Histological characteristics and clinical significance of fragile thrombus in endovascular thrombectomy for acute ischemic stroke

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"Non quia difficilia sunt, non audemus; sed quia non audemus, difficilia sunt."[*]

by *Lucius Annaeus Seneca*[**]

[*]"It is not because things are difficult that we do not dare; it is because we do not dare that they are difficult."
[**]A Roman philosopher of Stoicism (4 BC – AD 65)

Completing my dissertation, "Histological characteristics and clinical significance of fragile thrombus in endovascular thrombectomy for acute ischemic stroke," has been an extraordinary experience. It has been honored to have the guidance and support of several exceptional individuals who have played crucial roles in this accomplishment.

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ABSTRACT

Histological characteristics and clinical significance of fragile thrombus in endovascular thrombectomy for acute ischemic stroke

Background and purposes:

Fragile thrombi, which are prone to fragmentation during endovascular thrombectomy, pose significant challenges in achieving complete recanalization in patients with acute ischemic stroke. Fragmentation of these thrombi often results in distal migration, which complicates the procedure and reduces the likelihood of a successful outcome. Understanding the histological characteristics of thrombi that predispose them to fragility could provide insights into better management strategies for thrombectomy. This study investigated the relationship between thrombus fragility and its histological composition and evaluated the impact of this fragility on endovascular and clinical outcomes.

Materials and methods:

In this retrospective study, we included patients with acute ischemic stroke who underwent mechanical thrombectomy, from whom thrombi were successfully retrieved. The retrieved thrombi underwent immunohistochemical staining using antibodies specific to various markers, including erythrocytes, platelets, fibrin, lymphocytes, neutrophils, monocytes, tissue factors, neutrophil extracellular traps (NETs), and cathelicidin. The compositions of these thrombi were quantitatively analyzed using a semi-automated software to determine the fraction of each component. Thrombus fragility was defined as the occurrence of downstream occlusion due to thrombus fragmentation or the presence of multiple initial intracranial occlusions observed on conventional angiography.

Results:

This study included 310 patients, of whom 115 (37.1%) exhibited fragile thrombi. Through both univariable and multivariable analyses, atrial fibrillation (adjusted odds ratio [OR], 2.42; 95% confidence interval [CI], 1.08–5.41; $P = 0.032$) and a higher erythrocyte composition in the thrombus (adjusted OR, 1.19 per 5% increase in composition; 95% CI, 1.05–1.35; $P = 0.006$) were identified as independent predictors of thrombus fragility. Notably, patients with fragile thrombi were less likely to achieve complete recanalization (modified Thrombolysis In Cerebral Infarction [mTICI] grade 3; 32.2% vs. 80.0%; $P < 0.001$). Despite this, the overall rate of successful recanalization (mTICI grade 2b or 3) did not differ significantly between patients with fragile and non-fragile thrombi (90.4% vs. 93.8%; $P = 0.268$). Among those experiencing thrombus fragmentation, 61.0% initially achieved mTICI 2b, and subsequent thrombectomy attempts

improved mTICI grade in 72.0% of these patients. Functional independence at 3 months post-procedure was similar between the two groups (40.9% for fragile thrombi vs. 45.6% for non-fragile thrombi; $P = 0.413$).

Conclusion:

This study found that a higher erythrocyte composition within a thrombus is independently associated with increased thrombus fragility. However, this fragility did not significantly affect the clinical outcomes despite the challenges posed by thrombus fragmentation during the procedure. These findings suggest that although thrombus composition can influence procedural difficulties, it does not necessarily predict long-term functional outcomes in patients. Future research should continue to explore strategies to manage thrombus fragility and optimize procedural success and patient recovery.

Key words : stroke, thrombectomy, thrombus, fragility, fragmentation, histology

I. INTRODUCTION

Endovascular thrombectomy (EVT) is the standard of care in acute stroke due to large intracranial artery occlusion.[1] Since the successful introduction of EVT in stroke care, efforts have been made to expand its benefit to more patients by broadening the indications.[2-6] Although up to 90% of affected patients experience partial or complete recanalization, functional independence is achieved by only approximately half.[7-8] Complete recanalization significantly increases the likelihood of functional independence, prompting the development of procedural devices and techniques to improve recanalization.[9,10]

The process of EVT is dependent on the mechanical interaction between thrombectomy devices and the thrombus, which may have a significant impact on the success of the procedure.[11-15] Understanding the mechanical properties of a thrombus could be crucial for securing optimal endovascular results. One of the factors that contribute most to the mechanical properties of thrombi is their histopathologic composition.[16,17] Furthermore, advancements in thrombectomy devices have allowed the collection of retrieved thrombus samples, which can be analyzed for research purposes. A better understanding of thrombus composition may help overcome the current limitations of endovascular thrombectomy and provide new insights into the optimal endovascular strategy.

Fragile thrombi may undergo fragmentation during growth and embolization. It is also susceptible to fragmentation during EVT procedures, resulting in distal embolization and migration, which diminishes the likelihood of achieving complete recanalization. Studies have shown that 15–58% of patients experience thrombus fragmentation, distal embolization, or distal migration during EVT.[18-22] Thrombus fragility may be predominantly affected by its structure and composition. Thrombus fragmentation during EVT may also be associated with a mechanical interaction between the thrombectomy device and the characteristics of the thrombus.[23] Histological attributes have been pivotal in experimental studies to ascertain these characteristics.[24-27] However, the correlation has been insufficiently explored and remains inconsistent in clinical settings.[18,20,28] Furthermore, the potential impact of thrombus fragility on clinical outcomes, despite its theoretical significance, has not been thoroughly assessed.[18]

We hypothesized that the specific histological composition of the thrombus is responsible for determining its mechanical properties, which in turn affect its propensity to fragment. In particular, traditional histological components such as erythrocytes, fibrin, and platelets are likely to contribute to thrombus fragmentation. Moreover, we also hypothesized that thrombus fragmentation would have negative consequences on endovascular and clinical outcomes.

Accordingly, this study focused on determining how the composition of the thrombus affects its mechanical properties and the likelihood of fragmentation. In this study, we sought to explore the relationship between thrombus fragility and histology and examine its implications for both endovascular and clinical outcomes. Specifically, we aimed to investigate the association between thrombus fragility and histology, and assess its significance in terms of endovascular and clinical

outcomes.

II. MATERIALS AND METHODS

1. Study population

This study included patients from a prospective, hospital-based registry that enrolled patients with acute ischemic stroke within seven days of symptom onset. Consecutive patients who underwent successful thrombus retrieval during EVT at the Severance Hospital between January 2016 and December 2021 were included. Eligible participants were patients primarily treated with a stent retriever, whereas we excluded patients who underwent only contact aspiration thrombectomy. The Institutional Review Board of Severance Hospital approved this study (4-2023-1010). We obtained written informed consent from the patients or their next of kin for enrollment into the cohort and for utilizing retrieved thrombi in research.

2. Endovascular thrombectomy procedure

EVT was performed under local anesthesia according to common recommendations.[29] A stent retriever was usually used as a front-line endovascular modality. An 8- or 9-F balloon guide catheter (BGC) was routinely employed, whereas a distal access catheter was reserved for severely tortuous arteries. A stent retriever was delivered and deployed over the thrombus using a 0.021- or 0.027-inch microcatheter. The stent retriever was deployed for a few minutes before retrieval. For retrieval, the balloon of the BGC was inflated, and the stent retriever and microcatheter were carefully withdrawn with continuous aspiration through the BGC using a 20- or 50-mL syringe. The use of concurrent contact aspiration with a stent retriever for thrombectomy was reserved for rare, challenging cases that did not respond to standard approaches. This thrombectomy procedure was repeated until modified Thrombolysis In Cerebral Infarction (mTICI) 2b or 3 was achieved. Decisions to cease attempts or transition to an alternative endovascular technique were at the discretion of the operating physician.

3. Thrombus collection and immunohistochemical staining

Retrieved thrombi were immediately fixed in 4% paraformaldehyde, sent to the laboratory, embedded in paraffin, and stored until use.[30,31] The 4-μm-thick sections were treated with xylene and passed through an ethanol gradient. Then, the sections underwent heat-induced epitope retrieval, except for erythrocytes and fibrin. Subsequently, the sections were soaked in a solution containing

10 mM glycine in phosphate-buffered saline, and nonspecific bindings were blocked using a mixture of 1% horse serum and 5% nonfat milk in Tris-buffered saline for 20 minutes. The thrombi reacted with primary antibodies against erythrocytes, platelets, fibrin, lymphocytes, neutrophils, monocytes, tissue factors, neutrophil extracellular traps, and cathelicidin (Table 1). The sections were incubated at 37°C for 2 hours for monocytes or overnight at 4°C for others, followed by a secondary antibody reaction at 37°C for 30 minutes with 1:200-diluted biotin-conjugated Horse Anti-Mouse IgG antibody (BA-2000, Vector Laboratories, Peterborough, UK) for monocytes or biotin-conjugated Goat Anti-Rabbit IgG antibody (BA-1000, Vector Laboratories). After the secondary antibody reaction with an avidin/biotin/horseradish peroxidase complex, the color of positive signals was developed by incubating the slides in 3,3'-diaminobenzidine solution (D5637; Sigma-Aldrich, Burlington, MA, USA). Slides were counterstained with hematoxylin and mounted using Permount Mounting Medium (Fisher Scientific, Waltham, MA, USA).

4. Imaging analysis of thrombi

Images of stained thrombi were acquired using a whole-slide scanner (Leica Biosystems, Richmond, IL, USA) or Stereo Investigator Imaging system (MBF Bioscience, Williston, VT, USA) equipped with a light microscope (Axio Imager D2; Carl Zeiss Co., Ltd., Jena, Germany). The whole-slide scanner captured the images at a resolution of 0.2528 μM/pixel. Meanwhile, the Stereo Investigator Imaging system utilized the Virtual Slice module to acquire images at 400× magnification. The module automatically collected a series of contiguous images of a specimen using a motorized stage and merged them into a single-image montage representing the entire thrombus.

The acquired images were analyzed using the Automated Region-of-interest-based Image Analysis (ARIA) software program designed for automated composition analysis.[32,33] ARIA streamlines all traditional processes necessary for the imaging analysis of immunohistochemistry, allowing for rapid and less operator-dependent analysis. Briefly, ARIA automatically draws a contour around the thrombus area with adjustable handles for contour optimization. Following contouring, color deconvolution was performed to separate the colors into an immunohistochemistry color space for quantitative analysis. The ARIA then calculates the pixel densities for the total and stained areas of the thrombus. For quantitative analysis, each thrombus composition fraction (%) was determined by calculating pixel density as a percentage of the total thrombus area.

5. Determination of thrombus fragility

The presence of fragile thrombus was assessed using conventional angiography and defined as thrombus fragmentation during EVT or the presence of initially multiple intracranial occlusions.

Table 1. Primary antibodies for histological components of thrombus

Target	Immunogen	Clonality	Dilution	Catalog number*
Erythrocyte	Glycophorin A	Rabbit monoclonal	1:400	ab129024
Platelet	CD42b	Rabbit monoclonal	1:100	ab134087
Fibrin	Fibrinogen	Rabbit polyclonal	1:200	ab34269
Lymphocyte	CD3	Rabbit monoclonal	1:200	ab16669
Neutrophil	Neutrophil elastase	Rabbit polyclonal	1:200	ab68672
Monocyte	CD68	Mouse monoclonal	1:200	MA5-13324
Tissue factor	CD142	Rabbit polyclonal	1:100	PA5-27278
Neutrophil extracellular trap	Histone H3	Rabbit polyclonal	1:100	ab5103
Cathelicidin	Cathelicidin/CLP	Rabbit polyclonal	1:800	ab180760

*Antibodies were supplied by Abcam (Cambridge, UK) for erythrocyte, platelet, fibrin, lymphocyte, neutrophil, neutrophil extracellular trap, and cathelicdin and by Fisher Scientific (Waltham, MA, USA) for monocyte and tissue factor

Thrombus fragmentation was defined as downstream occlusion occurring after thrombectomy attempts. This downstream occlusion was any angiographical occlusion distal to the original target site, coupled with recanalization of the initial target.[18-20] All angiographic images taken immediately after each thrombectomy attempt were meticulously reviewed to assess the downstream occlusion. Additionally, the presence of initial multiple intracranial occlusions was considered to indicate fragile thrombus, as this may reflect fragmentation already incurred during thrombus growth or embolism.

6. Endovascular and clinical outcome variables

Successful recanalization was defined as achieving a final mTICI 2b or 3. The first-pass effect refers to near-complete or complete revascularization after the first pass of a thrombectomy device.[34] Two independent neurointerventionalists, blinded to the clinical information and follow-up imaging, assessed thrombus fragmentation and recanalization results. The κ-values for inter-rater reliability were 0.79 for thrombus fragmentation, 0.82 for successful recanalization, and 0.91 for first-pass effect, respectively. Discrepancies were resolved through consensus.

Functional outcomes were assessed based on modified Rankin Scale (mRS) scores 3 months after stroke onset. Stroke neurologists or clinical research assistants assessed the mRS scores during face-to-face interviews at the outpatient clinic. When patients could not visit the clinic, the mRS scores were assessed through telephone interviews with patients or their caregivers. Functional independence was defined as mRS scores of 0, 1, or 2.

7. Statistical analysis

First, patient demographics, stroke risk factors, endovascular details, and fractions of thrombus composition were compared between patients with and without fragile thrombus using the Student's t-test, Mann–Whitney U test, $\chi 2$ test, or Fisher's exact test. A multivariable logistic regression analysis was performed by incorporating variables with a $P < 0.1$ in univariable analyses to identify histological factors linked to fragile thrombus (Model 1). Second, we performed a trend analysis to investigate the changes in clinical findings based on the level of significant thrombus composition. Significant thrombus composition was classified into three groups based on tertile values. To address potential confounding factors more comprehensively, multivariable analysis was performed by adjusting for variables that showed significance in the trend analysis (Model 2). The area under the receiver operating characteristic curve (AUC) value determined the predictive accuracy of the significant thrombus composition for fragile thrombi, with the optimal cutoff identified via the Youden index. Third, immediate endovascular outcomes and treatment decisions after thrombus fragmentation were depicted in a Sankey diagram, and the benefit of additional thrombectomy

attempts was assessed. The advantage of endovascular therapy in terms of additional thrombectomy attempts following thrombus fragmentation was quantified. Another multivariable logistic regression analysis evaluated the relationship between fragile thrombus and functional independence, adjusting for variables with $P < 0.1$ in univariable analyses.

Statistical significance was set at $P < 0.05$, with a 95% confidence interval (CI). Analyses were performed using the R software (version 4.0.1; R Foundation, https://www.r-project.org).

III. RESULTS

1. Participants and general characteristics

Among the 376 patients with retrieved thrombi during EVT, immunohistochemistry of thrombi was performed in 340 (90.4%) cases (Figure 1), and 310 patients (mean age, 70.9 ± 13.6; men, 49.4%) were finally included in the study after excluding those who underwent aspiration thrombectomy alone. Intravenous tissue plasminogen activator (tPA) was administered before EVT in 114 (36.8%) patients (Table 2). The most common location of occlusion was the M1 segment of the middle cerebral artery (38.7%), followed by the internal carotid artery (27.1%). Occlusion was initially multiple in 15 (4.8%) patients. The BGC was used in 278 (89.7%) patients. The majority of patients who were not treated with BGC experienced posterior circulation stroke (30 of 32; 93.8%). Among the stent retrievers, Solitaire® (Medtronic, Minneapolis, MN, USA) was the most commonly used (258 patients, 83.2%). The most prevalent thrombus components were erythrocytes (35.6% [interquartile range, 20.3–46.7]) and fibrinogen (32.3% [21.2–47.2]) (Table 3).

2. Clinical and histological factors associated with fragile thrombus

Fragile thrombi were observed in 115 (37.1%) of the 340 patients. Atrial fibrillation (67.8% vs. 51.3%; $P = 0.004$; Table 4) and internal carotid artery occlusion (40.9% vs. 19.0%; $P < 0.001$) were more frequent in patients with fragile thrombi than in those without. No other clinical conditions were significantly associated with fragile thrombi.

Regarding thrombus composition, fragile thrombus was significantly associated with higher fractions of erythrocytes (40.2% [30.7–51.4] vs. 31.0% [16.6–43.3]; $P < 0.001$) and cathelicidin (6.9% [3.9–11.6] vs. 5.5% [2.0–9.4]; $P = 0.029$) (Table 5). A higher fraction of erythrocytes relative to platelets, platelets × fibrinogen, or whole common thrombus components (erythrocytes, platelets, fibrin, and leukocytes) was also significantly associated with fragile thrombi (for platelets, 4.6 [1.6–10.7] vs. 2.8 [1.2–6.6] under $P = 0.002$; for platelet × fibrinogen, 0.2 [0.1–0.4] vs. 0.1 [0.0–0.3] under $P = 0.003$; for whole common thrombus components, 44.0% [35.9–54.2] vs. 33.3% [20.2–

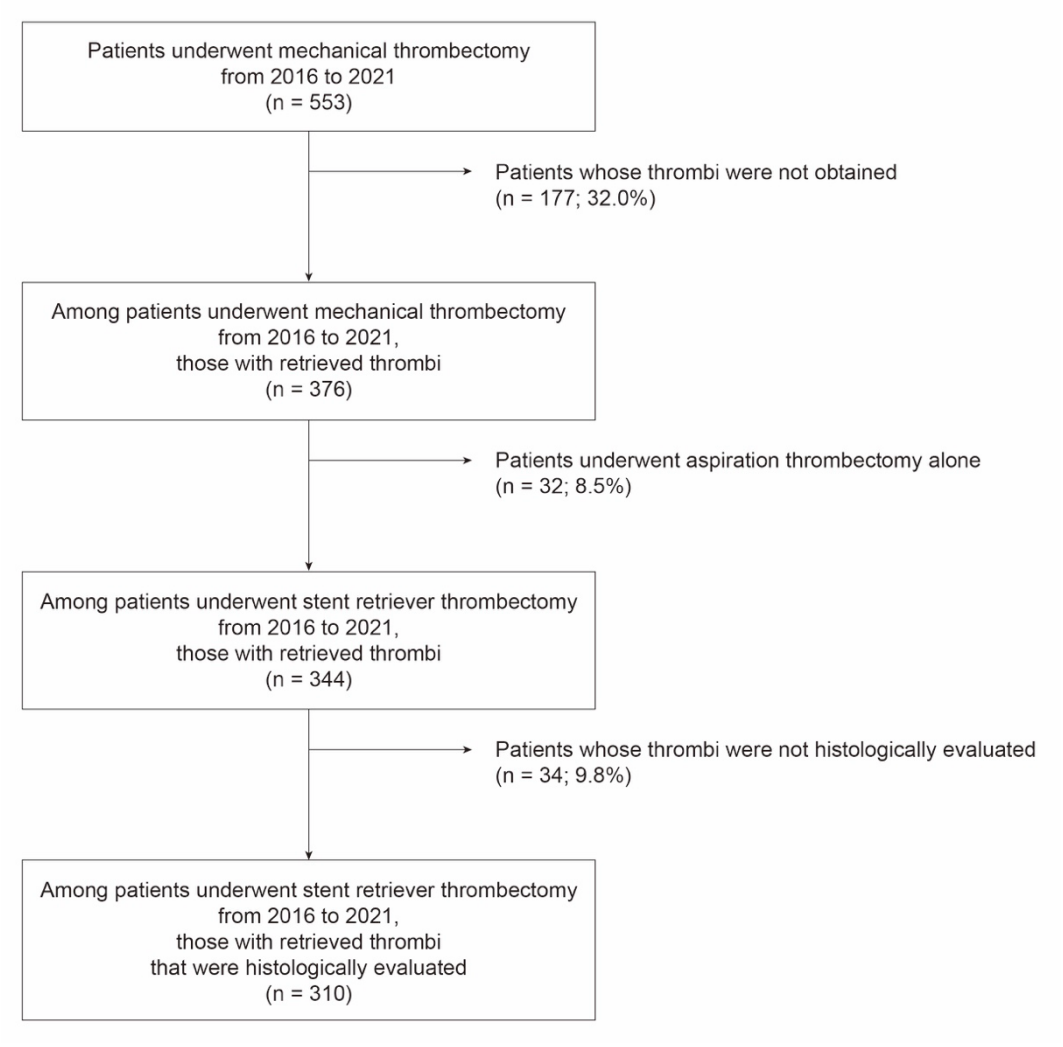

Figure 1. Patient selection flow chart.

Table 2. Demographics and clinical characteristics of the study population

Demographics and stroke risk factors	
Age (years)	70.9 (±13.6)
Men	153 (49.4)
Hypertension	224 (72.3)
Diabetes	100 (32.3)
Dyslipidemia	81 (26.1)
Current smoking	40 (12.9)
Coronary artery occlusive disease	46 (14.8)
Atrial fibrillation	178 (57.4)
Clinical conditions	
Initial NIHSS score	14.0 [9.0–18.0]
Intravenous tPA administration	114 (36.8)
Location of occlusion	
Internal carotid artery	84 (27.1)
M1 segment of middle cerebral artery	120 (38.7)
M2 segment of the middle cerebral artery	75 (24.2)
Anterior cerebral artery	1 (0.3)
Vertebrobasilar artery	26 (8.4)
Posterior cerebral artery	4 (1.3)
Tandem occlusion	26 (8.4)
Onset-to-puncture time (mins)	292.0 [167.0–610.0]
Use of balloon guide catheter	278 (89.7)
Endovascular outcomes	
Recanalization status	
Successful recanalization	287 (92.6)
Complete recanalization	193 (62.3)
First-pass effect	102 (32.9)
Puncture-to-recanalization time (mins)	31.0 [20.0–49.5]
Number of passes of stent retriever	2.3 (±1.4)
Clinical outcomes	
Functional independence	136 (43.9)

Values represent mean (±standard deviation), number (%), or median [interquartile range]; NIHSS = National Institutes of Health Stroke Scale; tPA = tissue-type plasminogen activator.

Table 3. Thrombus composition of the study population

Common thrombus components (%)	
Erythrocyte	35.6 [20.3–46.7]
Platelet	9.7 [4.3–19.5]
Fibrin	32.3 [21.2–47.2]
Leukocytes	8.5 [4.2–15.7]
Lymphocyte	0.3 [0.2–0.5]
Neutrophil	0.6 [0.2–2.1]
Monocyte	5.0 [2.2–11.1]
Specific components associated with thrombosis (%)	
Tissue factor	12.8 [1.5–42.7]
Neutrophil extracellular trap	2.7 [1.1–5.1]
Cathelicidin	6.0 [2.7–10.2]

Values represent median [interquartile range].

Table 4. Comparison of clinical findings according to thrombus fragility

	Fragile thrombus (*n* = 115)	No fragile thrombus (*n* = 195)	*P*-value
Demographics and stroke risk factors			
Age (years)	70.8 (±13.7)	70.9 (±13.6)	0.951
Men	57 (49.6)	96 (49.2)	0.955
Hypertension	80 (69.6)	144 (73.8)	0.416
Diabetes	34 (29.6)	66 (33.8)	0.436
Dyslipidemia	24 (20.9)	57 (29.2)	0.106
Current smoking	15 (13.0)	25 (12.8)	0.955
Coronary artery occlusive disease	14 (12.2)	32 (16.4)	0.311
Atrial fibrillation	78 (67.8)	100 (51.3)	0.004
Clinical conditions			
Initial NIHSS score	15.0 [10.0–18.0]	12.0 [8.0–18.0]	0.089
Intravenous tPA administration	47 (40.9)	67 (34.4)	0.251
Location of occlusion			<0.001
Internal carotid artery	47 (40.9)	37 (19.0)	
M1 segment of middle cerebral artery	41 (35.7)	79 (40.5)	
M2 segment of middle cerebral artery	21 (18.3)	54 (27.7)	
Anterior cerebral artery	0 (0.0)	1 (0.5)	
Vertebrobasilar artery	4 (3.4)	22 (11.3)	
Posterior cerebral artery	2 (1.7)	2 (1.0)	
Tandem occlusion	10 (8.7)	16 (8.2)	0.880
Onset-to-puncture time (mins)	267.0 [138.0–571.0]	308.0 [176.0–624.0]	0.210
Use of balloon guide catheter	108 (93.9)	170 (87.2)	0.060

Values represent mean (±standard deviation, number (%), or median [interquartile range]; NIHSS = National Institutes of Health Stroke Scale; tPA = tissue-type plasminogen activator.

Table 5. Comparison of thrombus composition according to thrombus fragility

	Fragile thrombus (n = 115)	No fragile thrombus (n = 195)	P-value
Common thrombus components (%)			
Erythrocyte	40.2 [30.7–51.4]	31.0 [16.6–43.3]	<0.001
Platelet	8.9 [3.8–17.4]	10.9 [4.5–20.7]	0.057
Fibrin	31.6 [21.0–48.1]	32.7 [21.4–45.7]	0.875
Leukocytes			
Lymphocyte	0.3 [0.2–0.5]	0.2 [0.1–0.4]	0.135
Neutrophil	0.7 [0.2–2.5]	0.6 [0.2–2.0]	0.813
Monocyte	5.1 [2.3–10.7]	4.9 [2.1–11.6]	0.989
Specific components associated with thrombosis (%)			
Tissue factor	13.9 [1.9–45.1]	11.8 [1.1–41.0]	0.608
Neutrophil extracellular trap	3.0 [1.1–6.4]	2.7 [1.1–4.6]	0.368
Cathelicidin	6.9 [3.9–11.6]	5.5 [2.0–9.4]	0.029
Relative composition of erythrocyte			
Erythrocyte / platelet	4.6 [1.6–10.7]	2.8 [1.2–6.6]	0.002
Erythrocyte / (platelet × fibrinogen)	0.2 [0.1–0.4]	0.1 [0.0–0.3]	0.003
Erythrocyte / whole common components*	44.0 [35.9–54.2]	33.3 [20.2–42.3]	<0.001

*Values represent percentage (%); Values represent median [interquartile range].

42.3] under $P < 0.001$). Multivariable analysis identified atrial fibrillation (adjusted odds ratio [aOR], 2.42; 95% CI, 1.08–5.41; $P = 0.032$) and erythrocyte composition in the thrombus (aOR, 1.19 per 5% composition; 95% CI, 1.05–1.35; $P = 0.006$) as independent factors for fragile thrombus. (Model 1 in Table 6).

3. Thrombus fragility and erythrocyte composition in thrombus

As the tertile of erythrocyte composition in the thrombus increased, the number of fragile thrombi increased significantly (P for trend < 0.001; Table 7). Additionally, a higher proportion of patients showed functional independence along with increased erythrocyte composition in the thrombus (P for trend < 0.022). Several other variables, such as atrial fibrillation, intravenous tPA administration, location of occlusion, tandem occlusion, and time from onset to groin puncture, were also associated with the erythrocyte composition in the thrombus. In the additional multivariable analysis, adjusting for those variables (Model 2 in Table 6), erythrocyte composition in the thrombus (aOR, 1.19 per 5% composition; 95% CI, 1.05–1.36; $P = 0.007$; Figure 2) was an independent factor associated with fragile thrombus. Erythrocyte composition in the thrombus remained independent even in the multivariable analysis without considering the use of BGC as a sensitivity analysis (Model 3 in Table 6). Representative examples of fragile thrombi are shown in Figures 3, 4, and 5.

Erythrocyte composition in the thrombus could predict fragile thrombus (AUC value, 0.655; cutoff, 26.9%; sensitivity, 84.4%; specificity, 42.1%; $P < 0.001$; Figure 6). The predictive power improved when considering the relative composition of erythrocytes to the whole common thrombus components (AUC value, 0.728; cutoff, 40.7%; sensitivity, 69.7%; specificity, 68.9%; $P < 0.001$).

4. Endovascular and clinical outcomes associated with fragile thrombus

Patients with fragile thrombus experienced a lower rate of first-pass effect (4.4% vs. 49.7%; $P < 0.001$; Table 8), required more passes of stent retriever (2.8 ± 1.3 vs. 2.0 ± 1.4; $P < 0.001$), and were less likely to achieve a final mTICI 3 (32.2% vs. 80.0%; $P < 0.001$). However, the rate of successful recanalization did not differ significantly between patients with and without fragile thrombus (90.4% vs. 93.8%; $P = 0.268$). Similarly, the proportion of patients achieving functional independence did not differ significantly between the two groups (40.9% vs. 45.6%; $P = 0.413$).

5. Endovascular trajectory after thrombus fragmentation

Among the 100 patients who had downstream occlusion due to thrombectomy attempts, 61

Table 6. Clinical and histological factors associated with fragile thrombus

	Model 1	Model 2	Model 3
	aOR (95% CI)	aOR (95% CI)	aOR (95% CI)
Clinical factors			
Atrial fibrillation	2.42 (1.08–5.41)*	2.13 (0.93–4.88)	2.10 (1.92–4.79)
Initial NIHSS score	1.01 (0.95–1.06)	1.00 (0.95–1.06)	1.00 (0.95–1.06)
Intravenous tPA administration		1.42 (0.66–3.07)	1.42 (0.66–3.08)
Internal carotid artery occlusion	2.09 (0.91–4.79)	2.40 (0.99–5.81)	2.51 (1.07–5.93)*
Tandem occlusion		0.48 (0.12–1.91)	0.48 (0.12–1.89)
Onset-to-puncture time (per 30 mins)		1.00 (0.99–1.01)	1.00 (0.99–1.01)
Use of balloon guide catheter	1.36 (0.37–4.99)	1.33 (0.36–4.96)	
Histological factors			
Erythrocyte (per 5%)	1.19 (1.05–1.35)**	1.19 (1.05–1.36)**	1.19 (1.05–1.35)**
Platelet (per 5%)	1.01 (0.88–1.16)	1.01 (0.89–1.16)	1.01 (0.88–1.16)
Cathelicidin (per 5%)	1.29 (0.93–1.80)	1.27 (0.91–1.77)	1.27 (0.91–1.78)

*P-value < 0.05; **P-value < 0.01; aOR = adjusted odds ratio; CI = confidence interval, NIHSS = National Institutes of Health Stroke Scale; tPA = tissue-type plasminogen activator.

1) Model 1 included atrial fibrillation, initial NIHSS score, location of occlusion, use of a balloon guide catheter, and histological composition of erythrocytes, platelets, and cathelicidin, all of which had P-value < 0.1 in univariable analyses.

2) Based on Model 1, Model 2 additionally included clinical factors with a significant trend association with erythrocyte composition of the thrombus: intravenous tPA administration, tandem occlusion, and onset-to-puncture time.

3) Model 3 was a sensitivity analysis that did not consider the use of balloon guide catheter.

Table 7. Characteristics according to erythrocyte composition in thrombus

	Tertile 1 13.5% [4.0–20.3] (*n* = 104)	Tertile 2 35.6% [31.5–39.0] (*n* = 103)	Tertile 3 50.9% [46.8–56.4] (*n* = 103)	*P*-value*
Demographics and stroke risk factors				
Age (years)	68.5 (±17.4)	72.6 (±11.0)	71.4 (±11.2)	0.126
Men	46 (44.2)	52 (50.5)	55 (53.4)	0.188
Hypertension	75 (72.1)	74 (71.8)	75 (72.8)	0.911
Diabetes	33 (31.7)	37 (35.9)	30 (29.1)	0.691
Dyslipidemia	31 (29.8)	23 (22.3)	27 (26.2)	0.555
Current smoking	12 (11.5)	13 (12.6)	15 (14.6)	0.517
Coronary artery occlusive disease	20 (19.2)	14 (13.6)	12 (11.7)	0.280
Atrial fibrillation	46 (44.2)	66 (64.1)	66 (64.1)	0.004
Clinical conditions				
Initial NIHSS score	13.0 [10.0–16.0]	14.0 [8.0–18.0]	14.0 [8.5–19.0]	0.416
Intravenous tPA administration	28 (26.9)	40 (38.8)	46 (44.7)	0.008
Location of occlusion				
Internal carotid artery	19 (18.3)	30 (29.1)	35 (34.0)	0.011
M1 segment of middle cerebral artery	41 (39.3)	39 (37.9)	40 (38.8)	
M2 segment of middle cerebral artery	32 (30.7)	25 (24.2)	18 (17.4)	
Anterior cerebral artery	1 (1.0)	0 (0.0)	0 (0.0)	
Vertebrobasilar artery	10 (9.7)	8 (7.8)	8 (7.8)	
Posterior cerebral artery	1 (1.0)	1 (1.0)	2 (2.0)	

(continued)

(continued)

Multiple occlusions	5 (4.8)	7 (6.8)	3 (2.9)	0.528
Tandem occlusion	3 (2.9)	11 (10.7)	12 (11.7)	0.023
Onset-to-puncture time†	351.0 [176.0–684.0]	309.0 [176.0–630.0]	258.0 [145.0–522.0]	0.038
Use of balloon guide catheter	93 (89.4)	94 (91.3)	91 (88.3)	0.801
Endovascular outcomes				
Recanalization status				
Successful recanalization	95 (91.3)	97 (94.2)	95 (92.3)	0.806
mTICI grade				0.820
0	1 (1.0)	2 (1.9)	2 (1.9)	
1	0 (0.0)	1 (1.0)	0 (0.0)	
2a	8 (7.7)	3 (2.9)	6 (5.8)	
2b	27 (26.0)	37 (35.9)	29 (28.2)	
3	68 (65.3)	60 (58.3)	66 (64.1)	0.844
First-pass effect	39 (37.5)	30 (29.1)	33 (32.0)	0.402
Puncture-to-recanalization time†	31.0 [21.0–49.0]	34.0 [20.0–54.0]	31.0 [20.0–49.0]	0.782
Number of passes of stent retriever	2.2 (±1.5)	2.5 (±1.5)	2.1 (±1.1)	0.665
Fragile thrombus	20 (19.2)	47 (45.6)	48 (46.6)	<0.001
Clinical outcomes				
Functional independence	38 (36.5)	44 (42.7)	54 (52.4)	0.022

**P*-value for trend; †Minutes; Values represent mean (±standard deviation), number (%), or median [interquartile range]; NIHSS = National Institutes of Health Stroke Scale; tPA = tissue-type plasminogen activator; mTICI = modified Thrombolysis In Cerebral Infarction.

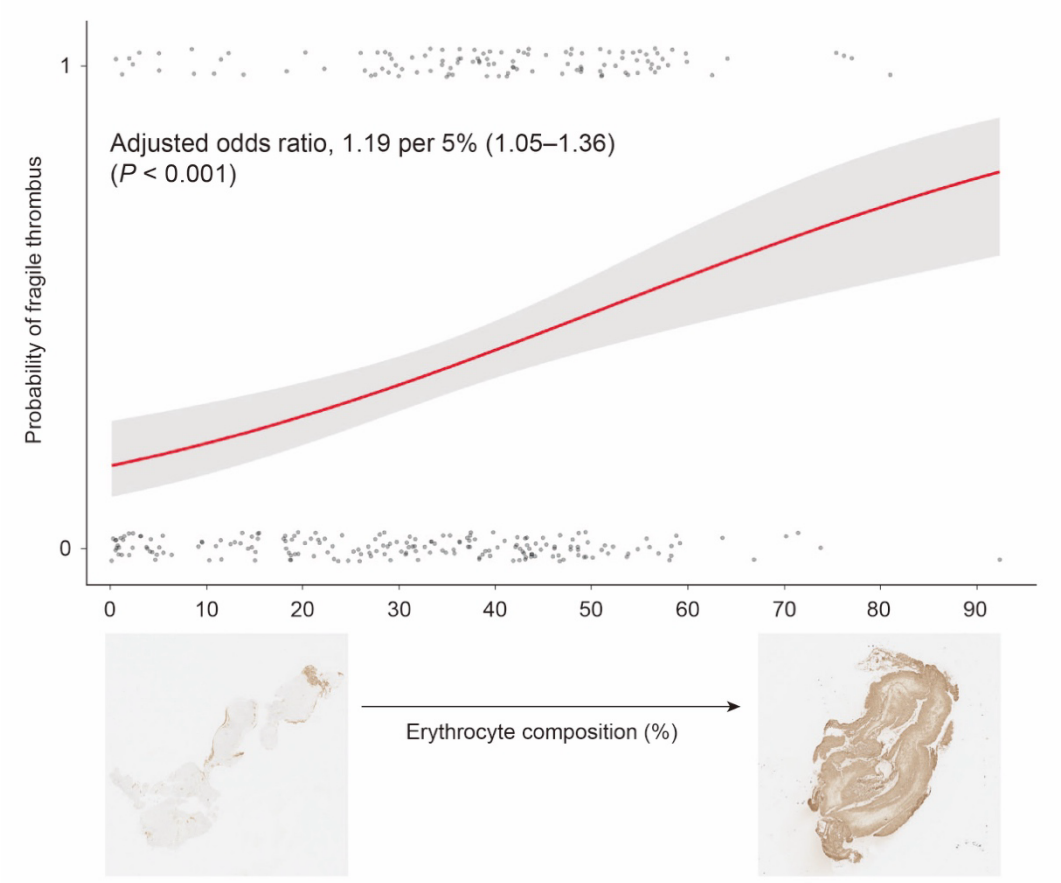

Figure 2. Probability of fragile thrombus by erythrocyte composition

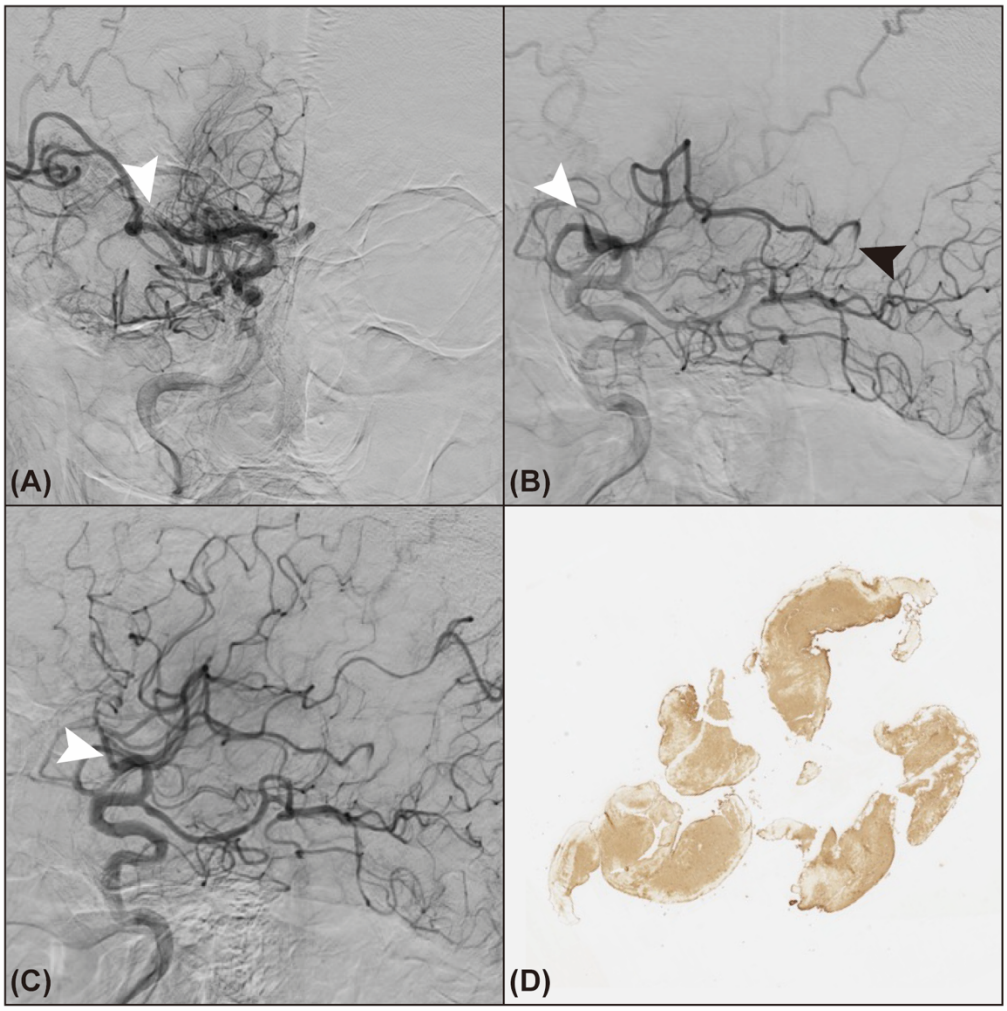

Figure 3. A 59-year-old man presented with multiple intracranial occlusions. On the first angiography of the right internal carotid artery (A, anteroposterior view; B, lateral view), multiple intracranial occlusions were observed, involving the superior division of the right middle cerebral artery (white arrowhead) and a distal branch of the inferior division of the right middle cerebral artery (black arrowhead). Following two stent retriever thrombectomy attempts, the superior division was completely recanalized (C, lateral view, arrowhead). The erythrocyte composition of the retrieved clot was 54.9% (D).

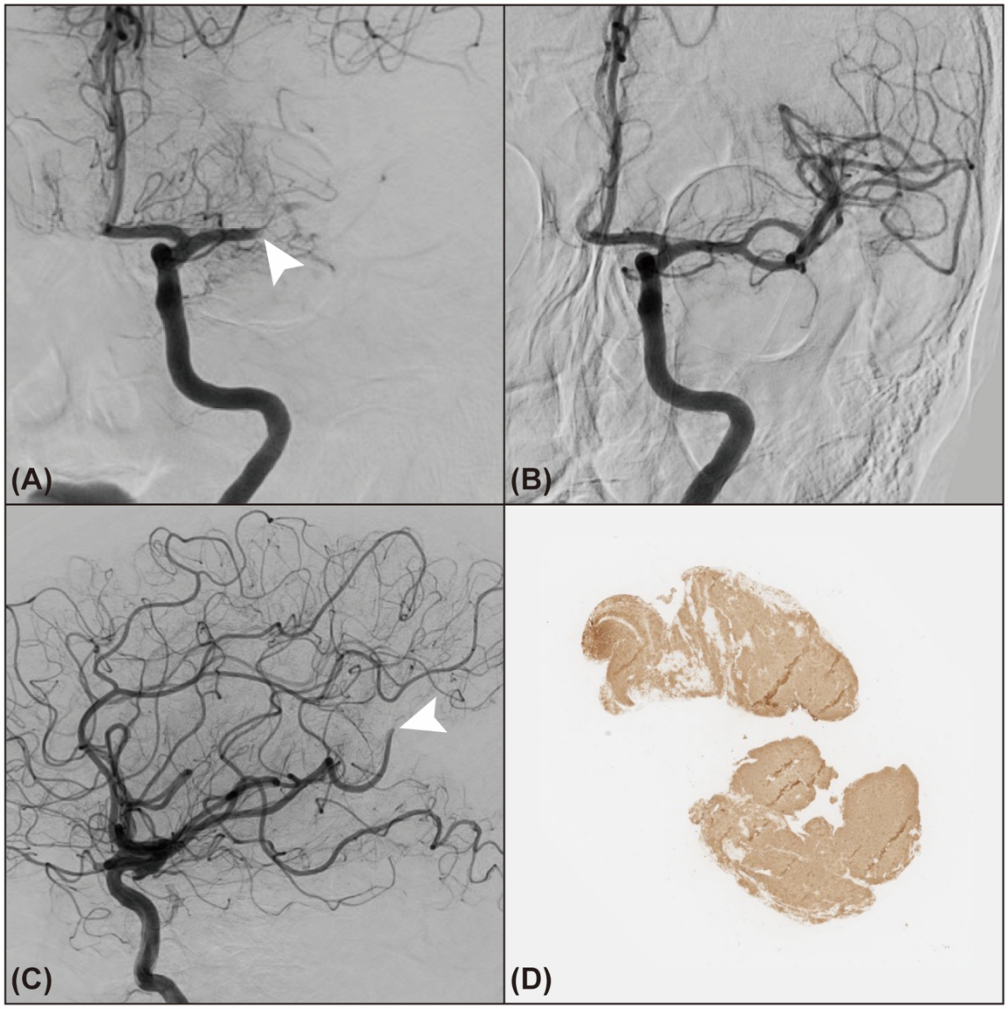

Figure 4. A 75-year-old woman experienced downstream occlusion during a stent retriever thrombectomy. Occlusion of the left middle cerebral artery (A, anteroposterior view, arrowhead) is fully recanalized using a stent retriever (B, anteroposterior view). However, downstream occlusion was noted at the far distal branch of the middle cerebral artery (C, lateral view, arrowhead) after the thrombectomy. No additional attempts were made to remove the distal thrombus. The erythrocyte composition of the retrieved clot was 66.7% (D).

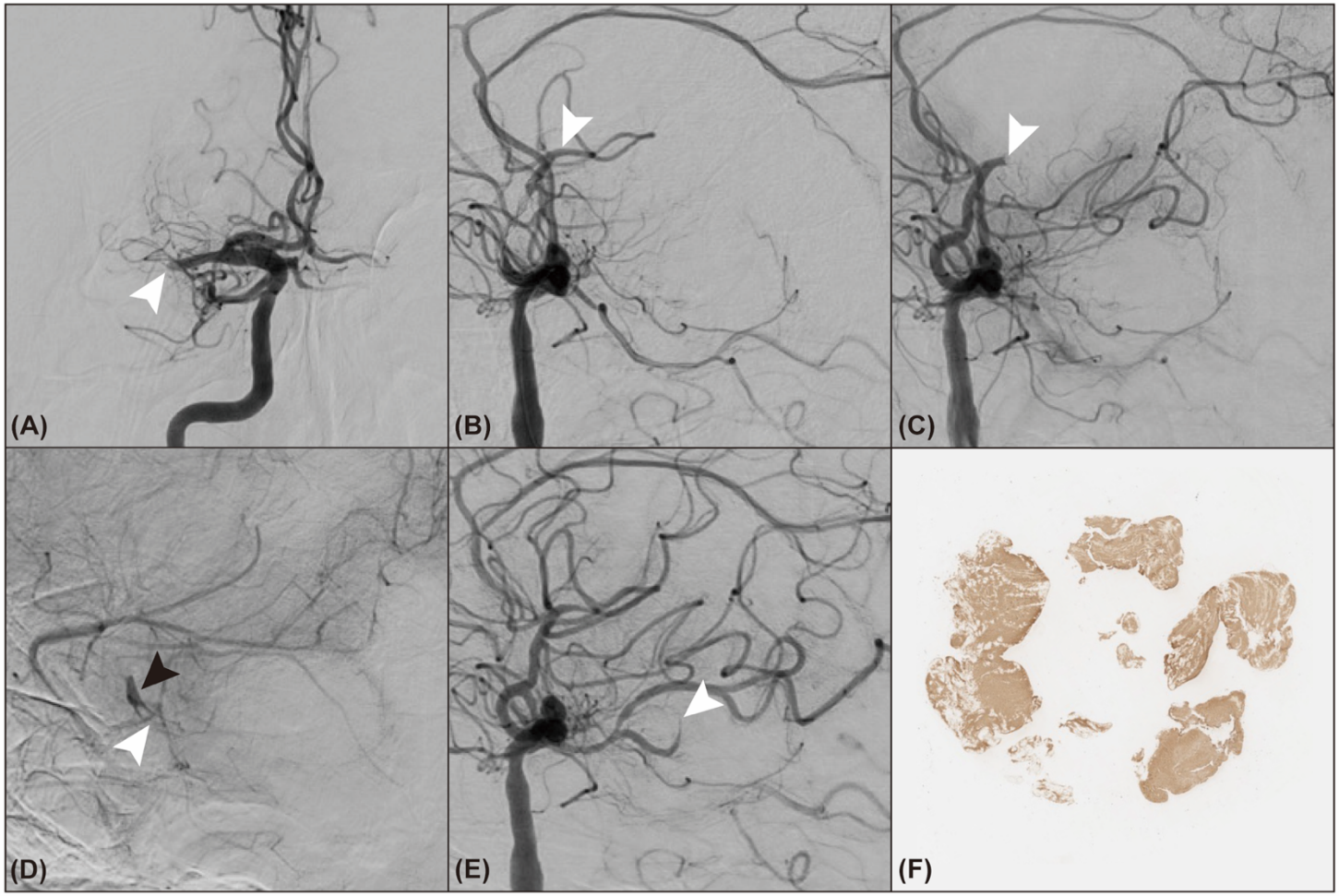

Figure 5. An 81-year-old man experienced downstream occlusion during a stent retriever thrombectomy. A stent retriever was used to treat occlusion of the right middle cerebral artery (A, anteroposterior view, arrowhead). After deployment of the stent retriever, the superior division of the middle cerebral artery was clearly visible (B, lateral view, arrowhead). However, following the removal of the stent retriever, downstream occlusion occurred in the superior division of the middle cerebral artery (C, lateral view, arrowhead). Furthermore, in the venous phase, contrast stagnation was observed in the inferior division of the middle cerebral artery (D, lateral view, black arrowhead), and a distal filling defect was identified (D, lateral view, white arrowhead), indicating another downstream occlusion. Occlusions in the superior and inferior divisions were successfully recanalized by additional stent retriever thrombectomies (E, lateral view). The other downstream occlusion was also noted on the final angiogram in the inferior division of the middle cerebral artery (E, lateral view, arrowhead). The erythrocyte composition of the retrieved clot was 55.7% (F).

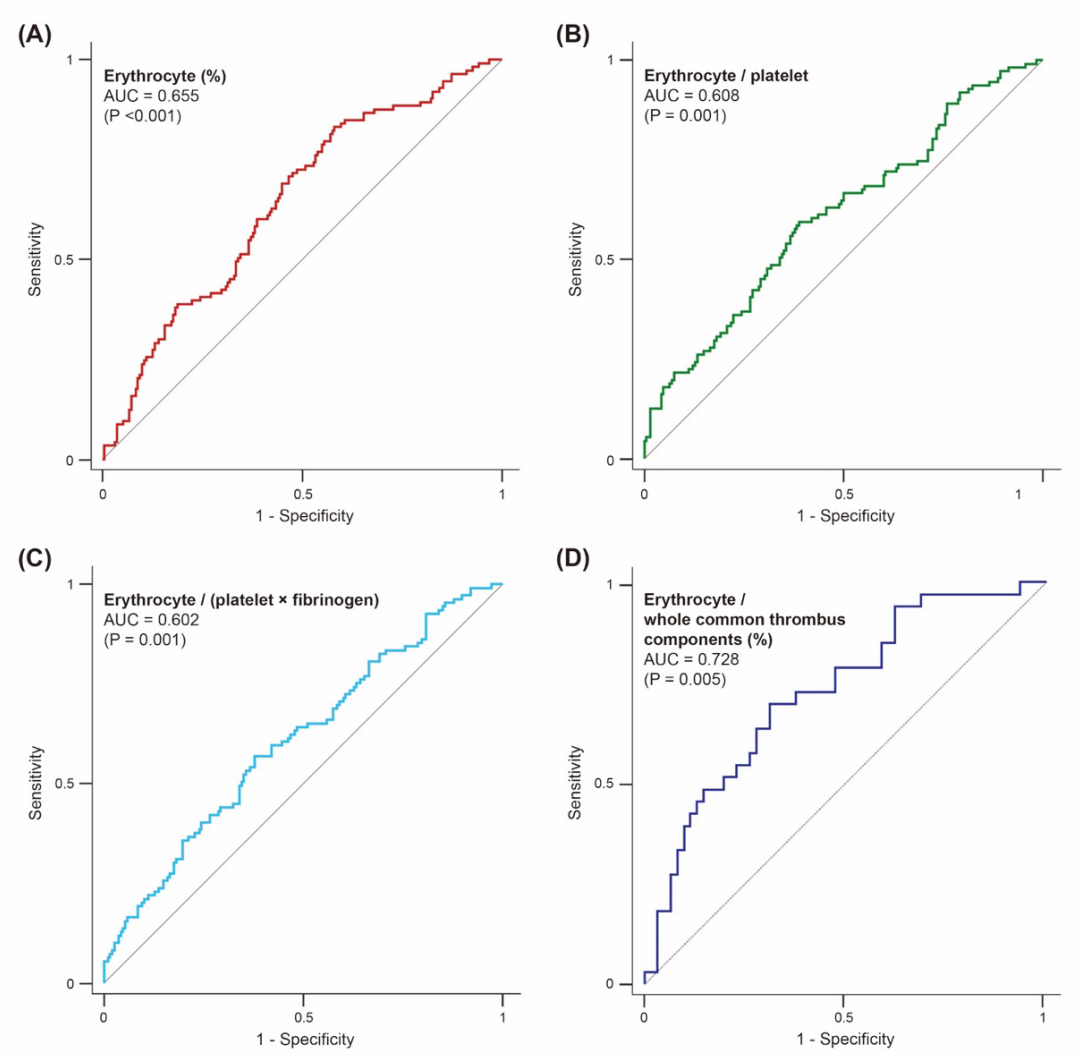

Figure 6. Prediction of fragile thrombus by erythrocyte composition in the thrombus. Receiver operating characteristic curves of erythrocyte (A), erythrocyte / platelet (B), erythrocyte / (platelet × fibrinogen), and its relative composition to the whole common thrombus components (D) for the prediction of fragile thrombus.

Table 8. Comparison of endovascular and clinical outcomes according to thrombus fragility

	Fragile thrombus (n = 115)	No fragile thrombus (n = 195)	P-value
Endovascular outcomes			
Successful recanalization	104 (90.4)	183 (93.8)	0.268
mTICI grade			<0.001
0	1 (0.8)	4 (2.1)	
1	0 (0.0)	1 (0.5)	
2a	10 (8.7)	7 (3.6)	
2b	67 (58.3)	27 (13.8)	
3	37 (32.2)	156 (80.0)	<0.001
First-pass effect	5 (4.4)	97 (49.7)	<0.001
Puncture-to-recanalization time*	33.5 [24.0–53.0]	29.0 [19.0–49.0]	0.128
Number of passes of stent retriever	2.8 (±1.3)	2.0 (±1.4)	<0.001
Clinical outcomes			
Functional independence	47 (40.9)	89 (45.6)	0.413

*Minutes; Values represent mean (±standard deviation), number (%), or median [interquartile range]; mTICI = modified Thrombolysis In Cerebral Infarction.

(61.0%) achieved immediate mTICI 2b despite their downstream occlusions (Table 9 and Figure 7). Further thrombectomy was performed in 62 patients with downstream occlusions. The attempts were conducted in over 95.2% of cases with downstream occlusions in the M1, M2, A3, P1, or P2 segments, but not in 89.5% of cases for downstream occlusions in the M3, M4, or P3 segments (Table 10). Of the 62 patients who underwent further thrombectomy, 44 (72.0%) achieved the improved mTICI grade. Finally, 57 (91.9%) patients achieved successful recanalization following further thrombectomy attempts (Table 9 and Figure 7).

Patients with downstream occlusion to the M1 segment of the middle cerebral artery had the lowest functional independence (9.5%) compared with the other segments (M2, M3, and M4), which were 60.0%, 55.6%, and 55.6%, respectively ($P < 0.001$; Table 11). For downstream occlusions in the M4 segment, functional independence was lower (42.9%) for occlusions in critical branches (precentral, central, and angular) than for non-critical branches (frontal and temporal branches; 69.2%), although this difference was not statistically significant ($P = 0.168$).

IV. DISCUSSION

1. Summary of key results

In this study, approximately 37% of the patients with intracranial vessel occlusion undergoing stent retriever treatment exhibited fragile thrombi. Higher erythrocyte composition in the thrombus was significantly associated with fragility. Although patients with thrombus fragmentation were less likely to achieve complete recanalization (mTICI 3), additional thrombectomy attempts enabled them to achieve successful recanalization (mTICI 2b or 3) at a rate similar to that without thrombus fragmentation. Functional independence did not show a significant difference between patients with and without fragile thrombus.

2. Thrombus fragility and erythrocyte composition in thrombus

In this study, thrombus fragility was higher in patients with erythrocyte-rich thrombi, and atrial fibrillation was the only clinical factor associated with fragile thrombus. Atrial fibrillation was also associated with other indications of fragile thrombus, such as secondary embolism and embolism to distal territories.[18,35] This study showed an estimated 20% increase in the frequency of fragile thrombus for every 5% increase in erythrocyte composition. In preclinical studies, erythrocyte-rich thrombi were less stiff, more fracture-labile, and less frictional, suggesting that they were likely to be softer.[24-26,36] In experimental conditions, stent retriever struts penetrated erythrocyte-rich thrombi more deeply.[23] Additionally, retrieving such thrombi resulted in more embolic fragments.[28] These findings in experimental conditions support our findings of an association between fragility and

Table 9. The fate of thrombus fragmentation in recanalization outcome

Location of downstream occlusion		Immediate mTICI grade	Total	Further attempts				Stop			
				Final mTICI grade				Final mTICI grade			
				3	2b	2a	Total	3	2b	2a	Total
MCA	M1	2b	1	1	0	0	1	0	0	0	0
		2a	19	9	5	3	17	0	0	2	2
		1	1	0	1	0	1	0	0	0	0
	M2	2b	16	9	6	0	15	0	1	0	1
		2a	17	8	6	2	16	0	0	1	1
		1	2	1	1	0	2	0	0	0	0
	M3	2b	9	1	2	0	3	0	6	0	6
	M4	2b	27	0	0	0	0	0	27	0	27
ACA	A3	2b	4	1	3	0	4	0	0	0	0
PCA	P1	2b	2	1	1	0	2	0	0	0	0
	P2	2b	1	0	1	0	1	0	0	0	0
	P3	2b	1	0	0	0	0	0	1	0	1
Total			100	31	26	5	62	0	35	3	38

Analysis of 100 patients who had a thrombus fragmentation after thrombectomy attempts. Although downstream occlusions occurred following thrombus fragmentation, 61 (61.0%) patients had an immediate modified Thrombolysis In Cerebral Infarction (mTICI) 2b (purple cells). Among 62 patients who underwent further thrombectomy attempts, 44 (72.0%) could achieve the improved mTICI grade (yellow cells). Finally, 57 (91.9%) patients achieved a final mTICI 2b or 3 following the further thrombectomy attempts (blue cells); MCA = middle cerebral artery; ACA = anterior cerebral artery; PCA = posterior cerebral artery.

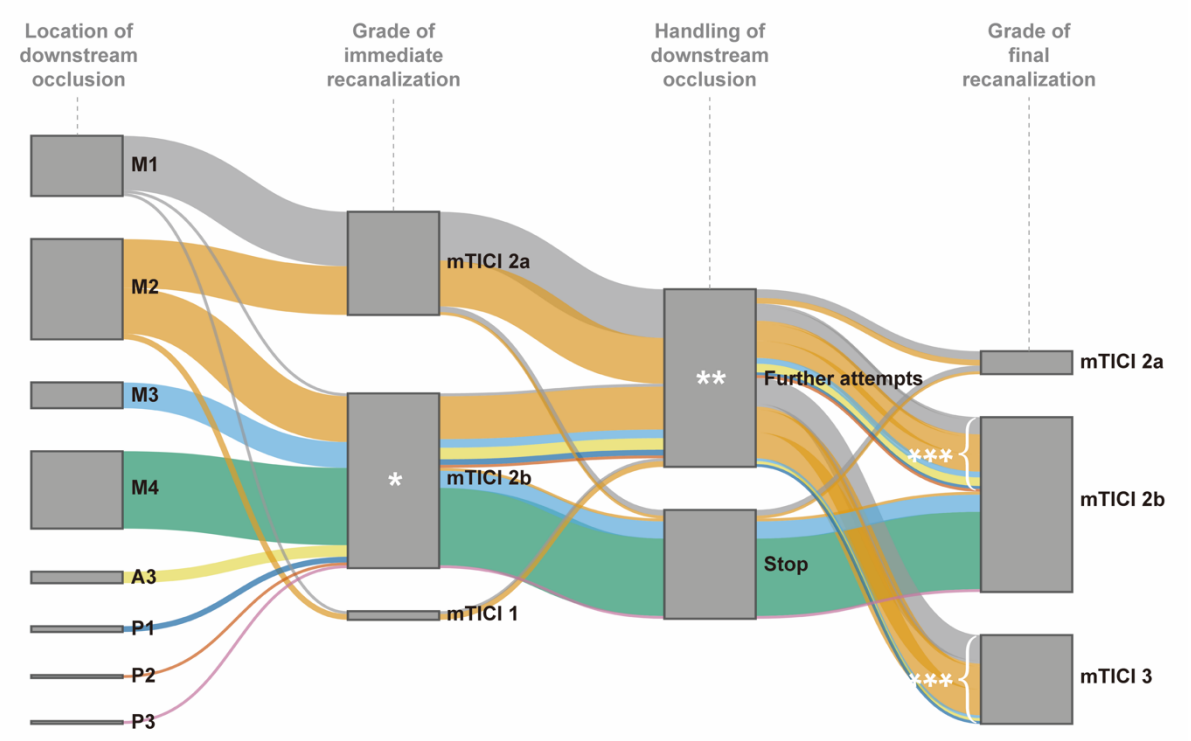

Figure 7. The fate of thrombus fragmentation in recanalization outcome. Sankey diagram is depicted by the analysis from 100 patients who had a thrombus fragmentation after thrombectomy attempts.
*Although downstream occlusions occurred following thrombus fragmentation, 61 (61.0%) patients had an immediate modified Thrombolysis In Cerebral Infarction (mTICI) 2b.
**Sixty-two patients (62.0%) were managed by further thrombectomy attempts.
***Finally, 57 patients (91.9%) could get a successful recanalization by further thrombectomy attempts.

Table 10. Handling of downstream occlusions after thrombus fragmentation

Location of downstream occlusion	Total (n = 100)	Further attempts (n = 62)	Stop (n = 38)
M1	21	19 (90.5)	2 (9.5)
M2	35	33 (94.3)	2 (5.7)
M3	9	3 (33.3)	6 (66.7)
M4	27	0 (0.0)	27 (100.0)
A3	4	4 (100.0)	0 (0.0)
P1	2	2 (100.0)	0 (0.0)
P2	1	1 (100.0)	0 (0.0)
P3	1	0 (0.0)	1 (100.0)

From the analysis from 100 patients who had a thrombus fragmentation after thrombectomy attempts. Dominancy of management was represented in gray cells. Values represent the number of patients and its percentage (%)

Table 11. Functional independence according to the specific location of thrombus fragmentation in the middle cerebral artery

Location of downstream occlusion*	Functional independence (n = 43)	Functional dependence (n = 49)	*P*-value
M1 segment	2 (9.5)	19 (90.5)	<0.001
M2 segment	21 (60.0)	14 (40.0)	
M3 segment	5 (55.6)	4 (44.4)	
M4 segment	15 (55.6)	12 (44.4)	
Critical branches	6 (42.9)	8 (57.1)	0.168
Precentral or central branches	3 (50.0)	3 (50.0)	
Angular branch	3 (37.5)	5 (62.5)	
Non-critical branches	9 (69.2)	4 (30.8)	
Frontal branches	3 (100.0)	0 (0.0)	
Temporal branches	6 (60.0)	4 (40.0)	

*First downstream occlusions occurred by the initial thrombectomy attempts.

From the analysis from 92 patients who had a thrombus fragmentation to the middle cerebral artery. Functional independence was defined as a modified Rankin Scale score 0–2 at 3 months after stroke; Values represent the number of patients and its percentage (%).

erythrocyte-rich thrombi in this study.

However, previous studies on stroke patients have reported inconsistent findings. One study, including 54 patients, noted that secondary embolism during EVT was associated with erythrocyte-rich thrombi, yet the association was not statistically significant.[18] In a study of 198 patients with middle cerebral artery occlusion, 36 (22%) exhibited clot migration, which was associated with a higher erythrocyte content.[22] Conversely, another study examining thrombi from 168 patients found secondary embolism in 27 (15%) patients, which was associated with high-fibrin and low-erythrocyte contents.[20] A study involving 85 patients undergoing EVT suggested that higher lymphocyte content in thrombi was associated with thrombus fragmentation.[37]

These discrepancies could be due to the different definitions of fragile thrombi and the smaller sample sizes in previous studies. Additionally, variations in the staining methods for erythrocytes, fibrin, and platelets may have contributed to these disparities. Immunohistochemistry was used in this study, whereas hematoxylin-eosin staining and histochemical staining, such as Martius Scarlet Blue staining, have been employed in previous studies. Exact differentiation between major thrombus components may occasionally be challenging with hematoxylin-eosin and histochemical stains.

Erythrocytes, fibrin, and platelets are major thrombus components that may be differently associated with its stability. Platelets aggregate via integrin $\alpha_{2b}\beta_3$, which binds to fibrinogen.[38] Consequently, platelets are tightly attached to each other via adhesive proteins. Platelet-driven clot contraction further tightens the fibrin network and expels water from the thrombus, leading to a compact and stiff thrombus.[25,39] In contrast, erythrocytes primarily aggregate via passive packing during thrombus formation; therefore, erythrocyte aggregates may be more susceptible to external forces. In experimental settings, erythrocyte composition is typically reciprocal to that of platelets. Thus, platelet-rich thrombi are significantly stiffer and consequently poor in erythrocytes.[24,25] In this study, patients with a higher proportion of erythrocytes had a lower platelet proportion, and fragile thrombi tended to have lower platelet counts. This may explain why erythrocyte-rich thrombi were more fragile in the present study.

3. Literature review—Thrombus composition and its mechanical property

Several in vitro and ex vivo studies have demonstrated a relationship between the thrombus composition and mechanical properties. Although various physical concepts can be used to understand the mechanical properties of thrombi, such as stiffness, hardness, deformability, viscosity, elasticity, toughness, fragility, and stickiness, "stiffness" is the most commonly represented mechanical property of thrombus. The stiffness of a thrombus can be analyzed in two different ways: compression and tension (stretching). Tensional characteristics are related to viscoelasticity and fragility, which contribute to thrombus fractures.

(1) Stiffness

① Compression

Boodt et al. (2021) conducted an unconfined compression test on 41 thrombi obtained through the thrombectomy procedure.[24] They discovered that the tangent modulus at 75% strain (E_{t75}), a marker of thrombus stiffness, was significantly correlated with the presence of fibrin and/or platelets (fibrin/platelets), erythrocytes, and platelets. The study revealed that the fibrin/platelet composition in thrombi was associated with increased stiffness, whereas erythrocytes had the opposite effect. Johnson et al. (2020) prepared two types of thrombus analogs: platelet-contracted clots (PCCs) and non-contracted clots (NCCs).[25] PCCs were prepared by combining blood mixtures of various hematocrit values with platelet-rich plasma, while NCCs were prepared under the same conditions but with platelet-poor plasma. The mechanical properties of thrombi are influenced by platelet-driven contractions, which tighten the fibrin network, expel serum from the thrombus, and reduce its volume. In their experiment, PCCs exhibited an earlier onset point on the stress-strain curve, indicating that they were deformed earlier under the applied force and resisted further deformation sooner. Overall, PCCs were found to be stiffer than NCCs, possibly because of platelet-driven contraction. Cruts et al. (2023) also discovered similar stress–strain relationships for thrombus analogs.[40] Compressive stiffness was higher in fibrin-rich thrombi (0% erythrocyte composition) than in erythrocyte-rich thrombi (> 90%).

Weafer et al. (2019) investigated the extent to which stent struts could indent thrombi, which is a measure of the compressive stiffness of clots.[23] To conduct their research, the authors prepared various thrombus analogs with hematocrit levels ranging from 0% to 80%. They used a steel indenter tip to indent the thrombi, and the indentation depth increased as the hematocrit level increased. The results showed that the stiffness of thrombi decreased significantly as erythrocyte content increased. In contrast, strut indentation during the embedding period decreased as erythrocyte content increased. This suggests that the benefit of embedding time is more pronounced for erythrocyte-poor thrombi. Thrombus imaging analysis showed a trend of increasing integrated thrombus volume with increasing erythrocyte content. Machi et al. (2017) also reported that larger white thrombi were not penetrated by the stent struts and remained on the side of the stent compressed against the vessel wall.[41]

② Tension, viscoelasticity, and fracture toughness

The rupture and fracture of thrombi can reveal their tensional characteristics and viscoelasticities. Tutwiler et al. (2020) investigated the mechanism and toughness of thrombus rupture with human blood plasma-derived fibrin thrombi, quantifying toughness through the critical energy release rate during cracked fibrin gel rupture.[42] Liu et al. (2021) calculated the fracture energy

for whole blood thrombi and platelet-poor thrombi.[43] The fracture energy was lower for platelet-poor thrombi, indicating that the fracture toughness was lower in erythrocyte-rich thrombi. Fereidoonnezhad et al. (2021) conducted experiments to investigate the relationship between thrombus composition and fracture toughness, specifically focusing on composition-dependent fracture behavior.[26] They prepared three different types of thrombi with varying fibrin content: high fibrin (5% of hematocrit), medium fibrin (20%), and low fibrin (40%). The results showed that fracture toughness significantly increased with higher fibrin content, indicating that thrombi with a higher erythrocyte content were more susceptible to tearing than those with a higher fibrin content.

Cahalane et al. (2023) investigated the tensile properties of thrombi using a tensile mold.[17] They prepared thrombus analogs with different erythrocyte compositions (5%, 10%, 20%, 40%, 60%, and 80%) from human blood. The tensile stiffness was quantitatively measured and showed a decrease with increasing erythrocyte composition in the thrombi. Gersh et al. (2009) previously demonstrated that the erythrocyte composition of thrombi affects the formation of thicker fibrin fibers, which consequently impacts the viscoelastic properties.[44] With thrombus analogs of varying erythrocyte levels, a higher erythrocyte composition increased the viscous component of thrombi relative to the elastic component, indicating a less elastic thrombus.

Thrombus fragmentation exhibits tensile characteristics associated with reduced elasticity and low fracture toughness, which are also observed in erythrocyte-rich thrombi. In a study conducted by Freiherr von Seckendorff et al. (2021), in vitro thrombectomy was performed on erythrocyte-rich and platelet-rich thrombi using a commercially prepared flow model equipped with a distal thrombus filter.[28] The results revealed a significantly higher number of embolic fragments deposited on the output filter membrane after thrombectomy for erythrocyte-rich thrombi.

(2) Friction

Friction between the thrombus and vessel wall can have a significant impact on the success of thrombectomy. Thrombus with high friction or a strong adhesion to the vessel wall may act as a sticky thrombus, making it more challenging to retrieve it.[45] Gunning et al. (2018) studied the friction of thrombus using an angle-changeable stainless-steel plate and calculated the coefficient of friction to determine the friction characteristics of thrombus with varying erythrocyte compositions (0%, 5%, 20%, 40%, and 80%).[36] Their findings revealed that fibrin-rich thrombi (< 20% erythrocytes) had a significantly higher coefficient of friction than those with a composition of ≥ 20% erythrocytes. This suggests that fibrin-rich thrombi are more frictional than erythrocyte-rich. The reproducibility of these results was demonstrated through experiments on bovine aortic surfaces.

(3) Summary of literatures

The relationship between thrombus composition and mechanical properties was significant. In the majority of experiments, fibrin or platelets are typically employed as a counterpart to erythrocytes. However, fibrin/platelet-rich thrombi, which were erythrocyte-poor, were stiff, tough, elastic, and less deformable. Additionally, the stent strut did not penetrate deeply into or integrate well with the fibrin/platelet-rich thrombi. Fibrin-rich thrombi were sticky and had higher friction with the vessel wall. These mechanical properties are believed to be the reason why fibrin/platelet-rich thrombi are difficult to retrieve during thrombectomy.

4. Clinical significance of thrombus fragmentation

In this study, the complete recanalization (mTICI 3) rate was significantly lower in patients with thrombus fragmentation. However, the rate of successful recanalization (mTICI 2b or 3) was similar between patients with and without thrombus fragmentation. Achieving complete recanalization with mTICI 3 may be associated with better outcomes than those with successful recanalization with mTICI 2b.[9] Nevertheless, in this study, functional outcomes were not different between patients with and without thrombus fragmentation.

Despite thrombus fragmentation, immediate recanalization of mTICI 2b was achieved in over 60% of the patients in this study. Additional thrombectomy procedures could effectively overcome downstream occlusions caused by thrombus fragmentation, with approximately 70% of the patients showing improved mTICI grades. Consequently, the final recanalization was not adversely affected by the thrombus fragmentation. In addition, most patients without further thrombectomy attempts had distal segment occlusions, such as M3, M4, and P3. Their occlusions were far distal and the area of the perfusion defect was not so large. Furthermore, although downstream occlusions and mTICI were assessed immediately after EVT procedures, thrombi in the distal arteries might be prone to spontaneous resolution sometime after EVT owing to their fragile nature. These findings suggest that thrombus fragmentation does not significantly affect functional outcomes. This is in line with a previous study, which demonstrated a comparable rate of functional independence between patients with secondary embolism (32%) and those without (48%) following EVT.[18]

However, the quality of the downstream occlusion could be crucial. Despite achieving improved mTICI grades after thrombus fragmentation, downstream occlusion to the M1 segment of the middle cerebral artery was significantly associated with lower functional independence. Downstream occlusion to the M1 segment is only possible in patients with internal carotid artery occlusion whose stroke was more severe. In addition, the immediate mTICI grade for the M1 occlusion was relatively poor (mTICI 2a in 90.5%) compared with other downstream occlusions. Subsequently, the final successful recanalization rate was also lower in the M1 occlusion (76.2%) than in the other downstream occlusions (95.8%; $P = 0.014$), which might negatively affect functional independence. Furthermore, patients with downstream occlusion to critical M4 branches tended to have lower functional independence. However, the influence of different M4 occlusions

on functional independence may be mitigated by individual collateral status, which was not adequately reflected in this study.

5. Literature review—Thrombus composition and endovascular outcomes

(1) Endovascular outcomes in animal and in vitro models

Yuki et al. (2012) conducted thrombectomy procedures on a swine animal model using an outdated device (Merci retriever) to remove two types of thrombi, erythrocyte-rich and fibrin-rich thrombi.[46] The composition of thrombi was not quantitatively analyzed; rather, their histology was examined grossly. The results showed that the number of attempts with the device was significantly higher for the fibrin-rich thrombi. Furthermore, successful recanalization based on Thrombolysis In Myocardial Infarction grades II and III was much lower for fibrin-rich thrombi, and it took significantly longer to achieve. An in vitro experiment demonstrated a similar association between thrombus composition and endovascular outcomes. Additionally, Freiherr von Seckendorff et al. (2021) used a flow model to show that a higher number of stent retriever passes and longer procedural times were necessary for platelet-rich thrombi.[28]

(2) Recanalization

The impact of thrombus composition on recanalization outcomes can be assessed using two methods. The first involves comparing the composition of thrombi based on the recanalization results. Typically, the mean or median values of specific thrombus components were examined between dichotomized recanalization end points. For example, Hashimoto et al. (2016) compared the mean erythrocyte composition between patients who achieved successful recanalization (mTICI 2b or 3) and those who did not.[47] The results showed that erythrocyte composition was significantly higher in the successful recanalization group (57% vs. 47%; $P = 0.042$). Additionally, erythrocyte composition was independently associated with successful recanalization (aOR, 4.35; 95% CI, 1.19–19.36; $P = 0.026$). A cutoff value for erythrocyte composition of > 64% was identified as predictive of successful recanalization. In contrast, fibrin/platelet composition did not differ significantly based on successful recanalization (42% vs. 48%; $P = 0.166$). Shin et al. (2018) investigated thrombus composition based on the Arterial Occlusive Lesion score.[48] The study revealed that erythrocyte composition had a higher mean value (37% vs. 20%; $P = 0.001$) in patients with better recanalization (Arterial Occlusive Lesion score 2–3).

Delvoye et al. (2022) concentrated on the platelet composition within the thrombus.[49] Using an immunoassay to measure glycoprotein VI levels, they quantitatively determined the platelets in the thrombus. Notably, the researchers compared the platelet composition between patients who

experienced the first-pass effect (extended TICI 2c or 3 achieved by a single pass of the device) and those who did not. The study found that the number of platelets was significantly lower in cases with the first-pass effect (0.098 vs. 0.111 ng/mg; $P < 0.001$), suggesting that platelet-poor thrombi are associated with the likelihood of the first-pass effect. Additionally, a higher platelet count was significantly associated with lower mTICI grades (aOR, 0.69; 95% CI, 0.53–0.89). In contrast, Sporns et al. (2017) discovered that the primary component of the thrombus did not have an effect on the success of recanalization.[20] The composition of erythrocytes (31.0% vs. 13.5%; $P = 0.096$) and fibrin (57.0% vs. 71.5%; $P = 0.128$) were similar between patients who successfully underwent recanalization and those who did not.

In a meta-analysis of six related studies, a higher erythrocyte composition was associated with a better angiographic outcome, with a mean difference of erythrocyte composition 9.6% (95% CI, 3.9–15.3; $P = 0.008$).[50]

The second approach involves comparing recanalization results based on the thrombus type. To achieve this, thrombi are typically classified according to their dominant composition. However, because there is no universally recognized method for determining dominance, this classification may be somewhat arbitrary. Shimizu et al. (2022) divided thrombi into platelet-rich/poor and erythrocyte-rich/poor categories based on platelet and erythrocyte composition, respectively.[51] The presence of platelets in the thrombus was confirmed by immunohistochemistry, with thrombi containing more than 24.3% platelets designated as platelet-rich. Complete recanalization (mTICI 3) was lower in platelet-rich thrombi than in platelet-poor thrombi (32.4% vs. 59.5%; $P = 0.035$). However, there was no significant difference in complete recanalization based on erythrocyte composition, with rates of 48.6% for erythrocyte-rich and 43.2% for erythrocyte-poor thrombi ($P = 0.816$), as determined by a cutoff value of 34.7%. Maekawa et al. (2018) divided thrombi into erythrocyte-rich and fibrin-rich categories, but did not provide a specific criterion for this classification.[52] Successful recanalization (mTICI 2b or 3) was comparable between the two types of thrombi, with 100% for erythrocyte-rich thrombi and 96% for fibrin-rich thrombi ($P = 0.999$).

A unique per-pass analysis can provide additional insights into the impact of thrombus composition on the outcomes of thrombectomy treatment. In a study conducted by Duffy et al. (2019), thrombus fragments were examined after each thrombectomy device pass.[53] Notably, a higher concentration of erythrocytes was observed in thrombi retrieved during passes 1 and 2 than in those retrieved during passes 3 to 6 ($P = 0.001$). Conversely, fibrin composition exhibited an inverse relationship ($P = 0.001$). The interpretation of these findings suggests that thrombi with a higher erythrocyte content are more easily retrieved and removed earlier during the procedure.[16,27,54,55]

(3) Number of thrombectomy device passes

Maekawa et al. (2018) discovered that there was a higher number of thrombectomy device passes in fibrin-rich thrombi compared to erythrocyte-rich thrombi (2.9 vs. 1.8; P = 0.020).[52] Shimizu et al. (2022) found that the number of device passes was greater in platelet-rich thrombi than in platelet-poor thrombi (1.5 vs. 1.0; P = 0.019).[51] However, there was no significant difference in the number of device passes between erythrocyte-rich and erythrocyte-poor thrombi (1.0 vs. 1.0; P = 0.375). Delvoye et al. (2022) also demonstrated a link between platelet composition and the number of device passes (OR, 1.11; 95% CI, 1.01–1.23); however, this association was not significant in the multivariable analysis (aOR, 1.09; 95% CI, 0.99–1.21).[49]

Kitano et al. (2022) conducted a study to compare the endovascular outcomes between fresh (< 1 day) and older (1–5 days) thrombi.[56] The results showed that patients with older thrombi required more device passes (2.0 vs. 1.0; P < 0.001). Additionally, the study found that first-pass recanalization (achieving mTICI 2b or 3 with a single device pass) was less likely in older thrombi (45% vs. 72%; P = 0.003). Researchers suggest that the higher number of device passes in older thrombi may be due to their higher platelet count and lower erythrocyte composition, although this is an indirect finding. Furthermore, a previous study by Duffy et al. (2019) found that thrombus fragments from passes 1 and 2 had higher erythrocyte and lower fibrin compositions, which may explain the lower number of device passes required for erythrocyte-rich (or fibrin-poor) thrombi.[53]

(4) Time to successful recanalization and procedural time

The research conducted by Simons et al. (2015) investigated the connection between the time from stroke onset to successful recanalization (onset-to-recanalization time) and procedural time based on erythrocyte and fibrin dominance in thrombi.[57] The thrombus was categorized as erythrocyte-dominant (erythrocyte > fibrin), equally dominant (erythrocyte = fibrin), or fibrin-dominant (erythrocyte < fibrin). The procedural time was significantly longer for equally dominant thrombi (70.3 min), and no relationship was observed between any type of thrombus and onset-to-recanalization or procedural time.

According to Sporns et al. (2017), there was a significant correlation between fibrin composition of the thrombus and procedural time (r = 0.484; P < 0.001), while erythrocyte composition of the thrombus was inversely correlated with procedural time (r = 0.491, P < 0.001).[20] Maekawa et al. (2018) analyzed time profiles according to thrombus composition and found that procedural (24.5 vs. 44.0 minutes; P < 0.010) and door-to-recanalization (86.5 vs. 110.0 minutes; P = 0.040) times were significantly shorter in patients with erythrocyte-rich thrombi.[52] Shimizu et al. (2022) also observed that puncture-to-recanalization time was significantly shorter for erythrocyte-rich thrombi (45.0 vs. 75.0 minutes; P = 0.007).[51] However, in that study, the puncture-to-recanalization time was not significantly longer for platelet-rich thrombi (71.0 vs. 53.0 minutes; P = 0.160). Delvoye et al. (2022) showed that a higher platelet composition is associated with a longer procedural time.[49] In a meta-analysis of five related studies, higher erythrocyte composition was

associated with shorter procedural time with a mean difference of procedural time 13.2 minutes (95% CI, 1.3–25.1; $P = 0.037$).[50]

(5) Secondary embolism

Thrombus fragmentation is a key observation derived from its mechanical properties and is the most direct indicator of thrombus fragility. This phenomenon can have negative effects on endovascular outcomes by causing downstream occlusion through secondary embolisms. This is a common occurrence during thrombectomy. In the research field, secondary embolism is defined as any occlusion that occurs distal to the original occlusion site after thrombectomy attempts.[19]

The relationship between thrombus composition and secondary embolism remains debatable. For 54 patients, Ye et al. (2020) found that patients with secondary embolism had a higher erythrocyte composition (42.9% vs. 26.8%; $P = 0.045$), but this was not statistically significant when comparing dichotomized values (erythrocyte-rich or not; 63.2% vs. 42.9%; $P = 0.154$) or in multivariable analysis (aOR, 35.4; 95% CI, 0.73–1718.99; $P = 0.072$).[18] Other thrombus compositions, such as fibrin (33.6% vs. 40.2%; P = 0.273) and platelets (16.2% vs. 22.4%; $P = 0.130$), were not significantly different between patients with and without secondary embolism. In contrast, from a study of 180 patients, Sporns et al. (2017) reported that secondary embolism was associated with lower erythrocyte (10.0% vs. 35.0%; $P < 0.001$) and higher fibrin (80.0% vs. 55.0%; $P < 0.001$) levels.[20] This finding appears to contradict the results of in vitro studies, which showed that erythrocyte-rich thrombi are less stiff and more deformable, potentially resulting in less fragmentation.

Thrombus migration is a noteworthy clinical observation that likely indicates fragility or other mechanical properties of the thrombus. This suggests that if thrombus migration is caused by spontaneous fragmentation, the thrombus may have a histological background similar to that of fragile thrombus. Thrombus migration can be directly detected by comparing pre-procedural images with procedural angiography. If the thrombus's location on the preprocedural computed tomography angiography has moved to a more distal position on the first procedural angiography, it can be considered a migrated thrombus.[21]

A study conducted by Maegerlein et al. (2018) revealed that the composition of erythrocytes and fibrin/platelets did not differ between patients with and without thrombus migration in the middle cerebral artery occlusion (41% vs. 37% for erythrocytes, $P = 0.230$; 54% vs. 57% for fibrin/platelets, $P = 0.240$).[21] However, upon further analysis, the researchers found that erythrocyte-rich thrombi (> 60% erythrocyte composition) were more prevalent in the thrombus migration group (36.4% vs. 5.7%, $P = 0.003$). This finding was consistent with the results reported by Sporns et al. (2019 and 2021), who also observed higher erythrocyte composition (50.0% vs. 26.0%, $P < 0.001$) and lower fibrin composition (43.5% vs. 62.0%, $P < 0.001$) in thrombi with migration.[22,58] Moreover,

higher erythrocyte composition was found to be an independent factor for thrombus migration (aOR, 1.03; 95% CI, 1.02–1.05; $P < 0.001$).

(6) Summary of literatures

The relationship between erythrocyte composition and successful recanalization was positive, while lower platelet composition was associated with specific outcomes, such as the first-pass effect and complete recanalization (Table 12). Furthermore, the number of thrombectomy device passes was possibly related to erythrocyte, platelet, and partly fibrin composition. Although limited, a smaller number of passes was associated with erythrocyte-rich (or platelet- and fibrin-poor) thrombi. The procedural time was consistently related to thrombus composition, with shorter times observed for erythrocyte-rich thrombi. However, it remains inconclusive whether thrombus composition is associated with secondary embolism, whereas thrombus migration is consistently associated with higher erythrocyte composition.

6. Heterogeneity in thrombus research

The study of thrombus composition is a complex task because of the presence of various heterogeneities in related research. First, one reason for this is that thrombi contain a range of components, including erythrocytes, platelets, and fibrin, which have traditionally been regarded as the main components of thrombi.[27] However, recent research has highlighted additional components beyond traditional ones, such as leukocytes, neutrophil extracellular traps, von Willebrand factor, tissue factor, and a few coagulation factors.[45,56,59-62] The mechanical properties of thrombi can be affected by individual components or by interactions between them, leading to unexpected heterogeneity in thrombus characteristics based on histological composition.

Second, although researchers have made progress in identifying and staining thrombus components, there is still no standardized approach to this task.[16] In the early days of research, classical staining methods such as Hematoxylin and Eosin staining and Martius Scarlet Blue staining were commonly used. However, recent studies have focused on immunohistochemical staining to identify specific thrombus components. The choice of staining method can vary depending on laboratory conditions.

Third, there is currently no universally accepted method for quantifying thrombus composition. Thrombi can be described quantitatively as a fraction, which represents the ratio of a specific component to the total thrombus area, or qualitatively, in terms of dominance.[16] For instance, thrombi can be characterized as erythrocyte-rich, platelet-rich, or fibrin-rich based on the dominant component. However, there is no standard criterion for determining the dominance of specific components, and this approach may overlook the role of other components.

Table 12. Summary of study results about the association between thrombus composition and endovascular outcomes

	Recanalization ↑	Pass number ↓	Procedural time ↓	Secondary embolism ↑
Qualitative studies				
Yuki, 2012[*46]	RBC-rich	RBC-rich	RBC-rich	
Freiherr von Seckendorff, 2021[†28]		RBC-rich	RBC-rich	RBC-rich
Simons, 2015[57]			NA	
Hashimoto, 2016[47]	RBC-rich			
Maegerlein, 2018[21]				RBC-rich
Maekawa, 2018[52]	NA	RBC-rich	RBC-rich	
Ye, 2020[18]				NA
Shimizu, 2022[51]	PLT-poor	PLT-poor	RBC-rich	
Quantitative studies				
Hashimoto, 2016[47]	↑RBC			
Sporns, 2017[20]	NA		↑RBC; ↓FIB	↓RBC; ↑FIB
Maegerlein, 2018[21]				↑RBC
Shin, 2018[48]	↑RBC			
Duffy, 2019[53]	↑RBC; ↓FIB			
Sporns, 2019 (also, 2021)[22,58]				↑RBC; ↓FIB
Ye, 2020[18]				↑RBC
Delvoye, 2022[49]	↓PLT	↓PLT	↓PLT	
Kitano, 2022[56]	Fresh	Fresh		

[*]Experimental study for animal model; [†]Experimental study of in vitro model; RBC = erythrocyte; NA = no association; PLT = platelet; FIB = fibrin; Fresh = fresh thrombus.

7. Study limitations and strengths

The strengths of this study include its relatively large sample size and precise measurement and analysis of each thrombus component. Immunohistochemistry was employed for thrombus component staining, enabling accurate determination of each component. Furthermore, a semi-automated software program was utilized for reliable analysis of thrombus composition, minimizing inter-observer variation.[32]

However, this study has several limitations. First, additional thrombectomy attempts in patients with downstream distal occlusions after thrombus fragmentation were performed at the discretion of the neurointerventionalists. Without specific guidelines for these cases, treatment strategies could differ between physicians and centers. Therefore, clinical outcomes after thrombus fragmentation should be interpreted with caution. The retrospective design of this study may restrict the generalizability of its findings to the clinical relevance of thrombus fragmentation.

Second, we included patients who underwent EVT primarily using stent retrievers. Consequently, endovascular and clinical outcomes are unknown for patients with potentially fragile thrombi but were primarily treated with mechanical devices other than stent retrievers.

Third, thrombus fragmentation may not always result in downstream occlusions. Fragile thrombus can be fragmented at the tip of the guide catheter during retrieval, potentially leading to embolism in a new territory, not downstream occlusion. However, fragmentation at the guide catheter tip appears to be distinct from fragmentation resulting from a stent retriever. When a thrombus is drawn into the guide catheter, it is subjected to significant compression from the orifice and walls of the catheter. This seems to be an extreme condition for assessing the mechanical properties of thrombi. Furthermore, embolism in a new territory is rare and may not significantly affect study outcomes in this population.

Fourth, this study does not offer a specific practical application for thrombus histology in an endovascular strategy. This is because histological analysis of a thrombus can only be performed after the completion of endovascular procedures, which is time consuming. Despite this limitation, the significance of thrombus histology in endovascular procedures requires further investigation. Future studies should explore specific preprocedural clinical findings that could potentially serve as surrogates for thrombus histology, such as thrombus imaging. Overall, more research is needed to develop better endovascular strategies based on the thrombus histology.

V. CONCLUSION

Fragile thrombus was independently associated with a higher erythrocyte composition in the

thrombus. Usually, downstream occlusions following thrombus fragmentation were endovascularly manageable using additional thrombectomy procedures. The fragile thrombus and its fragmentation during EVT were not significantly associated with functional independence.

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ABSTRACT IN KOREAN

급성 뇌경색에 대한 혈전 제거술 중 관찰되는 분쇄 취약 혈전의 조직학적 특성과 임상적 의의

배경 및 목적:

급성 뇌혈관 폐색에 대한 혈전 제거술은 회수성 스텐트(stent retriever)와 같은 혈전 제거 도구와 혈전 간의 기계적 상호 작용을 근간으로 한다. 혈전의 조직학적 특성은 혈전의 기계적 성질에 영향을 미칠 수 있는 주요한 요인이며, 임상적 관점에서 가장 직접적으로 관찰되는 혈전의 기계적 성질은 혈전의 분쇄 취약성(fragility)이다. 그럼에도 불구하고, 분쇄 취약 혈전(fragile thrombus) 대한 조직학적 특성과 그 임상적 의의는 잘 알려져 있지 않다. 이에 본 연구를 통해 혈전의 조직학적 구성이 혈전의 기계적 성질을 결정지을 것이라는 추정과 가설을 보다 임상적인 관점으로 확장하고자 하였다. 본 연구는 분쇄 취약 혈전이라는 꽤 흔한 임상적 현상에 대한 전(前)과 후(後)를 다루는 연구로, 혈전의 조직학적 구성과 분쇄 취약성과의 관계를 규명하고, 분쇄 취약 혈전과 시술 결과, 더 나아가 환자 예후에 미치는 영향을 알아보고자 하였다.

대상 및 방법:

급성 뇌혈관 폐색으로 내원하여 혈전 제거술을 시행 받은 환자들을 후향적으로 분석하였다. 얻어진 혈전은 적혈구, 혈소판, 피브린, 림프구, 호중구, 단핵구, 조직인자, 호중구 세포외 덫(neutrophil extracellular trap), 그리고 카델리시딘(cathelicidin)의 총 9개 혈전 구성 성분에 대한 면역조직화학염색을 시행하였다. 각 혈전 성분은 자동화된 혈전 분석 소프트웨어를 이용해 정량적으로 계산하였는데, 전체 혈전에서 해당 구성 성분이 차지하는 비율(%)을 산출하였다. 분쇄 취약 혈전은 시술 중 혈관 촬영 소견을 이용하여 정의하였다. 회수성 스텐트 시술로 본 폐색 부위가 개통된 이후, 해당 혈관 영역에 원위부 분지 폐색(downstream occlusion)이 관찰되는 경우 분쇄 취약 혈전으로 정의하였다. 또한, 혈전 제거술 이전에 다발성 혈관 폐색이 관찰되는 경우도 분쇄 취약 혈전으로 정의하였다.

결과:

최종적으로 310명의 환자들을 분석하였으며, 분쇄 취약 혈전은 115명에게서 관찰되었다(37.1%). 다변수 분석 결과, 심방 세동과 혈전내 적혈구 조성(적혈구 5% 당 보정 오즈비 1.19; 95% 신뢰구간 1.05−1.35; P= 0.006)이 분쇄 취약 혈전에 대한 독립 인자였다. 분쇄 취약 혈전을 가진 경우 완전 재개통(complete recanalization; mTICI 3)을 얻는 비율은 유의하게 적었지만(32.2% ↔ 80.0%; P < 0.001), 최종 재개통 성공(successful recanalization; mTICI 2b-3)에는 차이가 없었다(90.4% ↔ 93.8%; P = 0.268). 회수성 스텐트에 의해 혈전 분쇄가 일어난 100명의 환자 중 61명(61.0%)은 혈전 분쇄에도 불구하고 이미 좋은 재개통 등급(mTICI 2b)을 보였다. 혈전 분쇄 이후 시도된 추가 혈전 제거 시술을 통해 72.0%의 환자에게서 재개통 등급의 호전이 관찰되었다. 혈전 분쇄 발생은 환자의 3개월 기능적 독립(수정랭킹척도 0−2)에는 특별한 영향을 미치지 못하였다(40.9% ↔ 45.6%; P = 0.413).

결론:

특정 혈전 구성 성분은 혈전의 분쇄취약성과 관련이 있었으며, 특히 혈전 내 높은 적혈구 조성은 분쇄 취약 혈전과 독립적인 관련이 있었다. 혈전 분쇄에 의해 발생한 원위부 분지 폐색은 추가적인 혈전 제거 시술로 상당 부분 극복이 가능했다. 분쇄 취약 혈전과 시술 중 발생한 혈전 분쇄는 환자의 기능적 예후에 영향을 미치지는 않았다. 본 연구는 혈전 조성을 바탕으로 한 혈전의 기계적 및 임상적 특성에 대한 기초적 관점을 제시하였으며, 이는 효과적인 혈전 제거 치료 전략 수립에 중요한 치료적 토대가 될 수 있겠다.

핵심되는 말 : 뇌졸중, 혈전 제거술, 혈전, 분쇄, 조직학적 구성

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