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Research Article

Histopathological Profile of Thrombi Differs Depending on Pathogenesis of Embolic Cerebral Infarction and Evidence of Intracardiac Thrombus

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Abstract

Background and Objectives: Determining the etiopathogenesis of stroke is a cornerstone for correct secondary prophylaxis, however, in up to 40% the precise source of embolism cannot be determined. The aim of our study was to investigate histopathological differences of emboli retrieved during mechanical thrombectomy, and to investigate how radiological findings and ischemic stroke outcomes correlate with histopathological profile of thrombi. **Materials and Methods:** In total 40 stroke patients were included in the study. Histopathological examination was performed using Hematoxylin and Eosin staining. Subsequently, on the basis of hematoxylin-and-eosin staining, the percentage and area of erythrocytes, leukocytes, and fibrin in investigated thrombi were measured. Thrombi were stained immunohistochemically, according to manufacturer`s instructions. The results were compared in supposed etiopathological stroke subtype groups as cardioembolic, atheroembolic and cryptogenic. Correlation between histopathology of thrombi and stroke outcomes, as well as radiological findings, that is hyperdense artery sign and presence of thrombi in left atrium appendage on non-enhanced thoracic Computed Tomography, was also performed. **Results:** There were 26 cardioembolic thrombi, 7 atheroembolic and 7 cryptogenic thrombi. Atheroembolic thrombi had a higher percentage of the fibrin area versus total thrombi area than cryptogenic thrombi (p=0.038) and similar tendency comparing with cardioembolic thrombi (p=0.099). Cryptogenic thrombi had statistically lower fibrin/leukocytes ratio than atheroembolic thrombi (p=0,026). As to radiological findings, hyperdense artery sign did not correlate with any specific histopathological composition of thrombi, but patients with CT visualized thrombus in the heart had larger erythrocyte area in thrombus than those without mentioned finding (p=0.047). The ischemic stroke outcome in patients after performed mechanical thrombectomy did not correlate with thrombus histopathology. **Conclusion:** We found partial histologic similarities of cryptogenic thrombi with cardioembolic thrombi. Therefore patients with cryptogenic stroke should probably undergo extensive cardiac examination including long-term heart rhythm monitoring.

Keywords: Thrombus; Histology; Stroke; Computed Tomography (CT); Thrombectomy, Hematoxylin and Eosin (H&E) staining

Introduction

Determining the etiopathological source of stroke is a cornerstone for correct secondary prophylaxis. The majority of cerebral ischemic strokes are thromboembolic [1]. Although, their etiologic un pathogenetic mechanisms can differ significantly. The main embolic cerebral ischemic stroke causes are arterioarterial embolism from atherosclerotic plaque and cardioembolism [2]. The proportion of thromboembolic infarctions in Latvia is reaching 75% (Pauls Stradiņš Clinical University Hospital local stroke registry). Nevertheless, in up to 40% the precise source of embolism cannot be determined even after extensive evaluation [3]. For clinicians cryptogenic strokes are a major problem and are an important research focus [4,5]. Several studies have shown high rates of recurrent stroke episodes of ≤30% in the first year after suffered cryptogenic stroke [6].

With the development of endovascular therapy, it became possible to analyze the thrombus material histologically, thus providing additional diagnostic opportunities [7]. Some authors have hypothesized that it is histologically possible to differentiate between cardioembolus and arterioarterial embolus using the classical hematoxylin-eosin staining method [2]. The pattern of each thrombus component distribution often differs between patients with cardiac thrombi and those with arterial thrombi, and the efficacy of endovascular thrombectomy is different according to the thrombus composition [8]. Thus, it can be possible to clarify cryptogenic stroke etiology basing on thrombus histology. Although it may be possible, that histopathology of retrieved thrombi may be affected in patients who received intravenous thrombolysis procedure before endovascular mechanical thrombectomy was performed.

There is also little data on the correlation of radiological results with the histological qualitative structure of the thrombus. It is proposed that the presence of hyperdense artery sign (HAS) indicates more red blood cells (RBC`s) components and better revascularization outcomes [9]. The presence of thrombus in the heart and its impact on histopathology of cardioembolic thrombi has never been investigated, although it can provide an important clinical information about the thrombus composition on acute pre-treatment stage.

However, only a few studies have analyzed the outcome of endovascular therapy recanalization and the neurological clinical outcome in correlation with the histological composition of the thrombus. Successful reperfusion is routinely achieved in 70-80% of cases [10]. That's why a major and unanswered question in vascular neurology is about the poor clinical outcome of stroke patients with endovascular therapy. Of course, there are several already known factors such as time, collaterals, and vascular anatomy, however, it cannot be ruled out that thrombus structure is also important. For instance, it has been proposed that thrombi with a high white blood cells (WBC`s) fraction are related to more organized thrombi of cardioembolic origin associated with less favorable recanalization and clinical outcome in acute ischemic anterior circulation stroke [11]. A better understanding of thrombus type and properties can significantly improve stroke treatment outcomes.

The aim of this study was to investigate histopathological features of different stroke emboli subtypes retrieved during mechanical thrombectomy and compare radiological findings and stroke outcomes with histological profile of thrombi.

Materials and Methods

The study is classified as a single-center analytical observational study. Forty patients with cerebral infarction and large magistral artery occlusion, during a period of three months (13 January 2021 – 1 July 2021), have received acute reperfusion therapy with endovascular thrombectomy in Pauls Stradiņš Clinical University Hospital (Riga, Latvia). Immediately after clot retrieval, thrombus material was fixed in phosphate-buffered (10%) formalin. The total of 24 included patients were treated with intravenous recombinant tissue-type plasminogen activator (IV tPA) before endovascular thrombectomy.

Study Population

Patient data (age, gender, stroke subtype) were extracted from the medical records. Major clinical characteristics of patients included in the study could be found in Table S1 (see supplementary materials).

Patients clinical data	
Age, median (Q1; Q3)	71,5 (64,3; 78,0)
Gender	
Males, n (%)	16, (40,0%)
Females, n (%)	24, (60,0%)
Modified Rankin Scale before stroke	
0, n (%)	32, (80,0%)
1, n (%)	3, (7,5%)
2, n (%)	1, (2,5%)
3, n (%)	2, (5,0%)
4, n (%)	2, (5,0%)
Risk factors	
Arterial Hypertension	
Present, n (%)	35 (87,5%)
Not present, n (%)	5 (12,5%)
Atrial Fibrillation	
Present, n (%)	24 (60,0 %)
Not present, n (%)	16 (40,0 %)
Coronary Heart Disease	
Present, n (%)	10 (25,0 %)
Not present, n (%)	30 (75,0 %)
Dislipidemy	
Present, n (%)	12 (30,0 %)
Not present, n (%)	28 (70,0 %)
Diabetes Mellitus Type 2	
Present, n (%)	8 (20,0 %)
Not present, n (%)	32 (80,0 %)
Drug use before stroke	
Antiplatelet drugs	
Used, n (%)	6 (15%)
Did not use, n (%)	34 (85,0%)
Anticoagulants	
Used, n (%)	9 (22,5%)
Did not use, n (%)	31 (77,5%)
Severity of Stroke on arrival	
National Institutes of Health Stroke Scale (NIHSS) score, median (Q1, Q3)	15,0 (9,0; 18,0)
Modified Rankin Scale	
1, n (%)	1 (2,5 %)
2, n (%)	1 (2,5 %)
3, n (%)	3 (7,5 %)

4, n (%)	8 (20 %)
5, n (%)	27 (67,5 %)
Received therapy with intravenous recombinant tissue-type plasminogen activator (IV tPA)	
Received, n (%)	24 (60,0 %)
Did not receive, n (%)	16 (40,0 %)
Outcome on discharge	
National Institutes of Health Stroke Scale (NIHSS) score, median (Q1, Q3)	9,5 (3,3; 16,0)
Modified Rankin Scale, median (Q1, Q3)	
0, n (%)	1 (2,5 %)
1, n (%)	6 (15,0 %)
2, n (%)	1 (2,5 %)
3, n (%)	5 (12,5 %)
4, n (%)	8 (20,0%)
5, n (%)	14 (35,0 %)
6, n (%)	5 (12,5%)

Table S1: Major clinical characteristics of patients included in the study.

Pre-interventional Imaging

Unenhanced brain Computed Tomography (CT) and contrast-enhanced Computed Tomography Angiography (CTA) of the brachiocephalic arteries were performed in all patients either in our center or in the regional hospital. Brain Computed Tomography Perfusion (CTP) imaging was performed, if time from symptoms onset to hospital arrival exceeded 4,5 hours (26 patients). Unenhanced brain CT retrospectively was analyzed for presence or absence of the HAS. HAS was determined by an increased radiodensity of an artery and Hounsfield (HU) value was not specifically used for measurement of clot density. 29 patients during the acute stroke CT protocol have received unenhanced thoracic CT examination. Unenhanced thoracic CT was retrospectively analyzed for the presence or absence of intracardiac thrombi.

Endovascular Techniques

All procedures were performed using arterial access under sedation or general anesthesia according to present clinical status and stroke severity. The procedures were performed in a dedicated angio-suite (biplane Artis Zee, Siemens). Femoral access was obtained with an 8F vascular sheath and 6F long vascular sheath (Neuro max 088, Penumbra Inc.) The guide was advanced distally, as far as safely possible, into the internal carotid or in the vertebral artery, according to previous imaging findings. The aspiration catheter (Ace 60 or 68, Penumbra) was advanced to the level of the thrombus over any microcatheter and microwire the operator chooses. Under road map guidance, the wire and microcatheter were navigated to or past the thrombus. Over this platform, the larger aspiration catheter was delivered and positioned immediately adjacent to the site of occlusion. The microcatheter and wire were removed, and aspiration was applied via the aspiration pump that is part of the Penumbra separator system. If aspiration failed, the Solumbra technique as rescue switching strategy was performed advancing a stent-retriever (Solitare, Covidien) through the 0.025-inch microcatheter distal to the clot with an aspiration catheter (Ace 60 or 68, Penumbra) at the clot face. The microcatheter was then removed till stent retrieval proximal markers. Intermediate catheter was connected to a negative pressure suction pump (Penumbra) for continuous aspiration. Three to five minutes later, the aspiration catheter was slowly withdrawn until the blood flow velocity returned to normal in the connecting pipe of the negative pressure pump.

Histological and Immunohistochemical Analysis of Retrieved Thrombi

Histopathologically 40 thrombi were investigated. The size of each thrombus was registered. The formalin-fixed specimens were embedded in paraffin, cross-sectioned at 4-µm thickness and stained with haematoxylin-and-eosin (H&E) with Dako Autostainer link 48.

Subsequently, based on H&E staining, the percentage of erythrocytes, leukocytes and fibrin was measured in investigated thrombi, as well as microscopically was measured the area of these cells in mm2. The area of the thrombus components was measured with

Nikon's program Nis Elements D. From the H&E image of each thrombus, each component was outlined and the area obtained, which was then converted to a percentage of the total thrombus area. All thrombi were stained immunohistochemically, according to manufacturer`s (Dako) instructions, for CD3 positive T cells, CD68 positive monocytes, von Willebrand factor positive thrombocytes and CD15 positive leukocytes. Their amount was categorically measured ranging "+, "++" and "+++". Where "+" presence of cells occupying < 25% of the tissue, "++" presence of cells occupying 25 to 50% of the tissue and "+++" presence of cells occupying > 50% of the tissue.

Stroke Subtypes with Characteristic Histopathological Profile

Ischemic stroke patients, included in this study, were divided into three groups – cardioembolic, atheroembolic and cryptogenic group. In our study groups were defined as follows: cardioembolic group – patients with atrial fibrillation and/or proved thrombi in left atrium appendage. Atheroembolic group – patients with large artery atherosclerosis and/or artery dissection. Cryptogenic pathogenesis group – patients with ischemic stroke of unknown etiology – either fully investigated without any pathological findings or incompletely investigated. Histopathological findings of retrieved thrombi were compared between three mentioned groups.

The distribution of ischemic stroke patients by pathogenetic groups was as follows: cardioembolic (26 patients; 65,0%), atheroembolic (7 patients; 17,5%) and cryptogenic group (7 patients; 17,5%), see Figure 1.

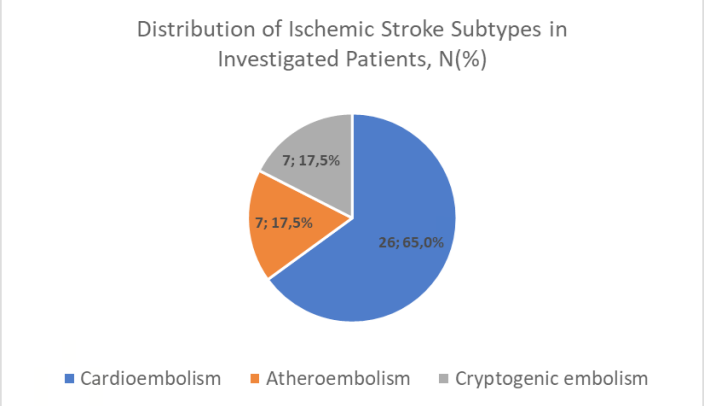

Figure 1: Distribution of Ischemic Stroke Subtypes in Included Patients.

The hystologic composition of each trombi group analysed by Hematoxylin and Eosin technicque and their size is present in Table S2 (see supplementary materials).

Characteristic	Cardioembolism group, median (Q1; Q3)	Atheroembolism group, median (Q1; Q3)	Cryptogenic group, median (Q1; Q3)	Test	Test Statistics, H-value	p value
Thrombus length, mm	7,0 (4,0; 8,3)	9,0 (3,0; 11,0)	7,7 (6,1; 9,0)	Kruskal-Wallis Test	0,617	0,735
Thrombus width, mm	4,0 (3,0; 5,0)	4,0 (2,0; 5,0)	4,6 (3,6; 5,0)		1,506	0,471
Thrombus area, mm2	30,0 (14,0; 39,2)	30,0 (4,0; 44,0)	32,9 (26,4; 45,0)		0,761	0,683
Fibrin area, mm2	20,0 (11,2; 30,0)	29,7 (3,4; 32,3)	19,8 (13,3; 31,9)		0,462	0,793
Percentage of fibrin area in thrombus, %	82,5 (67,3; 90,5)	85,0 (85,0; 93,0)	79,0 (51,1; 84,0)		4,902	0,086
Leukocytes area, mm2	1,6 (1,1; 3,0)	1,3 (0,2; 3,3)	4,0 (2,1; 4,6)		3,275	0,194
Percentage of leukocytes area in thrombus, %	6,5 (4,0; 11,0)	7,0 (3,0; 9,0)	12,0 (5,0; 16,0)		2,161	0,339
Erythrocytes area in thrombus, mm2	2,2 (0,6; 5,1)	0,7 (0,0; 2,2)	2,0 (1,2; 2,5)		2,136	0,344
Percentage of erythrocytes area in thrombus, %	8,5 (3,0; 27,8)	3,0 (0,0; 13,0)	5,0 (4,0; 12,0)		1,947	0,378
Fibrin area:Leukocyte area ratio	11,5 (7,0; 19,3)	14,0 (10,0; 34,0)	5,8 (4,0; 7,0)		5,515	0.063

Table S2: The hystologic composition of each trombi group analysed by Hematoxylin and Eosin technicque and their size.

Immunohystochemical profile of each ischemic stroke group is represented in Table S3 (see supplementary materials).

Characteristic	Cardioembolism group, N (%)	Atheroembolism group, N (%)	Cryptogenic group, N (%)	Test	p value
The amount of macrophages in thrombus, %				Fisher`s Exact Test	
less than 25%	14 (53,8%)	3 (42,9%)	4 (57,1%)		0,903
more than 25%	12 (46,2%)	4 (57,1%)	3 (42,9%)		
The amount of T lymphocytes in thrombus, %					
less than 25%	15 (57,7%)	5 (71,4 %)	5 (71,4%)		0,711
more than 25%	11 (42,3%)	2 (28,6%)	2 (28,6%)		
The amount of segmental leukocytes in thrombus, %					
less than 50%	15 (57,7%)	5 (71,4%)	2 (28,6%)		0,305
more than 50%	11 (42,3%)	2 (28,6%)	5 (71,4%)		
The amount of platelets in thrombus, %					
less than 50%	19 (73,1%)	5 (71,4%)	5 (71,4%)		>0,999
more than 50%	7 (26,9%)	2 (28,6%)	2 (28,6%)		

Table 3: Immunohystochemical analysis of composition of thrombi in each ischemic stroke group.

The Histologic Composition of each Group of Thrombi Analysed by Hematoxylin and Eosin Technique and their Size

Statistical Analysis

Statistical analysis was performed using SPSS® (IBM SPSS Statistics for Windows, Version 25.0). All tests were statistically significant if p value was less than 0,05. As the data did not fit the normal distribution, only non-parametric statistical methods were used. For the analysis of dependencies between nominal and scale data, were applied Mann-Whitney U test and Kruskal-Willi's test. For finding correlations between nominal data was used Fisher`s exact test. Standard deviation as an additional descriptive parameter was not applied, as data did not fit a normal distribution. As additional descriptive parameters were chosen median with quartiles.

Results

Impact of IV tPA on Thrombus Composition

The total of 24 patients have received IV tPA before mechanical thrombectomy. Only 16 patients underwent mechanical thrombectomy procedure alone. There were no statistically significant differences in thrombus composition comparing data in both groups (with and without IV tPA)– see Table S4 (see supplementary materials). From those, who received IV tPA, 14 had cardioembolic thrombi, 4 had atheroembolic thrombi and 6 had cryptogenic thrombi.

Characteristic	Patients without received intravenous thrombolysis, median (Q1; Q3)	Patients with received intravenous thrombolysis, median (Q1; Q3)		Test Statistics, Mann-Whitney U value; Z-test	p
Thrombus length, mm	6,5 (4,3; 10,5)	7,9 (5,3; 9,0)	Mann-Whitney U Test	183,500; -0,235	
Thrombus width, mm	4,2 (3,3; 5,0)	4,0 (3,0; 5,0)		173,000; -0,531	
Thrombus area, mm2	31,0 (16,0; 42,5)	29,4 (16,1; 39,5)		187,000; -0,138	
Fibrin area, mm2	23,9 (12,1; 31,3)	20,1 (9,9; 31,4)		182,500; -0,262	
Percentage of fibrin area in thrombus, %	81,5 (48,5; 91,5)	84,0 (75,8; 86,8)		175,000; -0,470	
Leukocytes area, mm2	1,5 (0,8; 2,9)	2,3 (1,2; 4,1)		157,500; -0,953	
Percentage of leukocytes area in thrombus, %	5,5 (3,3; 9,0)	7,5 (5,0; 12,0)		141,500; -1,399	
Erythrocytes area in thrombus, mm2	2,4 (0,7; 7,7)	1,7 (0,5; 2,9)		156,500; -0,981	
Percentage of erythrocytes area in thrombus %	10,0 (3,0; 39,8)	6,5 (2,3; 12,8)		159,500; -0,900	
Fibrin area:Leukocyte area ratio	12,5 (8,5; 21,0)	7,5 (7,0; 16,5)		145,500; -1,288	
Characteristic	Patients without received intravenous thrombolysis, N (%)	Patients with received intravenous thrombolysis, N (%)			p
The amount of macrophages in thrombus			Fisher`s Exact Test		
less than 25%	8 (50,0%)	13 (54,2%)			
more than 25%	8 (50,0%)	11 (45,8%)			
The amount of T lymphocytes in thrombus					
less than 25%	13 (81,25%)	12 (50,0%)			
more than 25%	3 (18,75%)	12 (50,0%)			
The amount of segmental leukocytes in thrombus					
less than 50%	7 (43,75%)	15 (62,5%)			
more than 50%	9 (56,25%)	9 (37,5%)			
The amount of platelets in thrombus					
less than 50%	11 (68,75%)	18 (75,0%)			
more than 50%	5 (31,25%)	6 (25,0%)			

Table S4: Differences in composition of thrombi in patients with and without performed intravenous thrombolysis before mechanical thrombectomy.

Although there was a tendency to be less T lymphocytes in the thrombi of patients, who did not receive IV tPA (p = 0,056), see Table 5.

Characteristic	Thrombi from patients without IV t-PA, N (%)	Thrombi from patients with IV t-PA, N (%)
T lymphocytes occupying less than 25% of thrombus area	13 (81,25%)	12 (50,00%)
T lymphocytes occupying more than 25% of thrombus area	3 (18,75%)	12 (50,00%)

Table 5: Distribution of thrombi by amount of T lymphocytes depending on received intravenous (IV) thrombolysis therapy with recombinant tissue plasminogen activator (rt-PA).

Histopathological Differences in Thrombus Composition in Different Ischemic Stroke Groups

Cardioembolic, atheroembolic and cryptogenic thrombi groups were classified as erythrocyte rich or fibrin rich according to their predominant composition. Histological examination revealed, that all of the investigated atheroembolic and cryptogenic thrombi were fibrin rich, whereas among cardioembolic thrombi 11,5% (n=3) were erythrocyte rich (see Table 6). Even so, using such simplified classification of retrieved thrombi, some differences were found in their histopathological composition (Bonferroni correction was not applied for the tests).

	Fibrin rich thrombi	Erythrocyte rich thrombi
Cardioembolism group, n (%)	23 (88,5%)	3 (11,5%)
Atheroembolism group, n (%)	7 (100,0%)	0 (0,0%)
Cryptogenic group, n (%)	7 (100,0%)	0 (0,0%)

Table 6: Distribution of thrombi type depending on the cerebral infarction pathogenesis.

Thrombi in patients with cryptogenic strokes had slightly larger leukocytes area (median - 4,0 mm2) comparing with cardioembolic thrombi (median – 1,6 mm2; p=0,099), although this result was not statistically significant. No differences were found between cryptogenic and atheroembolic thrombi (median – 1,3 mm2; p=0,165), (Figure 2), as well as between cardioembolic and atheroembolic thrombi (p=0,590).

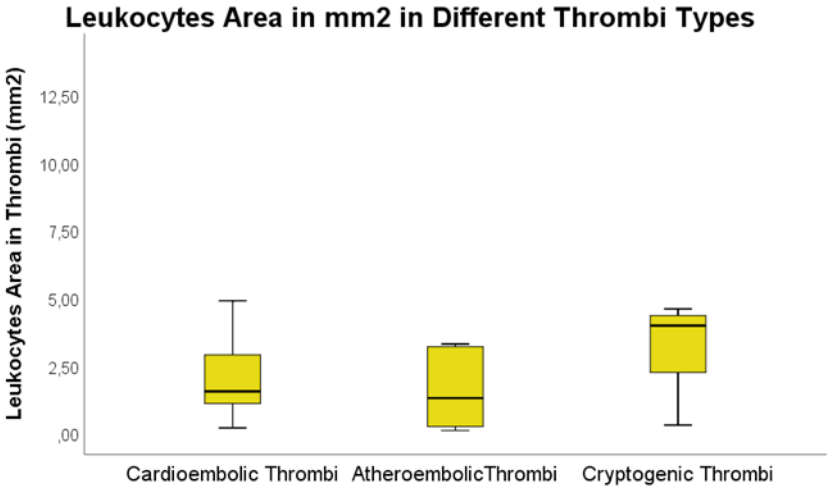

Figure 2: Leukocytes area in mm2 in different thrombi types.

Cryptogenic thrombi had statistically significantly lower fibrin/leukocytes ratio than atheroembolic thrombi (median - 5,8 and 14,0 respectively; p=0,026), the same tendency, but without statistical significance, comparing cryptogenic thrombi and cardioembolic thrombi (median - 5,8 and 11,5 respectively; P=0,067). No statistically significant differences were found comparing cardioembolic and atheroembolic thrombi (p=0,330), see Figure 3.

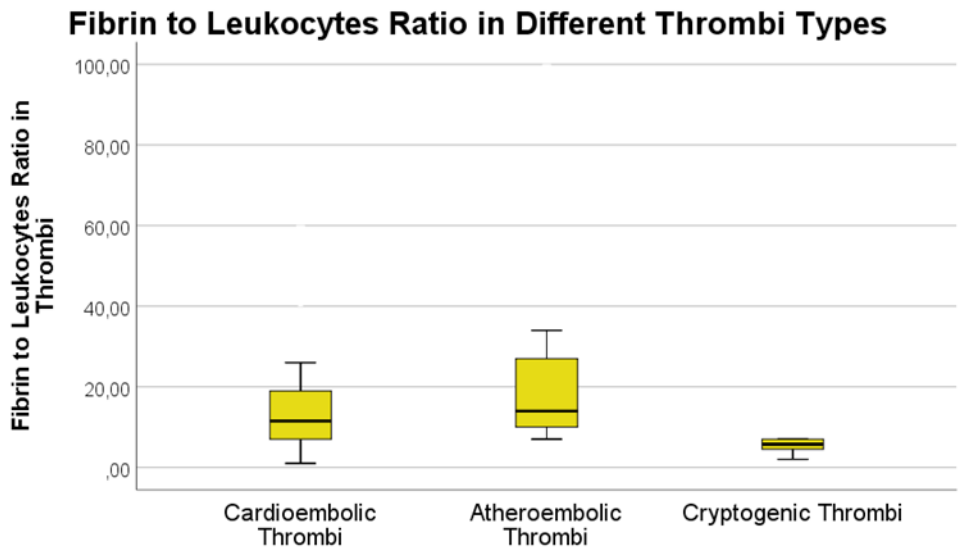

Figure 3: Fibrin to leukocytes ratio in different thrombi types.

Atheroembolic thrombi had a higher percentage of the fibrin area versus total thrombi area than cryptogenic thrombi (median - 85,0% and 79,0% respectively; P=0,038). The same tendency, but not statistically significant was found between atheroembolic and cardioembolic thrombi (median - 85,0% and 82,5% respectively; P=0,099). There were no any statistically significant differences comparing both cryptogenic and cardioembolic thrombi (p=0,288), see Figure 4.

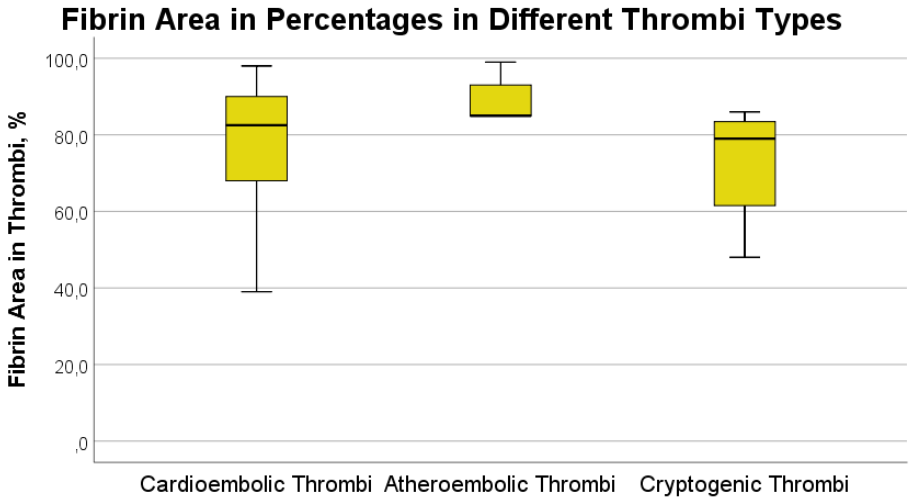

Figure 4: Fibrin area in percentages in different thrombi types.

There were no statistically significant differences in the proportion of other thrombus components between three mentioned groups, see Table S7 (see supplementary materials).

Characteristic	Comparison of Cryptogenic and Cardioembolic Groups, p value	Comparison of Cryptogenic and Atheroembolic Groups, p value	Comparison of Cardioembolic and Atheroembolic Groups, p value
Thrombus length, mm	0.269	0.282	0.233
Thrombus width, mm	0.738	0.146	0.167
Thrombus area, mm2	0.249	0.117	0.127
Fibrin area, mm2	0.107	0.148	0.110
Percentage of fibrin area in thrombus, %	0.288	*0,038	**0,099
Leukocytes area, mm2	**0,099	0.165	0.590
Percentage of leukocytes area in thrombus, %	0.354	0.331	0.442
Erythrocytes area in thrombus, mm2	0.705	0.210	0.413
Percentage of erythrocytes area in thrombus, %	0.502	0.438	0.418
Fibrin area:Leukocyte area ratio	**0,067	*0,026	0.330
	Mann-Whitney Test was used in statistical analysis.		
			* - statistically significant value
			** - tendency for statistically significant result

Table S7: Comparison of the histological composition between etiological stroke groups.

Impact of Radiological Findings on Thrombus Histopathology

In total, 23 patients had HAS on non-enhanced head CT and 17 patients did not. Histopathological findings in both patients` groups, that is with and without HAS, is seen in Table S8 (see supplementary materials). The mentioned information shows, that HAS did not correlate with thrombus size, histological composition of thrombus, as well as immunohistochemical profile. There was no statistically significant correlation between HAS and outcomes.

Characteristic	Composition of Thrombi in Patients without HAS, median (Q1; Q3)	Composition of Thrombi in Patients with HAS, median (Q1; Q3)	Test	Test Statistics, Mann-Whitney U value; Z-test	p value
Thrombus length, mm	8,0 (4,5; 11,1)	7,0 (5,0; 8,8)	Mann-Whitney U Test	170,500; -0,685	0,498
Thrombus width, mm	5,0 (3,0; 5,0)	4,0 (3,0; 4,2)		150,000; -1,261	0,221
Thrombus area, mm2	32,0 (22,5; 46,5)	28,8 (14,7; 37,2)		151,000; -1,218	0,232
Fibrin area, mm2	25,6 (13,3; 34,6)	19,7 (11,8; 29,9)		155,000; -1,108	0,277
Percentage of fibrin area in thrombus, %	82,9 (62,5; 91,0)	84,0 (72,0; 87,0)		191,000; -0,123	0,914
Leukocytes area, mm2	2,1 (1,1; 2,9)	1,9 (0,9; 4,0)		194,000; -0,041	0,978
Percentage of leukocytes area in thrombus, %	5,0 (3,0; 12,0)	7,0 (5,0; 11,0)		173,500; -0,604	0,551
Erythrocytes area in thrombus, mm2	1,5 (0,6; 4,0)	1,9 (0,5; 3,9)		187,000; -0,233	0,829
Percentage of erythrocytes area in thrombus, %	5,0 (2,5; 15,0)	8,0 (3,0; 21,0)		183,500; -0,329	0,745
Fibrin area:Leukocyte area ratio	15,0 (7,0; 20,5)	10,0 (7,0; 14,0)		177,000; -0,508	0,626
Characteristic	Composition of Thrombi in Patients without HAS, N (%)	Composition of Thrombi in Patients with HAS, N (%)	Test		p value
The amount of macrophages in thrombus, %			Fisher`s Exact Test		
less than 25%	9 (52,9%)	12 (52,2 %)			>0,999
more than 25%	8 (47,1%)	11 (47,8%)			
The amount of T lymphocytes in thrombus, %					
less than 25%	10 (58,8%)	15 (65,2%)			0,749
more than 25%	7 (41,2%)	8 (34,8%)			
The amount of segmental leukocytes in thrombus, %					
less than 50%	9 (52,9%)	13 (56,5%)			>0,999
more than 50%	8 (47,1 %)	10 (43,5%)			
The amount of platelets in thrombus, %					
less than 50%	12 (70,6%)	17 (73,9%)			>0,999
more than 50%	5 (29,4%)	6 (26,1%)			

Table S8: Composition of thrombi in patients with and without hyperdense artery sign (HAS).

In total, 29 patients have performed non-enhanced thoracic CT, and 11 patients did not receive this examination. From those, who had this investigation performed, 21 had thrombus in heart, whereas 8 had not.

Patients with visualised thrombus on CT in heart had larger erythrocyte area in thrombus, than those without visualised thrombus in heart (p=0,047). Other parameters did not differ statistically significantly and may be seen in Table S9 (see supplementary materials).

Characteristic	Composition of Thrombi in Patients without CT visualised thrombi in heart, median (Q1; Q3)	Composition of Thrombi in Patients with CT visualised thrombi in heart, median (Q1; Q3)	Test	Test Statistics, Mann-Whitney U value; Z-test	p value
Thrombus length, mm	8,5 (3,8; 9,2)	6,0 (4,0; 8,5)	Mann-Whitney U Test	72,000; -0,587	0,582
Thrombus width, mm	3,0 (2,3; 4,8)	4,2 (3,4; 5,0)		55,000; -1,427	0,168
Thrombus area, mm2	32,7 (7,5; 43,4)	30,0 (13,4; 37,4)		84,000; 0,000	>0,999
Fibrin area, mm2	21,1 (3,7; 35,7)	22,5 (11,9; 30,1)		77,000; -0,342	0,756
Percentage of fibrin area in thrombus, %	89,5 (85,0; 93,0)	83,0 (73,5; 88,3)		51,000; -1,613	0,114
Leukocytes area, mm2	1,7 (0,5; 3,0)	1,5 (1,0: 3,2)		79,500; -0,220	0,830
Percentage of leukocytes area in thrombus, %	6,5 (5,0; 8,5)	7,0 (4,0; 11,5)		81,000; -0,147	0,905
Erythrocytes area in thrombus, mm2	0,6 (0,1; 1,9)	1,8 (0,7; 4,0)		43,000; -2,004	0,047*
Percentage of erythrocytes area in thrombus, %	4,0 (0,0; 6,0)	8,0 (3,0; 17,5)		48,500; -1,740	0,083
Fibrin area:Leukocyte area ratio	13,5 (7,8; 19,3)	11,0 (7,0; 20,0)		78,500; -0,269	0,793
Characteristic	Composition of Thrombi in Patients without CT visualised thrombi in heart N, %	Composition of Thrombi in Patients with CT visualised thrombi in heart N, %	Test		p value
The amount of macrophages in thrombus, %			Fisher's Exact Test		0,215
less than 25%	2 (25%)	12 (57,1%)			
more than 25%	6 (75%)	9 (42,9%)			
The amount of T lymphocytes in thrombus, %					0,683
less than 25%	4 (50%)	13 (61,9%)			
more than 25%	4 (50%)	8 (38,1%)			
The amount of segmental leukocytes in thrombus, %					>0,999
less than 50%	4 (50%)	11 (52,4%)			
more than 50%	4 (50%)	10 (47,6%)			
The amount of platelets in thrombus, %					0,675
less than 50%	6 (75%)	13 (61,9%)			
more than 50%	2 (25%)	8 (38,1%)			

Table S9: Composition of thrombi in patients with (21 patients) and without (8 patients) CT visualised thrombi in heart.

Impact of Thrombus Histopathology on Ischemic Stroke Outcome

Ischemic stroke outcome was measured with National Institutes of Health Stroke Scale (NIHSS) and modified Rankin Scale (mRS). NIHSS score was analyzed as scale data and mRS score as ordinal data with 4 groups: first group – mRS score 0-3, second group – mRS score 4, third group – mRS score 5, fourth group – mRS score 6. Analyzing ischemic stroke outcomes depending on each thrombus histopathology, there were not found any statistically significant differences in histopathological thrombus composition.

Discussion

Impact of IV tPA on Composition of Thrombi

Analyzing our study results we have found, that IV tPA did not affect the results significantly, but there was a tendency to be less T lymphocytes in the thrombi of patients, who did not receive this treatment. It is known from previous research, that T cells are among the first cells to be recruited within the atheroma, and they are enriched in unstable plaques [12,13], it is known from cardiovascular studies that unstable plaques easier dissolve with IV tPA. Thrombi retrieved after IV tPA showed decreased number of T lymphocytes on histopathological analysis which confirms beforementioned hypothesis. It should be mentioned that there is lack of similar evidence in the stroke medicine. Other research has shown that RBC-rich thrombi respond better to r-tPA than platelet-rich or white thrombi [14]. Nevertheless, in our study investigated thrombi with and without received IV tPA did not had statistically significant differencies concerning other thrombus components.

Histopathological Differences of Thrombi by Stroke Etiology

In our study, thrombi in cryptogenic strokes demonstrated slightly larger leukocytes area (median - 4,0 mm2) comparing with cardioembolic thrombi (median – 1,6 mm2; p=0,099), although this result was not statistically significant. No differences were found between cryptogenic and atheroembolic thrombi, as well as between cardioembolic and atheroembolic thrombi. Nevertheless, we have found, that cryptogenic thrombi showed statistically significantly lower fibrin/leukocytes ratio than atheroembolic thrombi (median - 5,8 and 14,0 respectively; p=0,026), the same tendency, but not statistically significant, comparing cryptogenic thrombi and cardioembolic thrombi (median - 5,8 and 11,5 respectively; P=0,067). No statistically significant differences were found comparing cardioembolic and atheroembolic thrombi (p=0,330). That means that fibrin area to leukocyte area ratio in cryptogenic and cardioembolic thrombi was almost similar and at the same time lower than in atheroembolic thrombi, which in turn means that as leukocytes area almost do not differ between groups, fibrin area in both cryptogenic and cardioembolic thrombi is lower than in atheroembolic thrombi. That is also proved by our study following results: atheroembolic thrombi had a higher percentage of the fibrin area versus total thrombi area than cryptogenic thrombi (median - 85,0% and 79,0% respectively; P=0,038). The same tendency, but not statistically significant was found between atheroembolic and cardioembolic thrombi (median - 85,0% and 82,5% respectively; P=0,099). There were no any statistically significant differences comparing both cryptogenic and cardioembolic thrombi. That means, we can propose fibrin/leukocytes ratio as a new precise index for defining the fibrin area to total thrombus area in thrombi retrieved in stroke patients. One previous study came to the conclusion, that most large artery atherosclerosis thrombi are fibrin and platelet rich at plaque site and become fibrin and RBCs rich while occluding brain arteries [15]. Other study proposed, that cardioembolic thrombi are RBCs rich and have lower fibrin content compared with large artery atherosclerosis thrombi [16]. This corresponds to the traditional concept that cardioembolic thrombi forming in regions of stasis or slow flow are mainly composed of entrapped RBCs, and thrombi occurring in the context of atherosclerotic large arteries are mainly composed of fibrin and platelets [17,18]. In our study, making comparison between quantitative data of fibrin and erythrocytes content in thrombi, the conclusions were close to the traditional concept, but analysing retrieved material by fibrin or erythrocyte predominance in the thrombi, we had to admit, that not only atheroembolic, but also cardioembolic thrombi predominantly were fibrin rich. Some studies came to the similar conclusion - for instance, Niesten et al, 2014 [2] investigated 22 thrombi retrieved after mechanical thrombectomy in patients with acute stroke. They reported that there were no significant differences in the proportion of fi-brin and platelets between different stroke subtypes and even that clots originating from large-artery atherosclerosis (LAA) had the highest percentage of RBC`s compared with other stroke subtypes. But it is important to know, that in their study, the patient population included a small number of patients with LAA (n = 8) and cardioembolism (n = 6). This factor was the significant limitation. The same was about Maekawa et al, 2018 [19] research results, where they had found, that the proportion of fibrin in retrieved thrombi was higher in patients with cardioembolism than in those with no cardioembolism. But in this study there was only 5 patients with LAA, from whom 3 had erythrocyte rich and 2 fibrin rich thrombi. Sporns et al, 2017 [20] had larger study, where it was proved, that cardioembolic thrombi had higher proportions of fibrin/platelets and less erythrocytes. It is important to admit, that traditional concept about pathophysiology of cerebral thrombi has mainly been derived from coronary circulation studies, but in the era of mechanical thrombectomy, new results were shown. Our study had proved, that majority of cardioembolic thrombi were fibrin rich, although some of them had predominance of erythrocytes, as well as in quantitative measuring the amount of fibrin was higher in atheroembolic thrombi.

Data from other authors` studies show, that the infiltration of thrombi with leukocytes increases as the thrombus matures [8]. In another study the WBC`s proportion was similar in thrombi of cryptogenic and non-cardioembolic stroke patients (7,1% and 6,5% accordingly; p=0.487), while cardioembolic had a higher WBC`s proportion (9,1%) [21]. Boeckh-Behrens et al also concluded, that a higher percentage of WBC`s in the thrombus was associated with a cardioembolic etiology [11]. Although aged forms of thrombus may be more common in cardiac thrombi [8], some other studies did not found any statistically significant differences in leukocyte amount among different stroke subtype groups [16,19]. Our study also did not find statistically significant differences in leukocyte amount among different stroke subtype groups, which could mean that studied thrombi were of similar age and stage of the organization.

Impact of Radiological findings on Histopathology of Thrombi

In general, HAS corresponds to the thrombus composition to some degree [22] and is associated with RBC rich thrombi [9]. In addition, patients with a HAS are more likely to have a good angiographic outcome than those without [23]. Better results also depend not only on the composition of thrombi, but also on HU value - higher thrombus HU values are predictive of successful recanalization [24]. In our study HAS did not correlate neither with the histopathological composition of thrombi nor stroke outcomes. This may be due to the low number of included patients.

Our study has confirmed that patients with CT visualized thrombus in the left atrium appendage had larger erythrocyte area in thrombus than those without mentioned finding (p=0,047). This group is defined as cardioembolic group. It is widely accepted, that fresh red thrombi, containing mixtures of fibrin and RBCs, originate from low flow-regions while white thrombi, existing mainly out of platelets and fibrin, arise in regions of fast moving blood [25,26]. In cerebral stroke, thrombi from LAA are thought to originate from regions of high velocity blood stream, while most cardioembolic thrombi are assumed to develop from low flow-regions, such as the left atrial appendage [2]. The results of our study fully correspond to conventional point of view. But nevertheless, there may be absolutely opposite results - in other studies, patients with LAA had the highest percentage of RBCs [2,21,27]. Such different results may be due to some limitations - interpretations about the pathophysiology of cerebral thrombi have mainly been derived from coronary circulation studies [2], as well as usage of IV tPA before mechanical thrombectomy and following thrombi retrieval, that may have an impact on the composition of thrombi. Our study finding makes us consider including unenhanced thoracic CT examination in stroke patients` investigation protocol.

Impact of Histopathology of Thrombi on Stroke Outcome

Thrombi could roughly be divided into fibrin- and RBCs–rich clots [28]. In literature, it is highlighted, that RBCs-rich clots are associated with significantly higher recanalization rates, reduced number of maneuvers and a shorter mean recanalization time than fibrin-rich clots [29]. That may be explained with the fact that fibrin-rich clots have a significantly higher coefficient of friction than RBC-rich clots and therefore have a stronger interaction with the vessel wall and are harder to remove from the vessel wall [30]. Higher percentage of WBC`s in the thrombus are associated with less favorable recanalization (TICI<3) and clinical outcome (NIHSS score at discharge and mRS scores up to 90 days) [11]. The amount of WBC`s represents a marker of the level of organization, this is the age of the thrombus. The age, as well as stage of the organization of the thrombus are important for the success of the mechanical thrombectomy, as the stability and strength of adherence to the vessel wall of the targeted clot are thought to increase with time, making the removal of the thrombus more difficult [11]. In our study, the ischemic stroke outcome in patients after performed endovascular mechanical thrombectomy did not have any correlations with thrombus histopathology. Such results may be due to limited study population sample.

Study Limitations

Part of the patients, that were included in the research, before mechanical thrombectomy procedure had treatment with IV tPA. Although there was not statistically significant impact of this procedure on the results, the distribution of patients by performed IV tPA was not homogenous.

The limited number of patients was included – only 40 patient stroke thrombi were investigated.

The spreading of patients by stroke etiology was heterogenous, making it difficult to compare the groups.

Although Mann-Whitney test has shown statistically significant differences between atheroembolic and cryptogenic thrombi groups by the following parameters - percentage of fibrin area in thrombus and fibrin area to leukocyte area ratio in thrombus, Kruskal-Wallis test had not shown statistically significant differerence comparing three groups simultaneously (although it had shown tendency to be statistically significant). Also Bonferroni correction was not applied.

Conclusions

There was found, that cryptogenic thrombi had partial histologic similarities to cardioembolic thrombi, i.e., lower fibrin percentage in thrombi area. Therefore, patients with cryptogenic stroke should probably undergo cardiac examination including long-term heart rhythm monitoring.

As to radiological findings, patients with CT visualized thrombus in the left atrium appendage had larger erythrocyte area in thrombus than those without a mentioned finding. It may be useful to consider the inclusion of unenhanced thoracic CT examination in stroke patients` investigation protocol.

Histopathology of thrombi was not affected by IV rt-PA therapy which may rise questions about usefulness of rt-PA in patients with large vessel occlusion.

The ischemic stroke outcome in patients after performed endovascular mechanical thrombectomy did not depend on thrombus histology or its immunohistochemical profile.

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