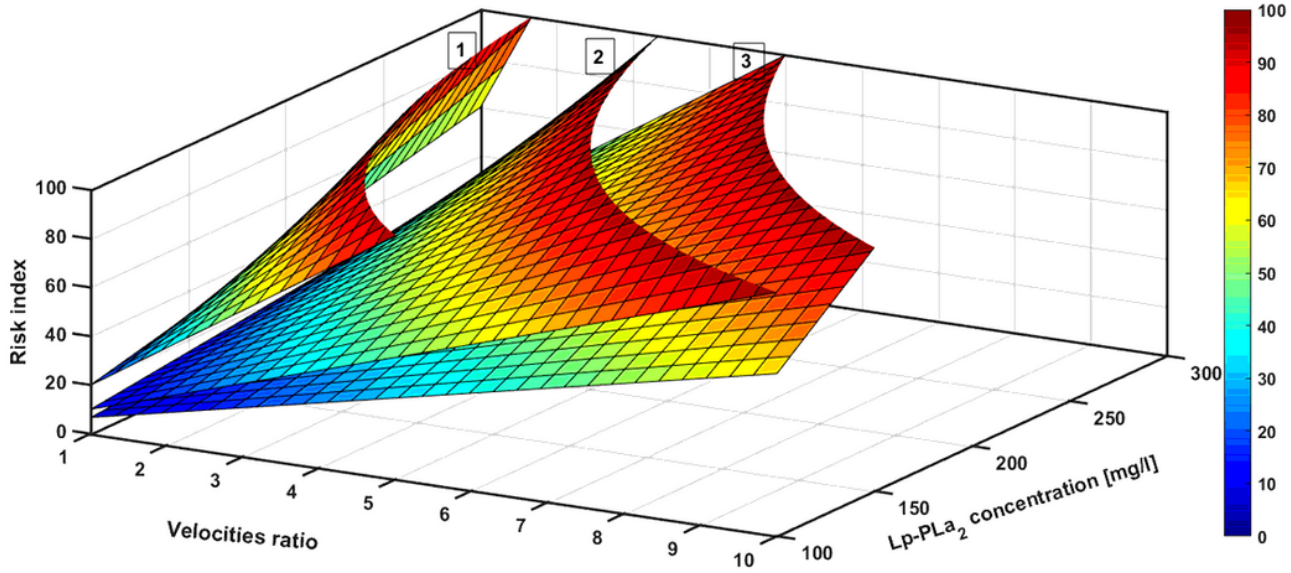

Figure 5

Ratio of ICA stenosis, atherosclerotic plaque structure and Lp-PLA2 concentration 1 – soft atherosclerotic plaque, 2 – mixed atherosclerotic plaque, 3 – hard atherosclerotic plaque

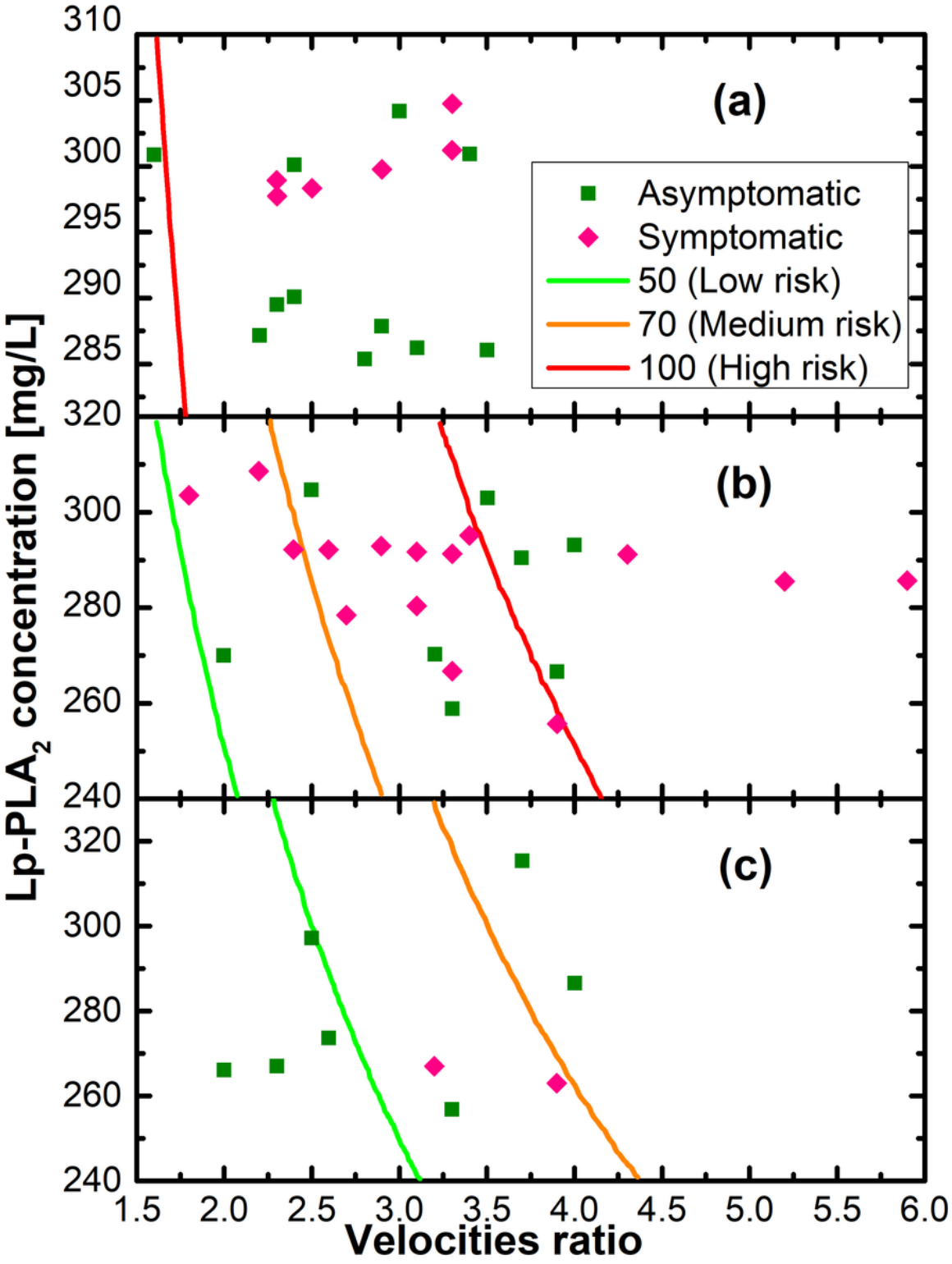

Figure 6

Correlation between concentration of Lp-PLA2 and velocities ratio according to plaque stability (a) Risk of stroke development in patients with soft atherosclerotic plaque; (b) Risk of stroke development in patients with mixed atherosclerotic plaque; (c) Risk of stroke development in patients with hard atherosclerotic plaque

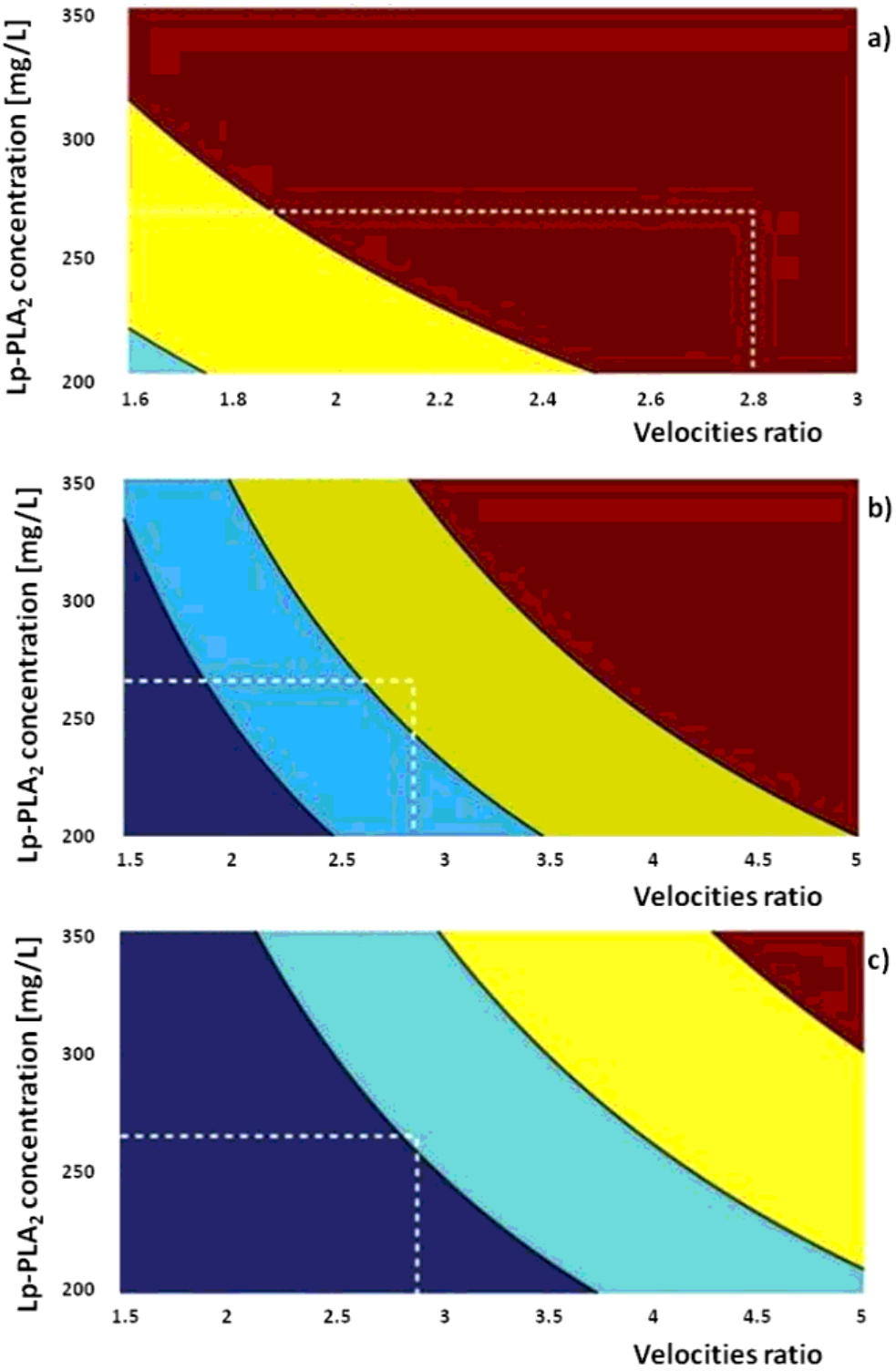

Figure 7

The graphical manifestation of risk of stroke calculated with “Stroke-Stop” formula (a) Risk of stroke development in patients with soft atherosclerotic plaque; (b) Risk of stroke development in patients with mixed atherosclerotic plaque; (c) Risk of stroke development in patients with hard atherosclerotic plaque