Stroke

CLINICAL AND POPULATION SCIENCES

Coagulation Factor Expression and Composition of Arterial Thrombi in Cancer-Associated Stroke

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BACKGROUND: Cancer is associated with an increased risk of stroke. Tumor cells activate platelets, induce a coagulation cascade, and generate thrombin. The composition of thrombi may reflect the mechanism of thrombosis, aiding the determination of the treatment strategy. Here, we investigated the composition and expression of coagulation factors in the thrombi of patients with cancer-associated stroke.

METHODS: Patients with stroke who underwent endovascular thrombectomy between September 2014 and June 2020 and whose cerebral thrombi were obtained were divided into those with cancer-associated stroke (cancer group) and propensity score–matched patients without cancer (control group), using 1:1 matching based on age and sex. Immunohistochemistry was performed of the thrombi, and the composition and expression of coagulation factors were compared between groups.

RESULTS: Among the 320 patients who underwent endovascular thrombectomy and who had thrombi obtained, this study included 23 patients with cancer and 23 matched controls. In both groups, the median age was 65 years, and 12 patients (52.2%) were men. Platelet composition was significantly higher in the cancer group than in the control group (median [interquartile range], 51.3% [28.0%–61.4%] versus 9.5% [4.8%–14.0%]; $P<0.001$). Among coagulation factors, thrombin (26.2% [16.2%–52.7%] versus 4.5% [1.3%–7.2%]; $P<0.001$) and tissue factors (0.60% [0.34%–2.06%] versus 0.37% [0.22%–0.60%]; $P=0.024$) were higher and factor X was lower (1.25% [0.39%–3.60%] versus 2.33% [1.67%–4.48%]; $P=0.034$) in the cancer group. 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; $P=0.627$).

CONCLUSIONS: Cerebral thrombi in patients with cancer-associated stroke showed higher proportions of platelets, thrombin, and tissue factors, suggesting their key roles in arterial thrombosis in cancer and providing a therapeutic perspective for preventing stroke in patients with cancer-associated stroke.

GRAPHIC ABSTRACT: A graphic abstract is available for this article.

Key Words: blood platelets ■ neoplasms ■ stroke ■ thrombin ■ thrombosis

Cancer and cerebrovascular diseases are major causes of death and disability worldwide. As cancer treatments improve, survival time is improving as well; accordingly, the incidence of cerebral infarction in patients with cancer is gradually increasing.[1,2] Cancer may be associated with an increased risk of cerebral infarction 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] Tumor cells activate platelets and the coagulation cascade, which are responsible for thrombus formation in cancer.[3,4]

Since the successful introduction of endovascular thrombectomy (EVT) in patients with acute ischemic stroke, fresh arterial thrombi can be obtained.[5–8] The analysis of thrombi may provide insight into the mechanism of thrombosis, thereby aiding the determination of the

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Nonstandard Abbreviations and Acronyms

EVT	endovascular thrombectomy
IQR	interquartile range
NBTE	nonbacterial thrombotic endocarditis
PAR	protease-activated protease

treatment strategy.[6,9–12] Histological analysis of thrombi in patients with cancer-associated stroke showed a high proportion of platelets with few red blood cells.[13,14] These findings confirm that tumor cells activate platelets and suggest that platelet activation plays an important role in arterial thrombosis.

Although tumor cells can induce hypercoagulability by generating and activating coagulation factors, the expression of coagulating factors in the arterial thrombi of patients with cancer-associated stroke is not well known. Here, we investigated the composition of thrombi and the expression of coagulation factors in the thrombi of patients with cancer-associated stroke.

METHODS

Deidentified study data are available from the corresponding author upon reasonable request.

Study Populations

This retrospective analysis used data from a prospective hospital-based stroke registry (Yonsei Stroke Cohort; https://www.clinicaltrials.gov; NCT03510312) and enrolled consecutive patients with acute ischemic stroke within 7 days after symptom onset.[15] Patients were routinely evaluated with brain imaging studies (magnetic resonance imaging or computed tomography), angiographic studies (magnetic resonance angiography, computed tomography angiography, or conventional angiography), cardiac evaluations (echocardiography, continuous electrocardiography monitoring at the stroke unit 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 1 day after EVT.

This study included patients who underwent EVT between September 2014 and June 2020 at Severance Hospital, Yonsei University, South Korea, and whose extracted thrombi were obtained. 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 patients with stroke who had active cancer and no other identified etiology; they did not have 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 were closely related to stroke, even if they had another cause.[16]

Patients were excluded if evaluation was incomplete, including cases lacking continuous electrocardiography monitoring and echocardiography. Active cancer was defined as any cancer that was newly diagnosed within 6 months of the stroke event or during hospitalization for the stroke event, required chemotherapy or surgical treatment within 6 months before the stroke event, or was recurrent, metastatic, or inoperable.[17] The control group included patients without a history of cancer who were selected by propensity score matching. Informed consent was obtained from patients or their caregivers for use of thrombi for research. This study was approved by the institutional review board of Yongin Severance Hospital (number 2021-0140-001). This article follows the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) reporting guideline.

Clinical Parameters and Outcome Measures

We collected data from the registry on demographics such as age, sex, risk factors, and comorbid diseases including hypertension, diabetes, dyslipidemia, atrial fibrillation, patent foramen ovale, NBTE, venous thrombosis (pulmonary embolism and deep vein thrombosis), and the use of tissue-type plasminogen activator before EVT. Data regarding the type of cancer and the presence of metastasis were obtained from the registry and through a review of medical records. All patients underwent regular follow-up after discharge by neurologists and clinical research assistants in the outpatient clinics through face-to-face interviews or via telephone interviews using a structured questionnaire.[18]

Immunohistochemistry of Thrombus

The retrieved thrombi were immediately immersed in 4% paraformaldehyde for fixation, embedded in paraffin, and stored until use.[19] 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. Following this, the sections were soaked in a solution containing 10 mmol/L glycine in PBS, 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 were reacted with primary antibodies against erythrocytes, platelets, neutrophils, monocytes, neutrophil extracellular traps, fibrin, thrombin, tissue factors, and coagulation factors X, XI, XII, and XIII (Table S1). The sections were incubated at 37 °C for 2 hours for monocytes and thrombin and 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, United Kingdom) for monocytes and thrombin or biotin-conjugated goat anti-rabbit IgG antibody (BA-1000; Vector Laboratories) for the others. Positive signals were developed using a 3,3′-diaminobenzidine (D5637; Sigma-Aldrich, Inc, St. Louis, MO) solution. After being counterstained with hematoxylin, the sections were mounted with Permount Mounting Medium (Fisher Scientific, Fair Lawn, NJ). The stained thrombi were scanned using a digital scanner (Aperio AT2; Leica Biosystems, Wetzlar, Germany).

Imaging Analysis of Thrombi

The imaging analysis was semiautomated using the open-source software automated region-of-interest–based image

analysis.[20] The analysis was performed by investigators who were blinded to the patients' clinical information. The percentage represents the area fraction of pixels exceeding a predefined density threshold (equivalent to 160 in ImageJ; National Institutes of Health, Bethesda, MD) to the total thrombus area.

Statistical Analysis

To create a control group, we performed propensity score matching using the R package of MatchIt by matching age and sex at a 1:1 ratio for the cancer group with patients without a history of cancer. Continuous variables are reported as median and interquartile range (IQR), while categorical variables are reported as n (%). Owing to the small number of patients included in each group, a nonparametric statistical analysis was performed. The Wilcoxon rank-sum test was used to examine continuous variables, while the χ^2 or Fisher exact test was used to examine categorical variables. We performed a sensitivity analysis after excluding 3 patients with NBTE and coexisting atrial fibrillation from the cancer group. All statistical analyses were performed using the R software (version 4.2.0; R Foundation for Statistical Computing, Vienna, Austria; http://www.R-project.org/). Statistical significance was set at $P<0.05$.

RESULTS

During the study period, thrombus samples were obtained from 320 patients who underwent EVT. Of them, 84 had a history of cancer or were newly diagnosed with cancer during hospitalization. After the exclusion of 9 patients who did not undergo echocardiography and 38 patients with inactive cancer, 37 patients with active cancer were identified. After the exclusion of 14 patients with other identified causes of stroke, 23 patients were included in the cancer group (Figure 1). Among the 7 patients with NBTE included in the cancer group, 3 had atrial fibrillation. In the control group, 23 matched patients with no history of cancer were included in the analysis (Figure 1). Among the 23 control group patients, 15 had cardioembolism (13 with atrial fibrillation, 1 with congestive heart failure featuring a left ventricular thrombus, and 1 with patent foramen ovale), 5 had large artery atherosclerosis, 1 had multiple causes (cardioembolism and large artery atherosclerosis), 1 had other determined etiology (aortic dissection), and 1 was classified as having cryptogenic etiology.

Among the 23 patients with cancer, lung cancer was the most common diagnosis (6 patients), followed by colorectal cancer (4 patients), gall bladder/bile duct cancer (3 patients), gastric cancer, bladder and ureter cancer, hematologic malignancy (2 patients each), and others (4 patients). Metastasis was identified in all 21 patients with solid cancer, except for the 2 patients with hematologic malignancy. During the 6-month follow-up period, cerebral infarction recurred in 9 patients (39.1%)

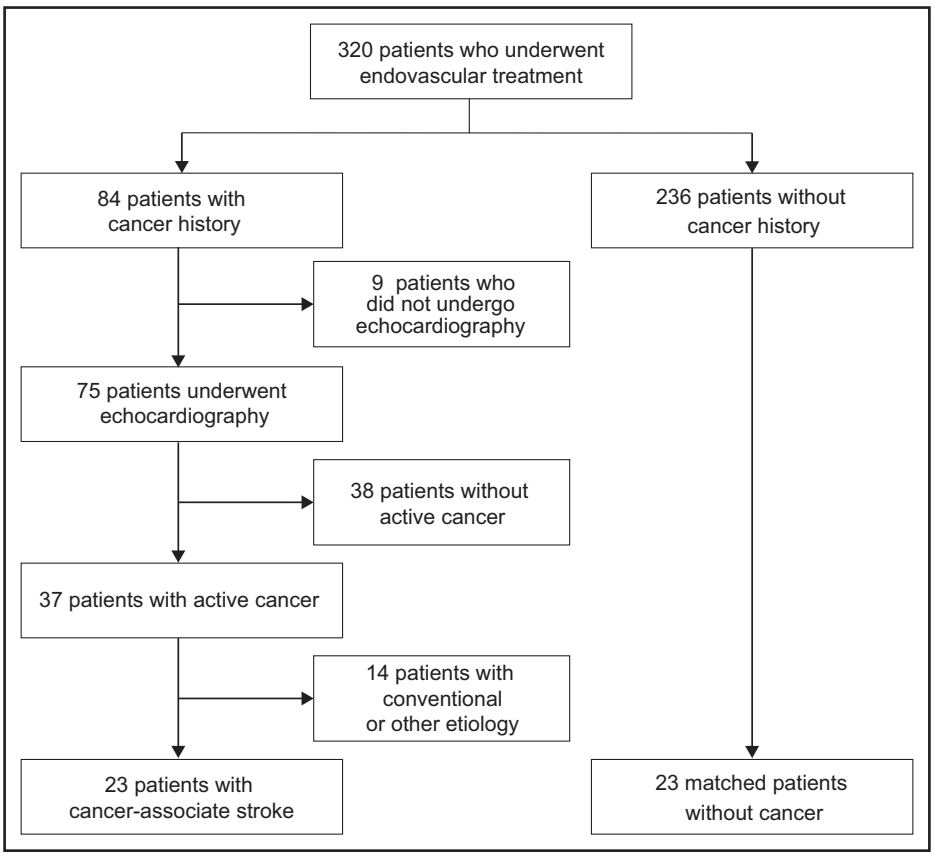

Figure 1. Flowchart of the study population.

and 15 patients died (65.2%) in the cancer group. In the control group, no patients had recurrent cerebral infarction and 3 patients died (13.0%).

Clinical Characteristics

In both groups, the median age was 65 years with an equal proportion of men (52.2%). Atrial fibrillation was less common in the cancer group compared with that in the control group (13.0% versus 56.5%; *P*=0.005). Venous thrombosis was more common in the cancer group (52.2% versus 4.3%; *P*=0.001). There was no significant intergroup difference in the presence of patent foramen ovale. The cancer group had a lower mean hemoglobin level (11.6 [IQR, 9.7–12.6] versus 12.6 [IQR, 11.1–14.5] g/dL; *P*=0.024) and a lower mean platelet count (118 [IQR, 76–164] versus 225 [IQR, 184–274]×10^9/L; *P*=0.001) than that of the control group. The mean D-dimer level was significantly higher in the cancer group (3.3 [IQR, 2.6–10.4] versus 0.8 [IQR, 0.4–2.3] mg/L; *P*<0.001). Intravenous thrombolysis was used less frequently in the cancer group compared with the control group (8.7% versus 43.5%; *P*=0.017; Table 1).

Thrombus Composition

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

Coagulation Factors in Thrombi

The cancer group showed significantly higher expression of thrombin (26.2% [IQR, 16.2%–52.7%] versus 4.5% [IQR, 1.3%–7.2%]; *P*<0.001) and tissue factor (0.60% [IQR, 0.34%–2.06%] versus 0.37% [IQR, 0.22%–0.60%]; *P*=0.024) than the control group (Figure 2). The expression of factor X was significantly lower in the cancer group (1.25% [IQR, 0.39%–3.60%] versus 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 3). 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.

Table 1. Baseline Characteristics of Patients by Study Group

	Cancer group (n=23)	Control group (n=23)	*P* value
Demographics			
Age, y	65.0 (55.5–70.0)	65.0 (54.5–70.0)	0.956
Sex, male	12 (52.2)	12 (52.2)	1.000
Clinical risk factors			
Hypertension	11 (47.8)	17 (73.9)	0.070
Diabetes	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, ×10^9/L	9.0 (5.9–9.9)	6.8 (4.9–9.0)	0.247
Platelets, ×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, s	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
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 indicates international normalized ratio; and PTT, partial thromboplastin time.

Sensitivity Analysis

In the sensitivity analysis after excluding 3 patients with NBTE and coexisting atrial fibrillation from the cancer group, the proportions of platelets, thrombin, and tissue factor were found to be higher in the cancer group

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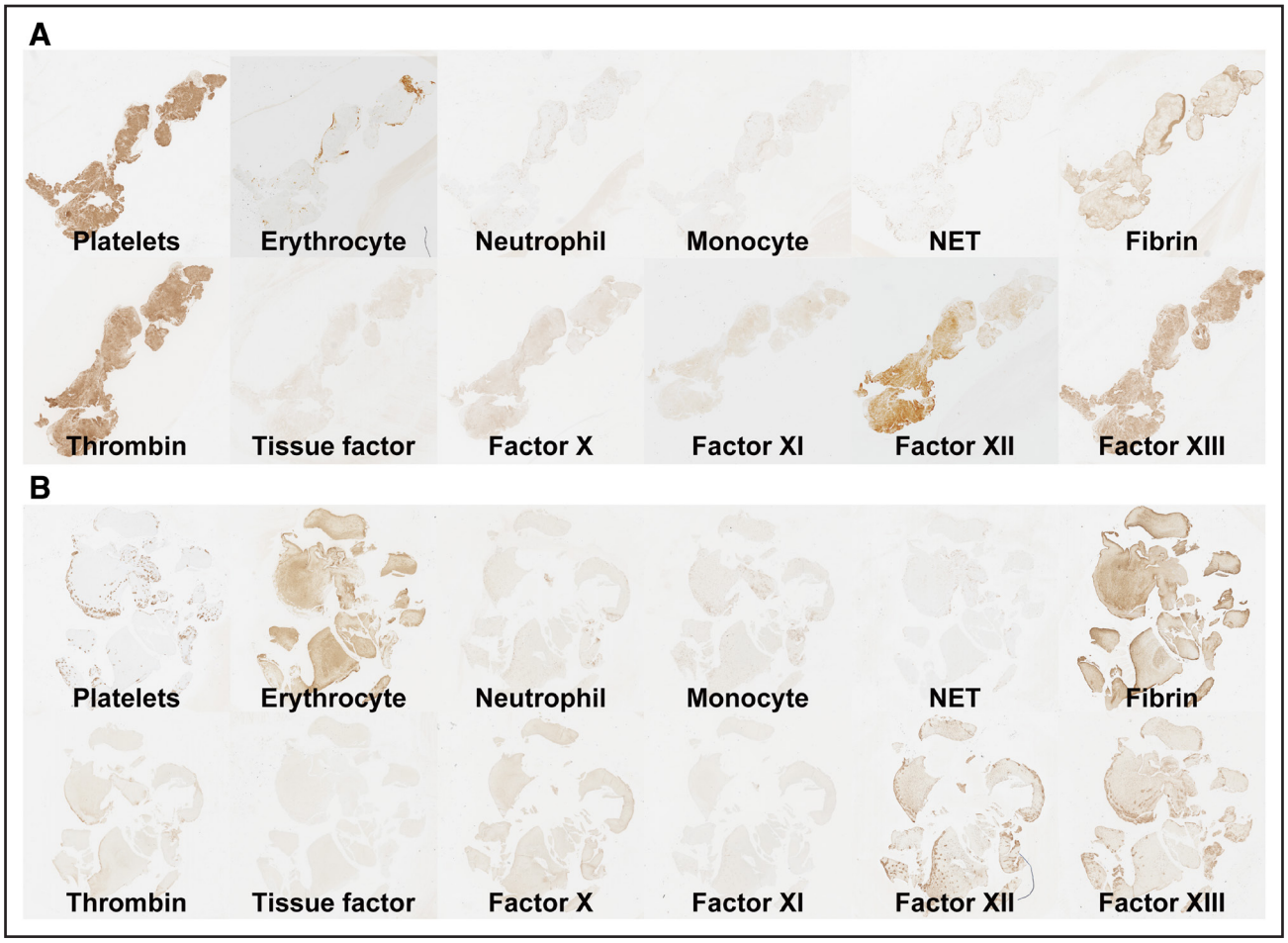

Figure 2. Representative images of immunohistochemistry staining.
A, The immunohistochemistry images of thrombi retrieved from the cancer group, and (**B**) those from the control group. The primary antibodies for immunohistochemistry are described in Table S1. NET indicates neutrophil extracellular trap.

compared with the control group. However, the proportions of erythrocytes, neutrophils, monocytes, and neutrophil extracellular traps were lower in the cancer group (Table S2). Thrombin and platelet compositions also showed a positive correlation (r=0.662; *P*=0.001; Figure S1). There was no difference in thrombus composition between patients with antithrombotic use and those without in both groups (Table S3). The thrombus composition did not differ between 5 patients with large artery atherosclerosis and the 13 patients with atrial fibrillation in the control group (Table S4). We also compared thrombus composition between the cancer group and patients with large artery atherosclerosis in the control group. Main findings were similar to those of comparison between the cancer group and the control group (Table S5).

DISCUSSION

In this study, 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 was correlated with the proportion of platelets in the cancer group but not in the control group. The control group included patients with various etiologies, but regardless of these etiologies, the thrombus composition in the cancer group was distinct from that in the control group.

This study showed that arterial thrombi in the cancer group were platelet rich and erythrocyte poor, consistent with the findings of previous studies.[13,14,21] Tumor cells induce platelet activation and aggregation to promote cancer progression and metastasis.[3,22–24] Tumor cells also enhance thrombocytosis via tumor-derived cytokines and platelet-activating factor 4.[25,26] Tumor cells and platelets form tumor cell–induced platelet aggregates. By forming these aggregates, 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.[3] In fact, the long-term use of aspirin reduces the risk of various cancers, distant metastasis, and death by ≈31% to 46%.[27–29]

Table 2. Thrombus Composition by Study Group

	Cancer group (n=23)	Control group (n=23)	*P* value
Platelet	51.3 (28.0–61.4)	9.5 (4.8–14.0)	<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	26.0 (14.7–37.3)	24.6 (15.0–31.1)	0.711

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

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.[4,30,31] In this study, we evaluated the expression of various coagulation factors in the extrinsic (tissue factor), intrinsic (factors XI and 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 PAR (protease-activated protease)-1, PAR-4, and glycoproteins Ib to IX.[32] Thrombin also converts fibrinogen into fibrin and amplifies the coagulation cascade by activating coagulation factors V, VIII, and XIII.[33,34] Tumor cells directly generate thrombin and indirectly generate it by secreting tissue factor that initiates the extrinsic coagulation pathway.[35,36] Thrombin generation and the thrombin-antithrombin complex can serve as biomarkers reflecting hypercoagulability in patients with cancer.[36] Tissue factor can be expressed directly by some tumor cells and transported by tumor-derived extracellular vesicles.[37–40] In the initial stage of metastasis, fibrin deposition and platelet recruitment by thrombin are crucial to the survival and adherence of tumor cells to the endothelium.[41] 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× higher than that in patients without cancer. Thrombin is generated directly by tumor cells and indirectly by the coagulation pathway. Our findings 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.[42] Reciprocally, the extracellular vesicles released from activated platelets contribute to further thrombin activation.[39] 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.[43,44] 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.[45] 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.[46] However, this needs to be tested in clinical trials.

This study has several limitations. First, it included patients with thrombotic occlusion of large cerebral arteries. While the thrombus composition may differ, potentially being more platelet-rich, in patients with smaller artery atherosclerosis, the small thrombi that occluded the distal cerebral arteries could not be examined. Therefore, the small thrombi that occluded the distal cerebral arteries could not be examined. In addition, this study excluded patients with cancer and coexisting etiologies such as atrial fibrillation because analyses of thrombi exclusively from cancer-associated stroke were necessary. Therefore, the findings of this study should be interpreted with consideration of the characteristics of the study population. Second, we could not compare histological features by cancer type because of the small sample size. Further studies are necessary to investigate thrombus characteristics according to cancer type. Finally, for the sensitivity analysis examining the impact of antithrombotics on thrombus composition, we combined patients with various anticoagulants and antiplatelets into the antithrombotic user category due to the limited number of cases. Although there were numerical differences in some thrombus compositions between patients with antithrombotic use and those without, they were statistically insignificant. However, these insignificances might be attributed to the small sample size.

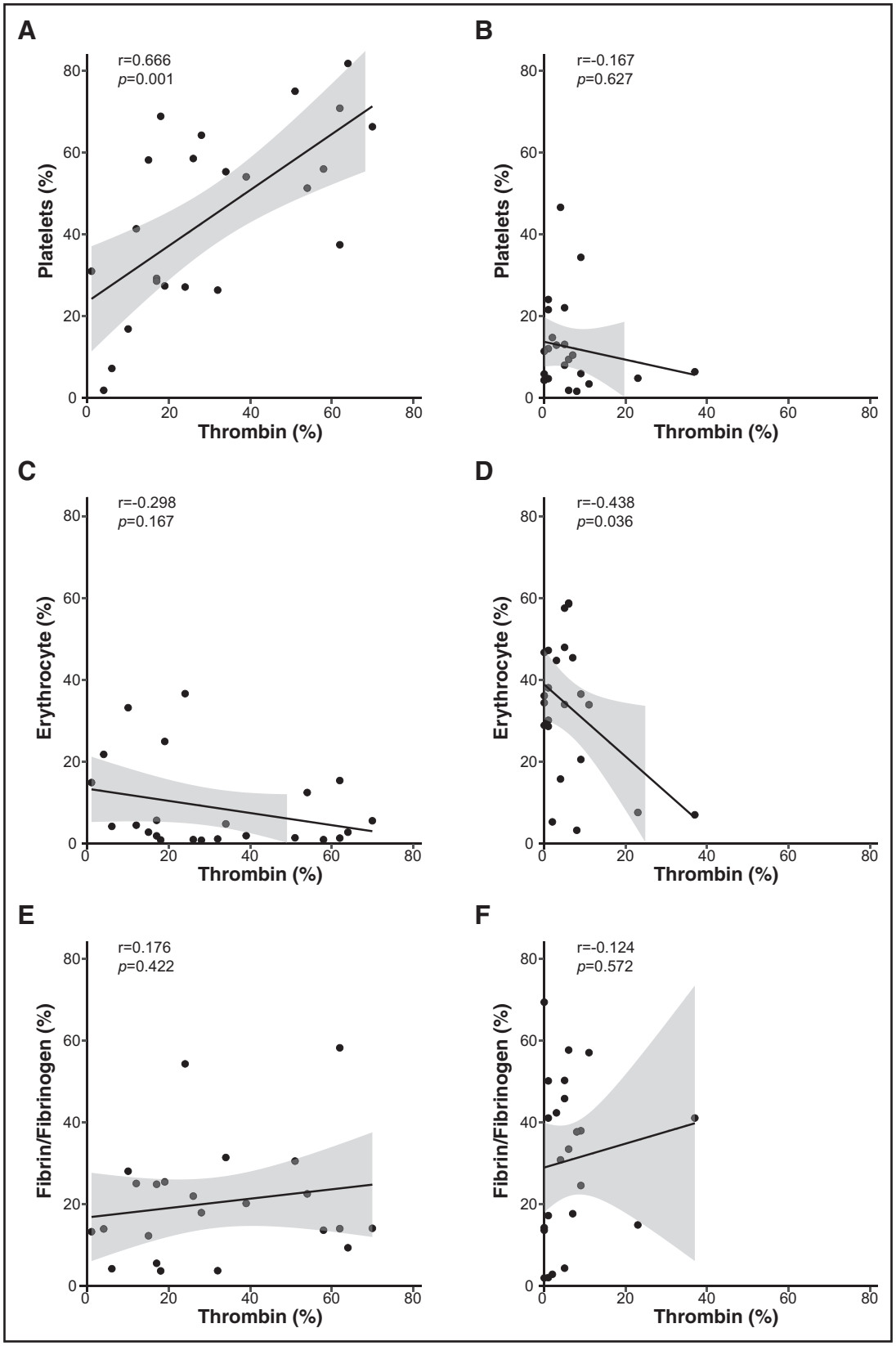

Figure 3. Correlation between thrombin and various thrombus components, involving platelet, erythrocyte, and fibrin. **A**, **C**, and **E**, Correlations in the cancer group. **B**, **D**, and **F**, Correlations in the control group.

CONCLUSIONS

Cerebral thrombi of patients with cancer-associated stroke had a significantly higher proportion of platelets and thrombin and showed a positive correlation between platelets and thrombin. These findings suggest crucial and interactive roles of platelets and thrombin in arterial thrombosis in cancer. Our findings also provide a perspective for developing strategies to prevent stroke recurrence in patients with cancer-associated stroke.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

STROBE Checklist
Tables S1–S5
Figure S1

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