JoS JOURNAL OF STROKE

Journal of Stroke 2024;26(3):454-457
https://doi.org/10.5853/jos.2024.00787

Letter to the Editor

Thrombi With a Higher Erythrocyte Composition Are More Fragile in Acute Stroke

Jang-Hyun Baek,[1,2] Il Kwon,[2,3] Sungeun Kim,[3] Hyo Suk Nam,[2,3] Young Dae Kim,[2,3] Byung Moon Kim,[4] Dong Joon Kim,[4] Tae-Jin Song,[5] Ji Hoe Heo[2,3]

[1]Department of Neurology, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Korea
[2]Department of Neurology, Yonsei University College of Medicine, Seoul, Korea
[3]Integrative Research Center for Cerebrovascular and Cardiovascular Diseases, Seoul, Korea
[4]Department of Radiology, Yonsei University College of Medicine, Seoul, Korea
[5]Department of Neurology, Seoul Hospital, Ewha Womans University College of Medicine, Seoul, Korea

Dear Sir:

Endovascular thrombectomy (EVT) is the standard of care for acute stroke, wherein achieving complete recanalization may improve clinical outcomes.[1] However, fragile thrombi are susceptible to fragmentation during EVT, causing distal embolization and migration, which diminishes the likelihood of complete recanalization.[2-5] Thrombus fragility may be predominantly affected by its structure and composition. In this study, we investigated the association between thrombus fragility and tumor histology.

This study included consecutive patients who underwent successful thrombus retrieval by EVT between January 2016 and December 2021, had their retrieved thrombi histologically evaluated, and were enrolled in the prospective registry. Eligible participants were patients primarily treated with a stent retriever; we excluded those who underwent only contact aspiration thrombectomy. This study was approved by the Institutional Review Board of Severance Hospital (4-2023-1010). Written informed consent was obtained from the patients or their next of kin for enrollment in the cohort and for utilizing the retrieved thrombi in the study.

EVT was performed under local anesthesia, according to common recommendations (Supplementary Methods). The retrieved thrombi were immediately fixed in 4% paraformaldehyde. All thrombi were immunohistochemically stained for erythrocytes, platelets, fibrin, lymphocytes, neutrophils, monocytes, tissue factors, and neutrophil extracellular traps (Supplementary Methods and Supplementary Table 1).[6] Images of stained thrombi were acquired using a whole-slide scanner 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 acquired images were analyzed using an Automated Region-of-interest-based Image Analysis software (Supplementary Methods).[7] Each thrombus composition fraction (%) was determined by calculating the pixel density as a percentage of the total thrombus area.

All conventional angiographic images obtained immediately after each thrombectomy attempt were reviewed. The presence of a fragile thrombus was defined as either the thrombus fragmentation during EVT or the presence of initially multiple intracranial occlusions. Thrombus fragmentation manifests as downstream occlusion subsequent to thrombectomy attempts, characterized by angiographical occlusion distal to the original target site coupled with recanalization of the initial target.[2-4] Furthermore, the presence of initially multiple intracranial occlusions was considered indicative of a fragile thrombus, as it may reflect pre-existing fragmentation during thrombus growth or embolism. Two independent neurointerventionalists (JHB and BMK), blinded to the histological and clinical information, assessed the images (κ-values: 0.79 for thrombus fragmentation, 0.82 for successful recanalization, and 0.91 for first-pass effect). Discrepancies were resolved through consensus.

In a cohort of 376 patients who underwent EVT and had their thrombi retrieved, immunohistochemistry was performed on 340 (90.4%) (Supplementary Figure 1). Finally, 310 patients were included in the study (mean age, 70.9±13.6 years; men, 49.4%) (Table 1). Fragile thrombi were observed in 115 (37.1%) patients.

Table 1. Comparison of clinical and histological findings according to thrombus fragility

	Total (n=310)	Fragile thrombus (n=115)	No fragile thrombus (n=195)	*P*
Demographics and stroke risk factors				
Age (yr)	70.9±13.6	70.8±13.7	70.9±13.6	0.951
Male sex	153 (49.4)	57 (49.6)	96 (49.2)	0.955
Hypertension	224 (72.3)	80 (69.6)	144 (73.8)	0.416
Diabetes	100 (32.3)	34 (29.6)	66 (33.8)	0.436
Dyslipidemia	81 (26.1)	24 (20.9)	57 (29.2)	0.106
Current smoking	40 (12.9)	15 (13.0)	25 (12.8)	0.955
Coronary artery occlusive disease	46 (14.8)	14 (12.2)	32 (16.4)	0.311
Atrial fibrillation	178 (57.4)	78 (67.8)	100 (51.3)	0.004
Clinical conditions				
Initial NIHSS score	14.0 [9.0–18.0]	15.0 [10.0–18.0]	12.0 [8.0–18.0]	0.089
Intravenous tPA administration	114 (36.8)	47 (40.9)	67 (34.4)	0.251
Location of occlusions				<0.001
Internal carotid artery	84 (27.1)	47 (40.9)	37 (19.0)	
M1 segment of middle cerebral artery	120 (38.7)	41 (35.7)	79 (40.5)	
M2 segment of middle cerebral artery	75 (24.2)	21 (18.3)	54 (27.7)	
Anterior cerebral artery	1 (0.3)	0 (0.0)	1 (0.5)	
Vertebrobasilar artery	26 (8.4)	4 (3.4)	22 (11.3)	
Posterior cerebral artery	4 (1.3)	2 (1.7)	2 (1.0)	
Tandem occlusion	26 (8.4)	10 (8.7)	16 (8.2)	0.880
Onset-to-puncture time (min)	292.0 [167.0–610.0]	267.0 [138.0–571.0]	308.0 [176.0–624.0]	0.210
Use of balloon guide catheter*	278 (89.7)	108 (99.1)	170 (99.4)	0.747
Endovascular outcomes				
Recanalization status†				
Successful recanalization	287 (92.6)	104 (90.4)	183 (93.8)	0.268
Complete recanalization	193 (62.3)	37 (32.2)	156 (80.0)	<0.001
First-pass effect	102 (32.9)	5 (4.4)	97 (49.7)	<0.001
Number of passes of stent retriever	2.3±1.4	2.8±1.3	2.0±1.4	<0.001
Puncture-to-recanalization time (min)	31.0 [20.0–49.5]	33.5 [24.0–53.0]	29.0 [19.0–49.0]	0.128
Thrombus composition (%)				
Erythrocyte	35.6 [20.3–46.7]	40.2 [30.7–51.4]	31.0 [16.6–43.3]	<0.001
Platelet	9.7 [4.3–19.5]	8.9 [3.8–17.4]	10.9 [4.5–20.7]	0.057
Fibrin	32.3 [21.2–47.2]	31.6 [21.0–48.1]	32.7 [21.4–45.7]	0.875
Leukocytes	8.5 [4.2–15.7]			
Lymphocyte	0.3 [0.2–0.5]	0.3 [0.2–0.5]	0.2 [0.1–0.4]	0.135
Neutrophil	0.6 [0.2–2.1]	0.7 [0.2–2.5]	0.6 [0.2–2.0]	0.813
Monocyte	5.0 [2.2–11.1]	5.1 [2.3–10.7]	4.9 [2.1–11.6]	0.989
Tissue factor	12.8 [1.5–42.7]	13.9 [1.9–45.1]	11.8 [1.1–41.0]	0.608
Neutrophil extracellular trap	2.7 [1.1–5.1]	3.0 [1.1–6.4]	2.7 [1.1–4.6]	0.368

Values represent mean±standard deviation, number (%), or median [interquartile range].
NIHSS, National Institutes of Health Stroke Scale; tPA, tissue-type plasminogen activator.
*The calculation of the percentages of patients using a balloon-guided catheter was limited to those with anterior circulation stroke; †Successful recanalization was defined as achieving final modified Thrombolysis In Cerebral Infarction (mTICI) 2b or 3; Complete recanalization was defined as the attainment of a final mTICI score of 3.

Table 2. Clinical and histological factors associated with fragile thrombus

	Adjusted odds ratio (95% CI)	*P*
Clinical factors		
Atrial fibrillation	1.61 (0.95–2.73)	0.075
Initial NIHSS score	1.01 (0.97–1.06)	0.494
Internal carotid artery occlusion	2.45 (1.41–4.27)	0.001
Histological factors		
Erythrocyte, per 5%	1.14 (1.05–1.23)	<0.001
Platelet, per 5%	0.99 (0.90–1.07)	0.725

The analysis included variables with *P*<0.1 in the univariable analyses.
CI, confidence interval; NIHSS, National Institutes of Health Stroke Scale.

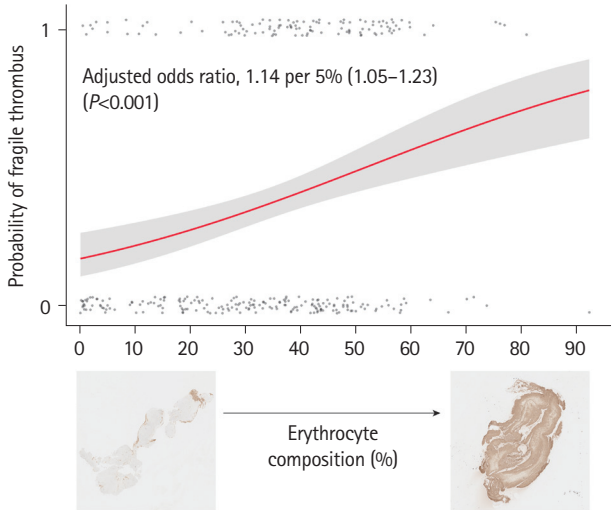


Figure 1. Probability of a fragile thrombus by erythrocyte composition.

Atrial fibrillation (67.8% vs. 51.3%; *P*=0.004) and internal carotid artery (ICA) occlusion (40.9% vs. 19.0%; *P*<0.001) were more frequent in patients with fragile thrombi than in those without (Table 1). Regarding thrombus composition, fragile thrombi were significantly associated with higher fractions of erythrocytes (40.2% [30.7–51.4] vs. 31.0% [16.6–43.3]; *P*<0.001) (Table 1). A higher fraction of erythrocytes relative to whole common thrombus components (i.e., erythrocytes, platelets, fibrin, and leukocytes) was also significantly associated with a fragile thrombus (44.0% [35.9–54.2] vs. 33.3% [20.2–42.3]; *P*<0.001). Multivariable analysis identified ICA occlusion (adjusted odds ratio, 2.45; 95% confidence interval, 1.41–4.27; *P*=0.001) and erythrocyte composition in thrombus (adjusted odds ratio, 1.14 per 5% composition; 95% confidence interval, 1.05–1.23; *P*<0.001) as independent factors for fragile thrombus (Table 2 and Figure 1). Erythrocyte composition in the thrombus could predict fragile thrombus (area under the receiver operating characteristic curve, 0.655; cutoff, 26.9%; sensitivity, 84.4%; specificity, 42.1%; *P*< 0.001) (Supplementary Figure 2). Platelet composition tended to be lower in a fragile thrombus (8.9% [3.8–17.4] vs. 10.9 [4.5–20.7]; *P*=0.057).

In this study, a fragile thrombus was observed in 37.1% of cases, a frequency comparable to 35.2%–57.5%, as stated in previous studies employing similar definitions to ours.[2,3] It was also associated with ICA occlusion and erythrocyte-rich thrombi. ICA occlusion has been suggested to correlate with distal and secondary embolisms.[2] Preclinical studies have shown that erythrocyte-rich thrombi are softer, less stiff, and more prone to fracture, suggesting their fragility.[8,9] Experimental retrieval of erythrocyte-rich thrombi resulted in the generation of more embolic fragments.[9] These findings support the association between fragility and erythrocyte-rich thrombi observed in our study.

Erythrocytes, fibrin, and platelets exhibit different degrees of stability. Platelets aggregate with each other by adhesively binding integrin $\alpha_{2b}\beta_3$ to fibrinogen. Consequently, platelets are tightly attached to each other. In contrast, erythrocytes primarily aggregate via passive packing, making them more susceptible to external forces. This may explain why erythrocyte-rich thrombi were more fragile, whereas those with higher platelet content tended to be less fragile.

However, previous clinical studies have reported inconsistent results. While one study demonstrated an association between clot migration and higher erythrocyte content,[5] others found associations of secondary embolism with high fibrin and low erythrocyte contents,[4] as well as an association between thrombus fragmentation and higher lymphocyte counts.[10] Such disparities may arise from the varying definitions of fragile thrombi and smaller sample sizes in previous studies. Additionally, previous studies utilized hematoxylin-eosin and histochemical stains, such as Martius Scarlet Blue, to detect erythrocytes and fibrin; this study employed immunohistochemistry. Precise differentiation between the major thrombus components may occasionally be challenging with histochemical staining.

Despite our strengths, such as a relatively large sample size, precise analysis of thrombus components through immunohistochemistry, and a semi-automated software program, our study has limitations. First, it focused on patients who underwent EVT primarily using stent retrievers. This was because patients who underwent only contact aspiration thrombectomy were excluded from this study, potentially having different mechanical impacts on thrombus. Second, although thrombus fragmentation may not always result in downstream occlusion, potentially leading to embolism in a new territory, such occurrences are rare and unlikely to significantly impact the outcome of the study. Finally, the definition of a fragile thrombus in this study may not accurately identify all such cases.

In conclusion, we found that thrombi with a higher erythrocyte composition were more fragile.

https://doi.org/10.5853/jos.2024.00787

Supplementary materials

Supplementary materials related to this article can be found online at https://doi.org/10.5853/jos.2024.00787.

Funding statement

This study was supported by a National Research Foundation of Korea (NRF) grant, funded by the Korean government (RNF-2021R1A2C2003658), and a grant from the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (RS-2023-00265497).

Conflicts of interest

The authors have no financial conflicts of interest.

Author contribution

Conceptualization: JHB, JHH. Study design: JHB, JHH. Methodology: JHB, IK, JHH. Data collection: all authors. Investigation: all authors. Statistical analysis: JHB. Writing—original draft: JHB. Writing—review & editing: JHB, JHH. Funding acquisition: YDK, JHH. Approval of final manuscript: all authors.

References

1. Aroor SR, Asif KS, Potter-Vig J, Sharma A, Menon BK, Inoa V, et al. Mechanical thrombectomy access for all? Challenges in increasing endovascular treatment for acute ischemic stroke in the United States. *J Stroke* 2022;24:41-48.
2. Ye G, Qi P, Chen K, Tan T, Cao R, Chen J, et al. Risk of secondary embolism events during mechanical thrombectomy for acute ischemic stroke: a single-center study based on histological analysis. *Clin Neurol Neurosurg* 2020;193:105749.
3. Kaesmacher J, Boeckh-Behrens T, Simon S, Maegerlein C, Kleine JF, Zimmer C, et al. Risk of thrombus fragmentation during endovascular stroke treatment. *AJNR Am J Neuroradiol* 2017;38:991-998.
4. Sporns PB, Hanning U, Schwindt W, Velasco A, Buerke B, Cnyrim C, et al. Ischemic stroke: histological thrombus composition and pre-interventional CT attenuation are associated with intervention time and rate of secondary embolism. *Cerebrovasc Dis* 2017;44:344-350.
5. Sporns PB, Jeibmann A, Minnerup J, Broocks G, Nawabi J, Schön G, et al. Histological clot composition is associated with preinterventional clot migration in acute stroke patients. *Stroke* 2019;50:2065-2071.
6. Yoo J, Kwon I, Kim S, Kim HM, Kim YD, Nam HS, et al. Coagulation factor expression and composition of arterial thrombi in cancer-associated stroke. *Stroke* 2023;54:2981-2989.
7. Heo J, Seog Y, Lee H, Lee IH, Kim S, Baek JH, et al. Automated composition analysis of thrombus from endovascular treatment in acute ischemic stroke using computer vision. *J Stroke* 2022;24:433-435.
8. Boodt N, Snouckaert van Schauburg PRW, Hund HM, Fereidoonnezhad B, McGarry JP, Akyildiz AC, et al. Mechanical characterization of thrombi retrieved with endovascular thrombectomy in patients with acute ischemic stroke. *Stroke* 2021;52:2510-2517.
9. Fereidoonnezhad B, Dwivedi A, Johnson S, McCarthy R, McGarry P. Blood clot fracture properties are dependent on red blood cell and fibrin content. *Acta Biomater* 2021;127:213-228.
10. Goebel J, Gaida BJ, Wanke I, Kleinschnitz C, Koehrmann M, Forsting M, et al. Is histologic thrombus composition in acute stroke linked to stroke etiology or to interventional parameters? *AJNR Am J Neuroradiol* 2020;41:650-657.

Correspondence: Ji Hoe Heo
Department of Neurology, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemoon-gu, Seoul 03722, Korea
Tel: +82-2-2228-1605
E-mail: jhheo@yuhs.ac
https://orcid.org/0000-0001-9898-3321

Received: February 22, 2024
Revised: April 29, 2024
Accepted: June 10, 2024

Supplementary Methods

Endovascular thrombectomy procedure

Typically, a stent retriever was used as the frontline endovascular modality. An 8- or 9-F balloon guide catheter (BGC) was routinely used; a distal access catheter was reserved for severely tortuous arteries. A stent retriever was delivered and deployed over the thrombus via a 0.021- or 0.027-inch microcatheter. The stent retriever was deployed a few minutes prior to retrieval. For retrieval, the balloon of the BGC was inflated; 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 and challenging cases that did not respond to the standard approaches. This thrombectomy procedure was repeated until modified Thrombolysis In Cerebral Infarction 2b or 3 was achieved. Decisions to cease attempts or to transition to an alternative endovascular technique were made at the discretion of the operating physician.

Thrombus collection and immunohistochemical staining

Retrieved thrombi were immediately fixed in 4% paraformaldehyde, sent to a laboratory, embedded in paraffin, and stored until use. The 4-μm-thick sections were treated with xylene and passed through an ethanol gradient. 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; non-specific binding was blocked using a mixture of 1% horse serum and 5% non-fat milk in Tris-buffered saline for 20 minutes. The thrombi were reacted with primary antibodies against erythrocytes, platelets, fibrin, lymphocytes, neutrophils, monocytes, tissue factors, and neutrophil extracellular traps. The sections were incubated at 37°C for 2 hours for monocytes or overnight at 4°C for the 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) for the others. After secondary antibody reaction with an avidin/biotin/horseradish peroxidase complex, the color of the positive signals was developed by incubating the slides in 3,3′-diaminobenzidine solution (D5637; Sigma-Aldrich, Burlington, MA, USA). The slides were counterstained with hematoxylin and subsequently mounted using Permount Mounting Medium (Fisher Scientific, Waltham, MA, USA).

Imaging analysis of thrombi

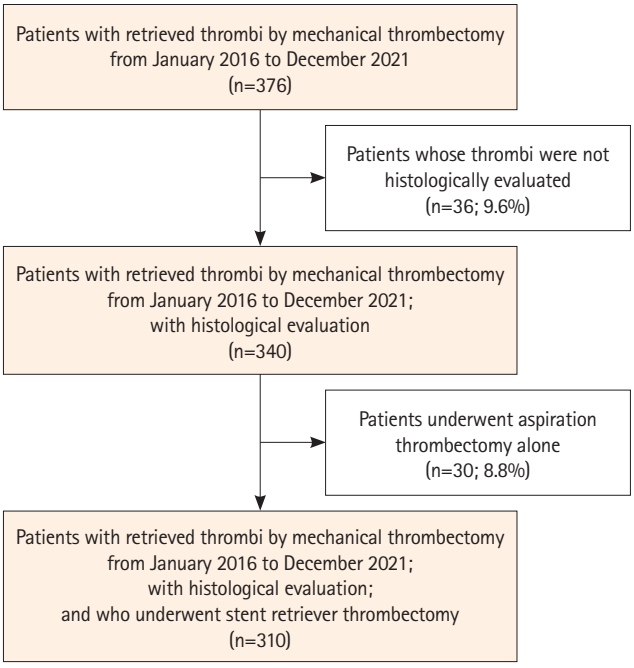
Images of the stained thrombi were acquired using a whole-slide scanner (Leica Biosystems, Richmond, IL, USA) or Stereo Investigator Imaging system (MBF Bioscience, Williston, VT, USA) equipped with a light microscope (Axio Imager D2; Carl Zeiss Co., Ltd., Jena, Germany). The whole-slide scanner captured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

The acquired images were analyzed using an Automated Region-of-interest-based Image Analysis (ARIA) software program designed for automated composition analysis. ARIA streamlines all traditional processes necessary for the imaging analysis of immunohistochemistry, allowing for rapid and less operator-dependent analysis. Briefly, the 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 the quantitative analysis, each thrombus composition fraction (%) was determined by calculating the pixel density as a percentage of the total thrombus area.

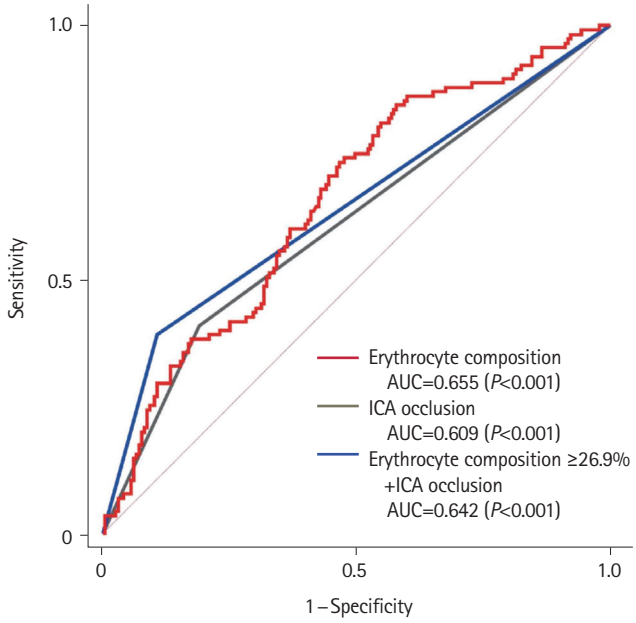
Supplementary Table 1. Primary antibodies for histological components of thrombus

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

*Abcam, Cambridge, UK; Fisher Scientific, Waltham, MA, USA.



Supplementary Figure 1. Patient selection flowchart.



Supplementary Figure 2. Receiver operating characteristic curves of erythrocyte composition in the thrombus and internal carotid artery (ICA) occlusion to predict a fragile thrombus. The erythrocyte composition in the thrombus could predict a fragile thrombus (area under the receiver operating characteristic curve [AUC], 0.655; cutoff, 26.9%; sensitivity, 84.4%; specificity, 42.1%; *P*<0.001). ICA occlusion could also predict fragile thrombi (AUC, 0.609; sensitivity, 40.9%; specificity, 81.0%; *P*<0.001). Considering both erythrocyte composition and ICA occlusion, it could predict fragile thrombi (AUC, 0.642; sensitivity, 39.1%; specificity, 89.2%; *P*<0.001).

https://doi.org/10.5853/jos.2024.00787