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T.C.
YEDİTEPE UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF NEUROSCIENCE

INVESTIGATION OF NEUROBIOLOGICAL AND BIOCHEMICAL CHANGES IN DETERMINATION OF REPERFUSION RESPONSE TO INTRAVENOUS THROMBOLYTIC AND MECHANICAL THROMBECTOMY TREATMENTS IN ACUTE ISCHEMIC STROKE PATIENTS

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APPROVAL

This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated ..... and numbered .....

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Director of Institute of Health Sciences

DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

15.12.2021

Sena Aksoy

DEDICATION

This thesis is dedicated to all my teachers who contributed to my education and gave me the chance to achieve my dreams.

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LIST OF SYMBOLS AND ABBREVIATIONS

ADAPT	A Direct Aspiration First Pass Technique
AHA	American Heart Association
ASA	American Stroke Association
ASPECTS	Alberta Stroke Program Early Computed Tomography Score
ATP	Adenosine triphosphate
BBB	Blood-brain barrier
CD	Cluster of differentiation
CNS	Central nervous system
CRP	C-reactive peptide
CT	Computed tomography
DAWN	DWI or CTP Assessment With Clinical Mismatch in the Triage of Wake-up and Late Presenting Strokes Undergoing Neurointervention With Trevo
DEFUSE	Diffusion and Perfusion Imaging Evaluation for Understanding Stroke Evaluation
DWI	Diffusion-weighted imaging
ECASS	European Cooperative Acute Stroke Study
ELISA	Enzyme-linked immunosorbent assay
EPITHET	Echo-Planar Imaging Thrombolytic Evaluation Trial
EXTEND	Extending the Time for Thrombolysis in Emergency Neurological Deficits
FLAIR	Fluid-attenuated inversion recovery imaging
HAS	Hyperdens artery sign
HDL	High-density lipoprotein
H&E	Hematoxylin-eosin
HI	Hemorrhagic infarction
HU	Hounsfield units
ICA	Internal carotid artery
ICAM	Intercellular adhesion molecule
ICH	Intracranial hemorrhage

IQR	Interquartile range
IL	Interleukin
iv tPA	Intravenous thrombolysis with tissue-type plasminogen activator
LDL	Low-density lipoprotein
MCA	Middle cerebral artery
MMP	Matrix metalloproteinase
MR	Magnetic resonance
MR CLEAN	Multicenter Randomized Clinical Trial of Endovascular Treatment for Acute Ischemic Stroke in the Netherlands
mRS	Modified Rankin Scale
MSB	Martius Scarlet Blue
MT	Mechanical thrombectomy
mTICI	Modified Thrombolysis in Cerebral Infarction
N-cadherin	Neural cadherin
NET	Neutrophil extracellular trap
NIHSS	National Institute of Health Stroke Scale
NINDS	National Institute of Neurological Disorders and Stroke
PCR	Polymerase chain reaction
PDGFR	Platelet-derived growth factor receptor
PECAM	Platelet-endothelial cell adhesion molecule
PH	Parenchymal hematoma
PMN	Polymorphonuclear cell
RBC	Red blood cell
REVASCAT	Randomized Trial of Revascularization with Solitaire FR Device versus Best Medical Therapy in the Treatment of Acute Stroke Due to Anterior Circulation Large Vessel Occlusion Presenting within Eight Hours of Symptom Onset
S1PR2	Sphingosine-1-phosphate receptor 2
TNF	Tumor necrosis factor
TOAST	Trial of ORG 10172 in acute stroke treatment
VCAM	Vascular cell adhesion molecule
VE-cadherin	Vascular endothelial cadherin

SD	Standard Deviation
WBC	White blood cell

ABSTRACT

Aksoy S. (2021). Investigation of Neurobiological and Biochemical Changes in Determination of Reperfusion Response to Intravenous Thrombolytic and Mechanical Thrombectomy Treatments in Acute Ischemic Stroke Patients. Yeditepe Univesity Institute of Health Sciences, Department of Neuroscience, Doctor of Philosophy Thesis, Istanbul.

Abstract: In this study, it was aimed to investigate the roles of MMP-9, MMP-2, VE-cadherin, N-cadherin, β2 integrin, S1PR2 and PDGFR-β in neuroinflammation and reperfusion after iv tPA and mechanical thrombectomy. In addition, thrombi removed by mechanical thrombectomy were examined to understand the underlying mechanisms of reperfusion.

101 patients admitted with acute ischemic stroke were examined in three groups as those who underwent iv tPA, mechanical thrombectomy and bridging therapy. Detailed clinical information was recorded. Infarct and hemorrhage volumes were calculated from cranial imaging performed at 24 hours. Blood samples were taken from the patients before and 24 hours after treatment, and the levels of MMP-9, MMP-2, VE-cadherin, N-cadherin, β2 integrin, S1PR2 and PDGFR-β were studied by ELISA. Thrombi were stained with hematoxylin-eosin and Martius Scarlet Blue for the analysis of fibrin and erythrocyte contents, and CD34 for determining endothelium in thrombus.

PDGFR-β levels were low in patients with smaller infarct and hemorrhage volume. S1PR2 levels were related with large artery atherosclerosis. From 56 thrombi, 41 were fibrin-dominant, 15 were RBC-dominant. Fibrin content was higher in thrombi removed with stent-retriever and combined methods. A significant relationship was found between RBC dominance and hyperdense artery sign. More CD34-stained areas were found in patients with longer symptom-recanalization time and thrombi removed from distal branches.

In conclusion, PDGFR-β and S1PR2 may be associated with ischemia-reperfusion injury and intracranial hemorrhage after acute ischemic stroke. Future studies on immunological examination of the thrombus may provide further insights.

Key words: acute ischemic stroke, intravenous thrombolysis, mechanical thrombectomy, reperfusion, neuroinflammation

ÖZET

Aksoy S. (2021). Akut İskemik İnmede İntravenöz Trombolitik ve Mekanik Trombektomi Tedavileri Sonucu Reperfüzyon Yanıtının Belirlenmesinde Biyokimyasal ve Nörobiyolojik Yanıtların Araştırılması. Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü, Sinir Bilim Ana Blim Dalı, Doktora Tezi, İstanbul.

Özet**:** Bu çalışmada, iv tPA ve mekanik trombektomi tedavileri sonucu nöroinflamasyon ve reperfüzyon yanıtının belirlenmesinde, MMP-9, MMP-2, VE-cadherin, N-cadherin, β2 integrin, S1PR2 ve PDGFR-β'nın rollerinin araştırılması amaçlanmıştır. Reperfüzyon mekanizmalarının anlaşılabilmesi için mekanik trombektomi ile elde edilen trombüsler incelenmiştir.

Akut iskemik inme tanısı ile hastaneye başvuran 101 hasta iv tPA, mekanik trombektomi ve köprüleme tedavisi uygulananlar olmak üzere üç grupta incelenmiştir. Hastaların detaylı klinik bilgileri kaydedilmiştir. Enfarkt ve kanama hacimleri 24. saatte yapılan kraniyal görüntülemelerden hesaplanmıştır. Hastalardan tedavi öncesinde ve tedaviden 24 saat sonra kan örnekleri alınarak MMP-9, MMP-2, VE-cadherin, N-cadherin, β2 integrin, S1PR2 ve PDGFR-β düzeyleri ELISA yöntemiyle çalışılmıştır. Trombüsler, fibrin ve eritrosit içeriğinin analizi için hematoksilen-eozin ve Martius Scarlet Blue, trombüs içindeki endotelizasyon için CD34 ile boyanmıştır.

Enfarkt ve kanama hacmi daha küçük olan hastalarda PDGFR-β seviyelerinin düşük olduğu görülmüştür. S1PR2 seviyeleri büyük arter aterosklerozu ile ilişkili olarak saptanmıştır. 56 trombüs örneğinden 41'i fibrinden zengin, 15'i eritrositten zengin olarak bulunmuştur. Stent-retriever ve kombine yöntemle elde edilen trombüslerde fibrin içeriğinin daha yüksek olduğu görülmüştür. Eritrosit içeriği ile hiperdens arter bulgusu arasında anlamlı bir ilişki tespit edilmiştir. CD34 ile boyanan alanların semptom-rekanalizasyon süresi uzun olan hastalarda ve daha distal dallardan çıkarılan trombüslerde daha fazla olduğu görülmüştür.

Sonuç olarak, PDGFR-β ve S1PR2, akut iskemik inme sonrası iskemi-reperfüzyon hasarı ve intrakraniyal kanama ile ilişkili olabilir. Trombüsün immünolojik incelemesi üzerine yapılacak olan çalışmalar bu mekanizmaların aydınlatılmasında daha fazla bilgi sağlayabilir.

Anahtar Kelimeler: akut iskemik inme, intravenöz tromboliz, mekanik trombektomi, reperfüzyon, nöroinflamasyon

1. INTRODUCTION and PURPOSE

Stroke is the leading cause of mortality and morbidity (1,2). Ischemic stroke accounts for 85% of all stroke cases and mainly caused by large vessel occlusion, small vessels disease and cardioembolism (3, 4). During ischemia, there is a core area, which is irreversibly damaged, and an area called penumbra, where blood flow is reduced to maintain normal function, but the damage is reversible. Several studies have been carried out on the treatment methods for acute ischemic stroke to rescue salvageable neuronal tissue, that are intravenous thrombolysis with tissue-type plasminogen activator (iv tPA) and mechanical thrombectomy (MT) (1,5).

The results of the multi-center studies demonstrated effectiveness of these treatments. But in some cases, although there is no hemorrhage that would worsen the clinical outcome, patients might not get benefit despite successful recanalization. In case of complete recanalization, tissue reperfusion might not be provided effectively, which can be the reason for the mismatch between recanalization and undesirable clinical outcome (6-9). Although there are several studies in the literature on the mechanisms of ischemia and reperfusion and potential biomarkers for stroke, the underlying exact mechanism has not been understood yet (10, 11).

It has been proven that excitotoxicity initiated by ischemia leads to inflammatory processes that may play role in the pathophysiology of acute ischemic stroke (12-16). The release of proinflammatory cytokines recruit infiltration of immune system cells such as neutrophils and monocytes into the brain tissue, and activation of microglia, endothelial cells and astrocytes. Proinflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) have been shown to cause increased matrix metalloproteinase (MMP) expression and disruption of the blood-brain barrier (BBB) (12, 14, 17). Disruption of the BBB structure worsens clinical results, causing both increased inflammation and tissue damage, and hemorrhagic transformation (12, 18, 19). Although there are studies in the literature on molecules related to neuronal damage, coagulation, oxidative stress and inflammation, which are used as biomarkers to predict treatment response and prognosis in cerebral ischemia, their mechanisms are not yet clearly understood (10, 11, 20, 21). Animal studies in understanding the pathophysiology of ischemic stroke and reperfusion are insufficient to evaluate the correlation of laboratory data with clinical responses and studies using human samples are limited (12, 22, 23).

Mechanical thrombectomy gives us an opportunity to examine retrieved thrombi. In the literature, there are several studies on the histopathological and immunohistochemical examinations of thrombi and their possible correlations with stroke type or success of the treatment (24-31). Therefore, understanding of the features of thrombi may be a future perspective for recanalization treatments to get better clinical outcomes in acute ischemic stroke.

In this thesis, we aimed to investigate the roles of MMP-9 and MMP-2, endothelial adhesion markers such as vascular endothelial cadherin (VE-cadherin) and neural cadherin (N-cadherin), leukocyte adhesion markers, β2 integrin and pericyte-related markers such as sphingosine-1-phosphate receptor 2 (S1PR2) and platelet-derived growth factor receptor β (PDGFR-β) in providing reperfusion after iv tPA and mechanical thrombectomy. At the same time, thrombus samples removed by mechanical thrombectomy were examined. The novel side of this study is that for the first time endothelial adhesion markers and pericyte-related markers in serum samples taken before and after treatment were analyzed together with clinical outcome and stroke type. When the pathways related to reperfusion and hemorrhage are clarified, it is thought that these markers will be useful in predicting the patients who will benefit from the treatment and reducing the complications.

2. LITERATURE REVIEW

Ischemic stroke is acute neurological deficit due to a vascular cause, resulting in reduced blood flow in a certain region of the central nervous system (CNS). It is the second most common cause of death and one of the most important causes of disability (2, 32). Considering the aging population and increase in cardiovascular risk factors such as diabetes, hypertension and hyperlipidemia, it is not inconceivable to predict that ischemic stroke will continue to be of importance in the future.

Due to the high incidence and disability rates, acute management of ischemic stroke with acute reperfusion has great importance to minimize mortality and morbidity (33). Recent studies have led to significant improvements in clinical outcomes with the use of intravenous thrombolysis and mechanical thrombectomy. Many studies have shown that these treatments reduce disability by restoring blood flow in salvageable brain tissue in selected patients (32, 33).

The only proven systemic treatment for acute ischemic stroke is intravenous recombinant tissue plasminogen activator. It was first approved by the Food and Drug Administration in 1996, based on the study of the National Institute of Neurological Disorders and Stroke (NINDS) tPA Stroke Trial in 1995, showing the efficacy by using modified Rankin Scale (mRS) at follow-up in acute ischemic stroke patients administered within 3 hours of stroke onset (Table 1) (34, 35). Although intracranial hemorrhage was more frequently seen in tPA group, there was no difference in mortality rates. Since then, increasing number of studies have shown that clinical benefit could be obtained in patients beyond three hours. After the results of European Cooperative Acute Stroke Study (ECASS) III trial were published, iv tPA was started to be recommended for the patients with 3-4.5 hours of onset by the American Heart Association/ American Stroke Association (AHA/ASA) (1, 36).

Alteplase acts by binding the surface of the clot and activating fibrin-bound plasminogen. This causes the activation of fibrinolysis, ending up with partial or complete recanalization of vessels, if the system works well. The half-life of tPA is about 4 minutes, but because of the interference on coagulation cascade, its effects can be seen up to 24 hours (37). The given dose is 0.9 mg/kg with the maximum dose of 90 mg (over 60 minutes with the first 10% of the dose given as bolus).

The main limitation of intravenous thrombolysis is patient selection. It can be used up to 4.5 hours after stroke onset because of the high intracranial hemorrhage of

fibrinolytic treatment when lately administrated. It is clearly known that the earlier it is administrated, the better outcomes can be held (4, 37). Researches demonstrated that the number of patients needed to be treated is four to get effectiveness, if tPA is given within 3 hours, whereas it is seven if it is applied between 3 and 4,5 hours (32). In order to evaluate the patients for tPA treatment, stroke severity should be assessed by using

Table 1: Modified Rankin Scale

	Modified Rankin Scale
0	No symptoms
1	No significant disability despite some symptoms, able to perform all usual activities
2	Mild disability, able to look after own affairs without assistance, but unable to carry out all previous activities
3	Moderate disability, needs some assistance but able to walk unassisted
4	Moderately severe disability, unable to unable to meet their own bodily needs without assistance and unable to walk unassisted
5	Severe disability requires constant nursing care and attention, bedridden, incontinent
6	Dead

NIHSS (Table 2) (34). According to the latest guideline for the acute management of acute ischemic stroke, published in 2019 by AHA/ASA, if NIHSS score is greater than 5, tPA is recommended. For the patients having NIHSS score between 0 and 5 with a disability, it is highly recommended to give tPA. Nevertheless, considering the complication risks, tPA is not recommended for the patients with nondisabling mild stroke having NIHSS score lower than 5 (36). The other contraindications of iv tPA are;

- Any type of hemorrhage (intracerebral, subarachnoid, subdural) in imaging, wide and demarcated hypodensity in computed tomography (CT) scan
- High systolic (>185 mmHg) and/or diastolic (>110 mmHg) blood pressure
- Thrombocytopenia (<100.000/mm3)
- INR> 1.7, aPTT>40 (34).

Table 2: National Institute of Health Stroke Scale

NIH Stroke Scale (NIHSS)		
1a- Level of Consciousness	Alert	**0**
	Arousable to minor stimulation	**1**
	Arousable only to painful stimulation	**2**
	Reflex response or un-arousable	**3**
1b- Level of Consciousness- Questions	Both correct	**0**
	One correct (or dysarthria, foreign language)	**1**
	Neither correct, aphasia or coma	**2**
1c- Level of Consciousness- Commands	Both correct	**0**
	One correct	**1**
	Neither correct	**2**
2- Gaze	Normal	**0**
	Partial gaze, abnormal gaze in one or both eyes	**1**
	Forced eye deviation, total paresis	**2**
3- Field of Vision	No visual loss	**0**
	Partial hemianopsia	**1**
	Complete hemianopsia	**2**
	Bilateral hemianopsia or blind	**3**
4- Facial Palsy	Normal	**0**
	Minor paralysis, flat nasolabial sulcus, asymmetrical smile	**1**
	Partial paralysis (lower face)	**2**
	Complete paralysis (upper and lower face) or coma	**3**
5- Motor (Arms) **5a- Motor left arm** **5b- Motor right arm**	Normal	**0**
	Drift but does not hit bed	**1**
	Some antigravity effort but can’t sustain	**2**
	Unable to overcome gravity, minimal proximal movement present	**3**
	No movement	**4**
	Amputation	**X**

6- Motor	Normal	**0**
(Legs)	Drift but does not hit bed	**1**
6a- Motor	Some antigravity effort but can't sustain	**2**
left leg	Unable to overcome gravity, minimal proximal movement	**3**
6b- Motor	present	
right leg	No movement	**4**
	Amputation	**X**
7- Ataxia	Normal	**0**
	In one extremity	**1**
	In upper and lower extremities	**2**
	Cannot be evaluated	**X**
8- Sensation	Normal	**0**
	Mild to moderate or aphasia or impaired consciousness	**1**
	Loss of sensation in one or both sides or unresponsive or quadriplegia	**2**
9- Aphasia	Normal	**0**
	Mild to moderate aphasia	**1**
	Severe aphasia	**2**
	No understanding or coma	**3**
10-	Normal	**0**
Dysarthria	Mild to moderate, intelligible	**1**
	Unitelligible, anartria or mute	**2**
11-	Normal none detected	**0**
Extinction/	Extinction or neglect in any modality	**1**
Neglect	Profound neglect in both visual and sensory modalities	**2**

Reviewing the more recent literature, the results of the Efficacy and Safety of Thrombolysis in Wake-up Stroke trial (WAKE-UP) showed that patients with a time of onset greater than 4.5 hours might benefit from iv tPA, when patients are selected if they have signal on diffusion-weighted imaging (DWI), but not on fluid-attenuated inversion recovery imaging (FLAIR), so called DWI-FLAIR mismatch (5). At the same time, Extending the Time for Thrombolysis in Emergency Neurological Deficits (EXTEND), ECASS-4 and Echo-Planar Imaging Thrombolytic Evaluation Trial (EPITHET) trials demonstrated that in patients with uncertain stroke onset beyond the 4.5 hours window, more sophisticated imaging methods such as CT perfusion, can be used to assess tissue viability (38, 39).

The evidence on possible clinical benefits of iv tPA has given an opportunity for treatment of the patients having wake-up stroke. Wake-up stroke can be identified as stroke which are noticed on awakening by the patients, so that the exact stroke onset remains unknown. Since these patients represent 20% of all stroke cases, this issue is more considerable (39, 40). It is clearly known that the time window for tissue viability varies between people due to different collateral status. Therefore, treatment decisions should be made as a result of more comprehensive evaluation rather than time window alone.

There are some relative exclusion criteria so the decision to proceed with this treatment should be taken by considering all risks and potential benefits (Table 3) (34).

Another treatment method for reperfusion is endovascular therapy with mechanical thrombectomy. In severe stroke cases with occlusion of proximal large vessels in anterior circulation, mechanical thrombectomy can be used without or with iv tPA as bridging therapy (41). MR CLEAN (Multicenter Randomized Clinical Trial of Endovascular Treatment for Acute Ischemic Stroke in the Netherlands) was the first trial. It showed that mechanical thrombectomy was superior, regarding functional independence on the modified Rankin scale at 90 days, to usual medical care in patients applied within 6 hours (1).

Endovascular therapy can be performed within 6 hours from stroke onset. The main principle is to find the patients who have small ischemic core infarct and large ischemic penumbra. There are several methods to select patients, but the most commonly used ones are CT-based ASPECTS (Alberta Stroke Program Early Computed Tomography Score) scoring and CT and/or magnetic resonance (MR)-angiography (4, 42). ASPECTS is a 10-point quantitative scoring system based on CT

Table 3: iv tPA Relative Exclusion Criteria

iv tPA Relative Exclusion Criteria
Undetermined stroke onset
Wake-up stroke
Cranio/spinal surgery or trauma in the last 3 months
Ischemic stroke in the last 3 months
Gastrointestinal hemorrhage in the last 3 weeks
Genitourinary hemorrhage in the last 3 weeks
Major surgery in the last 3 weeks
Major systemic trauma in the last 2 weeks
Arterial junction in the last week
The history of intracranial hemorrhage
The use of new oral anticoagulants (in 48 hours)
End-stage renal failure, dialysis
End-stage liver failure, cirrhosis
Aortic dissection
Infective endocarditis
Systemic malignancy
Intracranial tumor or arteriovenous malformation
ST-elevated myocardial infarction
Pericarditis
Dural punction in the last 7 days

scan imaging in middle cerebral artery (MCA) strokes. The vascular territory of MCA is divided into 10 regions as caudate, putamen, internal capsule, insular cortex, M1-3 regions at the level of basal ganglia, M4-6 regions at the level of the ventricles above basal ganglia (Figure 1). Each region without hypodensity gets one point, that giving the total of 10 points to a normal brain. Although it is developed for MCA strokes, there is an adjusted version for posterior circulation (43). The presence of proximal arterial occlusion is evaluated with CT and/or MR-angiography.

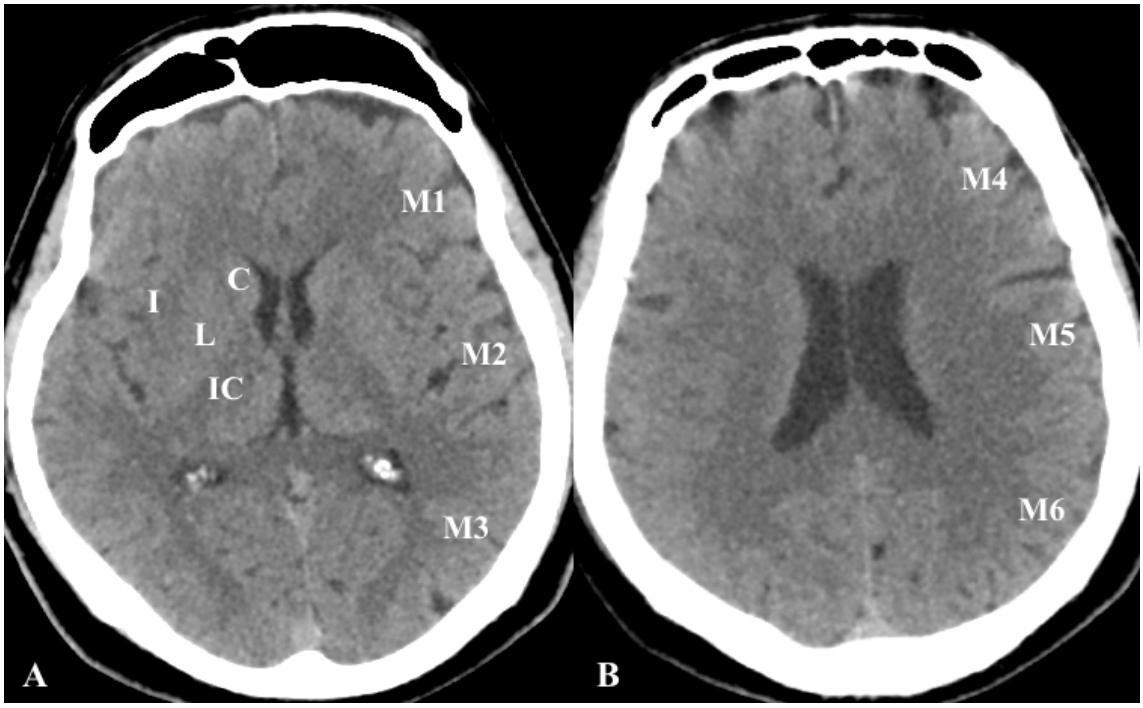

Figure 1: The ASPECTS scoring system. CT axial slice at the level of of thalamus and basal ganglia (A), CT axial slice at the level of superior margin of ganglionic structures (B). C-caudate, L-lentiform nucleus, IC-internal capsule, I-insular cortex, M1-anterior MCA cortex, M2-MCA cortex lateral to insular ribbon, M3-posterior MCA cortex, M4-anterior MCA territory superior to M1, M5-lateral MCA territory superior to M2, M6-posterior MCA territory superior to M3.

The patients with acute neurological deficit were evaluated for stroke severity with NIHSS and cranial imaging with non-contrast CT scan and CT and/or MR-

angiography (Figure 2), and those who meet following criteria are considered eligible for mechanical thrombectomy:

- Pre-stroke functional status and lack of serious comorbidities (pre-stroke modified Rankin score of 0-1)
- The causative arterial occlusion is in internal carotid artery (ICA) or proximal middle cerebral artery (Figure 2, 3 and 4)
- Age>18
- ASPECTS score>5
- Significant clinical deficit at time of treatment (NIHSS>5) (4, 44).

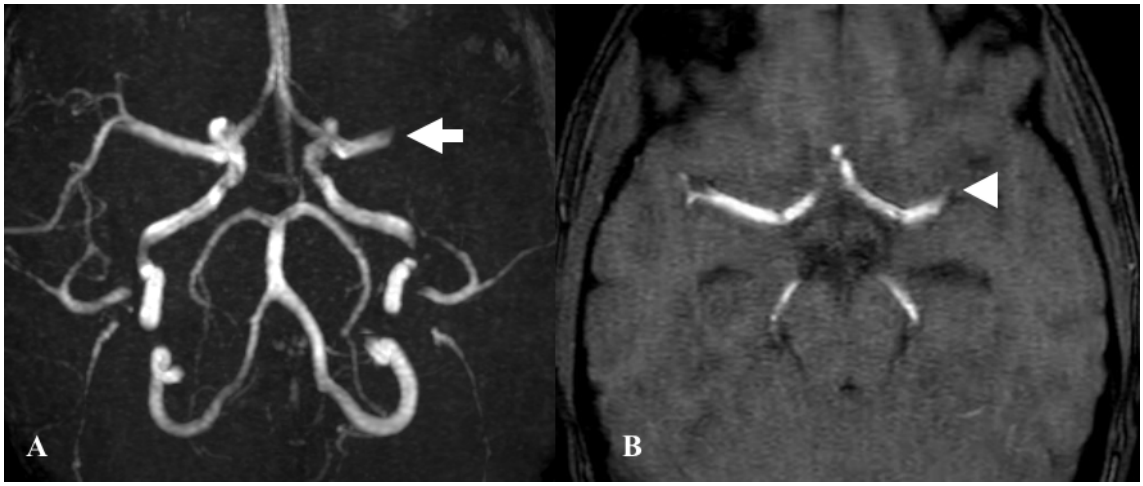

Figure 2: Three-dimensional time-of-flight maximal intensity projection (A) and source (B) images of unenhanced magnetic resonance angiography showing the occlusion of left middle cerebral artery.

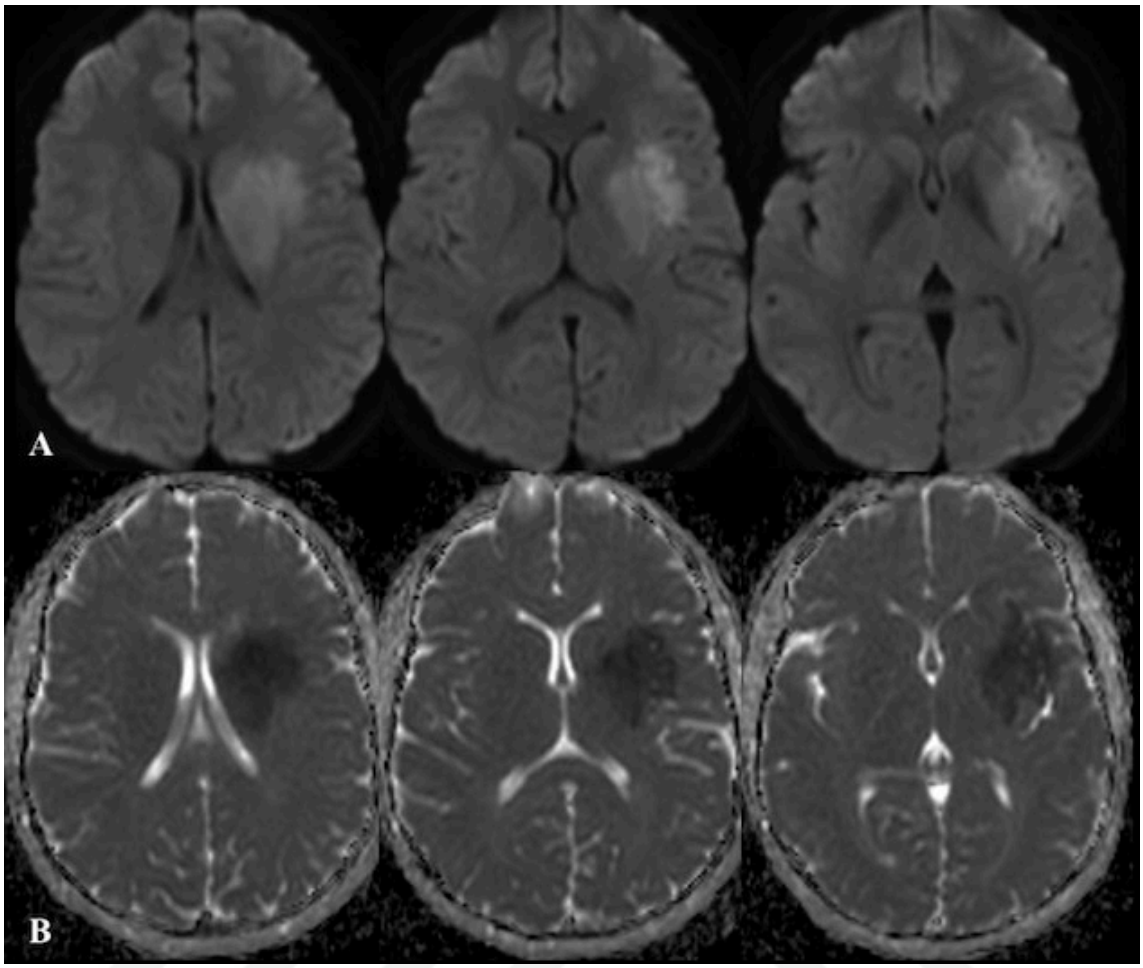

Figure 3: Magnetic resonance imaging showing acute ischemic infarction in left middle cerebral artery territory, the images of diffusion-weighted imaging (A) and corresponding apparent diffusion coefficient (B).

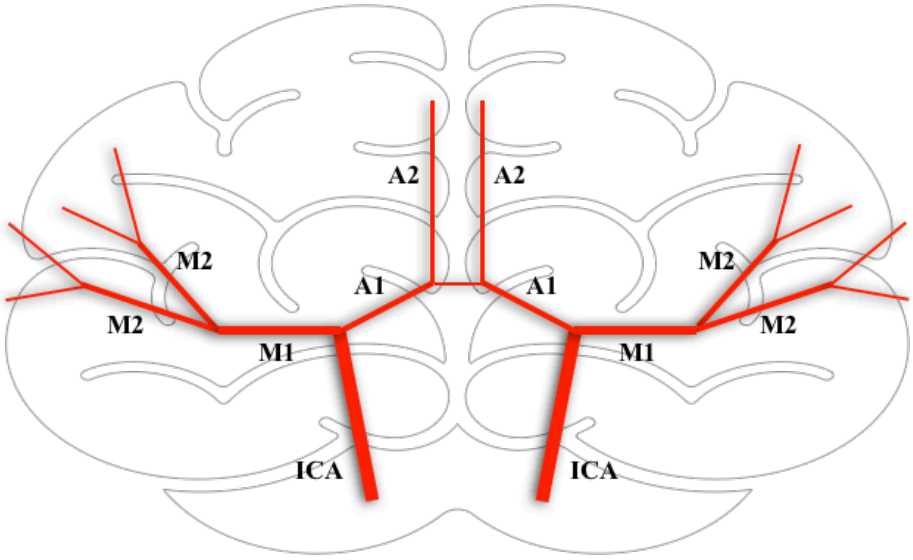

Figure 4: The schematic illustration of intracranial arteries. Internal carotid arteries terminate in middle and anterior cerebral arteries. The most proximal part of MCA is MCA M1 segment, followed by MCA M2 segment, which is the insular part of MCA.

More recent evidence revealed that, with the REVASCAT (Randomized Trial of Revascularization with Solitaire FR Device versus Best Medical Therapy in the Treatment of Acute Stroke Due to Anterior Circulation Large Vessel Occlusion Presenting within Eight Hours of Symptom Onset) trial, the period of time for anterior system circulation can be extended up to 8 hours (45). According to the latest data obtained from DAWN trial (DWI or CTP Assessment With Clinical Mismatch in the Triage of Wake-up and Late Presenting Strokes Undergoing Neurointervention With Trevo) and DEFUSE 3 trial (Diffusion and Perfusion Imaging Evaluation for Understanding Stroke Evaluation), it has been understood that, in selected patients with clinical-diffusion or diffusion-perfusion mismatch, or having large ischemic tissue compared to infarct volume on perfusion imaging, endovascular therapy can be applied up to 24 hours (46, 47).

Several different techniques and devices have been developed to increase the efficacy. There are three main techniques: stent-retriever, aspiration and combined method involving stent-retriever with concomitant aspiration. Much work on the selection of thrombectomy methods has been carried out, however it remains as a controversial issue. However, the general approach is to start with aspiration after the

results of ADAPT (A Direct Aspiration First Pass Technique) trial was published, because of shorter procedural time and better outcome (48, 49).

The success of recanalization after mechanical thrombectomy is measured by the modified Thrombolysis in Cerebral Infarction (mTICI) grading system (Table 3) (50). According to this grading system, 2b, 2c and 3 scores are accepted as successful recanalization. Moreover, recent studies proposed that mTICI 2c and 3 should be the new aim for mechanical thrombectomy due to better functional outcomes at 90 days (51).

In recent years, there has been considerable interest in endovascular therapy, better medical approaches about treatment window, patient selection, method, stroke in posterior circulation are still investigated (52-55).

With the increasing use of iv tPA and endovascular therapy, the complications of these treatments, which are intracranial hemorrhage (ICH) and malignant edema, have become an important issue (56, 57). Symptomatic ICH (sICH) is defined as any hemorrhage causing decline in NIHSS score and has a causal relationship between neurological deterioration and hemorrhage (56). However, there are several definitions of symptomatic ICH in the literature. The NINDS study defined sICH as neurological decline within 36 hours after iv tPA administration. Whereas, in DEFUSE study, sICH is also divided in minor and major hemorrhage, 2 to 3 points increase in NIHSS and >4 points increase in NIHSS, respectively (58). ICH can be classified as hemorrhagic infarction (HI) and parenchymal hematoma (PH) according to its size. In ECASS study, these terms were defined as HI1, small petechiae along the margins of the infarct, HI2, more confluent petechiae within the infarct without mass effect, PH1, a clot not exceeding 30% of the infarcted area, and PH2, a clot occupying more than 30% of the infarcted area, causing significant mass effect (59).

It has been reported that parenchymal hematoma with mass effect is associated with neurological decline and high mortality. For instance, in ECASS and ECASS II studies, PH2 was found to be related with early clinical deterioration and mortality in three months (59).

There are several studies in the literature to elucidate the relationship between iv tPA and/or mechanical thrombectomy and ICH, and possible risk factors. After ischemic stroke, the breakdown of the blood-brain barrier occurs within two weeks. It was proposed that because these treatments have rapid effect, intracranial hemorrhage in the first 24 hours could be a result of reperfusion (56, 59). Moreover, in patients treated

with iv tPA, if recanalization cannot be achieved, tPA may cause further damage due to its neurotoxic effects, leading to worse clinical outcome (60).

Table 4: Modified Thrombolysis in Cerebral Infarction

Modified Thrombolysis in Cerebral Infarction	
Grade	**Appearance on final angiographic image**
0	No perfusion or anterograde flow beyond site of occlusion
1	Penetration but not perfusion. Contrast penetration exists past the initial obstruction but with minimal filling of the normal territory
2	Incomplete perfusion wherein the contrast passes the occlusion and opacifies the distal arterial bed but rate of entry or clearance from the bed is slower or incomplete when compared to non-involved territories
2a	Some perfusion with distal branch filling of <50% of territory visualized
2b	Substantial perfusion with distal branch filling of ≥50% of territory visualized
2c	Near complete perfusion except for slow flow in a few distal cortical vessels, or presence of small distal cortical emboli
3	Complete perfusion with normal filling of all distal branches

It has recently been shown that tPA may disrupt blood-brain barrier through PDGF-CC, annexin A2, low density lipoprotein receptor-related protein 1 and N-methyl-D-aspartate receptor, and activate leukocytes and platelets (23, 61). Increased age, male sex, high systolic blood pressure, high blood glucose, severity of stroke symptoms at presentation, increased symptom-to-door time were found to be risk factors for both iv tPA and endovascular therapy, whereas poor collateral flow was thought to be a reason for ICH in endovascular therapy (62, 63).

The breakdown of BBB causes transvascular flow of plasma to tissue, ending up with cerebral edema. Unfortunately, cerebral edema results in severe clinical deterioration and the need for surgical intervention. Several studies have been conducted on the effect of iv tPA and mechanical thrombectomy on cerebral edema, but the results were not consistent. It has been hypothesized that reperfusion might enhance

the damage in blood-brain barrier and worsen cerebral edema. On the contrary, it was shown that recanalization decreases the risk of cerebral edema (64).

Poor clinical outcome remains a major problem in the management of acute ischemic stroke. It is known that, although successful recanalization rates after these treatments, functional independence at 90 days can be achieved in only half of the patients (65). In the EPITHET trial, it was demonstrated that four patients from 13 patients who was administrated tPA and showed recanalization by imaging, did not show reperfusion (6). Despite the huge interest on this result, the reasons and possible solutions have not been clearly revealed yet. Reviewing the literature, several clinical parameters such as greater age, high NIHSS, low ASPECTS, peripheral blood cell counts was found to be related with poor outcome (65, 66).

It has been revealed that recanalization does not always result in microvascular reperfusion, which indicates the restoration of microcirculatory blood flow (9). Thus, microcirculation means more than the reopening of an occluded vessel. In order to better understand how to provide microvascular blood flow, it is necessary to look at the cellular mechanism that develop after ischemia and reperfusion.

Ischemia is caused by thrombosis or embolism. The reduction of cerebral blood flow below certain values leads to cellular changes and ultimately neuronal death in brain tissue. Three different parts of ischemic brain were defined as ischemic core, where the damage is irreversible, penumbra, which is functionally impaired but still has viable tissue, and oligemia where the blood flow is recovered spontaneously. The penumbra is the target of these treatments. Studies on cerebral blood flow indicated that tissue vulnerability to ischemia differs, which is explained by the term differential neuronal vulnerability. The rate of conversion of the penumbra to the infarct core was found to be related to time, collateral status, patient factors such as diabetes, age, previous brain diseases, leukoaraiosis (67).

Ischemia and oxygen depletion have many cellular effects such as cellular acidosis, altered membrane potential, altered ion distribution, depletion of adenosine triphosphate (ATP), cytoskeletal disorganization and cellular swelling in early stages. Decreased blood flow leads to lack of energy for neurons to maintain their transmembrane potential (12). The dysfunction of ion channels by ATP depletion causes excessive glutamate release and excitotoxicity (13, 14, 16). As a result, glutamate excitotoxicity decreases the number and length of dendrites and tau hyperphosphorylation and causes neuronal apoptosis in the penumbra (12).

In recent years, the mechanisms of reperfusion injury have been investigated to understand insufficient functional outcome after the use of recanalization treatments. Reperfusion after ischemia causes an inflammatory reaction, resulting in microvascular dysfunction and impaired tissue function. The obstruction of microcirculation is thought to have a role in impaired reperfusion after successful recanalization, the phenomenon known as 'no-reflow' (9, 68). The major pathways explaining this phenomenon can be classified as:

- The disruption of neurovascular unit
- Constriction of pericytes due to oxidative-nitrative stress
- The activation of leukocytes and the infiltration of leukocytes and other blood cells in microvascular bed
- Overproduction of reactive oxygen species by rapid loss of adenosine triphosphate leading to oxidative stress
- Protein synthesis suppression due to inhibition of protein translation initiation
- The activation and aggregation of platelets as a result of encountering subendothelial extracellular matrix to blood cells because of endothelial disruption
- Complement activation (6, 18).

The BBB which is composed of endothelial cells, astrocyte end-feets, basement membrane and pericytes, limits the entrance of blood components into the cerebral parenchyma. The integrity of the BBB is of great importance to prevent extravasation of vascular inflammatory cells and proteins to brain tissue during ischemia (12). The functional unit for matching metabolic demand and blood supply in nervous system, connecting neural tissue to capillary vasculature is defined as the neurovascular unit, including neurons, non-neuronal cells (astrocytes, pericytes, microglia) and endothelium (Figure 5).

Cerebral endothelial cells are significantly different from non-cerebral ones with the presence of intercellular tight junctions and adherence junctions for restriction and regulation of the passage of plasma compounds to neural tissue as well as limited amount of transcytosis (12). Tight junction proteins are occludin and claudin, which are connected to cytoskeleton and construct a low paracellular permeability. Adherens junctions, where VE-cadherin and N-cadherin are expressed in endothelial cells are the

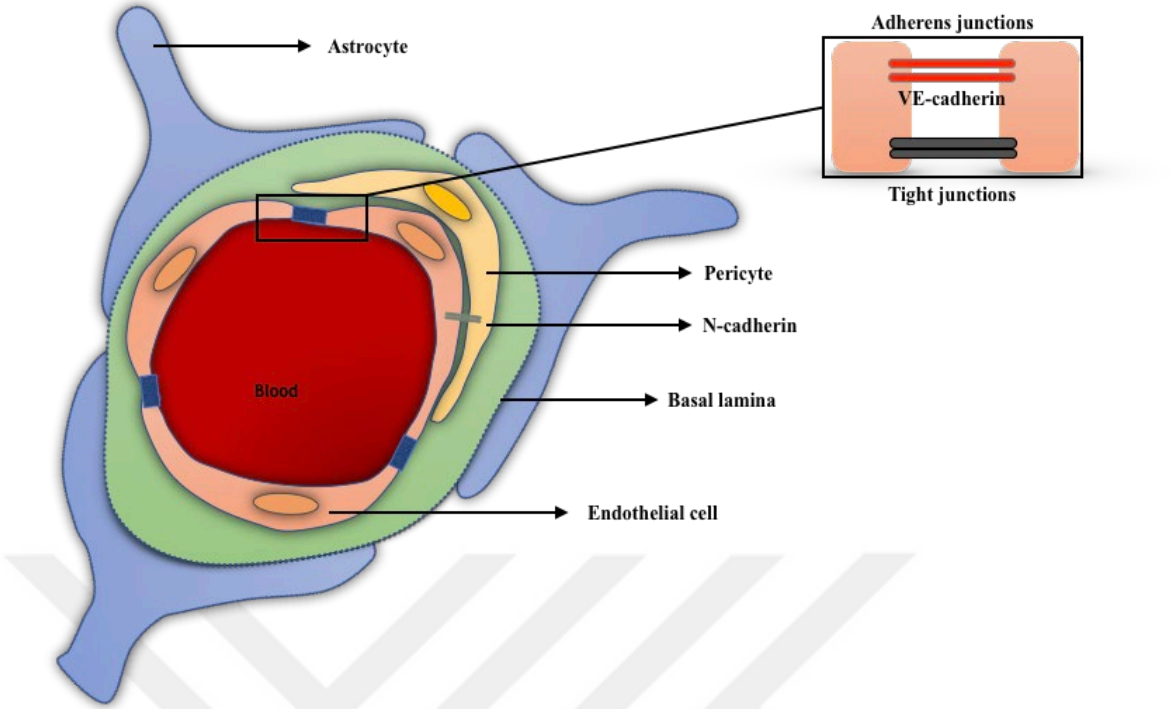

Figure 5: The schematic structure of blood-brain barrier, which is composed of endothelial cells, astrocyte end-feets, basement membrane and pericytes. Cerebral endothelial cells present tight junctions and adherens junctions, VE-cadherin and N-cadherin, to limit the entrance of blood components into the cerebral parenchyma.

other type of cell-cell adhesion contact, regulating endothelial permeability (69). The adherens junction may also modulate migration through intracellular interactions with integrin-based adhesion complexes (70). Integrin family, transmembrane proteins, anchors endothelium to extracellular matrix, and helps to maintain the integrity of blood-brain barrier (71).

Endothelium is impaired in hypoxia due to decrease in adenylate cyclase activity and increase in vascular permeability. Besides, ischemia promotes expression of proinflammatory gene products and bioactive agents in endothelium and induces apoptosis and autophagy-associated cell death. Thus, hypoxia affects the endothelium not only in cellular manner as barrier, but also activates polymorphonuclear cells (PMNs) trafficking, causing an inflammatory process, involving especially T cells (15). The activation of PMNs through β_2 integrins, in turn, stimulates endothelial gap formations and increases endothelial permeability (16).

Vascular endothelial cadherin is the primary endothelium-spesific member of cadherin family and only expressed in the endothelium (12, 69, 72). In ischemic conditions, increased intracellular concentration leads changes in signals regulating transcription of adherens junctions, in addition to the phosphorylation, cleavage and internalization of VE-cadherin. As a result, BBB permeability increases and leukocytes infiltrate into the CNS (12, 72, 73). Recent studies demonstrated that ischemia promoted VE-cadherin protein expression in mice (74). Similarly, in acute ischemic stroke patients, a higher level of VE-cadherin circulating endothelial cells were found, compared to healthy controls. VE-cadherin is also known as its function in angiogenesis. In adult mice, blocking it with antibodies resulted in microvascular dysfunction and impaired angiogenesis (69, 72).

Neural cadherin is expressed in vascular smooth muscle cells, vascular endothelium, astrocytes and neurons. It plays a role at heterotypic cell-cell contacts between endothelial cells and pericytes (72, 75). Animal studies suggested that N-cadherin mediates pericyte adhesion to cerebral vessels and the balance between N-cadherin and VE-cadherin may have an impact on ischemia in subacute phases, by affecting endothelium-pericyte interaction (75).

Proinflammatory cytokines, mainly TNF-α, IL-1 and IL-6 stimulate MMP production in ischemia (17). MMPs are zinc-containing endopeptidases, taking role in development, tissue repair and remodeling (76, 77). The activation of MMPs, especially

MMP-9, induces BBB disruption, ending up with hemorrhagic transformation and edema (78, 79).

MMP-9 is synthesized not only from glial cells and endothelium but also released from leukocytes, mainly neutrophils during reperfusion injury. It is known that occludin, tight junction protein of endothelium, is damaged by MMPs during ischemia (12). Studies on biomarkers about the risk for hemorrhagic transformation, an association between MMP-9 and hemorrhagic transformation was found regardless of tPA administration (78, 80). It is also found to be a potential biomarker to assess NIHSS score after 48 hours in ischemic stroke, due to its gradual increase in serum concentration over time (81, 82). Recent studies proposed that iv tPA, apart from its effect in reperfusion, has effect on the activation of MMPs, resulting in damage to BBB by several mechanisms such as releasing MMP-9 from neutrophil granules, induction of MMP-9 gene expression and conversion to the active form (79, 82, 83). MMP-2, another member of gelatins family, seems to have role in later stages of ischemic stroke during the formation glial scar (82). Although MMP-2 increased after stroke in animal models, the findings in human studies were controversial (57, 81, 84).

In the early stages of ischemia, many different cytokines, chemokines and prostaglandins are released and activate microglia. Activated microglia causes the induction of integrins and selectins, which results in leukocyte adhesion (14). In ischemia-reperfusion injury, leukocytes play a major role. Endothelial P-selectin expression increases with the induction of ischemia-reperfusion and interacts with P-selectin glycoprotein 1 on the surface of leukocyte, causing leukocyte-endothelial binding and leukocyte rolling. The process continues with leukocyte β_2 integrin and endothelial intercellular adhesion molecule 1 (ICAM 1), vascular cell adhesion molecule-1 (VCAM-1), platelet-endothelial cell adhesion molecule-1 (PECAM-1) interaction for adherence (6, 15, 16). It has been shown that ischemia-reperfusion injury was decreased in rodents with genetic deficiency of β_2 integrin or when antibodies against β_2 integrin were given, but, similar findings were not observed in clinical studies (85). Extravascular accumulation of PMNs results in increase in the breakdown of the BBB, increased microvascular permeability, hemorrhagic transformation, edema and further tissue damage (Figure 6). There are animal studies in the literature showing that neutrophil infiltration in damaged tissue is higher during reperfusion than permanent occlusion. In addition, with the administration of anti-neutrophil antibodies, smaller infarct size was observed in animals (42). It was proposed that as the number of passes

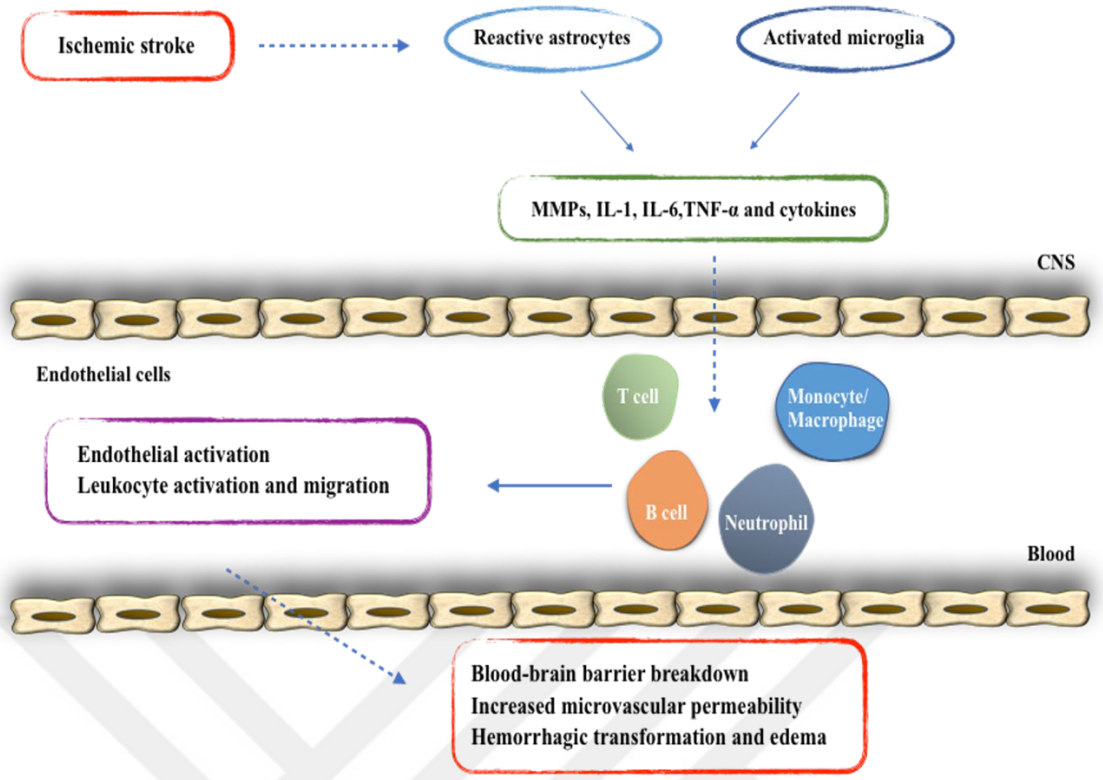

Figure 6: The schematic illustration of postischemic inflammatory response. During ischemia, reactive astrocytes and activated macrophages cause the release of cytokines, chemokines and MMPs, which results in the activation of leukocytes and endothelium, leukocyte adhesion and migration. Extravascular accumulation of leukocytes eventually causes the breakdown of blood-brain barrier, increased microvascular permeability, hemorrhagic transformation and edema.

increased during mechanical thrombectomy, disruption of the BBB and clinical outcome worsened (86).

Pericytes, a member of neurovascular unit, surround endothelium by their finger-like processes and act as smooth muscle cells, for the regulation of focal cerebral blood flow, in addition to their role in integrity of the BBB, angiogenesis, regulation in immune responses (9, 18). Recent studies have clearly shown that pericytes can contract or dilate according to tissue demand to regulate blood flow. It was shown in mice that despite successful recanalization after middle cerebral artery occlusion, pericytes around vessel wall remain contracted and restrict blood flow (18, 19). As capillary radius decreases because of pericyte contraction, erythrocytes and other blood cells can hardly pass vessel, causing entrapment of erythrocytes and platelet-fibrin deposits on vessel wall. This mechanism was previously explained via endothelial swelling and the swollen astrocyte endfeets resulted in luminal narrowing in capillaries of ischemic brain (6, 9). As a result, the constriction of capillaries by fibrin deposits, leukocytes and erythrocytes, the contraction of pericytes may be responsible for 'no-reflow' phenomenon and so, negative affect on tissue survival (6, 7, 8, 18). In addition to their parts in earlier stages of reperfusion injury, pericytes also have an important role in later stages for tissue repair because they are responsible for the release of vasoactive mediators, the growth and development of endothelial cells and angiogenesis (19).

Platelet-derived growth factor and its receptor are found in the CNS, mainly in neurons, astroglial cells, and pericytes, and plays an important role in pericyte functions such as migration, proliferation survival and angiogenesis (87, 88, 89). Experiments on PDGFR-β and pericytes was conducted and indicated that PDGFR-β was involved in functional recovery, local tissue responses and fibrosis after cerebral ischemia (87, 88, 90). Moreover, the increase in circulating PDGFR-β expressing cells in acute stroke patients and a correlation with neurologic impairment were found in previous studies (91).

Sphingosine-1 phosphate is a biolipid, modulating many cellular functions. It is found in platelets, erythrocytes and endothelial cells and acts through its receptor, sphingosine-1 phospate receptor (S1PR) 1-5. S1PR2 is found in vascular endothelium providing endothelial integrity. The activation of S1PR2 increases disruption of adherens junctions and BBB, vascular permeability in central nervous system after ischemic stroke, with the activation of MMP-9 (92, 93). In experimental stroke models,

the inhibition of S1PR2 potently decreased cerebral edema and hemorrhagic transformation (93).

The increase in reactive oxygen species during reperfusion results in further tissue damage by direct damage to cellular membranes, breakdown of the BBB, and stimulation of leukocyte activation (12, 14). The oxidative stress markers were found to be elevated in the patients with stroke.

During the early stages of reperfusion injury, misfolded proteins tend to accumulate in the lumen of endoplasmic reticulum, which results in the activation of some cellular factors and eventually leads to the downregulation of protein synthesis (6).

Platelets are activated by platelet-activating factor during reperfusion after ischemia and accumulates on endothelial cells through P-selectin. This interaction results in the release of proinflammatory molecules PDGF, arachidonic acid metabolites, thromboxane A2, serotonine, which cause further activation of leukocytes and further damage. Similarly, the complement system is also involved in the process through the mechanisms such as the production of proinflammatory factors and leukocyte activation (42).

Anti-inflammatory treatment has been applied to reduce damage in reperfusion. Enlimomab, anti-ICAM1, was given to the patients within 6 hours of stroke onset. However, it resulted in worse clinical outcome at 90 days compared to placebo, although anti-ICAM1 antibodies reduces infarct size in rats (12, 22). Two trials were conducted to investigate the possible effect of fingolimod in ischemic stroke. Fingolimod acts by sequestrating mature lymphocytes into lymph nodes, therefore, it is a very commonly used drug in multiple sclerosis. The studies concluded similarly that in patients treated with fingolimod had smaller infarct core, smaller hemorrhage volume and better clinical outcome, compared to placebo groups. Natalizumab, monoclonal antibody blocking the interaction of leukocyte and endothelium, is another candidate drug to limit neuroinflammation in stroke, however, the results were not successful to reduce infarct size. Due to neurotoxicity of tPA by disrupting the BBB through PDGF-CC, imatinib was given to mice with ischemic stroke and decrease in infarct size was observed. In both preclinical and clinical studies proposed that edaravone, free radical scavenger, reduced the rate of symptomatic intracranial hemorrhage when used as a combined treatment with tPA (23).

The widespread use of endovascular therapy allowed the investigation of thrombus that causes ischemic stroke. Several studies have been conducted to reveal whether there is a relationship between thrombus composition and stroke etiology or procedural outcome. In addition, the examination of thrombus was expected to understand 'tPA resistance' which explains why recanalization is achieved in only less than half of the cases (27).

Thrombus is mainly composed of erythrocytes, platelets, fibrin and leukocytes. The mechanism of thrombus formation may involve various features according to underlying etiology. To give an example, while endothelial damage, flow stasis and coagulation abnormalities play role in cardioembolic stroke, in atherosclerosis, platelet aggregation at stenotic site of the artery was followed by erythrocyte aggregation at poststenotic site due to turbulent flow (94). Looking at thrombus composition, there are two main areas: erythrocyte-rich area and platelet-fibrin-rich area, that can be examined after staining with Hematoxylin-eosin (H&E) or Martius Scarlet Blue (MSB). It was demonstrated that leukocytes tend to localize in platelet-fibrin-rich area, whereas T cells and monocytes were identified in erythrocyte-rich area with additional immunohistochemical staining (95, 96, 97). Besides, they showed the same opposite pattern in etiology that higher white blood cell (WBC) fraction was found in stroke of cardioembolic origin and related with poor recanalization rate and clinical outcome, whereas higher T cell content in atherosclerosis (24, 25).

Reviewing the literature, conflicting results about the predominant component of thrombus have been found in terms of stroke etiology. Recent studies have been demonstrated that thrombi in cardioembolic stroke contain more fibrin/platelets and less erythrocytes, whereas thrombi in atherosclerotic stroke are the opposite (94, 97). Moreover, in patients with cryptogenic stroke, thrombus composition was shown to be similar with the ones in cardioembolic stroke which indicates that cryptogenic strokes are of cardioembolic origin (26, 30, 94).

Leukocytes, neutrophils and neutrophil extracellular traps have been increasingly involved in thrombus studies. Neutrophils release decondensed chromatin which creates fibrous structures with granular components, that called as neutrophil extracellular traps. Neutrophil extracellular traps have both immunological and thrombotic properties. In recent studies, neutrophil extracellular traps were found to be related with more organized thrombus and cardioembolic stroke (27, 94, 97). About thrombus organization, it has been reported that endothelialization in the thrombus with

CD34 staining was demonstrated to be associated with a more organized fibrin structure (98).

Thrombus can be visualized by cranial imaging techniques. Cranial CT scan is the first imaging technique when the patients apply with acute neurological deficit. On CT scan, thrombus can be detected as hyperdensity along the vessel, which is named as hyperdense artery sign (HAS) (99, 100). Previous studies showed that as the fraction of red blood cell (RBC) increases, thrombus has higher density (28, 95). Recent studies have been focused to be able to differentiate thrombus composition with imaging to understand stroke etiology and to select the most suitable procedural technique for the patients (101).

In this study, the investigation of the roles of MMP-9, MMP-2, VE-cadherin, N-cadherin, β2 integrin, S1PR2 and PDGFR-β in neuroinflammation and reperfusion after iv tPA and mechanical thrombectomy was aimed. In addition, thrombi removed by mechanical thrombectomy were examined to understand the mechanisms underlying reperfusion.

3. MATERIALS and METHODS

3.1. Patient Selection

In this study, the samples of serum and thrombus were obtained from the patients who admitted to Bakırköy Prof. Dr. Mazhar Osman Mental Health and Neurological Diseases Training and Research Hospital with the diagnosis of acute ischemic stroke and treated with iv tPA and/or mechanical thrombectomy between August 2020 and March 2021. A total of 101 patients were included in the study within one-year period. Patients were evaluated in three groups:

1) Those receiving only iv tPA,

2) Those going through mechanical thrombectomy, and

3) Those receiving bridging therapy as mechanical thrombectomy after iv tPA.

Ischemic stroke cases in the middle cerebral artery supplying area are included in the study. Patients and/or their relatives were informed about the study, and only volunteers were recruited to the study after their written consent was obtained. The primary endpoint of the study was determined as reaching the target number of patients. For this study, approval for biological material transport was obtained from the Medical Specialty Education Board of Bakırköy Prof. Dr. Mazhar Osman Mental Health and Neurological Diseases Training and Research Hospital (Approval number: 2570). Ethical approval for this study was obtained from Yeditepe University Clinical Research Ethics Committee (Approval number: 1212).

Serum and thrombus samples to be used in this study were collected from the patients who underwent mechanical thrombectomy in Interventional Neuroradiology Unit, Bakırköy Sadi Konuk Training and Research Hospital, working in cooperation with Bakırköy Prof. Dr. Mazhar Osman Mental Health and Neurological Diseases Training and Research Hospital.

3.2. Clinical Information

The demographic characteristics of the patients were recorded such as age, sex, besides detailed clinical information including localization of infarction and occlusion, NIHSS score (Table 2) calculated according to the pretreatment neurological

examination by neurologists, the presence of comorbidities (diabetes, hypertension, atrial fibrillation, history of previous stroke), smoking, antiaggregant and anticoagulant drugs used before stroke, the numbers of leukocytes, neutrophils, lymphocytes, platelets, serum lipid profile (low-density lipoprotein, LDL, high-density lipoprotein, HDL, triglyceride), HbA1c and C-reactive peptide (CRP) levels. Moreover, because the study was conducted during the covid pandemic and all patients were tested for Covid-19 polymerase chain reaction (PCR), the presence of Covid-19 infection was also recorded. For the evaluation of the time interval between symptom onset and the start of particular treatments, symptom-to-door, door-to-needle, symptom-to-needle, symptom-to-puncture, symptom-to-recanalization times were recorded.

Diagnostic angiography and mechanical thrombectomy were performed by experienced neuroradiologists, on Philips Allura FD20/20 Interventional Biplane Angiography System (Philips Medical Systems, the Netherlands). During the procedure, treatment method was selected by neuroradiologists according to several conditions such as the site of occlusion, the structure of vessels and the appearance of the thrombi. 6F Sofia 0.070-inch catheter (MicroVention, Tustin, California) was used for aspiration, whereas Solitaire stent (Covidien/ev3, Irvine, California), Trevo stent (Stryker Medical, California) and the Catch thrombectomy device (Balt, Montmorency, France) for stent-retriever technique. In patients who underwent mechanical thrombectomy, procedural information such as the duration of procedure, the number of passes, the method used for recanalization, the use of general anesthesia, the need of stenting and the recanalization score after endovascular therapy by using modified treatment in cerebral ischemia score (Table 4) were recorded.

The clinical outcome was evaluated with NIHSS score 24 hours after treatment and at discharge from hospital, the modified Rankin Scale score (Table 1) calculated at discharge and 90 days after treatment. The assessment of mRS at 90 days was done by telephone calls and review of medical records. The duration of hospitalization and the complications during hospitalization were also recorded. Stroke etiology according to TOAST (trial of ORG 10172 in acute stroke treatment) classification was defined after etiological examinations such as electrocardiography, echocardiography, the examination of blood samples, MR-angiography, CT-angiography were completed. According to TOAST classification, stroke subtypes were defined as five categories: 1-large-artery atherosclerosis, 2-cardioembolism, 3-small-vessel disease, 4-stroke of other determined etiology, 5-stroke of undetermined etiology (102).

3.3. Neuroimaging Findings

All patients were imaged by CT scanner (Somatom Definition Flash, Siemens). MR imaging was performed by using 1.5 T scanners (Ingenia, Philips Healthcare, Best, the Netherlands or Magnetom Aera, Siemens).

The ASPECTS score and the presence of hyperdense artery sign were evaluated from the first cranial CT images of the patients before treatment. For the ones having hyperdense artery sign, the clot attenuation in Hounsfield units (HU) was measured from the images of nonenhanced cranial CT. Because differences in hematocrit levels may affect blood density, thrombus density was evaluated in relation to contralateral middle cerebral artery, by using the formula 'HU clot/ HU contralateral middle cerebral artery' (99). The presence of hemorrhage by cranial computed tomography after the treatment by using the ECASS protocol and the presence of occlusion or stenosis in related vessel at least 24 hours after treatment by MR-angiography, CT-angiography or carotid and vertebral artery doppler ultrasonography were evaluated and classified in three groups as no occlusion or stenosis, stenosis and occlusion (59). Infarct volume was calculated from cranial images of magnetic resonance imaging or computed tomography at least 24 hours after treatment by using ABC/2 formula (103). Likewise, the volume of cranial hemorrhage was calculated from cranial CT images obtained at 24 hours after treatment.

3.4. Analysis of Blood Samples

Blood samples were taken from the patients at two different times, before treatment and 24 hours after treatment. When the serum samples were taken, they were immediately centrifuged at 3000 rpm for 15 minutes and stored at -80°C after centrifugation until analysis (81).

The levels of MMP-9, MMP-2, N-cadherin, VE-cadherin, β2 integrin, S1PR2 and PDGFR-β were determined by commercially available enzyme-linked immunosorbent assay (ELISA), considering their low amounts in serum. All experiments were conducted in Koç University Research Center for Translational Medicine.

3.4.1. MMP-2

Human MMP 2, gelatinase A ELISA kit (BT Lab, E0901Hu) was used according to its instructions. The standard solutions were prepared with the

concentration of 1600 ng/ml, 800 ng/ml, 400 ng/ml, 200 ng/ml and 100 ng/ml. 50µl of standard solutions were added to each standard well and 40µl sample to sample wells. 10µl anti-MMP-2 antibody and 50µl streptavidin-HRP to sample wells and standard wells were added. The plate were incubated at 37°C for 60 minutes. After automated washing the plate for 5 times with wash buffer, 50µl of substrate solution A and 50µl of substrate solution B were added and the plate were further incubated for 10 minutes at 37°C in the dark. Within 10 minutes after adding 50µl stop solution to each well, optical density value was detected using a microplate reader set to 450 nm.

3.4.2. MMP-9

Human MMP 9 ELISA kit (BT Lab, E0936Hu) was used according to its instructions. The standard solutions were prepared with the concentration of 4800 ng/ml, 2400 ng/ml, 1200 ng/ml, 600 ng/ml and 300 ng/ml. 50µl of standard solutions were added to each standard well and 40µl sample to sample wells. 10µl anti-MMP-9 antibody and 50µl streptavidin-HRP to sample wells and standard wells were added. The plate were incubated at 37°C for 60 minutes. After automated washing the plate for 5 times with wash buffer, 50µl of substrate solution A and 50µl of substrate solution B were added and the plate were further incubated for 10 minutes at 37°C in the dark. Within 10 minutes after adding 50µl stop solution to each well, optical density value was detected using a microplate reader set to 450 nm.

3.4.3. N-cadherin

Human neural-cadherin ELISA kit (BT Lab, E0305Hu) was used according to its instructions. The standard solutions were prepared with the concentration of 240 ng/ml, 120 ng/ml, 60 ng/ml, 30 ng/ml and 15 ng/ml. 50µl of standard solutions were added to each standard well and 40µl sample to sample wells. 10µl anti-N-Cad antibody and 50µl streptavidin-HRP to sample wells and standard wells were added. The plate were incubated at 37°C for 60 minutes. After automated washing the plate for 5 times with wash buffer, 50µl of substrate solution A and 50µl of substrate solution B were added and the plate were further incubated for 10 minutes at 37°C in the dark. Within 10 minutes after adding 50µl stop solution to each well, optical density value was detected using a microplate reader set to 450 nm.

3.4.4. VE-cadherin

Human vascular endothelial-cadherin complex ELISA kit (BT Lab, E0272Hu) was used according to its instructions. The standard solutions were prepared with the concentration of 24 ng/ml, 12 ng/ml, 6 ng/ml, 3 ng/ml and 1.5 ng/ml. 50µl of standard solutions were added to each standard well and 40µl sample to sample wells. 10µl anti-VE-CAD antibody and 50µl streptavidin-HRP to sample wells and standard wells were added. The plate were incubated at 37°C for 60 minutes. After automated washing the plate for 5 times with wash buffer, 50µl of substrate solution A and 50µl of substrate solution B were added and the plate were further incubated for 10 minutes at 37°C in the dark. Within 10 minutes after adding 50µl stop solution to each well, optical density value was detected using a microplate reader set to 450 nm.

3.4.5. β2 integrin

Human integrin beta 2 ELISA kit (BT Lab, E0237Hu) was used according to its instructions. The standard solutions were prepared with the concentration of 800 ng/ml, 400 ng/ml, 200 ng/ml, 100 ng/ml and 50 ng/ml. 50µl of standard solutions were added to each standard well and 40µl sample to sample wells. 10µl anti-ITG B2/CD11+CD18 antibody and 50µl streptavidin-HRP to sample wells and standard wells were added. The plate were incubated at 37°C for 60 minutes. After automated washing the plate for 5 times with wash buffer, 50µl of substrate solution A and 50µl of substrate solution B were added and the plate were further incubated for 10 minutes at 37°C in the dark. Within 10 minutes after adding 50µl stop solution to each well, optical density value was detected using a microplate reader set to 450 nm.

3.4.6. PDGFR-β

Human beta-type platelet-derived growth factor receptor ELISA kit (BT Lab, E4265Hu) was used according to its instructions. The standard solutions were prepared with the concentration of 8 ng/ml, 4 ng/ml, 2 ng/ml, 1 ng/ml and 0.5 ng/ml. 50µl of standard solutions were added to each standard well and 40µl sample to sample wells. 10µl anti-PDGFRB antibody and 50µl streptavidin-HRP to sample wells and standard wells were added. The plate were incubated at 37°C for 60 minutes. After automated washing the plate for 5 times with wash buffer, 50µl of substrate solution A and 50µl of substrate solution B were added and the plate were further incubated for 10 minutes at

37°C in the dark. Within 10 minutes after adding 50µl stop solution to each well, optical density value was detected using a microplate reader set to 450 nm.

3.4.7. S1PR2

Human sphingosine 1-phospate receptor 2 ELISA kit (mybiosource, MBS762743) was used according to its instructions. The standard solutions were prepared with the concentration of 10 ng/ml, 5 ng/ml, 2.5 ng/ml, 1.25 ng/ml, 0.625 ng/ml, 0.312 ng/ml, 0.156 ng/ml and 100µl of standards and sample dilution buffer as blank were added into wells. All samples were diluted 1/2 with sample dilution buffer and 100µl of diluted samples were added into wells. The plate was covered and incubated at 37°C for 90 minutes. After automated washing the plate for 2 times with wash buffer, 100µl of biotin-labeled antibody working solution was added and incubated at 37°C for 60 minutes. After the incubation with antibody, washing was done for 3 times with wash buffer and 100µl of HRP-streptavidin conjugate was added and incubated at 37°C for 30 minutes. The plate was washed for 5 times with wash buffer. 90µl of TMB substrate was added and incubation at 37°C in dark for 10-20 minutes was performed. After adding 50µl stop solution into each well, optical density value was detected using a microplate reader set to 450 nm.

3.5. Analysis of Thrombi

Retrieved thrombus samples were collected and fixed in 10% formaldehyde solution, after gently washing them with saline, for at least 24 hours after endovascular therapy, performed in Bakırköy Dr. Sadi Konuk Training and Research Hospital, Interventional Neuroradiology Unit (Figure 7). Thrombi were transferred to Koç University Research Center for Translational Medicine for further analysis.

The samples were fixed in 10% formaldehyde solution in at least 24 hours to the maximum of seven days (104). After fixation, thrombi were processed for paraffin embedding, sectioned and stained with H&E for the examination of fibrin, red blood cells and white blood cells and MSB for better visualization of fibrin. The quantification of red blood cells, polymorphonuclear leukocytes and fibrin was performed by histopathological examination. For the analysis of thrombi, one section from each sample, representing whole thrombus, was stained for each staining method as suggested in the literature (96).

3.5.1. Hematoxylin & Eosin Staining

All thrombus samples were fixed with 10% formaldehyde solution. After fixation, tissue processing was done with formaldehyde-ethylene-xylene-paraffin by using automated tissue processing device (Tissue-Tek VIP 6 Al, Sakura Finetek USA). 4µm sections were prepared from paraffin blocks and taken onto slides. H&E staining was performed following the protocol as deparaffinization (incubation at 60 °C for 20 minutes and then xylene), alcohol, hematoxylin, washing with water, acid alcohol, lithium carbonate, alcohol, eosin, alcohol with increasing concentrations. H&E-stained slides were reviewed and images were obtained by for analysis.

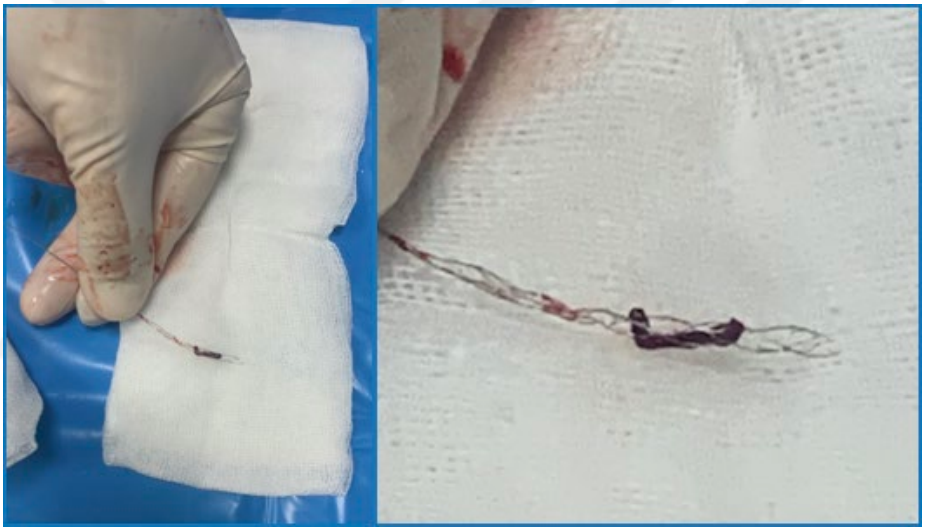

Figure 7: The macroscopic appearance of thrombus retrieved by stent-retriever technique.

3.5.2. Martius Scarlet Blue Staining

Paraffin-embedded samples were cut along the longest plane into 4µm-thick sections and deparaffinized. MSB staining kit (DiaPath, 010255) was used according to its instructions as follows. All steps were performed in a wet incubator box and humidity chamber. Deparaffinized sections were stained with Martius Yellow for two minutes. After washing the sections with distilled water, sections were covered with Ponceau Xilidine for 10 minutes, followed by washing in distilled water. Then, acid solution was added on sections for one minute and washed with distilled water. Lastly, sections were covered with Blue Methylene 0.5% for 30 seconds and washed in Acid

buffer. When the staining was completed, all sections were dehydrated quickly and mounted. MSB-stained slides were reviewed, and images were obtained by for analysis.

3.5.3. Immunohistochemical Staining of Thrombi

CD34 immunostaining was used to detect endothelial cells in thrombus. Automated staining system (Dako Omnis) was used for immunohistochemical staining. 3μm sections were prepared from paraffin blocks and taken onto slides. For deparaffinization, thrombus sections were incubated at 25 °C with Clearify Cleaning Agent and washed with distilled water for 5 seconds. Then, sections were incubated at 97 °C for 30 minutes with EnVision FLEX Target Retrieval Solution, High pH (50x, pH:9, Dako Omnis) for antigen retrieval. All sections were incubated with CD34 antibody (Dako Omnis, GA63261) for 20 minutes and washed with wash buffer for 2 minutes. Afterwards, incubation with EnVision FLEX Peroxidase-Blocking Reagent for 3 minutes and Mouse Linker for 10 minutes was applied and washed with wash buffer after each step. The sections were incubated with EnVision FLEX/HRP polymer for 20 minutes and washed with wash buffer 3 times for 2 minutes, distilled water for 31 seconds, followed by the incubation with EnVision FLEX DAB for 20 minutes and washing with Dab substrate buffer for 5 minutes and wash buffer for 2 minutes. Then, the sections were counterstained by incubating with Hematoxylin (Dako Omnis) for 4 minutes, washed with distilled water for 2 minutes and with wash buffer for additional 2 minutes. Slides were reviewed and images were obtained after being scanned into digital slides.

3.5.4. Analysis of Images

Orbit (Version 3.64) and Fiji (Version 1.0) programs were used for image analysis. The analysis of thrombi stained with H&E were performed by using color-based segmentation method to determine relative quantitative fraction of RBC, WBC and fibrin in both Orbit and Fiji. Likewise, the thrombi were analyzed to determine relative quantitative fraction of RBC, fibrin and old fibrin/collagen after staining with MSB by using Orbit. In the analysis of immunohistochemical staining with CD34, it was noted that, staining was only on the surface in some parts of the thrombus, while the ingrowth of endotelial cells was observed in some parts. For quantitative analysis of immunohistochemical staining with CD34, the number of all areas and parts of ingrowth stained with CD34 in whole thrombus was counted for each thrombus.

3.6. Statistics

Statistical analyzes were carried out using appropriate parametric and non-parametric tests according to the distribution of the data. Non-parametric data were expressed as median value and interquartile range, parametric data as mean and standard deviation. Differences between groups were analyzed by using Student's t-test or Mann-Whitney U, and correlation analyzes were performed with Pearson or Spearman tests. Fisher's exact test was utilized to evaluate categorical data. p value of <0.05 was considered statistically significant. SPSS (Version 20) and GraphPad (Version 9.1.2) programs were used as statistical analysis programs.

4. RESULTS

4.1. Clinical Information

A total of 101 patients were enrolled in this study. Patients were evaluated in three groups according to clinical treatment approach: iv tPA, mechanical thrombectomy, and bridging therapy. Alteplase was administered to the patients with disabling acute ischemic stroke, applied within 4.5 hours of symptom onset and without any contraindication for iv tPA use. Patients with proximal large vessel occlusion were evaluated for mechanical thrombectomy, if they applied within 6 hours of symptom onset or there was clinical-diffusion mismatch. 54 (53.5%) patients underwent mechanical thrombectomy, 31 (30.7%) were treated with iv tPA and 16 (15.8%) patients with both iv tPA and mechanical thrombectomy, indicated as bridging group.

Fifty-six (55.4%) of the patients were male and 45 (44.6%) of them were female. The mean age was 66.13 ± 13.99. While acute ischemic infarction was found in right MCA supplying area in 48 (47.5%) patients and left MCA in 48 (47.5%) patients, infarction was observed in multiple localizations in 5 (5%) patients. Twenty (19.8%) patients did not have proximal occlusion, whereas 27 (26.7%) had occlusion in ICA, 27 (26.7%) in MCA M1, 21 (20.8%) in MCA M2 and 6 (5.9%) patients had tandem occlusion.

Forty-one (40.6%) patients had diabetes, 64 (63.4%) had hypertension and 62 (61.4%) had hyperlipidemia. Atrial fibrillation was found in 38 (37.6%) patients. 22 (21.8%) patients had previous stroke. The number of the patients who had positive Covid-19 PCR results or accepted as infected because of very typical signs and symptoms for Covid-19 infection was 10 (9.9%). 75 (74.3%) patients were recorded as no use of previous antiaggregant, 89 (88.1%) patients with no use of anticoagulant drugs.

Patients were evaluated in three groups according to the time between symptom onset and admission to hospital: less than 270 minutes, more than 270 minutes, unknown symptom onset or wake-up stroke. Seventy-seven (76.2%) patients were admitted to hospital within 270 minutes after symptom onset, 14 (13.9%) patients beyond 270 minutes and 10 (9.9%) cases were wake-up stroke. In addition, symptom-

Table 5: The median and IQR values of symptom-to-door, door-to-needle, symptom-to-needle, symptom-to-puncture, symptom-to-recanalization and the duration of procedure times.

	iv tPA	***MT***	***iv tPA+MT***	***Total***
Symptom-to-door (min)	70.5 (IQR:52.5-118)	137.5 (IQR:50-283.25)	61 (IQR:40-87)	90 (IQR:50-202)
Door-to-needle (min)	79 (IQR:59-100)	-	72 (IQR:64.5-86)	73 (IQR:61-95)
Symptom-to-needle (min)	167 (IQR: 132.25-235)	-	148 (IQR:117-160)	160 (IQR:126-210)
Symptom-to-puncture (min)	-	313.5 (IQR:195-420)	177 (IQR:146-235)	240 (IQR:177-363)
Symptom-to-recanalization (min)	-	356 (IQR:229-442.5)	194 (IQR:184.75-245.75)	259 (IQR:210-429)
Duration of procedure (min)	-	28 (IQR:20-51.5)	30 (IQR:14.25-62.75)	28.5 (IQR:19.5-59)

to-door, door-to-needle, symptom-to-needle, symptom-to-puncture, symptom-to-recanalization times were recorded (Table 5). Symptom-to-door, symptom-to-needle, symptom-to-puncture, symptom-to-recanalization times were found significantly different between the groups ($p=0.015$, $p=0.047$, $p=0.001$, $p<0.001$). For the patients underwent mechanical thrombectomy, duration of procedure and the number of passes were also noted. The medians of the number of passes were 1 (IQR:1-3) in mechanical thrombectomy group and 1.5 (IQR:1-2.75) in bridging group. The number of passes and duration of procedure were significantly correlated ($p<0.001$).

The methods used during mechanical thrombectomy were recorded as stent retriever in 34 (49.3%) patients, aspiration in 22 (31.9%) patients and combined method in 13 (18.8%) patients. The recanalization scores of mechanical thrombectomy group were mTICI 0 in 5 (9.3%), mTICI 2a in 7 (13.0%), mTICI 2b in 20 (37.0%), mTICI 2c in 4 (7.4%) and mTICI 3 in 18 (33.3%) patients, whereas the scores in bridging group were found as mTICI 0 in 2 (12.5%), mTICI 2a in 1 (6.3%), mTICI 2b in 2 (12.5%), mTICI 2c in 1 (6.3%) and mTICI 3 in 10 (62.5%) patients (Figure 8). Therefore, successful recanalization was achieved in 55 (78.6%) patients, 42 (77.8%) patients from mechanical thrombectomy group and 13 (81.3%) from bridging group. During the procedure, general anesthesia was applied to 8 (50%) patients from bridging group and 32 (59.3%) patients from mechanical thrombectomy group.

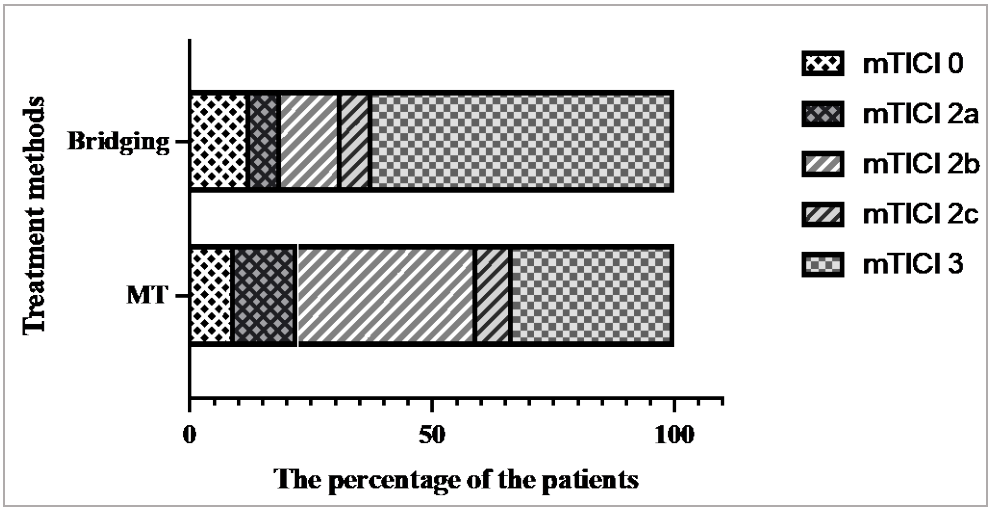

Figure 8: The percentages of mTICI scores of the patients according to treatment methods.

Stroke severity was assessed by using NIHSS. NIHSS scores were recorded on admission, at 24 hours and at discharge (Table 6). There was statistically significant difference between the groups in terms of NIHSS scores ($p<0.001$), mechanical thrombectomy group had the highest scores, whereas iv tPA groups had the lowest scores.

The modified Rankin scale was used to assess functionality of the patients for follow-up. The patients with mRS scores of 0-2 were evaluated as functionally independent. The number of functionally independent patients at 90 days after treatment was 51 (50.5%); 24 (77.4%) from iv tPA group, 22 (40.7%) from in mechanical thrombectomy group and 5 (31.3%) from bridging group. 21 (20.8%) patients died at 90 days (Figure 9).

Table 6: The median and IQR values of NIHSS on admission, at 24 hours, at discharge and the duration of hospitalization

	iv tPA	***MT***	***iv tPA+MT***	***Total***
NIHSS on admission	6 (IQR:4-9)	13 (IQR:11-15.25)	9.5 (IQR:7-12.75)	11 (IQR:7-14)
NIHSS at 24 hours	4 (IQR:3-7)	10.5 (IQR:7.75-14)	7 (IQR:4-14)	8 (IQR:4-12.5)
NIHSS at discharge	3 (IQR:0-5.25)	8 (IQR:4-13)	6 (IQR:3-9)	5 (IQR:2-11)
Duration of hospitalization (day)	9 (IQR:7-13)	14 (IQR:9-23.5)	13 (IQR:10-16)	12 (IQR:8-17.5)

According to TOAST classification, 23 (22.8%) patients had large artery atherosclerosis, 44 (43.6%) had cardioembolism, 14 (13.9%) had small vessel disease. 4 (%4) patients had other etiologies such as vasculitis and 16 (15.8%) patients were accepted as cryptogenic stroke.

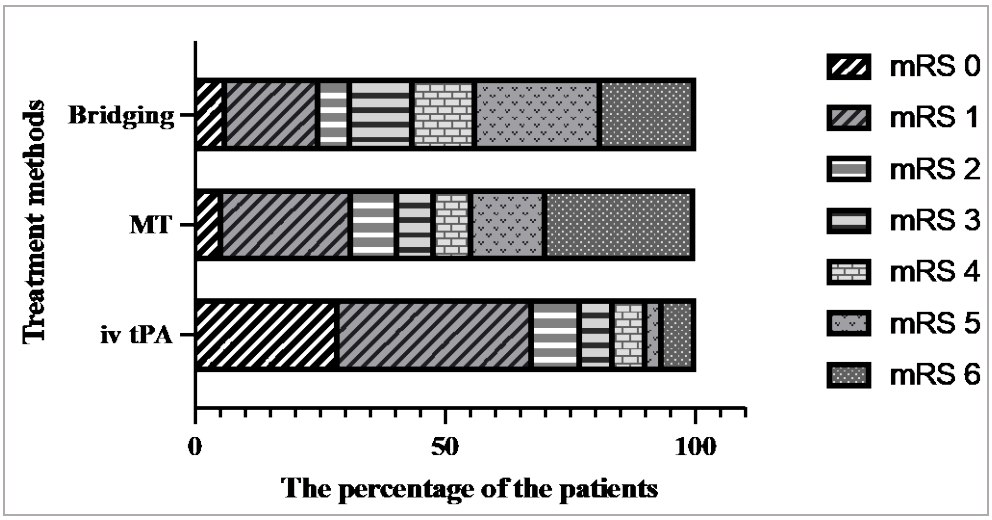

Figure 9: The percentages of mRS scores of the patients at 90 days according to treatment methods.

4.2. Neuroimaging Findings

All patients were evaluated with non-contrast cranial tomography on admission. Because one patient was referred from another hospital and CT images could not be reached, CT images of 100 patients were scored using ASPECTS. ASPECTS scores of all patients were between 6 and 10, as 6 in 9 (8.9%) patients, 7 in 27 (26.7%) patients, 8 in 24 (23.8%) patients, 9 in 32 (31.7%) patients, 10 in 8 (7.9%) patients.

Thirty-eight (70.4%) patients of mechanical thrombectomy group and 11 (68.8%) patients of bridging group had hyperdense artery sign on initial cranial CT.

The median lesion volume at 24 hours after treatment was calculated as 3857.68 mm^3 (IQR: 1322.19-35372.16) in iv tPA group, 106375.61 mm^3 (IQR: 20674.22-218992.67) in mechanical thrombectomy group and 42654.81 mm^3 (IQR: 11903.33-106176.53) in bridging group.

All patients underwent non-contrast cranial CT at 24 hours after treatment for the evaluation of intracranial hemorrhage. 75 (74.3%) patients had no hemorrhage. Intracranial hemorrhage was observed in 3 (9.7%) patients from iv tPA group, 18 (33.3%) patients from mechanical thrombectomy group and 5 (31.3%) patients from bridging group. The median volumes of lesion and hemorrhage were significantly different between groups ($p<0.001$, $p=0.044$).

The recanalization status after treatment was evaluated by imaging techniques. 34 patients could not be imaged due to their medical conditions incompatible with MRI,

such as intubation. One patient (3.2%) had occlusion and 4 (12.9%) had stenosis in related vessel in iv tPA group. In mechanical thrombectomy group, 5 (9.3%) patients had occlusion and 4 (7.4%) had stenosis, whereas occlusion was found in 2 (12.5%) patients in bridging group.

4.3. Analysis of Serum Samples

The levels of MMP-2, MMP-9, N-cadherin, VE-cadherin, β2 integrin, PDGFR-β, S1PR2 were examined in serum samples before and 24 hours after treatment.

4.3.1. MMP-2

The levels of MMP-2 was evaluated in two groups as less than 400 ng/ml and more than 400 ng/ml, according to the distribution of the data. In iv tPA group, the levels of MMP-2 at 24 hours after treatment were significantly different in the groups ~~of~~ according to lesion volume at 24 hours (p=0.033), that, MMP-2 levels were found lower in the patients with smaller lesion. Similar relationship was observed in the analysis of MMP-2 levels at 24 hours and functional dependency (p=0.012), lower MMP-2 levels in the group of mRS 0-2 was found (Figure 10). Moreover, a significant difference in the levels of MMP-2 at 24 hours and TOAST classification was found in iv tPA group (p=0.047), all values were less than 400 ng/ml in the group of small vessel disease. No such relationship was observed in mechanical thrombectomy and bridging groups.

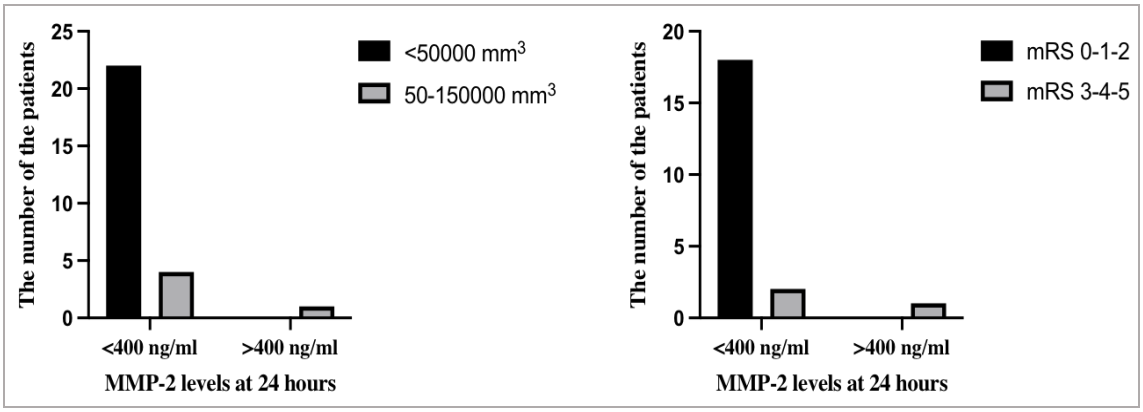

Figure 10: MMP-2 levels at 24 hours, in the groups of lesion volume at 24 hours and functional dependency at 90 days (p=0.033, p=0.012)

4.3.2. MMP-9

There was a significant difference in the levels of MMP-9 at 24 hours between treatment groups (p=0.022), iv tPA group had the lowest median compared to others (Figure 11). MMP-9 was not found to be related with the presence of hemorrhage, the volume of lesion and hemorrhage and the parameters of clinical outcome.

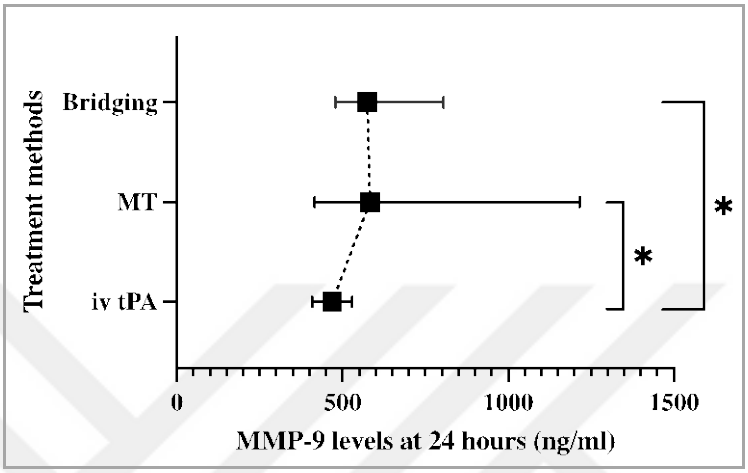

Figure 11: MMP-9 levels at 24 hours according to treatment methods, iv tPA group had the lowest median value compared to other groups (p=0.022).

4.3.3. VE-cadherin and N-cadherin

The levels of VE-cadherin and N-cadherin were evaluated in two groups: less than 7.5 ng/ml, and more than 7.5 ng/ml for VE-cadherin; less than 75 ng/ml, and more than 75 ng/ml for N-cadherin, according to the distribution of the data. There was a significant difference in the levels of VE-cadherin at 24 hours and TOAST classification (p=0.044). All values were found below 7.5 ng/ml in small vessel disease. Similar relation was found between N-cadherin levels on admission and TOAST classification (p=0.047), there was no value higher than 75 ng/ml in small vessel disease group.

4.3.4. β2 integrin

There was a significant difference in the levels of β2 integrin at 24 hours between treatment groups (p=0.022), iv tPA group had lower values compared to mechanical thrombectomy group (Figure 12). There was no similar relationship in the levels of β2 integrin before the treatment.

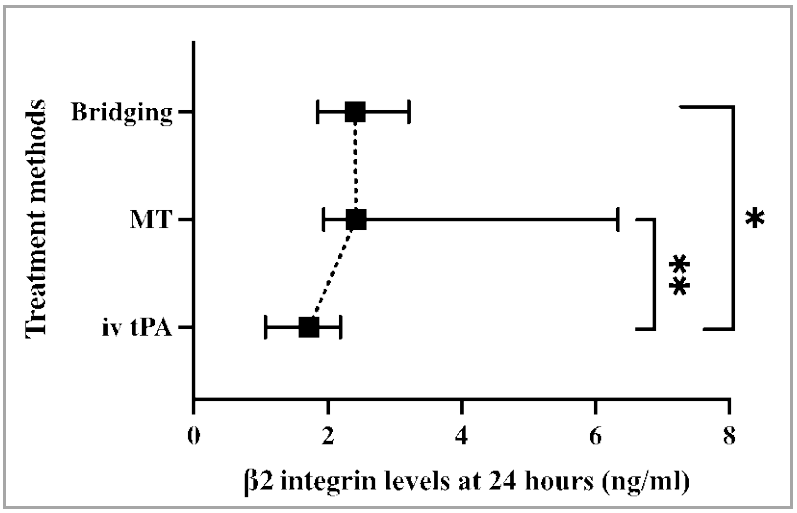

Figure 12: β2 integrin levels at 24 hours according to treatment methods, iv tPA group had the lowest median value compared to other groups (p=0.022).

4.3.5. PDGFR-β

The levels of PDGFR-β both before treatment and at 24 hours were significantly different between treatment groups (p=0.009, p=0.006), higher levels were found in mechanical thrombectomy group (Figure 13-A). Additionally, only in mechanical thrombectomy group, PDGFR-β levels both before treatment and at 24 hours after treatment were found as significantly related with the volume of infarction at 24 hours (p=0.047, p=0.016) (Figure 13-B). There was no similar relationship in iv tPA and bridging groups.

In the analysis of all patients to investigate the relationship with intracranial hemorrhage after treatment and the levels of PDGFR-β, higher PDGFR-β levels before treatment were found to be associated with lower rates of hemorrhage (p=0.043). However, no such relationship was seen in PDGFR-β levels at 24 hours (p=0.415). In subgroup analysis, in the patients who underwent mechanical thrombectomy and successful recanalization was achieved, the frequency of intracranial hemorrhage was evaluated and found that PDGFR-β levels before treatment were significantly high in the patients without hemorrhage (p=0.02).

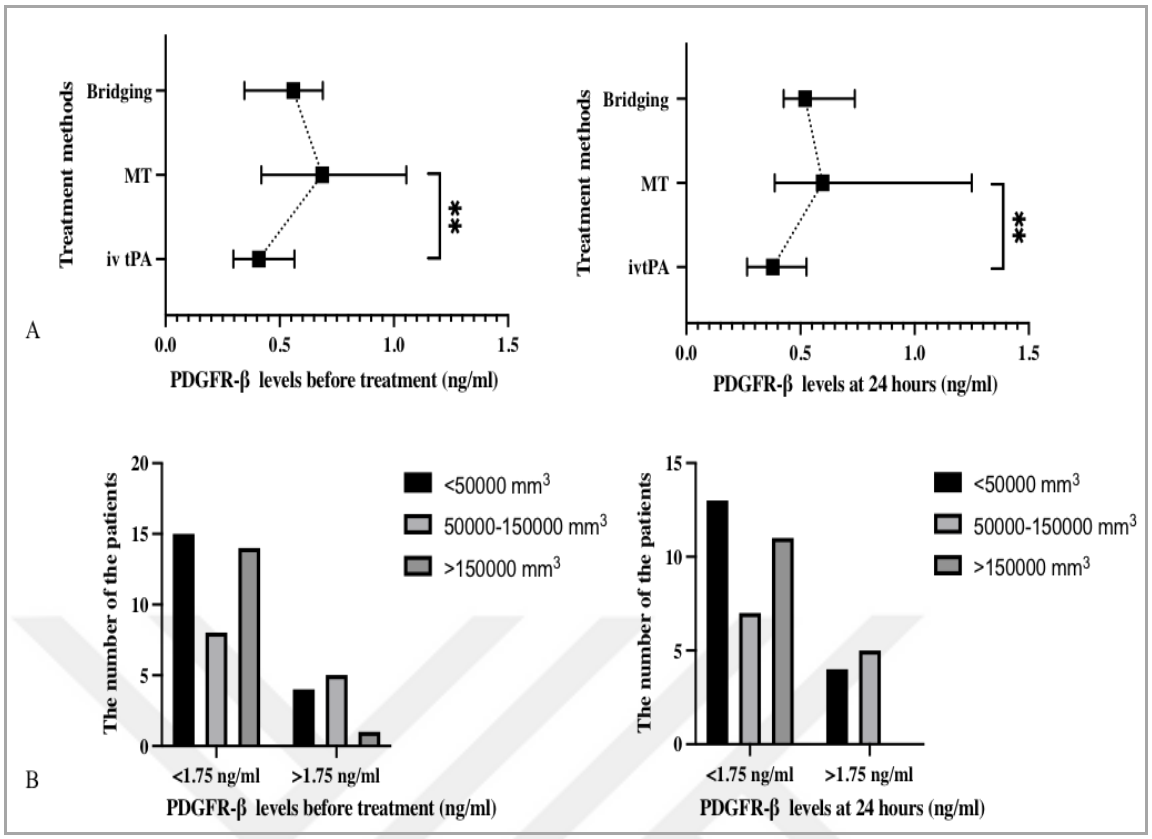

Figure 13: PDGFR-β levels before treatment and at 24 hours according to treatment methods, mechanical thrombectomy group had the highest median value compared to other groups (p=0.009, p=0.006) (A), PDGFR-β levels before treatment and at 24 hours after treatment according to the volume of infarction at 24 hours (p=0.047, p=0.016) (B).

4.3.6. S1PR2

The levels of S1PR2 were evaluated in three groups as less than 4 ng/ml, between 4 and 7 ng/ml and more than 7 ng/ml, according to the distribution of the data. S1PR2 levels before treatment were found as significantly related with the median times of symptom-to-door, symptom-to-puncture and symptom-to-recanalization (p=0.001, p=0.036, p=0.044), that S1PR2 increased with time. S1PR2 levels at 24 hours after treatment did not show such relationship.

According to TOAST classification, the patients were grouped as large artery atherosclerosis and the other etiologies. The levels of S1PR2 before treatment and at 24 hours after treatment were found significantly related with large artery atherosclerosis (p=0.008, p=0.002), that, the patients with large artery atherosclerosis had higher S1PR2 levels (Figure 14-A). On the contrary, the patients with cardioembolic etiology were found to have lower levels of S1PR2 at 24 hours after treatment (p=0.022), but there was no such relationship with S1PR2 levels before treatment (p=0.142).

The presence of intracranial hemorrhage after treatment was found to be related with the levels of S1PR2 before treatment and at 24 hours after treatment (p=0.041, p=0.025), that, higher S1PR2 levels were shown to be associated with lower rates of hemorrhage (Figure 14-B). In subgroup analysis, in the patients who underwent mechanical thrombectomy and successful recanalization could not be achieved, the frequency of intracranial hemorrhage was evaluated and found that S1PR2 levels before and after treatment were significantly high in the patients without hemorrhage (p=0.017).

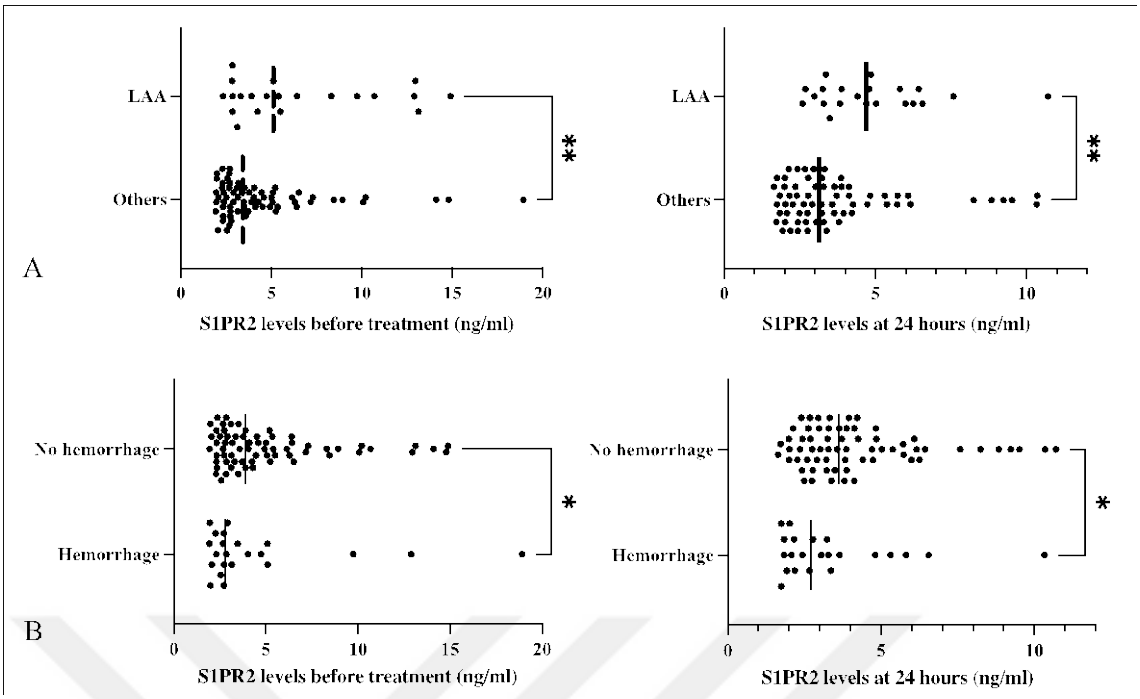

Figure 14: S1PR2 levels before treatment and at 24 hours according to TOAST classification, the levels of S1PR2 before treatment and at 24 hours after treatment were higher in large artery atherosclerosis (p=0.008, p=0.002) (A), higher S1PR2 levels before treatment and at 24 hours after treatment were shown to be associated with lower rates of hemorrhage (p=0.041, p=0.025) (B).

4.4. Leukocyte and neutrophil counts

The median of leukocyte count was calculated as 8355/µl (IQR: 6582.5-10697.5) in all patients, 6980/µl (IQR: 6020-9470) in iv tPA group, 9060/µl (IQR: 6590-11030) in mechanical thrombectomy group and 9125/µl (IQR: 7067.5-10847.5) in bridging group. The median values of neutrophil count and percentage were 4060/µl (IQR: 3120-6390), 61% in iv tPA group, 6270/µl (IQR: 4060-8455), 68.3% in mechanical thrombectomy group and 5880/µl (IQR: 4585-7105), 65.85% in bridging group, respectively. This difference in neutrophil count and percentage was statistically significant (p=0.007, p=0.003). To exclude infectious reasons causing the difference in neutrophil counts, CRP levels were compared between groups and did not show any significant relationship (p=0.089).

The analysis of the relationship of leukocyte and neutrophil counts and the etiology, it was found that the patient with large artery atherosclerosis had significantly higher leukocyte and neutrophil numbers, compared to other etiologies (p=0.004, p=0.006).

4.5. Clinical Outcome

The modified Rankin scale at 90 days was used to evaluate clinical outcome. The volumes of lesion and hemorrhage at 24 hours were found significantly different (p<0.001, p=0.049). The volume of hemorrhage was significantly higher in patients with mRS 6. Patients with no or mild symptoms had lower volumes of lesion at 24 hours. NIHSS scores before treatment and at 24 hours after treatment showed significant difference between mRS groups (p<0.001), the lower NIHSS scores, the better clinical outcome at 90 days.

The patients were evaluated in three groups according to functional dependency, that, patients with mRS 0-2 as functionally independent, 3-5 as functionally dependent and mRS 6 as dead. Like previous results, the volumes of lesion and hemorrhage at 24 hours were found significantly different between groups (p<0.001, p=0.003). NIHSS scores before treatment and at 24 hours showed very significant difference (p<0.001). The patients with mRS 0-2 showed significantly higher difference in between NIHSS scores before treatment and at 24 hours in comparison with the other groups (p=0.002). There was significant difference in the numbers of leukocyte and neutrophil before treatment (p=0.019, p=0.008), patients with mRS 6 at 90 days had higher counts. The other parameters found as related with functional dependency were CRP, HDL and

HbA1c levels (p=0.017, p=0.02, p=0.02, respectively). Higher CRP and HbA1c levels and lower HDL levels were found to be associated with poor prognosis.

When the procedural data of the patients who underwent mechanical thrombectomy were evaluated, the number of passes were found significantly different between the groups of functional dependency (p=0.039). It was determined that clinical outcome at 90 days was worse, as the number of passes increased, and the duration of procedure did not show such relationship (p=0.068). The status of recanalization determined by mTICI scoring system, was found to be related with the lesion volume at 24 hours (p=0.033), patients with successful recanalization (mTICI 2b-2c-3) had smaller lesions at 24 hours after treatment. Parallel to this finding, there was a significant relationship between recanalization status and mRS at 90 days (p=0.041). It was observed that the patient who could not be recanalized showed worse clinical outcome during follow-up.

Another important parameter to assess the outcome is the presence of intracranial hemorrhage. The levels of S1PR2 before treatment and at 24 hours, and PDGFR-β before treatment were found to be associated, as mentioned before. Leukocyte and neutrophil counts were significantly different in the patients with and without hemorrhage (p=0.022, p=0.029). Higher numbers of leukocytes and neutrophils were found to be related with intracranial hemorrhage. Moreover, HbA1c levels showed the similar relationship (p=0.04). The patients with higher HDL levels were found to have less intracranial hemorrhage at 24 hours (p=0.021), however, LDL did not show any significant difference between groups (p=0.27). Similar results were obtained in subgroup analysis in the patients who underwent mechanical thrombectomy and successful recanalization was achieved. Leukocyte and neutrophil counts, HDL and HbA1c levels were found significantly different between the groups of intracranial hemorrhage (p=0.026, p=0.041, p=0.009, p=0.046, respectively). From the clinical aspect, the patients who had higher NIHSS scores on admission were found to have increased rates of hemorrhage (p=0.007).

4.6. Analysis of Thrombi

Seventy patients underwent mechanical thrombectomy and 56 thrombi were obtained. All thrombi were stained with hematoxylin-eosin and Martius Scarlet Blue for the identification of fibrin and red blood cells; and CD34 for endothelial cells.

4.6.1. Hematoxylin-eosin staining

Fifty-six thrombi were stained with hematoxylin-eosin and analyzed by cell-based segmentation method with Fiji and Orbit programs and relative percentage of fibrin and RBCs were calculated (Figure 15). The thrombi were evaluated in two groups: thrombus containing fibrin more than 50% of its area was accepted as fibrin-dominant, whereas thrombus containing red blood cells more than 50% of its area was accepted as RBC-dominant (Figure 16).

From all 56 thrombi, 41 (73.2%) were fibrin-dominant, 15 (26.8%) were RBC-dominant. In mechanical thrombectomy group, 32 thrombi (72.7%) were fibrin-dominant, 12 (27.3%) were RBC-dominant, whereas, 9 thrombi (75%) were fibrin-dominant and 3 (25%) were RBC-dominant in bridging group. There was no significant difference between treatment groups ($p=0.595$).

There was no difference in the rates of fibrin or RBC-dominant thrombi between two different programs, Fiji and Orbit. Moreover, a strong correlation was found between the percentages of fibrin and red blood cells calculated with two different programs ($p<0.001$).

When the relationship between the etiology and the dominant component of thrombus was examined, there was no statistically significant difference ($p=0.21$). The dominant component of thrombus was found to be related with the occluded vessel ($p=0.027$), thrombi retrieved from MCA M2 had higher rate of fibrin dominance, compared to the thrombi retrieved from ICA and tandem occlusions. A significant relationship was found between thrombus histopathology and the method used during mechanical thrombectomy ($p=0.045$), fibrin content was higher in thrombi removed with stent-retriever and combined methods (Figure 17-A). However, it was shown that the dominant component of thrombus was not significantly related to symptom-to-door time, symptom-to-puncture time, symptom-to-recanalization time, the duration of procedure and the number of passes. The fibrin or RBC dominance did not show any difference according to the status of recanalization ($p=0.363$).

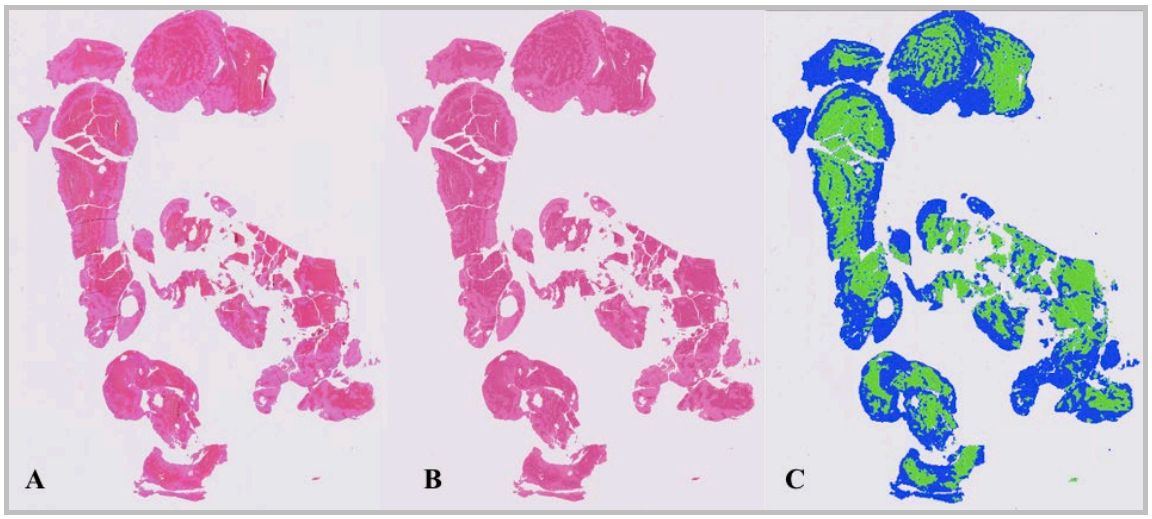

Figure 15: Thrombus stained with hematoxylin-eosin, pink-red area shows red blood cells, pale pink area shows fibrin-platelets (A), thrombus image after cell segmentation with Fiji (B), thrombus image after cell segmentation with Orbit, green area shows RBCs, blue area shows fibrin-platelets (C).

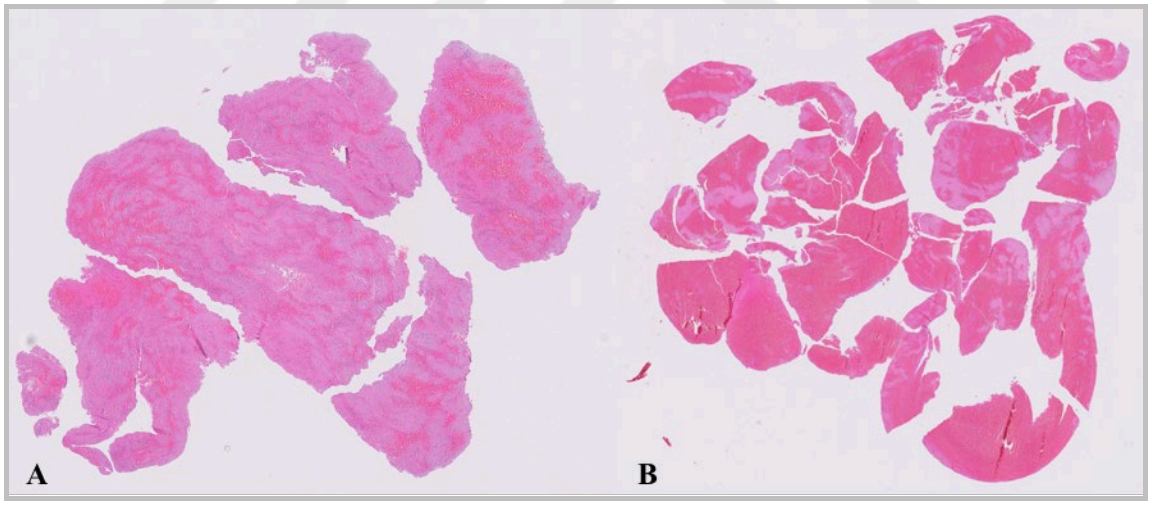

Figure 16: The examples of fibrin-dominant (A) and RBC-dominant (B) thrombi.

The parameters of clinical outcome such as NIHSS scores, the volume of lesion and hemorrhage, the presence of hemorrhage and the status of functional dependency were examined for their possible relationship with fibrin or RBC dominance, no significant relationship was found (p=0.15, p=0.226, p=0.622, p=0.299, p=0.129, respectively).

There was a significant relationship with RBC dominance and hyperdense artery sign (p=0.015). There were 14 (32.6%) RBC-dominant thrombi in the patients with hyperdense artery sign, whereas 1 (7.7%) in patients without hyperdense artery sign (Figure 17-B).

4.6.2. Martius Scarlet Blue staining

Forty-nine thrombi were stained with MSB. The analysis was done by cell-based segmentation method with Orbit program and relative percentage of fibrin, RBCs and collagen/old fibrin were calculated (Figure 18).

There was no significant difference in the rates of fibrin or RBC-dominant thrombi between two different staining methods, H&E and MSB. Moreover, a strong correlation was found between the percentages of RBCs in thrombi stained with H&E and MSB ($p<0.001$).

It was observed that there was a great difference in terms of the amount of fibrin and collagen/old fibrin in the structure of thrombi. To investigate the reasons for this difference, the ratio of collagen/old fibrin to fibrin was used for the analysis of the relevant parameters.

There was no significant relationship between the ratio of old fibrin to fibrin and TOAST classification (p=0.741). From the procedural aspect, it was observed that the duration of mechanical thrombectomy and the ratio of old fibrin to fibrin were negatively correlated (p=0.017). However, there was no such correlation with the number of passes (p=0.091). Similarly, no correlation was found with recanalization and hemorrhage status.

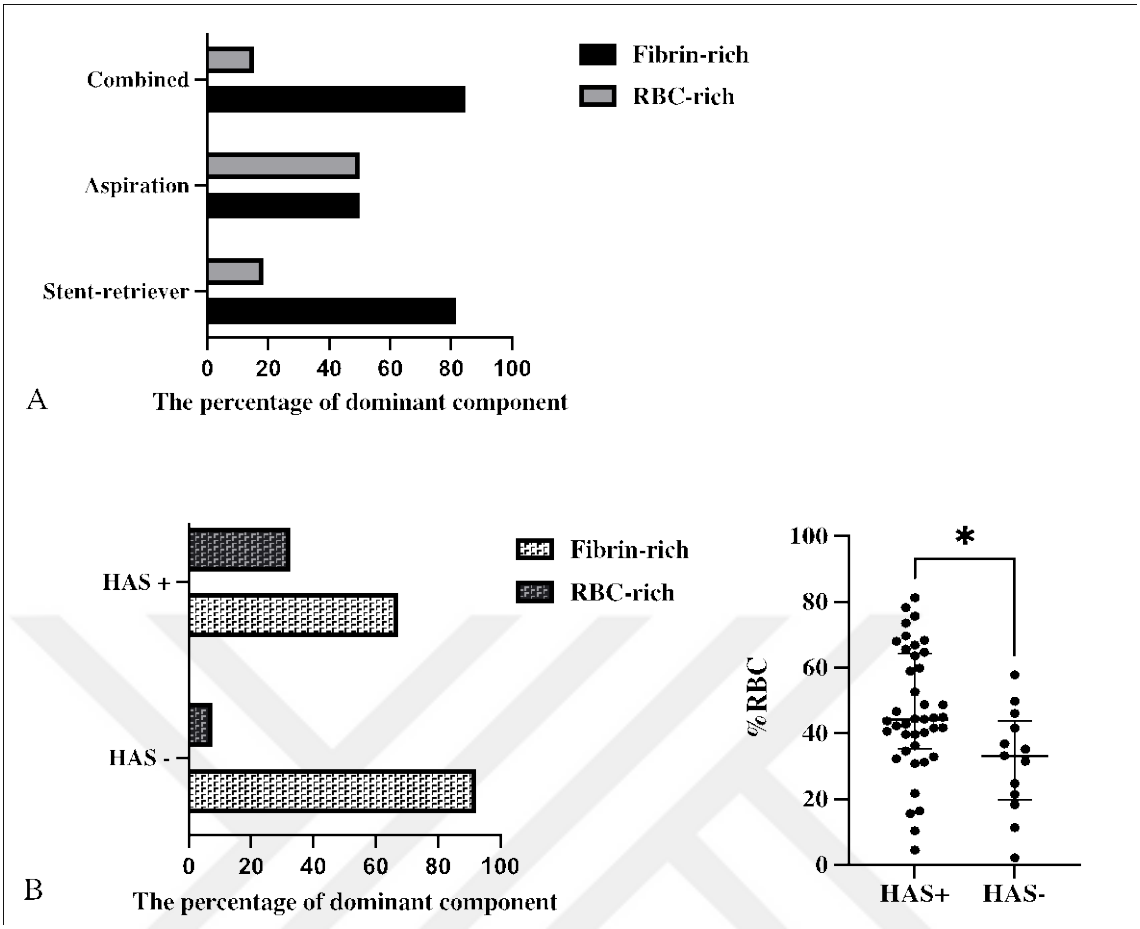

Figure 17: The relationship between thrombus histopathology and the method used during mechanical thrombectomy, fibrin content was higher in thrombi removed with stent-retriever and combined methods (p=0.045) (A), the relationship between RBC dominance and hyperdense artery sign, the patients with hyperdense artery sign had higher RBC-dominance than the patients without hyperdense artery sign (p=0.015).

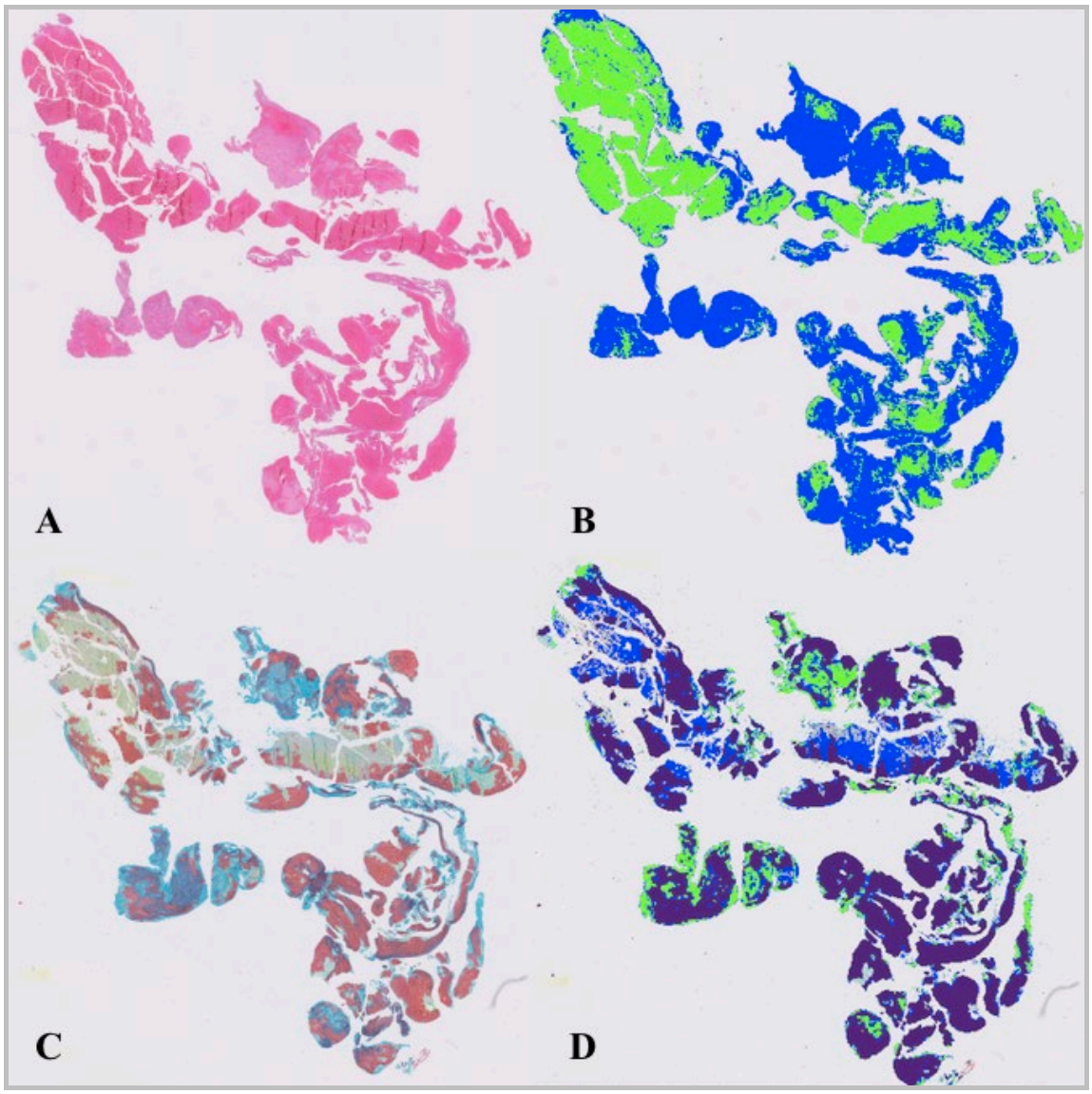

Figure 18: Thrombus stained with hematoxylin-eosin, pink-red area shows RBCs, pale pink area shows fibrin-platelets (A), thrombus image after cell segmentation with Orbit, green area shows RBCs, blue area shows fibrin-platelets (B), the same thrombus stained with MSB, red area shows fibrin, blue area shows old fibrin-collagen and yellow-green area shows RBCs (C), thrombus image after cell segmentation with Orbit, purple area shows fibrin, green area shows old fibrin-collagen and blue area shows RBCs (D).

4.6.3. Immunohistochemical staining

Fifty-four thrombi retrieved during mechanical thrombectomy were analyzed. For the analysis of CD34, the number of areas stained with CD34 in whole thrombus was counted for each thrombus (Figure 19).

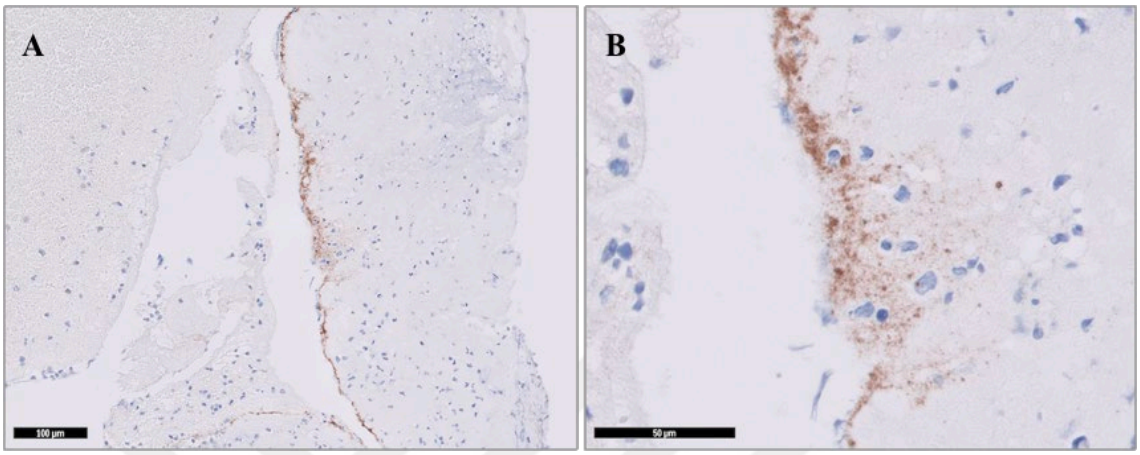

Figure 19: Thrombus stained with CD34 showing endothelial cells on the surface and ingrowth of endothelial cells towards inner part of the thrombus with x10 and x40 magnifications (A, B).

The number of areas stained with CD34 was found to be correlated with symptom-to-door time, symptom-to-puncture time, symptom-to-recanalization time ($p=0.017$, $p<0.001$, $p<0.001$). It was observed that the stained areas increased as time progressed (Figure 20). There was no such relationship with the duration of procedure and the number of passes.

When the thrombi were examined after CD34 staining, it was noted that, staining was only on the surface in some parts of the thrombus, while the ingrowth of endotelial cells was observed in some parts (Figure 19). The number of areas where the ingrowth of endothelial cells was found to be related with the occlusion site ($p=0.017$). The thrombi from more distal branches (MCA M2) had less CD34+ areas showing endothelial ingrowth (ICA, MCA M1 and tandem occlusions).

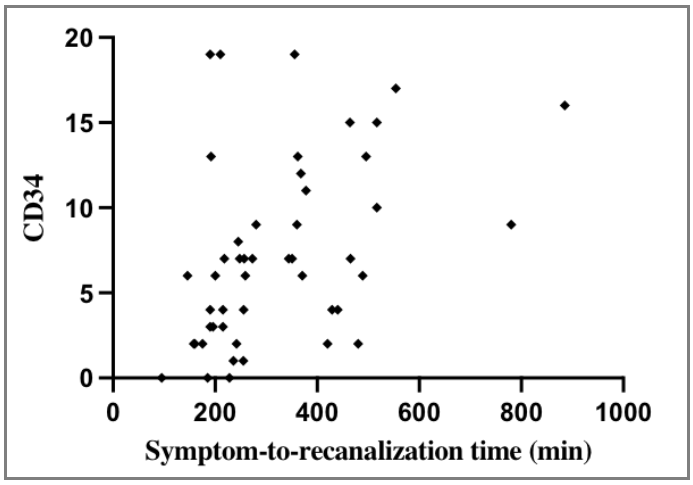

Figure 20: The correlation between the total number of areas stained with CD34 and symptom-to-recanalization time ($p<0.001$)

5. DISCUSSION and CONCLUSION

In this study, the investigation of the roles of MMP-2 and MMP-9, VE-cadherin, N-cadherin, β2 integrin, S1PR2 and PDGFR-β in providing reperfusion after iv tPA and mechanical thrombectomy was aimed and thrombus samples retrieved after mechanical thrombectomy were examined to understand the mechanisms of cerebral ischemia.

One of the novel findings in this study was increased plasma levels of S1PR2 in large artery atherosclerosis patients. There are various studies in the literature on the role of S1PR2 after ischemia (105-108). In an animal study with transient middle cerebral occlusion, S1PR2 was shown in the inner layer of vessels in ischemic core and peri-infarct lesion at acute phase of ischemia (105). Thus, increasing S1PR2 levels may be associated with the progression of ischemia. Supporting this idea, we have detected increased levels as the duration of symptom-to-door, symptom-to-puncture, and symptom-to-recanalization increases in our study. Previous experimental animal studies have been shown that S1PRs regulate the permeability of BBB by decreasing the endothelial adhesion molecules, microglial polarization and pericyte migration (106, 107). The inhibition of S1PR2 by its antagonist JTE013 resulted in improved neurological outcome and reduction in infarction and edema volume in a mouse model of transient focal cerebral ischemia (93). In our study, contrary to previous results, we have detected high S1PR2 levels in serum in non-hemorrhage associated cases at the admission and 24 hours, including all tPA and MT treatments. When we investigated in detail, we have detected increased S1PR2 levels in patients going through mechanical thrombectomy without successful recanalization and without hemorrhage. On the other hand, we couldn't detect a correlation with the hemorrhage in successfully recanalized patients. When the results are considered, we can argue that high levels of S1PR2 may reflecting the amount of ischemia rather than reperfusion injury. Hence progression of ischemia or unsuccessful reperfusion may cause higher levels.

Considering that S1PR2 levels are found higher in pericytes and fibroblasts in animal studies, it can be thought that these findings about S1PR2 are related to pericytes and perivascular fibroblasts. Additionally, it was suggested in the literature that S1PR2 plays a role in the activity of MMP-9 during cerebral ischemia (93, 108). It was also shown in a study in mice that $S1pr2^{-/-}$ mice had lower MMP-9, but not MMP-2 activity (93). In the present study, we could not detect a relation with MMP-9 and S1PR2. It

might be due to the technique used for MMP-9 detection. ELISA test detects all MMP-9, but active form can not be detected. This might be the reason for this result.

Recent studies in the literature have shown that S1P is associated with atherosclerosis, coronary artery disease and peripheral artery disease in humans. A human study suggested that in ischemic stroke, low serum S1P concentration contributed to abnormal lipid metabolism, promoted the formation of plaque and atherosclerosis and dyslipidemia (109). The patients with large artery atherosclerosis had higher S1PR2 levels before treatment and at 24 hours in our study, which supports previous reports about S1PR2 and large artery atherosclerosis in the literature. S1PR2 levels may be increased due to chronic atherosclerosis that leads decreased levels of S1P.

In the early stages after acute ischemic stroke, there is increased PDGFR-β expression in the peri-infarct area, which was demonstrated in animal studies. Pericytes through PDGFR-β promote maintenance of the BBB, peri-infarct astrogliosis and functional recovery (87, 88, 90). PDGFR-β knockout mice had larger infarct volume compared to wild-types. In our study, PDGFR-β levels both before treatment and at 24 hours were found higher in mechanical thrombectomy group, compared to other treatment groups. In patients who underwent mechanical thrombectomy, the larger affected brain tissue due to large vessel occlusion may have caused an increase in PDGFR-β level compared to the group receiving iv tPA. The longer symptom-to-door times compared to the bridging group may be associated with increased PDGFR-β levels in mechanical thrombectomy group. In addition, in mechanical thrombectomy group, lower PDGFR-β levels both before treatment and at 24 hours after treatment were found to be associated with higher infarction volume at 24 hours. This finding points out that time passing before recanalization as well as infarct volume are the determinants of levels of PDGFR-β in serum since MT patients had longer duration of ischemia. Higher PDGFR-β levels before treatment, but not at 24 hours, were found to be associated with lower rates of hemorrhage. Parallel to this finding, in the patients who underwent mechanical thrombectomy with successful recanalization, PDGFR-β levels before treatment were significantly high in the patients without intracranial hemorrhage. These findings suggested that pericyte activity, which increases in the early stages of acute ischemia, has a protective role in acute ischemia by reducing ischemia-reperfusion injury.

MMP-2 levels at 24 hours were found lower in the patients with smaller lesion and functionally independent at 90 days in iv tPA group. This finding is consistent with the research showing MMP-2-mediated blood-brain barrier damage in early stages of ischemic stroke (57). However, MMP-2 and MMP-9 levels were not found to be associated with intracranial hemorrhage and stroke severity assessed by NIHSS scores on admission and at 24 hours in our study, in contrast to the previous studies (81). When we analyzed subgroups according to TOAST classification, MMP-2 levels at 24 hours in the patients with small vessel disease were lower in iv tPA group. Our findigs does not appear to confirm the previous studies suggesting that MMP-2 levels are higher in patients with cardioembolic stroke (76, 82). These different results may be due to several reasons. In our study, the patient population presented with acute ischemic stroke in hyperacute period and treated with iv tPA and/or mechanical thrombectomy; while in other studies, patients who did not got these treatments and who got within the first 24-48 hours were included. Different time periods and treatment methods may have resulted in different results. Another reason might be that serum levels of MMPs were measured in our study, the activity of MMPs was not evaluated. It is thought that although total MMP-2 levels did not change, active MMP-2 levels may increase. Additionally, the serum samples of the patients were centrifuged and kept at -80 °C until testing, which may have altered the serum concentrations.

iv tPA group had the lowest levels of MMP-9 and β2 integrin at 24 hours after treatment compared to other treatment groups; this significantly differs from previous results reported in the literature, in which tPA increases MMP-9 level in plasma releasing it from neutrophil granules (79, 82). Considering the effect of β2 integrin and MMP-9 on neuroinflammation after ischemic stroke, it can be thought that larger affected brain tissue and worse clinical pathology in patients who underwent mechanical thrombectomy may have caused this difference since in that group reperfusion success rates were low (79, 85). However, MMP-9 and β2 integrin levels were not found to be associated with the volume of infarction and hemorrhage at 24 hours.

Endothelial cells are one of the main components of the BBB, and the disruption of tight junctions and adherens junctions in ischemic stroke is known to impair the integrity of endothelial cells. This study demonstrated that VE-cadherin and N-cadherin levels were not associated with lesion volume, intracranial hemorrhage and stroke severity. These findings correlate fairly well with an animal study in mice showing that

partial loss of VE-cadherin causes vascular remodeling and reduced chronic lesion size, but VE-cadherin and N-cadherin do not have any effect in early stages (110).

When the results of the complete blood count, which are frequently used in clinical practice, are evaluated in relation to neuroinflammation, neutrophil count and percentage were found higher in mechanical thrombectomy group. CRP levels were examined in order to exclude infectious causes that may cause an increase in neutrophil count, and no significant difference was observed. Among the patients included in the present study, there was no patient with hematological disease that could cause a change in complete blood count. This finding is in line with previous study highlighting that high WBC and neutrophil counts on admission are strongly associated with the presence of large vessel occlusion, regardless of occluded vessel and collateral status (111).

Leukocyte and neutrophil counts were found to be related with hemorrhage and clinical outcome. The patients with mRS 6 at 90 days and the ones with intracranial hemorrhage had higher numbers of leukocytes and neutrophils. Moreover, higher CRP and HbA1c levels and lower HDL levels were found to be associated with poor prognosis. As revealed in recent reports, in the patients successfully treated with endovascular therapy, high neutrophil counts and blood glucose were found to be related with poor prognosis. The mechanism causing this condition is thought to be thromboinflammation in the venous compartment of the microcirculation as well as insufficient collateral circulation (112, 113).

Patient selection for mechanical thrombectomy is still a controversial issue. Although some studies in the literature emphasize that successful recanalization should be targeted regardless of the number of passes in clinical prognosis, there are also some publications showing that increased numbers of passes and prolonged procedure time worsen the clinical outcome (114). When the procedural data of patients who underwent mechanical thrombectomy in this study were evaluated, it was found that clinical outcome at 90 days was worse, as the number of passes increased regardless of the duration of procedure. The patients with successful recanalization (mTICI 2b-2c-3) had smaller lesions at 24 hours and better clinical outcome and functional independence at 90 days. It was observed that the patient who could not be recanalized showed worse clinical outcome during follow-up.

Studies on thrombus histopathology have recently increased with the widespread use of mechanical thrombectomy. The relationship between thrombus structure and clinical findings has been tried to be understood by using different staining techniques

and computer programs. In this study, two different staining methods, H&E and MSB, were used to analyze the main components of thrombus. In H&E staining, images were analyzed with two different programs, Fiji and Orbit, and it was evaluated whether there was a methodological difference between them. There was no difference in fibrin or RBC predominant thrombus rates between the two different programs. In addition, a strong correlation was found between the percentages of fibrin and RBCs calculated with Fiji and Orbit. However, Orbit provides ease of use, as it allows different structures to be better visualized with different colors and allows for the creation of models with machine learning while performing the analysis, allowing the analysis of more thrombi in shorter period of time.

There was no difference in the dominant structure of the thrombus with the two different staining methods used in this study. However, on the contrary to the findings in the literature in MSB staining, it was noted that there was a higher rate of blue-stained areas. Considering that the blue color is associated with collagen or old fibrin in MSB staining and collagen is not found at a high rate in the structure of thrombus, it was thought that the blue-stained regions were organized fibrin, named as old fibrin (115). To exclude a methodological inaccuracy and to examine the correlation between the two staining methods, RBC percentages were evaluated and found highly correlated. The finding that the higher ratio of old fibrin to fibrin in thrombus was related to the duration of the procedure, might be associated with the easier removal of an organized thrombus. Further studies are needed to better analyze the blue-stained regions.

Recent studies on thrombus histopathology are generally focused on the relationship between thrombus structure and stroke etiology. In this context, no significant relationship was found in this study. Similarly, no relationship was revealed with clinical outcome parameters such as NIHSS scores, lesion and hemorrhage volume, the presence of hemorrhage, and functional dependence status. However, when the procedural data of mechanical thrombectomy was evaluated, the higher percentage of fibrin-rich thrombus in patients with stent-retriever and combined method was noted. On the contrary, aspiration used thrombi had more RBC-rich thrombi as percentage when compared to stent-retriever and combined group. This finding was thought to be related with the risk of fragmentation of RBC-rich thrombi, which was mentioned in the literature. Furthermore, the relationship between hyperdense artery sign on computed tomography and RBC-rich thrombus was shown, as indicated in the previous studies (116).

In the present study, CD34 positivity was observed only on the surface in some parts of the thrombus, and inward growth of endothelial cells was observed in some parts. For this reason, the number of staining on the surface and the ingrowth areas were evaluated separately. In previous studies on the histopathological analysis of thrombus, CD34 positivity in immunostaining was evaluated in relation to fibrin organization, and CD34+ thrombi were grouped as late-stage thrombi (98). Total number of areas stained with CD34 was found to be increased as symptom-to-door, symptom-to-puncture, symptom-to-recanalization times increased in the study. This finding is consistent with the literature, showing that the number of CD34+ areas increases in the late stages of thrombus. As the duration of the disease increases, the CD34+ areas in thrombus increase, indicating the endothelialization of thrombus and a more organized thrombus formation as time progresses. In addition, it was revealed that thrombus from more distal branches had more fibrin content and fewer CD34+ areas showing endothelial ingrowth. Although a statistically significant relationship between etiology and thrombus structure was not observed in our study, it is thought that thrombi with high fibrin content and low endothelialization in more distal vessels may be of embolic origin.

In conclusion, PDGFR-β and S1PR2 were associated conversely with the ischemia-reperfusion, and presence of intracranial hemorrhage after acute ischemic stroke. In addition, our findings suggest that S1PR2 may have a role in triggering inflammation in large artery atherosclerosis since at the admission patients having large artery atherosclerosis demonstrated higher levels of S1PR2. Further studies are needed to examine PDGFR-β and S1PR2 to elucidate the pathophysiology of ischemic stroke and in more detail.

From the clinical aspect, in large vessel occlusion, pre-treatment leukocyte and neutrophil counts seem to be associated with intracranial hemorrhage and clinical prognosis. We believe that understanding the inflammatory pathways in acute ischemic stroke will provide important information for treatment and patient selection.

In the histopathological examination of thrombus, MSB staining may be superior to H&E because it provides three different colors and higher contrast, and two types of fibrin content can be detected. Orbit, a machine learning-based image analysis method, has been found to be an effective tool for thrombus analysis. It has been determined that the endothelization of thrombi, as well as fibrin/erythrocyte contents, may be associated

with the pathogenesis of stroke. A more comprehensive immunological examination of the thrombus content in future studies may provide further insights.

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7. APPENDICES

7.1. Ethical Approval

T.C. YEDİTEPE ÜNİVERSİTESİ

Sayı : 37068608-6100-15- 1872 **08/05/2020**
Konu: Klinik Araştırmalar
Etik Kurul Başvurusu hk.

İlgili Makama (Sena Aksoy)

Yeditepe Üniversitesi Fizyoloji AD. Prof. Dr. Bayram Yılmaz'ın sorumlu araştırmacı olduğu **'Akut İstemik İnmede İntravenöz Tramolitik ve Mekanik Trombektomi Tedavileri Sonucu Reperfüzyon Yanıtının Belirlenmesinde Biyokimyasal ve Nörobiyolojik Yanıtların Araştırılması ''** isimli araştırma projesine ait Klinik Araştırmalar Etik Kurulu (KAEK) Başvuru Dosyası **(1834)** kayıtlı Numaralı KAEK Başvuru Dosyası , Yeditepe Üniversitesi Klinik Araştırmalar Etik Kurulu tarafından **07.05.2020** tarihli toplantıda incelenmiştir.

Kurul tarafından yapılan inceleme sonucu,yukarıdaki isim belirtilen çalışmanın yapılmasının etik ve bilimsel açıdan uygun olduğuna karar verilmiştir. **(KAEK Karar No:1212)**

Prof. Dr. Turgay Çelik

Yeditepe Üniversitesi
Klinik Araştırmalar Etik Kurul Başkanı

Yeditepe Üniversitesi 26 Ağustos Yerleşimi, İnönü Mahallesi Kayışdağı Caddesi 34755 Ataşehir / İstanbul
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7.2.Approval for Biological Material Transport

T.C.
İSTANBUL VALİLİĞİ
İL SAĞLIK MÜDÜRLÜĞÜ
S.B.Ü. BAKIRKÖY PROF. DR. MAZHAR OSMAN
RUH SAĞLIĞI VE SİNİR HASTALIKLARI
EĞİTİM ARAŞTIRMA HASTANESİ

11.02.2020

TIPTA UZMANLIK EĞİTİMİ KURULU KARARI

İlgi : Asist. Dr. Sena AKSOY'un 24.01.2020 tarih ve 2570 sayılı dilekçesi.

Hastanemizin Tıpta Uzmanlık Eğitim Kurulu, Hastane Başhekimi Doç. Dr. Murat ERKIRAN, Hastane Eğitim Koordinatörü Prof. Dr. Nesrin KARAMUSTAFALIOĞLU ve üyeler Doç. Dr. Bekir TUĞCU, Doç. Dr. H. Dilek ATAKLI ve Doç. Dr. Gül KARAÇETİN'in katılımları ile 11.02.2020 tarihinde toplanmış;

Hastanemizin Nöroloji Kliniği Uzmanlık Öğrencisi Dr. Sena AKSOY'un, Yeditepe Üniversitesi Tıp Fakültesi Sağlık Bilimleri Enstitüsü Sinirbilim Doktora Programı kapsamında yürüteceği "Akut İskemik İnmede İntravenöz Trombolitik ve Mekanik Trombektomi Tedavileri Sonucu Reperfüzyon Yanıtının Belirlenmesinde Biyokimyasal ve Nörobiyolojik Yanıtların Araştırılması" başlıklı araştırmasını hastanemizde uygulama talebi değerlendirilmiş, araştırmaya dair etik kurul onayı alınmasını takiben hastanemizde uygulanması oy birliği ile uygun görülmüştür.

BAŞKAN
Doç. Dr. Murat ERKIRAN

Prof. Dr. Nesrin KARAMUSTAFALIOĞLU
Eğitim Koordinatörü

Doç. Dr. Bekir TUĞCU
Beyin ve Sinir Cerrahisi Kliniği
Eğitim Sorumlusu

Doç. Dr. H. Dilek ATAKLI
Nöroloji Kliniği Eğitim Sorumlusu

Doç. Dr. Gül KARAÇETİN ÖZEN
Çocuk ve Ergen Ruh Sağlığı ve Hastalıkları
Kliniği Eğitim Sorumlusu

Dr. Sena, AKSOY

Prof. Dr. Nesrin Yılmaz

7.3. Curriculum Vitae

Personal Informations

Name	Sena	**Surname**	Aksoy

Education

Degree	Department	The name of the Institution Graduated From	Graduation year
University	Faculty of Medicine	Hacettepe University	2013
High school	-	İstanbul Atatürk Science High School	2006

	Languages	Grades (#)
1	English	Advanced
2	Spanish	Intermediate

All the grades must be listed if there is more than one (KPDS, ÜDS, TOEFL; EELTS vs)

Work Experience (Sort from present to past)

	Position	Institute	Duration (Year - Year)
1	Resident Doctor	Bakırköy Prof. Dr. Mazhar Osman Mental Health and Neurological Diseases Training and Research Hospital, Department of Neurology	2017- ..
2	Researcher	Yeditepe University, Department of Physiology	2016-2017
3	Resident Doctor	Marmara University Hospital, Department of Internal Medicine	2015-2015
4	Resident Doctor	Gazi University Hospital, Department of Internal Medicine	2014-2015
5	General practitioner	Bolu Abant İzzet Baysal State Hospital	2013-2013

Computer Skills

	Program	Level
1	Microsoft Office	Advanced
2	SPSS	Intermediate
3	Fiji	Intermediate
4	Orbit	Intermediate

***Excellent, good, average or basic**

Scientific works

The articles published in the journals indexed by SCI, SSCI, AHCI

Bastug Gul Z., Gozubatik Celik G., Selcuk B., Aksoy S., Gul M. and Soysal A. New Indicator of Inflammation in Migraine: Red Blood Cell Distribution. *Haydarpasa Numune Med J*. 2021;6(2):166–171.
Yildirim E., Aksoy S., Onel T. and Yaba A. Gonadal development and sex determination in Mouse. *Reprod Biol*. 2020;20(2):115-126.

Articles published in other journals

Bastug Gul Z., Gul G., Eren F., et al. Pregnancy and Epilepsy: Clinical Data and Adverse Outcomes of Pregnant Women with Epilepsy. *Epilepsi.* 2021;27(4):239-244.
Bastug Gul Z., Gozubatik Celik G., Uzulmez M., et al. The Impact of The Coronavirus Disease 2019 Outbreak on The Quality of Life of Patients with Epilepsy. *Epilepsi.* 2021;27(2):78-84.
Eren F., Guler S., Gul G., Ceyhan Dirican A., Ataklı D. and Soysal A. Clinical, Electrophysiological, Radiological Features and Prognosis of Creutzfeldt–Jakob Disease. *Epilepsi.* 2020;26(3):169-173.
Aksoy S., Uzulmez M. and Soysal A. Vgkc Encephalitis with Low-Titer Antibodies: Case Report. *J Neurol Res Rev Rep*. 2020;2(4):1-3.
Guler S., Selcuk H.H., Eren F., Apaydin E. and Soysal A. Leptomeningeal Metastasis as the First Presentation of Lung Adenocarcinoma. *J Neurol Neurophysiol*. 2018;9:6.

Others (Projects / Certificates / Rewards)

Certificate on Laboratory Animal Science

7.4. Patient Consent Form

AYDINLATILMIŞ HASTA ONAM FORMU

ARAŞTIRMANIN ADI: Akut İskemik İnmede İntravenöz Trombolitik ve Mekanik Trombektomi Tedavileri Sonucu Reperfüzyon Yanıtının Belirlenmesinde Biyokimyasal ve Nörobiyolojik Yanıtların Araştırılması

ARAŞTIRMANIN SÜRESİ: 1 YIL

ARAŞTIRMAYA KATILACAK GÖNÜLLÜ SAYISI: 100

ARAŞTIRMANIN AMACI: Beyne giden kan akımının, damarın pıhtıyla tıkanması nedeniyle azalmasına veya durmasına iskemik inme denir. İskemik inme nedeniyle hastaneye ilk 4.5 saat içinde başvuran uygun hastalara toplardamardan pıhtı açıcı tedavi verilebilir, ilk 6 saat içinde başvuran uygun hastalara kasıktan anjiyografi gibi girilerek ana atardamardan pıhtı çıkarılır. Böylece, kanlanması bozulan beyin dokusunda tekrar kan dolaşımı sağlanmaya çalışılır. Fakat bu tedavilerin, her hastada mevcut felç durumunu düzeltmediği, bazı hastalarda beyin kanamasına neden olarak hastanın durumunu kötüleştirebildiği bilinmektedir. Bu çalışmada, hastalardan alınacak serum ve pıhtı örneklerinden çalışılması planlanan bazı biyobelirteçlerin, dokunun tekrar kanlanması ile ilgili mekanizmalardaki rolünün incelenmesi hedeflenmektedir. Tedaviye yanıt ve olası komplikasyonlar ile ilişkili bu mekanizmaların aydınlatılmasının, tedaviden fayda görecek olan hastaların öngörülmesinde ve komplikasyonların azaltılmasında faydalı olacağı düşünülmektedir.

ARAŞTIRMADA İZLENECEK YÖNTEM: Hastalardan tedavi öncesi ve tedaviden 24 saat sonra olmak üzere 2 farklı zamanda kan örnekleri alınacak ve santrifüj edilerek serum örnekleri elde edilecektir. Mekanik trombektomi tedavisi yapılması halinde, işlem sonrası elde edilen pıhtı örnekleri uygun koşullarda saklanacaktır. Daha sonra bu örneklerin, Yeditepe Üniversitesi Tıp Fakültesi Fizyoloji Anabilim Dalı Beyin Araştırma Laboratuvarı ve Koç Üniversitesi Translasyonel Tıp Araştırma Merkezi Laboratuvarı'nda çalışılması planlanmaktadır.

OLASI RİSKLER VE RAHATSIZLIKLAR: Bu çalışma kapsamında hastaya rutin tedavi sırasında uygulanan girişimlerin haricinde herhangi bir invaziv girişimde bulunulmayacaktır.

ARAŞTIRMACILARIN KİMLİĞİ

Bu araştırma ile ilgili herhangi bir sorunuz veya endişeniz varsa, lütfen iletişime geçiniz:

Dr. Sena Aksoy
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Aydınlatılmış Hasta Onam Formu'ndaki tüm açıklamaları okudum. Bana, yukarıda konusu ve amacı belirtilen araştırma ile ilgili yazılı ve sözlü açıklama aşağıda adı belirtilen hekim tarafından yapıldı. Araştırmaya gönüllü olarak katıldığımı, istediğim zaman gerekçeli veya gerekçesiz olarak araştırmadan ayrılabileceğimi ve kendi isteğime bakılmaksızın araştırmacı tarafından araştırma dışı bırakılabileceğimi biliyorum. Söz konusu araştırmaya, hiçbir baskı ve zorlama olmaksızın kendi rızamla katılmayı kabul ediyorum.

_____

Katılımcı Adı-Soyadı ve İmzası Tarih

_____

Velayet veya vesayet altında bulunanlar için veli veya vasinin Adı-Soyadı ve İmzası Tarih

_____

Araştırmacının Adı-Soyadı ve İmzası Tarih

7.5. Data Collection Form

ARAŞTIRMA FORMU

"Akut İskemik İnmede İntravenöz Trombolitik ve Mekanik Trombektomi Tedavileri Sonucu Reperfüzyon Yanıtının Belirlenmesinde Biyokimyasal ve Nörobiyolojik Yanıtların Araştırılması"

DEMOGRAFİK VERİLER			
Adı- Soyadı		**TC Kimlik No**	
Yaşı		**Protokol No**	
Cinsiyeti		**Başvuru Tarihi**	
Mesleği		**Telefon No**	

KLİNİK- TEDAVİ İLE İLGİLİ VERİLER				
Lezyon Yeri				
Oklüzyon Yeri				
Uygulanan Tedavi	**iv tPA**		**Mekanik Trombektomi**	
Klinik				
Erken BT bulguları	**Dens MCA**	**Kortikal silinme**	**İnsular ribbon silinme**	**Derin yapılarda silinme**

KOMORBİDİTE- KULLANILAN İLAÇLAR			
Hipertansiyon		**Antiagregan**	
Diyabet		**Antikoagülan**	
Geçirilmiş inme		**Antihipertansif**	

Koroner Arter Hastalığı		**Antidiyabetik**	
Atriyal Fibrilasyon		**Antihiperlipidemik**	
Sigara		**Diğer**	
Hiperlipidemi			
Alkol			
Diğer			

SEMPTOM- TEDAVİ ZAMANI İLE İLGİLİ BİLGİLER			
Semptom Başlangıcı		**Anjiyo Laboratuvarına Geliş**	
Acile Başvuru		**Kasık Giriş**	
ivtPA Bolus		**Rekanalizasyon Zamanı**	
ivtPA İdame		**mTICI**	
NIHSS		**Deneme Sayısı**	
ASPECT		**Genel Anestezi**	

MODİFİYE RANKİN SKALASI (MRS)		**İnme Öncesi**	**7.gün**	**Tabur-culuk**	**90.gün**
0	Hiç semptom yok				
1	Belirgin sakatlık yok (Semptomlara rağmen hasta günlük aktivitelerini ve görevlerini yerine getirebiliyor)				
2	Hafif sakatlık (Hastalık öncesi yaptığı bütün görev ve aktiviteleri yapamıyor ama yardım olmaksızın kişisel işlerini yapabiliyor)				

3	Orta derecede sakatlık (Kendi işlerini görmek için kısmen yardıma ihtiyacı var ama kendi başına yardımsız yürüyebiliyor)				
4	Ağır sakatlık (Yardımsız yürüyemiyor ve yardımsız bedensel ihtiyaçlarını karşılayamıyor)				
5	Çok ağır sakatlık (Yatağa bağımlı, inkontinans, devamlı bakıma ve dikkate muhtaç)				
6	Ölüm				

iv tPA DAİMİ DIŞLAMA KRİTERLERİ	
Tedaviye semptom başlamasından sonraki 4.5 saat içinde başlayamamak	
Görüntülemede herhangi bir tip akut (intraserebral, subaraknoid, subdural) kanama	
BT'de demarke ve geniş hipodansite	
Sistolik kan basıncı > 185 mmHg veya Diastolik kan basıncı > 110 mmHg	
Trombositopeni (>100.000/mm3)	
INR > 1.7	
aPTT > 40 sn	

iv tPA GÖRECELİ DIŞLAMA KRİTERLERİ	**iv tPA DIŞLAMA KRİTERİ DEĞİLDİR**
Başlangıç zamanının belirlenememiş olması	BT'de hiperdens arter işareti
Uyanma esnasında fark edilen inme	Minör inme (NIHSS<5)
Son 3 ay içinde kranio/spinal cerrahi	Majör inme (NIHSS>22)
Son 3 ay içinde kranio/spinal travma	Hızlı düzelen hasta
Son 3 ay içinde iskemik inme	İnsidental intrakranial anevrizma
Son 3 hafta içinde gastrointestinal kanama	Ekstra-aksiyel intrakranial tümör
Son 3 hafta içinde genitoüriner kanama	Serviko-kranial arter diseksiyonu

Son 3 hafta içinde majör cerrahi	İleri yaş (>80 yaş)
Son 2 hafta içinde majör sistemik travma	Demans
Son 1 hafta içinde komprese edilemeyecek arterlere ponksiyon	Epileptik nöbet
İntrakranial kanama öyküsü	İnme öncesi mobiliteyi engellemeyen özürlülük
YOAK kullanımı (son 48 saatte)	Hiperglisemi/ Hipoglisemi
Son evre böbrek yetmezliği, diyaliz	Menstrüel kanama
İleri karaciğer yetmezliği, siroz	Hamilelik
Aort diseksiyonu	Akut miyokart infarktüsü (non-STEMI, posterior veya inferior STEMI
İnfektif endokardit	İntrakardiyak trombüs
Sistemik malignite	Son 7 gün içinde aspirin ve/ veya klopidogrel kullanımı
İntrakranial intraaksiyel tümör veya kitle	IV heparin kullanımı (son 24 saatte, aPTT< 40 sn)
İntrakranial AVM	Düşük molekül ağırlıklı heparin kullanımı (son 24 saatte, aPTT<40 sn, anti-faktör Xa normal
Yaygın ön duvar ST elevasyonlu miyokart infarktüsü	
Perikardit	
Son 7 gün içinde dural ponksiyon	

ETYOLOJİK TETKİKLER- KAN			
HbA1c		**WBC**	
LDL		**Hb**	
HDL		**Platelet**	
Total Kolesterol		**Lökosit Sayısı**	
Trigliserid		**Nötrofil Sayısı**	
Vitamin B12		**Lenfosit Sayısı**	

Serum Kreatinin		**aPTT/ INR**	
CRP		**Sedimentasyon**	
Diğer			

ETYOLOJİK TETKİKLER- GÖRÜNTÜLEME	
MR Anjiografi	
EKO	
Doppler USG	
EKG	
TEE	
24 saatlik Ritm Holter testi	
Vaskülit Belirteçleri	
Trombofili Paneli	
Diğer	

TEDAVİ SONRASI TAKİP İLE İLGİLİ BİLGİLER			
24. saat BT		**Yatış Süresi**	
24. saat NIHSS		**Komplikasyon**	
Diğer			

TABURCULUK BİLGİLERİ- PROGNOZ			
Çıkış Tedavisi			
Taburculukta NIHSS		**Diğer**	
Taburculukta mRS			