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Outcome of patients with nonbacterial thrombotic endocarditis and thrombus composition of patients with cancer-associated stroke

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Outcome of patients with nonbacterial thrombotic endocarditis and thrombus composition of patients with cancer-associated stroke

Directed by Professor Ji Hoe Heo

The Doctoral Dissertation
submitted to the Department of Medicine,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy in Medical Science

Joonsang Yoo

December 2023

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신경과학교실 / 유★상 / 2023-12-22 17:28:18

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ABSTRACT

Outcome of patients with nonbacterial thrombotic endocarditis and thrombus composition of patients with cancer-associated stroke

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The Graduate School, Yonsei University

(Directed by Professor Ji Hoe Heo)

Background

Cancer is associated with an increased risk of stroke. However, the outcomes and exact mechanism in cancer-associated stroke remain unclear. Additionally, the composition of thrombi can reflect the mechanism of thrombosis, which may guide the treatment strategies. Here we investigated the association of stroke etiology and outcome, and composition and expression of coagulation factors in the thrombi of patients with cancer-associated stroke.

Materials and Methods

This study was performed retrospectively using a hospital-based prospective cohort of patients with ischemic stroke. This study included consecutive stroke patients with active cancer who underwent echocardiography to assess etiology of stroke and outcomes. We compared clinical characteristics, the presence of metastasis, and clinical outcome among patients with nonbacterial thrombotic endocarditis (NBTE), those with cryptogenic etiologies, and those with determined etiologies. Furthermore, thrombi were analyzed in stroke patients who underwent endovascular thrombectomy and whose cerebral thrombi were obtained. For the thrombus analysis. patients were divided into two groups: those with cancer-associated stroke (cancer group) and matched stroke patients without cancer (control group). Immunohistochemistry was performed on the thrombi, and the composition and expression of coagulation factors were compared between the groups.

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Results

Among 245 patients, 20 had NBTE, 96 had cryptogenic etiologies, and 129 had determined etiologies. Metastasis was observed in all 20 patients with NBTE, 69.8% of patients with cryptogenic etiology, and 48.8% of patients with determined etiology. Patients with NBTE exhibited significantly higher rates of mortality and stroke than those with cryptogenic or determined etiologies. The presence of NBTE was independently associated with composite outcomes of mortality and stroke events. For the thrombus analysis, the study included 23 cancer patients and 23 matched controls. The cancer group had a significantly higher platelet composition than the control group (median [interquartile range], 51.3% [28.0–61.4] vs. 9.5% [4.8–14.0], $p<0.001$). Among coagulation factors, thrombin (26.2% [16.2–52.7] vs. 4.5% [1.3–7.2], $p<0.001$) and tissue factors were higher (0.60% [0.34–2.06] vs. 0.37% [0.22–0.60], $p=0.024$), and factor X was lower in the cancer group (1.25% [0.39–3.60] vs. 2.33% [1.67–4.48], $p=0.034$). There was a positive correlation between thrombin and platelets in the cancer group (r=0.666, $p=0.001$), but not in the control group (r=-0.167, $p=0.627$).

Conclusion

In patients with metastatic cancer, NBTE should be considered a potential cause of stroke. Patients with NBTE have a high risk of recurrent stroke and mortality. Cerebral thrombi in patients with cancer-associated stroke had higher proportions of platelets, thrombin, and tissue factors, suggesting their pivotal roles in arterial thrombosis in cancer and providing a therapeutic perspective for stroke prevention in these patients.

Key words : stroke, cancer, thrombus, platelets, thrombin, tissue factor

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Outcome of patients with nonbacterial thrombotic endocarditis and thrombus composition of patients with cancer-associated stroke

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I. INTRODUCTION

Cancer and cerebrovascular diseases are major causes of death and disability worldwide. As cancer treatments improve and survival times increase, the incidence of cerebral infarction in cancer patients is gradually increasing.[1,2] In addition, stroke patients with systemic malignancy have a poorer prognosis,[3] and they have a substantial short-term risk of recurrent stroke.[4]

Systemic malignancy is intrinsically associated with increased risk of ischemic stroke,[5-10] through several thrombotic mechanisms including nonbacterial thrombotic endocarditis (NBTE), paradoxical embolism of venous thrombi through arteriovenous shunt, and diffuse arterial thrombosis from disseminated intravascular coagulation.[2] NBTE is characterized by noninfectious vegetation on the cardiac valves.[11] NBTE is a cause of stroke and most frequently develops in patients with cancer.[2,12] Previous case reports and post-mortem studies have shown the frequent occurrence of metastasis in patients with NBTE.[13] These findings suggest a potential association between NBTE and metastasis. Moreover, given that stroke patients with metastasis have worse outcomes than those without metastasis, it can be inferred that patients with NBTE may also experience poor outcomes. However, information on the presence of metastasis is insufficient in many cases,[14-17] and the outcomes of stroke patients with NBTE and the association between NBTE and metastasis

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remains uncertain.

Meanwhile, since the successful introduction of endovascular treatment (EVT) in patients with acute ischemic stroke, fresh arterial thrombi can be obtained.[18-21] The analysis of thrombi may provide insight into the mechanism of thrombosis, thereby aiding the determination of the treatment strategy.[19,22-25] Histological analysis of thrombi from patients with cancer-associated stroke revealed a high proportion of platelets with few red blood cells, especially in patients with NBTE.[20,26,27] These findings suggest that tumor cells activate platelets, playing a crucial role in the arterial thrombosis of patients with cancer.[28,29] In addition, tumor cells can induce hypercoagulability by generating and activating coagulation factors. However, the expression of coagulating factors in the arterial thrombi of patients with cancer-associated stroke is not well known.

We hypothesized that development of NBTE is associated with metastasis and, subsequently, leads to an increased risk of recurrent stroke and mortality. In addition, we hypothesized that the composition and coagulation factor expression of thrombi in patients with cancer would differ from those in patients without cancer. Therefore, we investigated the presence of metastasis, mortality, and stroke recurrence in stroke patients with active cancer diagnosed with NBTE. Then, we investigated the composition and coagulation factor expression of arterial thrombi that were obtained during EVT.

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II. MATERIALS AND METHODS

1. Study population

This study was performed retrospectively using a hospital-based prospective cohort of consecutively enrolled patients with ischemic stroke within 7 days of symptom onset (Yonsei Stroke Cohort; Clinicaltrials.gov NCT03510312).[30] Patients were routinely evaluated with brain imaging studies (magnetic resonance imaging and/or computed tomography), angiographic studies (magnetic resonance angiography, computed tomography angiography, or conventional angiography), cardiac evaluations (echocardiography, continuous electrocardiography monitoring at the stroke unit and/or 24 hour-Holter monitoring, and cardiac computed tomography), and standard blood tests. Blood samples for complete blood counts, prothrombin time, and activated partial thromboplastin time were collected upon the patient's arrival at the emergency department or before EVT in the case of in-hospital stroke. Fibrinogen and D-dimer levels were measured within one day after EVT. Active cancer was defined as any cancer that was newly diagnosed within 6 months of the stroke event, required chemotherapy or surgical treatment within 6 months prior to the stroke event, or was recurrent, metastatic, or inoperable.[31]

To analyze the association between the presence of metastasis, outcome, and NBTE, consecutive patients with active cancer who were registered between January 2010 and December 2018 and who underwent echocardiography were included. Patients with brain malignancy or hematologic malignancy were excluded. Study participants were categorized into those with NBTE, those with cryptogenic etiology, and those with determined etiology.[11] NBTE was identified from formal reports by cardiologists who diagnosed vegetation through echocardiographic examinations, with no evidence of infective endocarditis. Patients with NBTE and co-existing atrial fibrillation were classified into the NBTE group. Patients with a patent foramen ovale (PFO) without venous thrombosis and other determinable etiologies were classified into cryptogenic etiology

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group.

For the histologic analysis of thrombi, we included patients who underwent EVT between September 2014 and June 2020 and from whom thrombi were extracted in the Yonsei Stroke Cohort. Patients were excluded if evaluation was incomplete, including cases lacking continuous electrocardiography monitoring and echocardiography. This study included patients with cancer-associated stroke (cancer group) and those without a history of cancer (control group). The cancer group was defined as stroke patients who had active cancer and no other identified etiology. These patients did not exhibit significant (>50%) stenosis of the relevant artery, high-risk cardioembolic sources, or other rare causes of stroke. However, patients with cancer-associated NBTE were included in the cancer group because they are very closely related to stroke, even if they had another cause.[13] The control group included patients without a history of cancer who were selected by propensity score matching.

The cohort and this study were approved by the Institutional Review Board of the Yonsei University Health System and Yongin Severance Hospital (IRB no. 2021-0140-001). Due to the retrospective nature of the study, informed consent for cohort study was waived. However, for the histologic analysis study, informed consent was obtained from patients or their caregivers to use the thrombi for research.

2. Clinical parameters and outcome measures

From the cohort, we collected demographic data, including age, sex, risk factors, and comorbid diseases. These diseases encompassed hypertension, diabetes, dyslipidemia, atrial fibrillation, PFO, NBTE, venous thrombosis (both pulmonary embolism and deep vein thrombosis), and the use of tissue plasminogen activator before EVT. We investigated the cancer type and presence of metastasis. Data on 6-month mortality and recurrence of cerebral infarction were also collected. After discharge, all patients were regularly followed

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up by neurologists and clinical research assistants in the outpatient clinic through face-to-face or telephonic interviews, using a structured questionnaire.[32] Medical records were reviewed to gather information on mortality and the cause of death. Causes of death were categorized as either stroke-related, cancer-related, or others/unknown.[33] We also extracted information from medical records regarding any new stroke events, such as cerebral infarction or intracerebral hemorrhage, following the initial cerebral infarction.

3. Immunohistochemistry of thrombus

Retrieved thrombi were immediately fixed in 4% paraformaldehyde, embedded in paraffin, and stored for subsequent use.[34] The 4-μm-thick sections were treated with xylene and passed through an ethanol gradient. All sections underwent heat-induced epitope retrieval, except those for erythrocytes and fibrin. We used IHC-Tek epitope retrieval solution and a steamer (ICH World, Inc., Woodstock, MD, USA) for platelets, monocytes, neutrophil extracellular traps, thrombin, and factor XIIIa. For neutrophils, factor X, factor XII, tissue factor, and factor XIa, a 0.01 M sodium citrate buffer (pH 6.0) was used. Subsequently, sections were immersed in a 10 mM glycine solution in phosphate buffered saline. Nonspecific bindings were blocked using a 1% horse serum and 5% nonfat milk mixture in Tris-buffered saline for 20 minutes. Thrombi were then exposed to primary antibodies targeting erythrocytes, platelets, neutrophils, monocytes, neutrophil extracellular traps, fibrin, thrombin, tissue factors, and coagulation factors X, XIa, XII, and XIIIa (Table 1). For monocyte and thrombin, sections were incubated at 37°C for 2 hours, while others were incubated overnight at 4°C. This was followed by a 30-minute secondary antibody reaction at 37°C. For monocytes and thrombin, 1:200-diluted biotin-conjugated Horse Anti-Mouse IgG antibody (BA-2000, Vector Laboratories, Peterborough, UK) was used and for the others, a biotin-conjugated Goat Anti-Rabbit IgG antibody (BA-1000, Vector Laboratories) was used. Positive signals were visualized using a 3,3′-diaminobenzidine (D5637; Sigma-Aldrich Inc., St. Louis, MO, USA) solution. After counterstaining with hematoxylin, sections were mounted using Permount Mounting Medium (Fisher Scientific, Fair Lawn,

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NJ, USA). Digital scans of the stained thrombi were obtained using the Aperio AT2 scanner (Leica Biosystems, Wetzlar, Germany). Imaging analysis was semi-automated using the open-source software, Automated Region-of-interest-based Image Analysis.[35] Analysis was performed by investigators who were blinded to the patients' clinical data. Percentages represent the area fraction of pixels exceeding a predefined density threshold (equivalent to 160 in ImageJ [National Institutes of Health, Bethesda, MD, USA]) relative to the total thrombus area.

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Table 1. Details of the primary antibodies used for the immunohistochemical analyses

Antibody target	Antibody name (lot number)	Supplier	Heat retrieval	Dilution
Erythrocyte	Anti-glycophorin A (ab129024)	Abcam	None	1:400
Platelet	Anti-CD42b (ab134087)	Abcam	40 min	1:100
Neutrophil	Anti-neutrophil elastase (ab68672)	Abcam	40 min	1:200
Monocyte	Anti-CD68 (MA5-13324)	Invitrogen	40 min	1:200
Neutrophil extracellular trap	Anti-histone H3 (ab5103)	Abcam	40 min	1:100
Fibrin	Anti-fibrinogen (ab34269)	Abcam	None	1:200
Thrombin	Anti-thrombin (ab17199)	Abcam	40 min	1:400
Tissue factor	Anti-CD142 (PA5-27278)	Invitrogen	5 min × 3	1:100
Factor X	Anti-factor X/Xa (PA5-29118)	Invitrogen	40 min	1:400
Factor XIa	Anti-factor XIa (ab232726)	Abcam	5 min × 3	1:100
Factor XII	Anti-factor XII (ab196670)	Abcam	40 min	1:100
Factor XIIIa	Anti-factor XIII (PA5-22110)	Invitrogen	40 min	1:400

Heat-induced epitope retrieval was performed using IHC-Tek epitope retrieval solution and a steamer (ICH World, Inc., Woodstock, MD, USA) for platelets, monocytes, neutrophil extracellular trap, thrombin, and factor XIIIa; or using 0.01 M sodium citrate buffer (pH 6.0) for neutrophil, factor X, factor XII, tissue factor, and factor XIa.

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4. Statistical analysis

Variables were expressed as mean±standard deviation, median (interquartile ranges [IQR]), or number (percentage), as appropriate. For the outcome analysis, baseline characteristics among three groups were compared using one-way analysis of variance or the Kruskal–Wallis test for continuous variables and the Chi-square test or Fisher's exact test for categorical variables. Post-hoc analysis was performed using Bonferroni's correction or Dunn's test. Kaplan–Meier estimates and log-rank tests were performed (adjusted by Bonferroni's correction) for mortality, occurrence of stroke, and composite outcomes of mortality and stroke. To determine whether the presence of NBTE was associated with composite events of mortality and stroke, we performed multivariate Cox proportional hazard regression analyses. As all patients with NBTE had metastasis and 90% of patients with NBTE had multiple territory infarctions, we constructed two different multivariate regression models to avoid multicollinearity. Model 1 included the presence of NBTE and Model 2 included metastasis and multiple territory infarctions.

For the histology analysis, a propensity score was used to establish a control group, we used the "MatchIt" R package to match the cancer group with patients without a history of cancer based on age and sex at a 1:1 ratio. Owing to the small number of patients included in each group, a non-parametric statistical analysis was performed. The Wilcoxon rank-sum test was used to examine continuous variables, while the chi-squared or Fisher's exact test was used to examine categorical variables. All statistical analyses were performed using the R statistical software (version 4.2.0; R Foundation for Statistical Computing, Vienna, Austria; http://www.R-project.org/). A p-value of <0.05 was considered statistically significant.

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III. RESULTS

1. Characteristics of the included patients in the outcome analysis

In the outcome analysis, 289 patients with acute ischemic stroke and active cancer were registered to the cohort. Of these, 245 patients (84.8%) underwent echocardiography and were included in this study (123 patients underwent both TEE and TTE, 25 patients underwent TEE only, and 97 patients underwent TTE only). The mean age of the included patients was 68.7±10.7 years, and 136 patients (55.5%) were men. Compared to patients with echocardiography, those without were younger (64.1±10.5 years vs. 68.7±10.7 years, p=0.010), had fewer atrial fibrillation (4.6% vs. 20.8%, p=0.018) and showed higher National Institutes of Health stroke scale (NIHSS) scores (6.5 [3–15] vs. 5 [2–11], p=0.041). Cancer types were not different between patients who underwent echocardiography and those who did not (Table 2).

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Table 2. Comparison between patients with and without transesophageal echocardiography

	Without transesophageal echocardiography (n=44)	With transesophageal echocardiography (n=245)	*p* value
Demographics			
Age, year	64.1±10.5	68.7±10.7	0.010
Sex, male	25 (56.8)	136 (55.5)	>0.999
Risk factors			
Hypertension	25 (56.8)	159 (64.9)	0.392
Diabetes mellitus	14 (31.8)	77 (31.4)	>0.999
Dyslipidemia	7 (15.9)	46 (18.8)	0.810
Atrial fibrillation	2 (4.6)	51 (20.8)	0.018
Current smoker	7 (15.9)	46 (18.8)	0.810
Body mass index (kg/m^2)	21.8±3.2	22.6±3.5	0.162
Metastatic cancer	34 (77.3)	150 (61.2)	0.062
Cancer type			0.556
Colorectal	4 (9.1)	32 (13.1)	
Gastric/esophageal	6 (13.6)	44 (18.0)	
Hepatobiliary	4 (9.1)	38 (15.5)	
Pancreas	8 (18.2)	22 (9.0)	
Lung	11 (25.0)	46 (18.8)	
Bladder/urinary tract	2 (4.5)	11 (4.5)	
Female genital organ	1 (2.3)	12 (4.9)	
Others	8 (18.2)	40 (16.3)	
Initial NIHSS score	6.5 (3–15)	5 (2–11)	0.041
Reperfusion therapy	8 (18.2)	46 (18.9)	>0.999
Multiple vascular territory	20 (45.5)	127 (51.8)	0.538

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involvement			
Laboratory findings			
Hemoglobin, g/dL	11.1±1.8	11.3±2.4	0.557
White blood cells, $\times 10^9$/L	9.1±5.0	8.2±4.2	0.285
Platelets, $\times 10^9$/L	219±120	214±117	0.806
D-dimer, mg/L	5.2±8.0	3.5±6.5	0.201
Fibrinogen, g/L	3.3±1.4	3.3±1.4	0.870

Values are presented as n (%) or mean±standard deviation.

NIHSS, National Institutes of Health Stroke Scale

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2. Comparison between the stroke etiologies in the outcome analysis

Of the 245 patients, 20 (8.2%) had NBTE, 96 (39.2%) had a cryptogenic etiology, and 129 (52.7%) had determined etiologies. In patients with determined etiologies, 51 (39.5%) had cardioembolism, 46 (35.7%) had large artery atherosclerosis, and 19 (14.7%) had two or more causes (Table 3). Of 150 patients (61.2%) with metastasis, 20 (13.3%) were diagnosed with NBTE. Compared with patients with determined etiologies, those with NBTE were younger, had more frequent multiple vascular territory involvement, lower platelet counts, lower fibrinogen levels, and higher D-dimer levels (Table 4).

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Table 3. Etiologic mechanisms of stroke in 129 patients with a determined etiology

Mechanism	Etiology
Cardioembolism	51
Atrial fibrillation	31
Patent foramen ovale (with venous thrombosis)	7
Akinetic left ventricular segment	3
Hypokinetic left ventricular segment	3
Valvular heart disease (Prosthetic valve replacement)	2
Cardiac thrombus	2
Atrial septal aneurysm	1
Status of inserted pacemaker (Sick sinus syndrome)	1
Infective endocarditis	1
Large artery atherosclerosis	46
Small artery disease	10
Other determined etiologies	3
Two or more causes	19
Large artery atherosclerosis + cardioembolism	14
Atrial fibrillation	11
Atrial flutter	1
Prosthetic valve replacement	1
Spontaneous echo contrast	1
Large artery atherosclerosis + small artery disease	3
Cardioembolism + small artery disease	2
Atrial fibrillation	2

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Table 4. Comparison of baseline characteristics according to stroke etiology

	NBTE (n=20)	Cryptogenic etiology (n=96)	Determined etiology (n=129)	*p* value
Demographics				
Age, year	61.5±8.7	68.1±10.1	70.3±10.9	0.002*,†
Sex, male	9 (45.0)	43 (44.8)	84 (65.1)	0.006‡
Risk factors				
Hypertension	10 (50.0)	57 (59.4)	92 (71.3)	0.062
Diabetes mellitus	5 (25.0)	25 (26.0)	47 (36.4)	0.204
Dyslipidemia	5 (25.0)	13 (13.5)	28 (21.7)	0.228
Atrial fibrillation	5 (25.0)	0 (0)	46 (35.7)	<0.001*,‡
Current smoker	1 (5.0)	12 (12.5)	17 (13.2)	0.581
Patent foramen ovale	3 (15.0)	21 (21.9)	26 (20.2)	0.782
Body mass index (kg/m^2)	22.3±2.9	22.8±3.5	22.5±3.6	0.676
Medications prior to admission				
Antiplatelets	3 (15.0)	19 (19.8)	39 (30.2)	0.129
Anticoagulants	3 (15.0)	4 (4.2)	15 (11.6)	0.066
Statin	7 (35.0)	18 (18.8)	37 (28.7)	0.138
Medications mainly used after admission				0.048‡
Antiplatelets	6 (30.0)	56 (58.3)	73 (56.6)	
Anticoagulants	12 (60.0)	31 (32.3)	49 (38.0)	
Both antiplatelets and anticoagulants	1 (5.0)	2 (2.1)	5 (3.9)	
No medication	1 (5.0)	7 (7.3)	2 (1.6)	
Initial NIHSS score	7 (3–12)	5 (2–10)	5 (1–12)	0.547

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Reperfusion therapy	6 (30.0)	12 (12.6)	28 (21.7)	0.095
Multiple territory involvement	18 (90.0)	62 (64.6)	47 (36.4)	<0.001†,‡
Laboratory findings				
Hemoglobin, g/dL	11.2±1.6	10.8±2.0	11.7±2.8	0.036‡
White blood cells, $\times 10^9$/L	10.4±5.1	7.8±3.9	8.1±4.2	0.041*
Platelets, $\times 10^9$/L	133±82	201±113	237±118	<0.001*,†
D-dimer, mg/L	7.4±6.5	4.8±9.0	1.9±2.8	<0.001†,‡
Fibrinogen, g/L	2.6±1.6	3.3±1.4	3.5±1.3	0.013†
Metastatic cancer	20 (100.0)	67 (69.8)	63 (48.8)	<0.001*,†,‡
Cancer type				0.118
Colorectal	2 (10.0)	11 (11.5)	19 (14.7)	
Gastric/esophageal	3 (15.0)	14 (14.6)	27 (20.9)	
Hepatobiliary	6 (30.0)	20 (20.8)	12 (9.3)	
Pancreas	4 (20.0)	9 (9.4)	9 (7.0)	
Lung	1 (5.0)	21 (21.9)	24 (18.6)	
Bladder/urinary tract	2 (10.0)	3 (3.1)	6 (4.7)	
Female genital organ	1 (5.0)	5 (5.2)	6 (4.7)	
Others	1 (5.0)	13 (13.5)	26 (20.2)	

Values are number (%) or mean±standard deviation.

NBTE, Nonbacterial thrombotic endocarditis; NIHSS, National Institutes of Health Stroke Scale.

*NBTE vs. Cryptogenic, $p<0.05$

†NBTE vs. Determined, $p<0.05$

‡Cryptogenic vs. Determined, $p<0.05$

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3. Metastasis, cancer type, and NBTE

All 20 patients diagnosed with NBTE had metastasis, while those without metastasis did not have NBTE. The presence of metastasis was significantly higher in patients with NBTE (100%) than in those with stroke with a cryptogenic etiology (69.8%; p=0.014 after Bonferroni's correction) or those with a determined etiology (48.8%; p<0.001 after Bonferroni's correction; Table 4). The cancer type was not significantly different among the three groups (Table 4).

4. Six-month mortality and stroke events

All patients were followed for 6 months after the initial stroke event. During follow-up, 127 patients (51.8%) either died or developed stroke events (110 deaths and 55 stroke events). The median survival for patients with NBTE was 44.5 days, and event-free survival of composite outcomes of mortality and stroke was 36 days. Ninety percent (18/20) of patients with NBTE died or developed stroke during the 6-month follow-up, which was significantly higher than that for those with a determined etiology (50/129, 38.8%, p<0.001) (Table 5). Compared to event-free patients, those with events (mortality or stroke) were younger and had lower body mass indexes and higher initial NIHSS scores. They also had lower hemoglobin levels, platelet counts, and fibrinogen levels; and higher white blood cell counts and D-dimer levels (Table 6). Metastasis and multiple vascular territory involvements were more common in patients with mortality or stroke events. Cancer type was also different between event-free patients and those with events (Table 6).

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Table 5. Causes of death and type of stroke event according to etiology

	NBTE (n=20)	Cryptogenic etiology (n=96)	Determined etiology (n=129)	*p* value
Composite outcomes of mortality and stroke	18 (90)	59 (61.5)	50 (38.8)	<0.001*,†,‡
Mortality	16 (80)	52 (54.2)	32 (32.6)	<0.001†,‡
Causes of death				0.307
Cancer-related	13 (81.2)	43 (82.7)	27 (64.3)	
Stroke-related	2 (12.5)	7 (13.5)	12 (28.6)	
Other/unknown cause	1 (6.3)	2 (3.8)	3 (7.1)	
Any stroke events	10 (50)	24 (25.0)	21 (16.3)	<0.003†
Stroke type				
Cerebral infarction	10 (100)	23 (95.8)	20 (95.2)	
Intracerebral hemorrhage	0 (0)	1 (4.2)	1 (4.8)	

Values are number (%).

NBTE, nonbacterial thrombotic endocarditis.

*NBTE vs. Cryptogenic, $p<0.05$

†NBTE vs. Determined, $p<0.05$

‡Cryptogenic vs. Determined, $p<0.05$

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Table 6. Comparison of characteristics between patients with and without events

	No events (n=118)	Events (n=127)	*p* value
Demographics			
Age, year	68.4±9.9	69.0±11.4	0.664
Sex, male	80 (67.8)	56 (44.1)	<0.001
Risk factors			
Hypertension	79 (66.9)	80 (63.0)	0.607
Diabetes mellitus	39 (33.1)	38 (29.9)	0.697
Dyslipidemia	27 (22.9)	19 (15.0)	0.155
Atrial fibrillation	26 (22.0)	25 (19.7)	0.768
Current smoker	20 (16.9)	10 (7.9)	0.049
Body mass index (kg/m^2)	23.2±3.2	22.0±3.8	0.010
Metastatic cancer	42 (35.6)	108 (85.0)	<0.001
Cancer type			0.020
Colorectal	19 (16.1)	13 (10.2)	
Gastric/esophageal	22 (18.6)	22 (17.3)	
Hepatobiliary	13 (11.0)	25 (19.7)	
Pancreas	5 (4.2)	17 (13.4)	
Lung	21 (17.8)	25 (19.7)	
Bladder/urinary tract	8 (6.8)	3 (2.4)	
Female genital organ	5 (4.2)	7 (5.5)	
Others	25 (21.2)	15 (11.8)	
Initial NIHSS score	3 (1–10)	6 (2–11.5)	0.007
Reperfusion therapy	25 (21.2)	21 (16.7)	0.460
Multiple vascular territory involvement	36 (30.5)	91 (71.7)	<0.001
Stroke etiology			<0.001
NBTE	2 (10.0)	18 (90.0)	

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Cryptogenic	37 (38.5)	59 (61.5)	
Determined	79 (61.2)	50 (38.8)	
Laboratory findings			
Hemoglobin, g/dL	11.6±2.1	11.0±2.7	0.033
White blood cells, $\times 10^9$/L	7.5±3.7	8.9±4.5	0.008
Platelets, $\times 10^9$/L	243±112	188±116	<0.001
D-dimer, mg/L	1.1±1.5	5.7±8.3	<0.001
Fibrinogen, g/L	3.6±1.2	3.2±1.5	0.021

Values are presented as n (%) or mean±standard deviation.

NIHSS, National Institutes of Health Stroke Scale; NBTE, nonbacterial thrombotic endocarditis.

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The Kaplan-Meier survival analysis revealed difference in 6-month mortality among the three groups (p<0.001). Patients with NBTE showed lower 6-month survival probability, stroke-free, and event-free probability compared to those with either cryptogenic etiology or determined etiologies (Figure 1). Mortality, stroke events, and composite outcomes were also more common in patients with a cryptogenic etiology than in those with a determined etiology (Figure 1).

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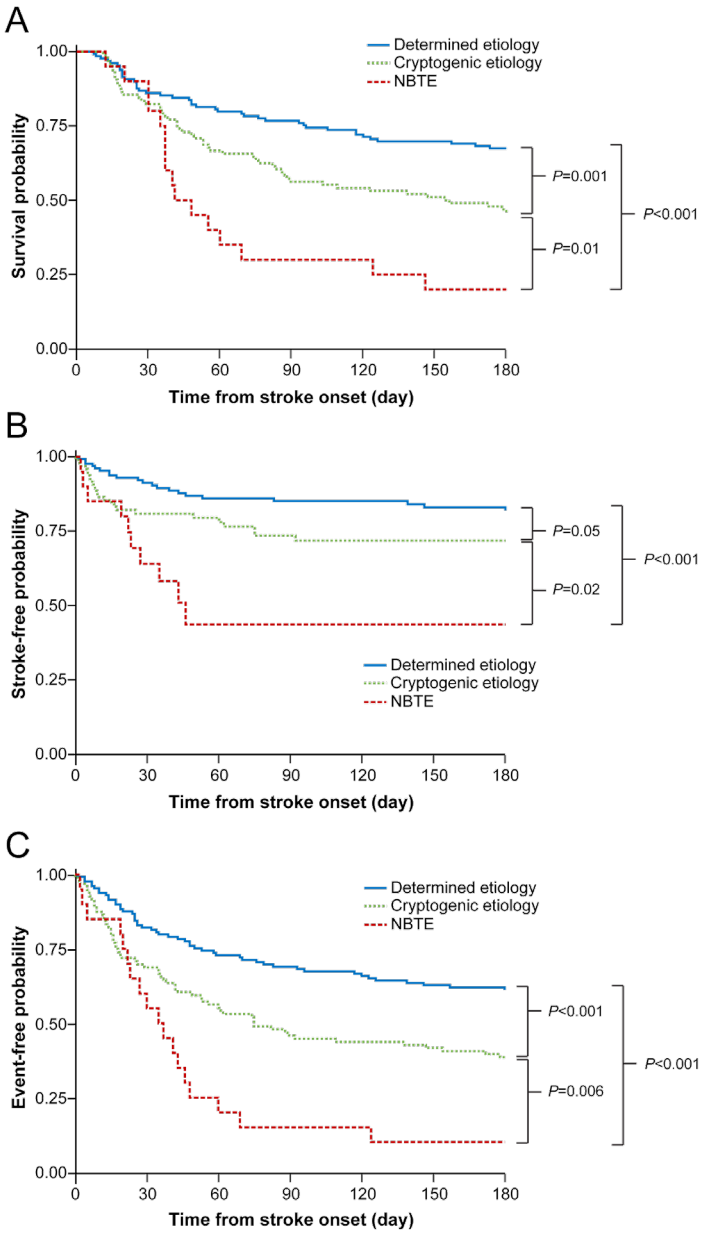

Figure 1. Kaplan-Meyer analysis of (A) mortality, (B) stroke events, and (C) composite outcomes of mortality and stroke events during the 6-month follow-up. Bonferroni's correction was performed for between-group comparisons. NBTE, nonbacterial thrombotic endocarditis

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5. Causes of death

Of the 110 patients (44.9%) who died, patients with NBTE were most common (16/20 [80.0%]), followed by those with a cryptogenic etiology (52/96 [54.2%]) and those with a determined etiology (32/129 [32.6%]) (p<0.001). Causes of death were cancer-related in 83 (75.5%), stroke-related in 21 (19.1%), and other/unknown in 6 (5.5%) patients. The cause of death did not significantly differ among the three groups (p=0.307; Table 5).

6. Stroke events

During the 6-month follow-up, stroke events occurred in 22.4% (55/245) of patients—53 (21.6%) with ischemic stroke and 2 (0.8%) with intracerebral hemorrhage (ICH). Stroke was diagnosed in 49 patients (89.1%) using diffusion-weighted imaging and in five patients (9.1%) using a CT scan. For one patient (1.8%) diagnosed in another hospital, the type of imaging used was uncertain. Stroke events occurred most frequently in patients with NBTE (10/20, 50%), followed by those with a cryptogenic etiology (24/96, 25.0%) and those with a determined etiology (21/129, 16.3%) (Table 5). Mortality was more frequent in patients with stroke events than in those without (69.1% [38/55] vs. 37.9% [72/190]; p<0.001).

7. Factors associated with mortality and stroke events

In the multivariate Cox proportional hazard analysis, the female sex and a lower body mass index were associated with composite outcomes of mortality and stroke in both Model 1 and in Model 2. The composite outcomes were independently associated with the presence of NBTE (hazard ratio [HR] 1.941, 95% confidence interval [CI] 1.052–3.690) (Table 7, Model 1). The presence of metastasis (HR 2.870, 95% CI 1.648–4.996) and the involvement of multiple vascular territories (HR 2.524, 95% CI 1.570–4.058) were also independently associated with the composite outcomes (Table 7, Model 2).

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Table 7. Cox models for 6-month composite outcomes of mortality/stroke events

	Multivariate analysis			
	Model 1		Model 2	
	Hazard ratio (95% CI)	*p* value	Hazard ratio (95% CI)	*p* value
Demographics				
Age, year	1.015 (0.996–1.034)	0.137	1.011 (0.992–1.030)	0.269
Sex, male	0.540 (0.347–0.851)	0.007	0.520 (0.333–0.810)	0.004
Risk factors				
Hypertension				
Diabetes mellitus				
Dyslipidemia	0.878 (0.519–1.463)	0.625	0.948 (0.561–1.601)	0.840
Atrial fibrillation				
Current smoker	0.938 (0.429–1.807)	0.862	0.856 (0.418–1.753)	0.670
Body mass index (kg/m^2)	0.926 (0.869–0.980)	0.012	0.929 (0.875–0.986)	0.015
Stroke etiology				
Determined	Ref			
Cryptogenic	1.447 (0.938–2.218)	0.090		
NBTE	1.941 (1.052–3.690)	0.038		
Medications mainly used after admission*				
Antiplatelets	Ref		Ref	
Anticoagulants	1.353 (0.915–1.999)	0.130	0.958 (0.644–1.427)	0.834
Presence of metastasis			2.870 (1.648–4.996)	<0.001
Cancer type				
Colorectal	0.694 (0.326–1.565)	0.362	0.796 (0.360–1.761)	0.573
Gastric/esophageal	1.415 (0.675–2.839)	0.342	1.399 (0.682–2.868)	0.359
Hepatobiliary	1.522 (0.745–2.997)	0.236	1.312 (0.649–2.656)	0.450
Pancreas	1.093 (0.513–2.460)	0.826	0.767 (0.339–1.734)	0.523
Lung	1.330 (0.670–2.568)	0.404	1.084 (0.550–2.133)	0.816

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Bladder/urinary tract	0.686 (0.201–2.788)	0.576	1.120 (0.293–4.280)	0.869
Female genital organ	1.180 (0.477–3.289)	0.738	1.115 (0.424–2.936)	0.825
Others	Ref		Ref	
Initial NIHSS score	1.023 (0.994–1.056)	0.140	1.028 (0.998–1.060)	0.071
Reperfusion therapy				
Multiple vascular territory involvement			2.524 (1.570–4.058)	<0.001
Laboratory findings				
Hemoglobin, g/dL	0.960 (0.864–1.046)	0.415	1.007 (0.920–1.102)	0.879
White blood cells, $\times 10^9$/L	1.095 (1.040–1.145)	<0.001	1.094 (1.040–1.151)	0.001
Platelets, $\times 10^9$/L	0.997 (0.995–0.999)	0.003	0.997 (0.996–0.999)	0.006
D-dimer, mg/L	1.053 (1.028–1.078)	<0.001	1.033 (1.006–1.062)	0.018
Fibrinogen, g/L	0.999 (0.998–1.001)	0.480	0.999 (0.997–1.001)	0.174

NBTE, nonbacterial thrombotic endocarditis; NIHSS, National Institutes of Health Stroke Scale.

* Excluded 8 patients with both antiplatelet and anticoagulant, 10 patients without antithrombotics.

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8. Included patients in the histology analysis

During the study period, thrombus samples were obtained from 320 patients undergoing EVT. Of these, 84 were either previously diagnosed with cancer or received a new diagnosis during hospitalization. After excluding nine patients who did not undergo echocardiography and 38 patients with inactive cancer, 37 patients with active cancer remained. From this group, 14 patients with other identified causes of stroke were further excluded, leaving 23 patients in the cancer group (Figure 2). Of the seven patients with NBTE included in the cancer group, three had atrial fibrillation. For the control group, 23 matched patients without a history of cancer were included in the analysis (Figure 2).

Among the 23 patients with cancer, lung cancer was the most common diagnosis (six patients), followed by colorectal cancer (four patients), gall bladder/bile duct cancer (three patients), gastric cancer, bladder and ureter cancer, hematologic malignancy (two patients each), and others (four patients). Metastasis was identified in all 21 patients with solid cancer, except for the two patients with hematologic malignancy. During the 6-month follow-up period, cerebral infarction recurred in nine patients (39.1%) and 15 patients died (65.2%) in the cancer group. In the control group, no patients had recurrent cerebral infarction and three patients died (13.0%).

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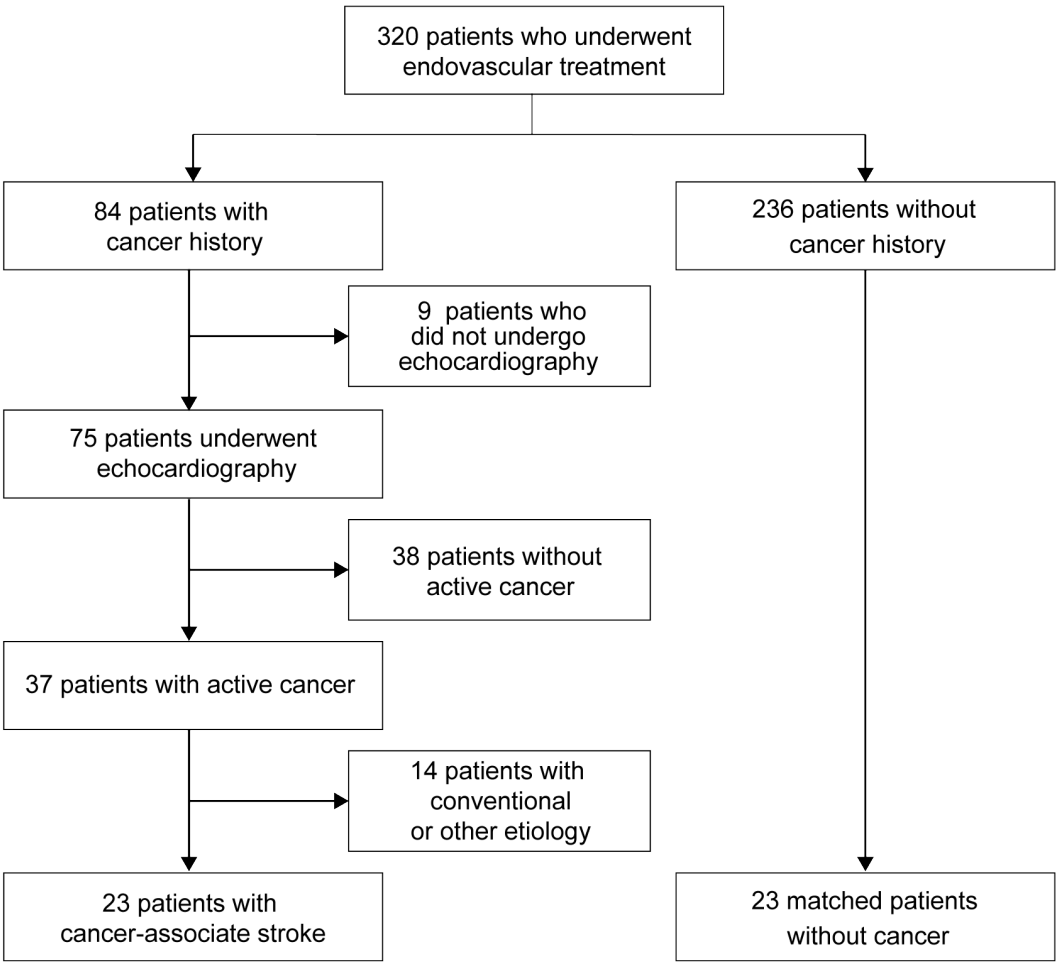

Figure 2. Flow diagram of the study population

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9. Clinical characteristics of patients in the histology study

The median age in both groups was 65 years, with males comprising 50% of each group. Atrial fibrillation was less prevalent in the cancer group than in the control group (13.0% vs. 56.5%, p=0.005). Venous thrombosis was more common in the cancer group (52.2% vs. 4.3%, p=0.001). There was no significant difference between the groups regarding the presence of PFO. The cancer group had a lower mean hemoglobin level (11.6 [IQR, 9.7–12.6] vs. 12.6 [IQR, 11.1–14.5] g/dL, p=0.024) and a lower mean platelet count (118 [IQR, 76–164] vs. 225 [IQR, 184–274] × 10^9/L, p=0.001) compared to the control group. The mean D-dimer level was significantly higher in the cancer group (3.3 [IQR, 2.6–10.4] vs. 0.8 [IQR, 0.4–2.3] mg/L, p<0.001). Intravenous thrombolysis was used less frequently in the cancer group compared to the control group (8.7% vs. 43.5%, p=0.017) (Table 8).

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Table 8. Baseline characteristics of patients by study group

	Cancer group (n=23)	Control group (n=23)	*p* value
Demographics			
Age, years	65.0 (55.5–70.0)	65.0 (54.5–70.0)	0.956
Sex, male	12 (50.0)	12 (50.0)	1.000
Clinical risk factors			
Hypertension	11 (47.8)	17 (73.9)	0.070
Diabetes mellitus	4 (17.4)	7 (30.4)	0.491
Dyslipidemia	5 (21.7)	4 (17.4)	0.770
Atrial fibrillation	3 (13.0)	13 (56.5)	0.005
Patent foramen ovale	3 (13.0)	3 (13.0)	1.000
Nonbacterial thrombotic endocarditis	7 (30.4)	0 (0)	0.009
Venous thrombosis	12 (52.2)	1 (4.3)	0.001
Laboratory findings			
Hemoglobin, g/dL	11.6 (9.7–12.6)	12.6 (11.1–14.5)	0.005
White blood cells, $\times 10^9$/L	9.0 (5.9–9.9)	6.8 (4.9–9.0)	0.247
Platelets, $\times 10^9$/L	118 (76–164)	225 (184–274)	0.001
Prothrombin time, INR	1.19 (1.03–1.33)	1.02 (0.95–1.15)	0.099
Activated PTT, sec	29.1 (26.4–31.7)	29.3 (27.5–31.4)	0.538
Fibrinogen, mg/dL	265 (162–314)	320 (262–362)	0.008
D-dimer, mg/L	3.3 (2.6–10.4)	0.8 (0.4–2.3)	<0.001
Intravenous thrombolysis	2 (8.7)	10 (43.5)	0.017
In-hospital stroke	3 (13.0)	4 (17.4)	0.793

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Antithrombotic use at admission	12 (52.2)	12 (52.2)	1.000
Oral anticoagulant	6 (25.0)	3 (12.5)	
Low-molecular weight heparin	2 (8.3)	0 (0)	
Antiplatelet	2 (8.3)	7 (29.2)	
Antiplatelet + low-molecular weight heparin	2 (8.3)	0 (0)	
Antiplatelet + oral anticoagulant	0 (0)	2 (8.3)	

The data are represented as numbers (%) or median (interquartile range) of percentages.
INR: international normalized ratio; PTT: partial thromboplastin time

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10. Thrombus composition in the cancer and control groups

The cancer group exhibited a significantly higher platelet composition compared to the control group (51.3% [IQR, 28.0–61.4%] vs. 9.5% [IQR, 4.8–14.0%], $p<0.001$) (Figure 2). Conversely, the cancer group had significantly lower proportions of erythrocytes (4.2% [IQR, 1.4–13.7%] vs. 34.4% [IQR, 24.6–46.1%], $p<0.001$), neutrophils (1.2% [IQR, 0.6–3.3%] vs. 3.8% [IQR, 1.7–6.7%], $p=0.021$), monocytes (1.6% [IQR, 1.3–2.6%] vs. 3.4% [IQR, 2.0–4.8%], $p=0.009$), and neutrophil extracellular traps (1.3% [IQR, 0.8–1.9%] vs. 2.3% [IQR, 1.5–3.8%], $p=0.047$) (Table 9).

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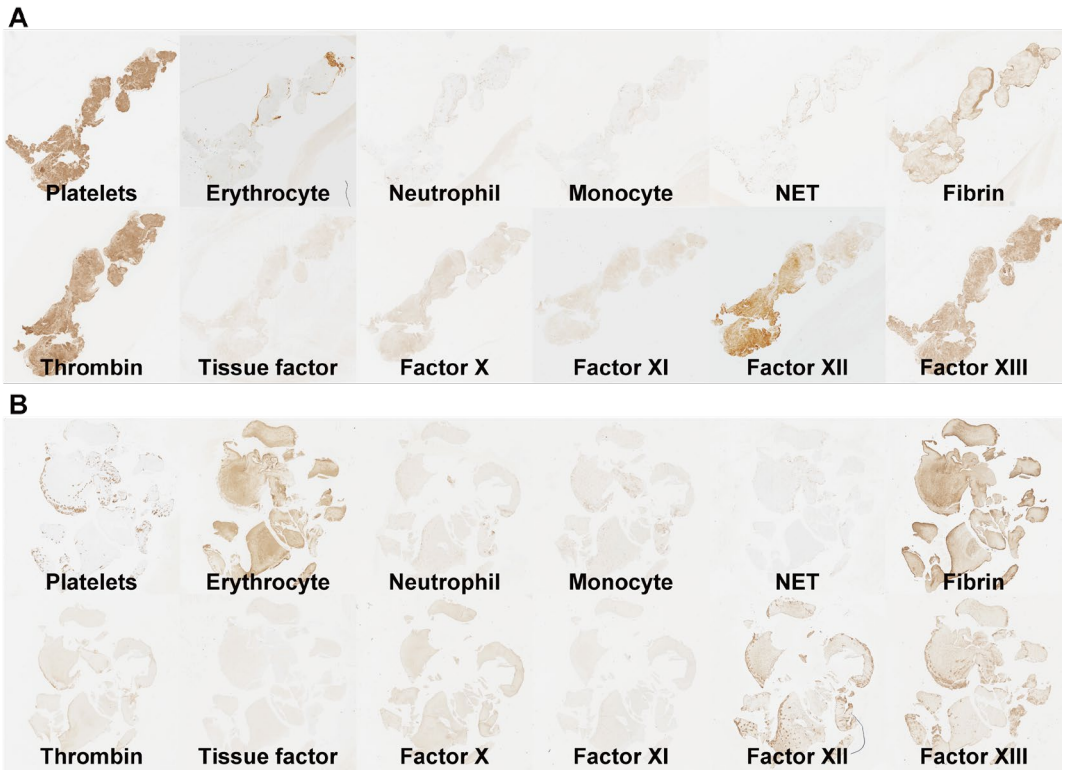

Figure 3. Representative images of immunohistochemistry staining of: (A) thrombi retrieved from the cancer group; and (B) thrombi retrieved from the control group. The primary antibodies for immunohistochemistry are described in Table 1. NET: neutrophil extracellular trap

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Table 9. Thrombus composition by study group

	Cancer group (n=23)	Control group (n=23)	*p* value
Platelet	39.2 (30.4–44.8)	11.7 (8.4–18.5)	<0.001
Erythrocyte	4.2 (1.4–13.7)	34.4 (24.6–46.1)	<0.001
Neutrophil	1.2 (0.6–3.3)	3.8 (1.7–6.7)	0.021
Monocyte	1.6 (1.3–2.6)	3.4 (2.0–4.8)	0.009
Neutrophil extracellular trap	1.3 (0.8–1.9)	2.3 (1.5–3.8)	0.047
Fibrin	17.9 (12.8–25.3)	33.5 (14.6–44.1)	0.079
Thrombin	26.2 (16.2–52.7)	4.5 (1.3–7.2)	<0.001
Tissue factor	0.60 (0.34–2.06)	0.37 (0.22–0.60)	0.024
Factor X	1.25 (0.39–3.60)	2.33 (1.67–4.48)	0.034
Factor XI	0.15 (0.08–0.51)	0.12 (0.05–0.22)	0.421
Factor XII	30.5 (19.2–45.7)	21.4 (14.7–32.5)	0.065
Factor XIII	29.7 (19.6–35.7)	26.3 (18.5–29.1)	0.157

The data are represented as numbers (%) or median (interquartile range) of percentages.

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11. Coagulation factors in thrombi

The cancer group showed significantly higher expression of thrombin (26.2% [IQR, 16.2–52.7%] vs. 4.5% [IQR, 1.3–7.2%], $p<0.001$) and tissue factor (0.60% [IQR, 0.34–2.06%] vs. 0.37% [IQR, 0.22–0.60%], $p=0.024$) than the control group (Figure 3). The expression of factor X was significantly lower in the cancer group (1.25% [IQR, 0.39–3.60%] vs. 2.33% [IQR, 1.67–4.48%], $p=0.034$). However, there were no significant intergroup differences in the expression of factors XI, XII, or XIII. We compared the correlation between thrombin levels and platelet composition and found a positive correlation in the cancer group (r=0.666, $p=0.001$) but not in the control group (r=-0.167, $p=0.627$) (Figure 4). There was also a negative correlation between thrombin and erythrocytes in the control group (r=-0.438, $p=0.036$), but not in the cancer group (r=-0.298, $p=0.167$). No significant correlation was observed in either group between the compositions of thrombin and fibrin.

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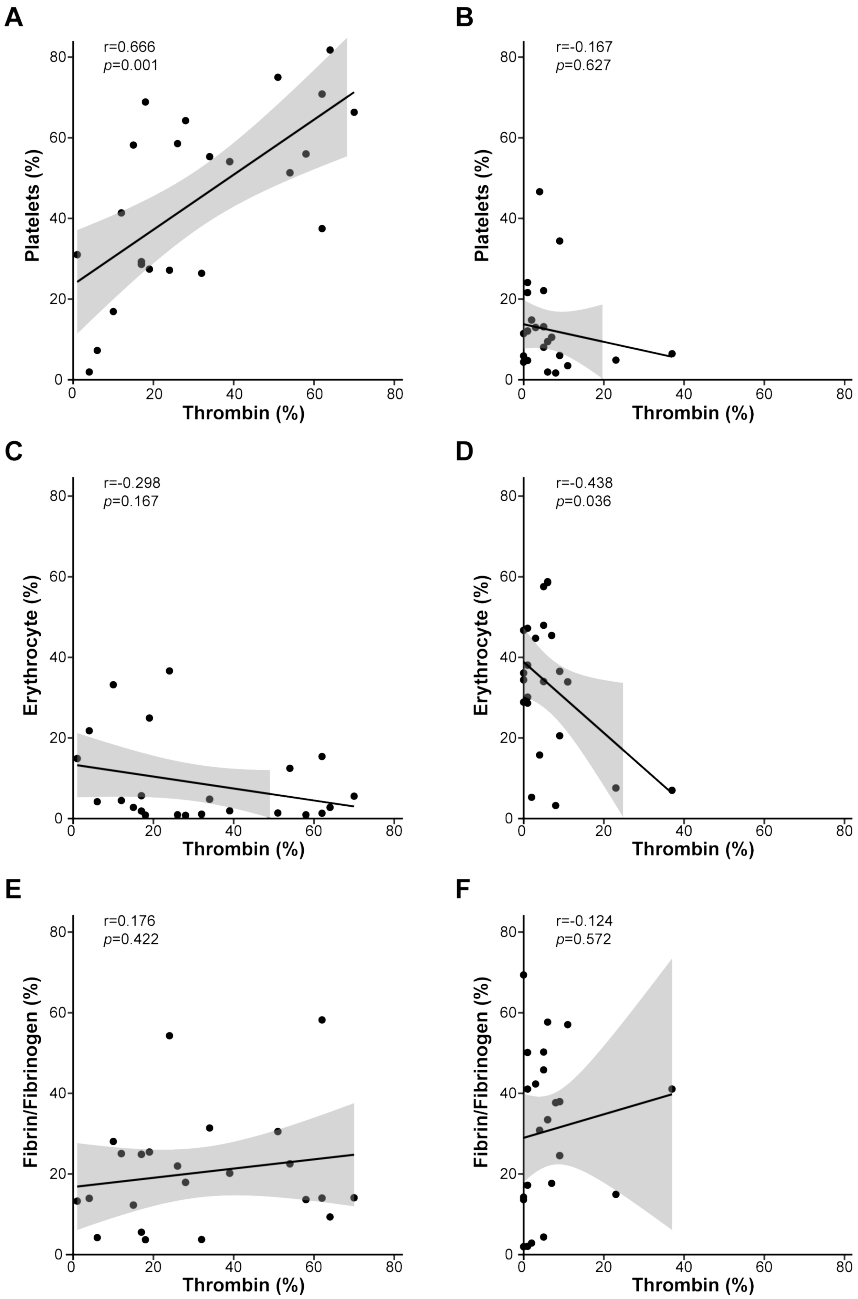

Figure 4. Correlation between thrombin and platelets (A and B), erythrocytes (C and D), and fibrin (E and F) in the thrombi of patients from the cancer group (A, C, and E) and the control group (B, D, and F).

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12. Sensitivity analysis

In a sensitivity analysis that excluded three patients with NBTE and co-existing atrial fibrillation from the cancer group, the proportions of platelets, thrombin, and tissue factor were found to be higher in the cancer group compared to the control group. However, the proportions of erythrocytes, neutrophils, monocytes, and neutrophil extracellular traps were lower in the cancer group (Table 10). Thrombin and platelet compositions also showed a positive correlation (r=0.662, p=0.001) (Figure 5). There was no difference in thrombus composition between patients using antithrombotic and those not using them in both groups (Table 11).

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Table 10. Thrombus composition by study group (after excluding patients with nonbacterial thrombotic endocarditis and co-existing atrial fibrillation)

	Cancer group (n=20)	Control group (n=23)	*p* value
Platelet	52.7 (28.0–61.4)	9.5 (4.8–14.0)	<0.001
Erythrocyte	4.3 (1.4–15.1)	34.4 (24.6–46.1)	<0.001
Neutrophil	1.2 (0.6–3.4)	3.8 (1.7–6.7)	0.032
Monocyte	1.6 (1.3–3.1)	3.4 (2.0–4.8)	0.025
Neutrophil extracellular trap	1.3 (0.8–1.7)	2.3 (1.5–3.8)	0.041
Fibrin	21.1 (13.4–26.7)	33.5 (14.6–44.1)	0.163
Thrombin	24.8 (13.5–52.7)	4.5 (1.3–7.2)	<0.001
Tissue factor	0.59 (0.34–2.06)	0.37 (0.22–0.60)	0.025
Factor X	1.53 (0.39–3.96)	2.33 (1.67–4.48)	0.101
Factor XI	0.14 (0.07–0.68)	0.12 (0.05–0.22)	0.507
Factor XII	33.6 (24.5–43.5)	29.4 (19.9–34.6)	0.073
Factor XIII	28.2 (12.1–37.3)	24.6 (15.0–31.1)	0.709

The data are represented as median (interquartile range) of percentages.

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Table 11. Comparison of thrombus composition by use of antithrombotics in both groups

	No antithrombotics (n=22)	Antithrombotics (n=24)	*p* value
Platelet	23.1 (11.5–41.4)	20.6 (6.2–58.4)	0.991
Erythrocyte	23.4 (5.7–34.4)	6.3 (2.3–36.3)	0.279
Neutrophil	3.0 (1.1–5.2)	1.7 (0.9–5.1)	0.609
Monocyte	2.7 (1.6–3.9)	1.9 (1.3–4.3)	0.164
Neutrophil extracellular trap	1.6 (1.0–3.7)	1.7 (0.9–3.0)	0.939
Fibrin	25.3 (14.9–42.3)	16.0 (10.8–32.1)	0.164
Thrombin	10.2 (3.5–22.8)	8.3 (3.0–30.1)	0.957
Tissue factor	0.62(0.29–1.6)	0.41 (0.22–0.61)	0.111
Factor X	2.9 (1.7–4.0)	1.4 (0.5–2.8)	0.127
Factor XI	0.13 (0.07–0.48)	0.15 (0.05–0.41)	0.870
Factor XII	33.6 (21.3–40.4)	29.9 (21.2–37.0)	0.535
Factor XIII	25.0 (13.7–40.1)	24.6 (16.4–33.4)	0.506

The data are represented as median (interquartile range) of percentages.

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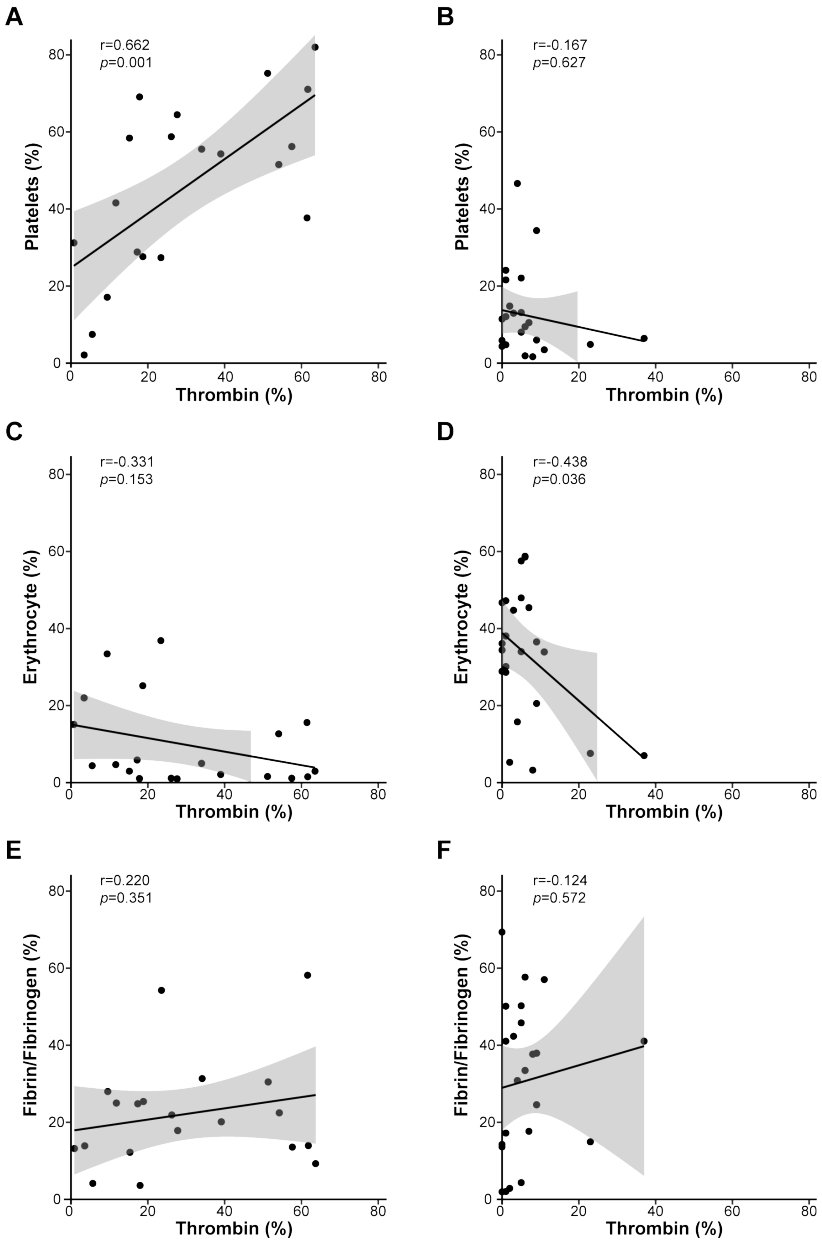

Figure 5. Correlation between thrombin and platelets (A and B), erythrocytes (C and D), and fibrin (E and F) in the thrombi of patients from the cancer group (A, C, and E) and the control group (B, D, and F), after excluding patients with nonbacterial thrombotic endocarditis and co-existing atrial fibrillation from the cancer group.

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IV. DISCUSSION

In this study, approximately one in 11 stroke patients with active cancer was diagnosed with NBTE. NBTE was also found exclusively in patients with metastasis. In addition, stroke patients with NBTE had a very high risk of mortality and stroke events during a 6-month follow-up. The thrombi obtained from the cancer group had higher platelet counts and lower erythrocyte proportions than those obtained from the control group. Among the coagulation factors, the expression levels of thrombin and tissue factors were higher in the cancer group. Thrombin expression correlated with the proportion of platelets in the cancer group, but this was not observed in the control group.

Patients with active cancer have a significant risk of recurrent strokes. In a retrospective study, ischemic stroke recurred in 16% of patients within 6 months after the initial stroke.[16] In the present study cohort, 21.6% experienced a recurrence of ischemic stroke, and 0.8% developed ICH. Particularly, 50% of patients with NBTE developed stroke within 6 months; significantly higher than those without NBTE. Ninety percent of patients with NBTE had lesions involving multiple vascular territories, which suggests multiple embolization from the heart. Patients with NBTE may be prone to a high risk of multiple thromboembolisms because the platelet-rich vegetation (thrombus) is attached to the fast-moving cardiac valves.

This study highlighted significant differences in mortality rates across the three etiological categories of stroke patients with active cancer. During the 6 months of follow-up, 80% of patients with NBTE died. However, less than one-third of the patients with a determined etiology died. Patients with a cryptogenic etiology had an intermediate risk of 6-month mortality. The high mortality in patients with NBTE may be associated with metastasis since all patients with NBTE had it. In fact, 67.1% of stroke patients with metastatic cancer died within 6 months in my previous study,[20] but the 6-month mortality in patients with NBTE was even higher. In the present study, mortality was also higher in patients who

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developed recurrent stroke; therefore, frequent stroke reoccurrence in patients with NBTE might also increase the risk of death.

In this study, patients with NBTE exhibited elevated D-dimer levels and a more frequent involvement of multiple vascular territories compared to patients with a determined stroke etiology. These are known characteristics of cancer-related stroke.[2] Findings of this study suggest that some features of cancer-related stroke may be associated with NBTE.[36,37] Patients with a cryptogenic etiology showed intermediate characteristics between those with NBTE and those with a determined etiology. Although NBTE is often not diagnosed despite TEE being performed,[38] NBTE has been suggested as the likely cause of cryptogenic stroke in many patients with cancer.[37] Therefore, undiagnosed NBTE may have been present in some patients with a cryptogenic etiology.

The underlying pathomechanism of NBTE remains elusive. Autopsy studies of patients with NBTE have shown that cardiac vegetation is mainly composed of platelets,[39] and examinations of thrombi retrieved during mechanical thrombectomy in stroke patients with NBTE indicated very high platelet and low erythrocyte fractions.[19,26] These studies suggest that platelet-mediated mechanisms play a key role in the development of cancer-associated NBTE. The role of platelets in cancer, especially their critical involvement in tumor growth and metastasis, has been extensively studied.[40]

This study revealed that NBTE occurred solely in patients with metastasis. Platelets can contribute to tumor invasion and metastasis via several mechanisms. Platelets are essential for the survival of circulating tumor cells,[28] and these cells rapidly associate with platelets via their receptors and induce tumor cell-induced platelet aggregation (TCIPA).[41] This TCIPA is critical for tumor cell survival as platelets form a physical shield around tumor cells, protecting them from recognition and lysis by natural killer cells and shear-induced damage.[30,31] These findings suggest that the association of tumor cells with platelets during

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metastasis may somehow contribute to the development of platelet-rich vegetation in NBTE.

This study also showed that arterial thrombi in the cancer group were platelet-rich and erythrocyte-poor, consistent with the findings of previous studies.[26,27,42] Tumor cells induce platelet activation and aggregation to promote cancer progression and metastasis.[28,41,43,44] Tumor cells also enhance thrombocytosis via tumor-derived cytokines and platelet-activating factor 4.[45,46] Tumor cells and platelets form TCIPA, therefore platelets physically protect tumor cells from natural killer cells and shear force in the circulation, which promotes tumor cell survival and metastasis, and facilitates tumor arrest at the endothelium.[28] In fact, the long-term use of aspirin reduces the risk of various cancers, distant metastasis, and death by approximately 31–46%.[47-49] Thus, platelet activation and the resulting thrombosis are the outgrowth of tumor cells' behavior for their growth and survival in the circulation.

Tumor cells also activate the coagulation system.[29,50,51] In this study, we evaluated the expression of various coagulation factors in the extrinsic (tissue factor), intrinsic (factors XI, XII), and common pathways (factors X, thrombin, and XIII). We found a significant increase in the expression of thrombin and tissue factor in the thrombi of the cancer group. Thrombin is a potent agonist for platelet activation via protease-activated protease (PAR)-1, PAR-4, and glycoprotein Ib-IX.[52] Thrombin also converts fibrinogen into fibrin and amplify the coagulation cascade by activating coagulation factors V, VIII, and XIII.[53,54] Tumor cells directly generate thrombin and indirectly generate it by secreting tissue factor that initiates the extrinsic coagulation pathway.[55,56] Thrombin generation and the thrombin-antithrombin complex can serve as biomarkers reflecting hypercoagulability in cancer patients.[56] Tissue factor can be expressed directly by some tumor cells and transported by tumor-derived extracellular vesicles.[57-60] In the initial stage of metastasis, fibrin deposition and platelet recruitment by thrombin is crucial to the survival and adherence of tumor cells

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to the endothelium.[61] Thus, tumor-induced coagulation system activation further enhances platelet activation and thrombosis and contributes to tumor growth, metastasis, and invasion.

This study found a significant correlation between platelet composition and thrombin expression in thrombi in the cancer group, but not in the control group. These findings suggest a tumor cell–specific role for the interaction between thrombin and platelets. In this study, the thrombin expression level was much higher than that of tissue factor, and it was 5.8 times higher than that in patients without cancer. Thrombin is generated directly by tumor cells and indirectly by the coagulation pathway. Findings of this study suggest that the direct generation of thrombin by tumor cells may greatly contribute to an increase in thrombin. Thrombin is the most potent activator of platelets, enhancing the formation of tumor cell-platelet aggregates.[62] Reciprocally, the extracellular vesicles released from activated platelets contribute to further thrombin activation.[59] Tumor cells may play a central role in direct platelet activation, excessive thrombin generation, and reciprocal interaction between platelets and thrombin, resulting in the formation of platelet-rich thrombi in cancer-associated stroke.

Although anticoagulants are often preferred for preventing cancer-associated stroke, evidence of their benefits is limited. Furthermore, the best treatment regimen for stroke prevention among anticoagulated patients remains unknown.[63,64] The findings of this study, in conjunction with the existing knowledge on tumor-induced thrombosis, suggest that platelets and thrombin play key roles in thrombosis in cancer-associated stroke. Given that thrombi in cancer-associated stroke are platelet-rich and tumor cells induce platelet activation and aggregation, antiplatelets may be beneficial in preventing arterial thrombosis. Antiplatelets may also suppress tumor growth and metastasis.[65] In this study, the composition of factor X was lower in the cancer group. Factor X plays a crucial role in the common pathway of the coagulation cascade. The control group comprised patients with

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various stroke etiologies, and the activation of the coagulation pathway might have contributed to many cases within this group. The findings in this study suggest that factor X might have played a less prominent role in the cancer group compared to the control group.[66] Among anticoagulants, a direct thrombin inhibitor may be more helpful than other upstream anticoagulants because thrombin is generated not only via the coagulation pathway but also directly by tumor cells. However, this needs to be tested in clinical trials.

This study has several limitations. First, while patients were enrolled prospectively, the study was conducted retrospectively at a single center. In addition, as the study hospital has a dedicated cancer center, the frequency of cancer, particularly that of advanced cancer or metastasis, might be higher than that in other more general hospitals. Second, while a majority of patients (85%) with active cancer underwent echocardiography, only 60.4% were evaluated using TEE, the gold standard for diagnosis of vegetation.[38,67] Although TEE is one of the routine evaluations in stroke evaluation protocol, it was impractical to perform TEE in all patients (it is semi-invasive and requires patient cooperation and informed consent). Vegetation is often invisible on TTE, and even on using TEE, a small vegetation may not be detected. Therefore, the presence of NBTE in this study might have been underestimated. For the thrombus histology study, included patients had thrombotic occlusion of large cerebral arteries. Therefore, the small thrombi that occluded the distal cerebral arteries could not be examined. In addition, this histologic study excluded patients with cancer and coexisting etiologies such as atrial fibrillation because analyses of thrombi exclusively from cancer-associated stroke were necessary. Lastly, due to the limited sample size, we could not compare histological features across different cancer types. Further studies are necessary to investigate thrombus characteristics according to cancer type.

V. CONCLUSION

In patients with metastatic cancer, especially those without determinable etiologies, NBTE should be suspected as a potential mechanism of stroke. This study also demonstrated that

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patients with NBTE have a significantly high short-term risk of both recurrent stroke and mortality. Furthermore, cerebral thrombi in patients with cancer-associated stroke not only had a significantly higher proportion of platelets and thrombin but also exhibited a positive correlation between them. These findings suggest crucial and interactive roles of platelets and thrombin in arterial thrombosis in cancer. The findings of this study also provide a perspective for developing strategies to prevent stroke recurrence in patients with cancer-associated stroke. Further research is needed to determine the optimal preventive treatment for patients with cancer-associated stroke.

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ABSTRACT(IN KOREAN)

비세균성 혈전성 심내막염환자의 예후와 암과 관련된 뇌졸중 환자에게서 얻어진 혈전의 특성

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유준상

배경
암은 뇌졸중의 위험을 증가시킨다. 그러나 암과 관련된 뇌졸중의 결과와 정확한 메커니즘은 명확하지 않다. 또한, 혈전의 구성은 혈전 형성의 메커니즘을 반영할 수 있으며, 이는 치료 전략을 수립하는데 도움을 줄 수 있다. 본 학위논문에서 우리는 암과 관련된 뇌졸중 환자의 뇌졸중의 원인과 예후의 연관성에 대하여 조사하였으며, 암과 관련된 뇌졸중 환자에게서 얻어진 혈전의 구성과 응고인자의 조성에 대하여 조사하였다.

재료 및 방법
이 연구는 허혈성 뇌졸중 환자의 병원 기반 전망적 코호트를 사용하여 후향적으로 수행되었다. 이 연구에는 뇌졸중의 원인과 결과를 평가하기 위해 심초음파를 받은 활동성 암을 가진 뇌졸중 환자를 포함하였다. 우리는 비세균성 혈전성 심내막염(Nonbacterial thrombotic endocarditis; NBTE)을 가진 환자와 뇌졸중의 확실한 원인이 밝혀지지 않은 환자, 그리고 확실한 뇌졸중의 원인을 가진 환자 사이에서 임상적 특성, 전이의 존재, 그리고 임상 결과를 비교하였다. 혈전 분석은 혈전 제거술을 받은 뇌졸중 환자 중 혈전이 얻어진 환자들을 대상으로 하였다. 혈전 분석에서 환자는 암과 관련된 뇌졸중(암 그룹)을 가진 환자와 암이 없는 일치된 뇌졸중 환자(대조 그룹)로 나누었다. 혈전에 대하여 면역조직화학을 수행하였으며, 그룹 간에 혈전의 구성과 응고인자의 조성이 비교되었다.

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결과
245명의 환자 중 20명이 NBTE를 가지고 있었고, 96명은 뇌졸중의 확실한 원인이 밝혀지지 않은 환자였고, 129명은 확실한 뇌졸중의 원인을 가지고 있었다. 전이는 NBTE를 가진 모든 20명의 환자에서, 뇌졸중의 확실한 원인이 밝혀지지 않은 환자의 69.8%에서, 그리고 확실한 뇌졸중의 원인을 가진 환자의 48.8%에서 관찰되었다. 혈전 분석을 위한 연구에는 23명의 암 환자와 23명의 일치된 대조군이 포함되었다. 암 그룹은 대조 그룹보다 현저히 높은 혈소판 구성을 가지고 있었다(중앙값 [사분범위], 52.7% [28.6–64.2] vs. 9.5% [4.8–14.0], p<0.001). 응고 인자 중에서는, 암 그룹에서는 트롬빈(26.2% [16.2–52.7] vs. 4.5% [1.3–7.2], p<0.001), 그리고 조직 인자(0.60% [0.34–2.06] vs. 0.37% [0.22–0.60], p=0.024)가 높았으나, 응고인자 10은 암 그룹에서 더 낮았다(1.25% [0.39–3.60] vs. 2.33% [1.67–4.48], p=0.034). 암 그룹에서 트롬빈과 혈소판은 양의 상관관계를 가졌으나(r=0.666, p=0.001), 대조군에서는 그러하지 않았다(r=-0.167, p=0.627).

결론
전이성 암을 가진 환자에서는 NBTE가 뇌졸중의 잠재적 원인으로 고려되어야 할 것이다. NBTE를 가진 환자는 높은 재발 뇌졸중과 사망 위험이 있었다. 암과 관련된 뇌졸중 환자의 뇌 혈전은 혈소판, 트롬빈, 그리고 조직 인자의 비율이 높았으며, 이러한 요소들은 암에서 동맥 혈전 형성에서 중요한 역할을 하고, 암 환자에서 뇌졸중 예방을 위한 치료적 관점을 제시한다.

핵심되는 말 : 뇌졸중, 암, 혈전, 혈소판, 트롬빈, 조직 인자

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