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Original Article

Thrombus formation in cancer patients using autopsy materials: an immunohistochemical analysis

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Abstract: Background: Cancer-associated thrombosis (CAT) is a well-known complication of malignant tumors. It has been predominantly reported in mucin (MUC)-producing adenocarcinomas, with MUC secreted by the tumor thought to be involved in the thrombotic mechanism. However, studies comparing tumor and thrombus areas are scarce. Methods: In this study, we examined the immunohistochemical characteristics of 18 autopsy specimens of tumor and thrombus sections (CAT) and control specimens of 25 tumors without thrombus and 16 thrombi without tumors. Immunohistochemistry was performed using antibodies associated with coagulation and MUC, including tissue factor (TF), thrombin, MUC2, MUC5AC, and MUC6. Results: It was revealed that TF was predominantly positive in the tumor sections of CAT compared with tumors without thrombus ($P<0.0001$), and MUC2 expression was significantly higher in thrombus sections of CAT than that in thrombi without tumors ($P<0.0001$). In the thrombus region, TF positivity was inversely correlated with MUC2 positivity. Conclusions: These results suggest that the expression of TF in tumor tissues is involved in the pathogenesis of CAT, and furthermore, that MUC2 contributes to thrombus formation in CAT via a pathway other than TF. In the future, these immunohistochemical analyses will help predict CAT in cancers and detect hidden cancers in patients with thrombosis.

Keywords: Cancer-associated thrombosis (CAT), thrombus, tissue factor (TF), mucin (MUC), immunohistochemistry

Introduction

It is well known that cancer-associated thrombosis (CAT) is caused by or associated with malignant tumors [1, 2]. The incidence of thrombosis occurring among cancer patients within one year is approximately 4.5% [3] and the risk of venous thromboembolism (VTE) is increased 12-fold compared with patients without cancer [4, 5]. Cancer patients with VTE had a 3-6-fold higher risk of mortality compared to those without thrombosis [6]. Histological types noted to most likely develop CAT include adenocarcinomas, especially mucin (MUC)-producing adenocarcinomas of the pancreas and gastrointestinal tract [7-10]. The cause of thrombosis in CAT might be abnormal hypercoagulability of the coagulation cascade including thrombin and tissue factor (TF) secreted from tumor cells [11, 12]. Interactions between circulating carcinoma MUC and leukocyte L-selectin as well as platelet P-selectin have also been reported to contribute to CAT [13].

However, the details of the *in vivo* mechanism of thrombus formation in CAT remain unknown. High TF immunopositivity was reported in primary tumors or tumor cells within thrombi in CAT autopsy cases [14, 15]. Furthermore, to the best of our knowledge, comparisons of the primary tumor site and thrombus, or immunohistochemical analysis of other coagulation markers and MUC-types have not been reported. Here, we analyzed the features of tumors and thrombi in the same autopsy cases of CAT by the immunohistochemical staining of various coagulation factors and MUC-types, along with controls containing tumors without thrombi and thrombi without tumors.

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Materials and methods

Case selection

The study protocol was approved by the Committee of Human Study of Tokyo Medical University (#T2022-0133). Signed consent forms are available from the homepage of Tokyo Medical University Hospital. CAT was defined as broad-sense Trousseau syndrome [1], namely a malignant tumor with thrombus formation in any part of the body at autopsy, regardless of the presence of cerebral infarction (CI).

Eighteen CAT cases were identified from autopsies performed during 2002-2021 (n=18) (T+Th+; tumor+thrombi+, Group 1). Because all these histologic types were adenocarcinoma, all control cases were also selected from adenocarcinoma. Thus, adenocarcinoma cases without thrombi (n=25) (T+Th-; Group 2) were identified from the same period. Non-tumor patients with thrombi (n=16) (T-Th+; Group 3) were identified from the same period. Inclusion criteria were: tumor diagnosis or cause of death confirmed histologically; known age, sex, organs with tumors, and thrombus sites; and paraffin-embedded samples available for specimen preparation.

From these selected cases, we evaluated their clinical characteristics including age, sex, location of the primary lesion, site of thrombus, and presence of CI. Two pathologists (YY and AK) carefully reevaluated the histological characteristics of the tumors and thrombi, especially the presence of intra-tumoral thrombi, intrathrombotic tumors, and poorly differentiated components. The thrombus diameter was measured based on histological observations of the specimens, which may not necessarily match the maximum thrombus diameter observed macroscopically. The presence of thrombus organization was determined when immunostaining showed CD31-positive elongated cells.

Immunohistochemistry

Representative tissue specimens of tumors were selected in Groups 1 and 2, and those of thrombi were selected in Groups 1 and 3. For each specimen, we performed immunohistochemical staining for TF, thrombin, MUC2, MUC5AC, and MUC6, which are involved in the formation of thrombi and mucus. Furthermore, for the thrombus, immunostaining with CD31 along with periodic acid-Schiff (PAS), alcian-blue, and phosphotungstic acid hematoxylin staining was performed.

Paraffin-embedded samples were cut into 4-μm-thick sections. A standard polymer method was used for immunohistochemical staining using antibodies to TF (rabbit, polyclonal, 1:2000 dilution, ab104513, Abcam), thrombin (rabbit, polyclonal, 1:500 dilution, ab92621, Abcam), CD31 (rabbit, clone EPR17259, 1:2000 dilution, Abcam), MUC2 (rabbit, clone EPR6145, 1:200 dilution, Abcam), MUC5AC (mouse, clone 45M1, 1:400 dilution, Abcam), and MUC6 (mouse, clone MUC6/916, 1:200 dilution, Abcam). Deparaffinized and hydrated sections were treated with 0.3% hydrogen peroxide in methanol for 20 min to block endogenous peroxidase activity. To expose antigens, sections were autoclaved in 10 mM citrate buffer (pH 6.0) at 121°C for 10 min and cooled for 30 min. After rinsing in 0.05 M phosphate-buffered saline (PBS) containing 0.1% Tween-20 (pH 7.6), sections were incubated for 60 min with primary antibodies at room temperature (RT). Samples were washed three times in PBS and incubated with Dako secondary antibody (horseradish peroxidase-labeled polymer conjugated to a mixture of goat anti-mouse and anti-rabbit immunoglobulin antibodies, Code: K5007, prediluted) for 15 min at RT. 3,3-diaminobenzidine tetrachloride was used for color development and sections were counterstained with hematoxylin.

Evaluation of tissue images

We evaluated the staining of each specimen microscopically under an optical microscope (Olympus BX50, magnification ×200). Two pathologists (YY, AK) independently evaluated the immunohistochemical results. Positivity was considered when both pathologists rated the staining as greater than 10% positive in the cancer or thrombus tissue of interest, and negative if both rated it as 0% positive. The cutoff was set as low as 10% because it was based on HER2 counts in breast cancer and stomach cancer, and also because tumors and thrombi have a mixture of components other than the tissue of interest. In addition, positivity was assumed when positive viable tumor cells were

Table 1. Clinicopathological features of study cases: Group 1 (n=18), Group 2 (n=25), and Group 3 (n=16)

	Group 1 (n=18)	Group 2 (n=25)	Group 3 (n=16)
Age, years	70±8	73±9	63±16
Sex (M/F)	14/4	13/12	7/9
Cerebral infarction (+/-)	3/15	0/25	0/16
Thrombosis in tumor site (+/-)	4/14	2/23	-
Poorly differentiated (+/-)	16/2	19/6	-
Thrombus diameter (mm)	6.1±4.4	-	7.1±3.5
Intra-thrombotic tumor cells (+/-)	7/11	-	-
Tumor primary site			
Pancreas	7	5	-
Lung	5	12	-
Stomach	3	6	-
Colon	2	2	-
Unknown	1	0	-
Site of thrombus			
Pulmonary artery	16	-	8
Femoral vein	1	-	0
Splenic artery	1	-	1
Portal vein	0	-	1
Coronary artery	0	-	3
Inside the left ventricle	0	-	2
Cerebral artery	0	-	1

M: male, F: female.

identified, even in areas where the tumor was undergoing necrosis. If at least one of the pathologists evaluated it as less than 10% positive, the two pathologists reviewed the specimen together, and the results were considered positive if a positive image was obtained for the tissue of interest, and negative if an object other than the tissue of interest was stained or considered an artifact.

Statistical analysis

The expression of immunohistochemical staining was evaluated as 0: negative (-), and 1: positive (+). These results were used as independent variables, and age and thrombus diameter are shown as numerical values. Sex (male or not) and the presence or absence of CI, intratumoral thrombus, intra-thrombotic tumor cells, poorly differentiated components, and thrombus organization determined based on endothelial cell proliferation assessed by CD31 staining are represented as 0 or 1. Statistical analyses were performed using an unpaired, two-tailed *t*-test for comparisons between two groups and one-way ANOVA with Tukey's multiple comparison test for comparisons between three groups, using Prism version 10 (GraphPad Software, San Diego, CA, USA). $P<0.05$ was considered statistically significant.

Correlation analyses (Spearman's rank correlation) were performed for immunohistochemical results between Group 1 and Group 2, between Group 1 and Group 3, and between tumors and thrombi within Group 1.

Results

Clinical and histopathological characteristics

The clinicopathologic characteristics of CAT (Group 1), along with the tumor control (Group 2) and thrombus control (Group 3) groups are shown in **Table 1**. Tumors in Groups 1 and 2 were predominantly pulmonary, gastrointestinal, or pancreatic in origin, and pulmonary artery emboli were predominant in Groups 1 and 3. Although the age and sex ratios of all the groups were not significantly different, the Group 3 cases were significantly younger than those in Group 2 (Tukey's test, $P=0.03$).

Correlation analysis across all tumor sites (Groups 1 and 2) revealed CI presence correlated with Group 1 (P=0.034), reflecting the results showing that CI was present in CAT cases only. Additionally, male sex correlated with the presence of poorly differentiated components ($P=0.014$), and the presence of intratumoral thrombus correlated with CI presence ($P=0.005$) (**Table 2A**).

Across all thrombus sites (Group 1 and 3), male sex ($P=0.04$) and the presence of intra-thrombotic tumors ($P=0.004$) correlated with Group 1. Furthermore, male sex correlated with the presence of intra-thrombotic tumors ($P=0.018$)

Table 2A. List of results of correlation analyses between tumors in Group 1 (n=18) and Group 2 (n=25)

	CAT (Group 1)	Male	Cerebral infarction	Poorly differentiated	Thrombosis in tumor	Immunohistochemical expression	
						TF	MUC2
CAT (Group 1)	-	NS	r=0.32 P=0.034	NS	NS	r=0.67 P<0.001	r=0.44 P=0.003
Male	-	-	NS	r=0.37 P=0.014	NS	NS	NS
Cerebral infarction	-	-	-	NS	r=0.41 P=0.005	NS	r=0.29 P=0.054
Poorly differentiated	-	-	-	-	-	NS	NS
Thrombosis in tumor	-	-	-	-	-	NS	NS
TF	-	-	-	-	-	-	NS
MUC2	-	-	-	-	-	-	-

CAT: cancer-associated thrombosis, NS: not significant, TF: tissue factor, MUC: mucin.

Table 2B. List of results of correlation analyses between thrombi in Group 1 (n=18) and Group 3 (n=16)

	CAT (Group 1)	Male	Intra-thrombotic tumors	Immunohistochemical expression		
				Thrombin	TF	MUC2
CAT (Group 1)	-	r=0.34 P=0.04	r=0.48 P=0.004	r=-0.65 P<0.001	NS	r=0.76 P<0.001
Male	-	-	r=0.40 P=0.018	r=-0.41 P=0.015	r=-0.39 P=0.020	NS
Cancer in thrombus	-	-	-	r=-0.42 P=0.012	NS	r=0.33 P=0.053
Thrombin	-	-	-	-	r=0.48 P=0.003	r=-0.52 P=0.001
TF	-	-	-	-	-	r=-0.46 P=0.005
PAS	-	-	-	-	-	r=1 P<0.0001

CAT: cancer-associated thrombosis, NS: not significant, TF: tissue factor, MUC: mucin, PAS: Periodic acid-Schiff.

(**Table 2B**). Within CAT cases (Group 1), CI presence correlated with aging (P=0.016) and intratumoral thrombosis (P=0.045) (**Table 2C**).

Immunohistochemical analyses

Significant differences between CAT (Group 1) and controls (Groups 2 and 3) are summarized in a bar graph (**Figure 1**) and **Table 2**, because many markers were assessed. When comparing poorly differentiated areas with moderate-to-well differentiated areas within the same case, no apparent differences in staining characteristics were observed for the various antibodies used.

Immunohistochemical findings of tumors were compared between Groups 1 and 2. The results showed that TF expression differed significantly between the groups: more than 80% of specimens in CAT (Group 1) were positive, whereas over 80% in Group 2 were negative (**Figures 1**, **2**) (P<0.001). In contrast, as for thrombin expression, most specimens from tumor sites in Group 1 and 2 were negative (**Figure 1**). For MUC-related molecules, the results showed that MUC2, MUC5AC, and MUC6 were positive in some tumor tissues (**Figures 1**, **2**), and the MUC2 positivity rates were significantly higher in Group 1 than in Group 2 (P=0.003). In addition, although statistically not significant, in the correlation analysis of the overall tumor area, MUC2 positivity tended to correlate with CI presence (P=0.054). These findings suggest that the expression of TFs and MUC2 in tumor

Table 2C. List of results of correlation analyses of immunohistochemical expression within Group 1 (n=18)

	Age	Male	CI	Por	Thrombus in tumor	Thrombin (th)	TF (th)	MUC2 (ca)	MUC2 (th)	MUC5AC (ca)	MUC5AC (th)	MUC6 (ca)
Age, years	-	NS	r=0.56 P=0.016	NS	NS	NS	NS	NS	NS	NS	NS	NS
Male	-	-	NS	NS	NS	NS	NS	NS	NS	NS	r=-0.66 P=0.002	NS
CI	-	-	-	NS	r=0.47 P=0.045	NS	r=1 P<0.0001	NS	NS	NS	NS	NS
Por	-	-	-	-	NS	NS	r=-0.5 P=0.034	NS	NS	NS	NS	NS
Thrombus in tumor	-	-	-	-	-	NS	NS	NS	r=-0.66 P=0.003	NS	NS	NS
Thrombin (th)	-	-	-	-	-	-	r=0.64 P=0.004	NS	NS	r=0.64 P=0.004	NS	NS
TF (th)	-	-	-	-	-	-	-	NS	r=-0.5 P=0.035	r=0.5 P=0.034	NS	NS
MUC2 (ca)	-	-	-	-	-	-	-	-	r=0.57 P=0.014	NS	NS	NS
MUC2 (th)	-	-	-	-	-	-	-	-	-	NS	NS	r=-0.68 P=0.002
MUC5AC (ca)	-	-	-	-	-	-	-	-	-	-	r=0.5 P=0.034	NS
MUC5AC (th)	-	-	-	-	-	-	-	-	-	-	-	NS
MUC6 (ca)	-	-	-	-	-	-	-	-	-	-	-	-

CI: Cerebral infarction, NS: not significant, TF: tissue factor, Por: poorly differentiated, th: thrombus, MUC: mucin, ca: carcinoma.

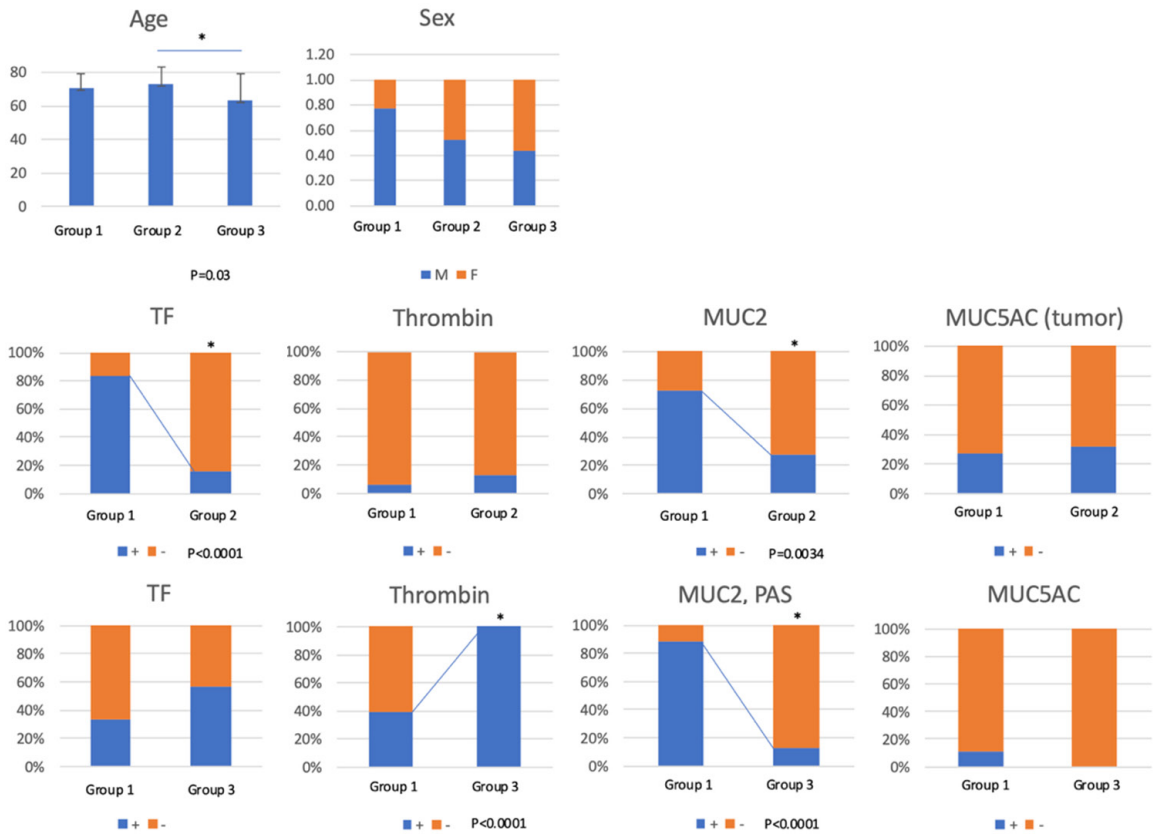

Figure 1. Clinical characteristics and immunohistochemical staining results. The clinical characteristics and immunohistochemical staining results are presented as a bar graph (statistical significances are based on *t*-tests and Tukey tests). TF: tissue factor, MUC: mucin, PAS: Periodic acid-Schiff.

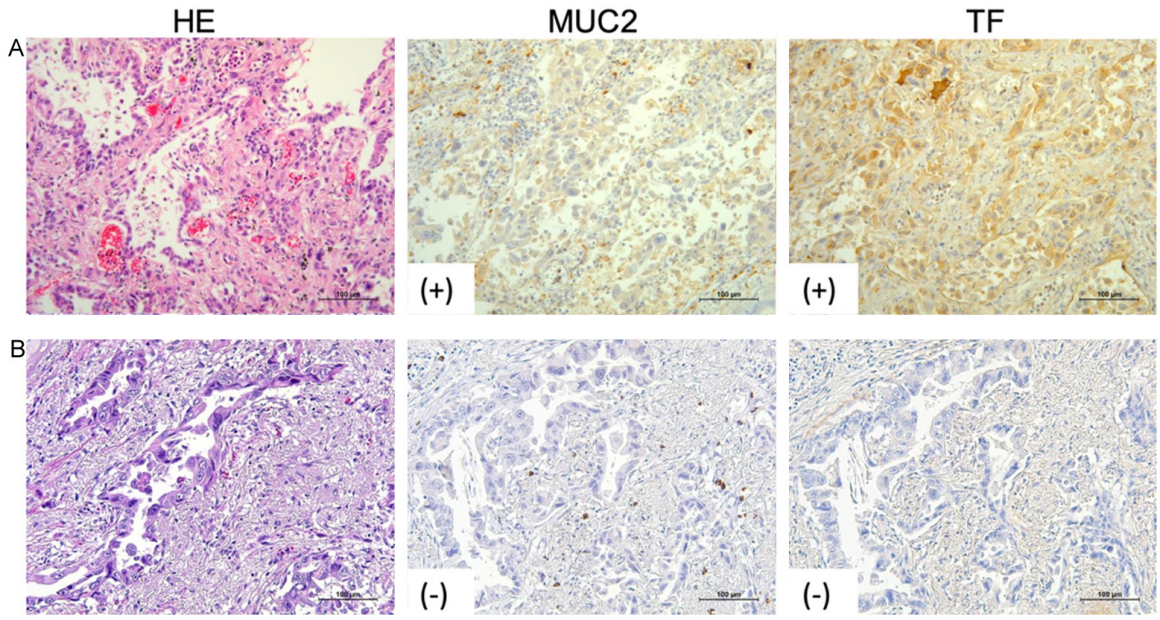

Figure 2. Representative histopathological images and immunohistochemical staining results of the tumor areas. (A) Cancer-associated thrombosis (CAT) and (B) a control specimen (no thrombus). From left: Hematoxylin and eosin (HE) staining, mucin (MUC) 2 immunostaining, tissue factor (TF) immunostaining. (A) Lung adenocarcinoma in a CAT case. Formation of glandular structures and cord-like structures with unclear glandular formation are shown. Tumor cells show positive staining for MUC2 and TF (magnification ×200). (B) Lung adenocarcinoma in a control case. Tumor cells show negative staining for MUC2 and TF (magnification ×200).

tissues is important for the development of CAT.

Next, immunohistochemical studies were performed to determine whether the thrombus with CAT was unique. MUC2$^+$ thrombi were found in 16 of 18 Group 1 specimens, but in only 2 of 16 in Group 3, which was significantly different (P<0.001) (**Figures 1**, **3A**, **3B**; **Table 2B**). Positive and negative findings for MUC2 and PAS were consistent in all cases (r=1, P<0.0001). Furthermore, in the correlation analysis of the overall thrombus region, MUC2 expression and PAS tended to correlate with intra-thrombotic tumors (P=0.053) (**Figure 3A**). Although not statistically significant (P>0.05), MUC5AC$^+$ thrombi were found in 2 of 18 Group 1 specimens, and in none of the 16 Group 3 specimens. There was no significant difference in the positivity of MUC5AC and MUC6 between Groups 1 and 3. Regarding thrombin, all samples in Group 3 were positive, whereas 6 of 16 samples in Group 1 were positive, indicating a significant difference in expression between the two groups (P<0.001) (**Figure 1**). These findings indicate that CAT thrombi are characterized by high positivity for MUC2 and low positivity for thrombin.

In the thrombus region, correlation analysis revealed that TF positivity correlated with female sex (P=0.02), thrombin positivity (P=0.003), and negativity for MUC2 and PAS (P=0.005 each). The results of thrombin positivity were similar: it correlated with female sex (P=0.015) and inversely correlated with MUC2 and PAS (P=0.001) along with cancer in thrombus (P=0.012) (**Table 2B**). Regarding thrombus organization, CD31 positivity was found in 7 of 18 Group 1 cases and 5 of 16 Group 3 cases, with no significant group differences (t-test) nor correlations with other factors.

A comparison of immunopositivity between tumors and thrombi within CAT showed that in Group 1, all 10 cases with MUC2$^+$ tumors had MUC2$^+$ thrombi. However, MUC2$^+$ thrombi were observed in 4 other cases, despite having MUC2$^-$ tumors. Of 5 MUC5AC$^+$ tumors, 2 had MUC5AC$^+$ thrombi, whereas all 13 cases with MUC5AC$^-$ tumors had MUC5AC$^-$ thrombi. Thus, the correlation analysis revealed that MUC2$^+$ tumors had a significantly higher rate of MUC2$^+$ thrombi (P=0.014), and the same was true for MUC5AC$^+$ tumors (P=0.034) (**Tables 2C**, **3**). Other significant correlations included CI presence with TF$^+$ thrombi (P<0.0001), poorly di-

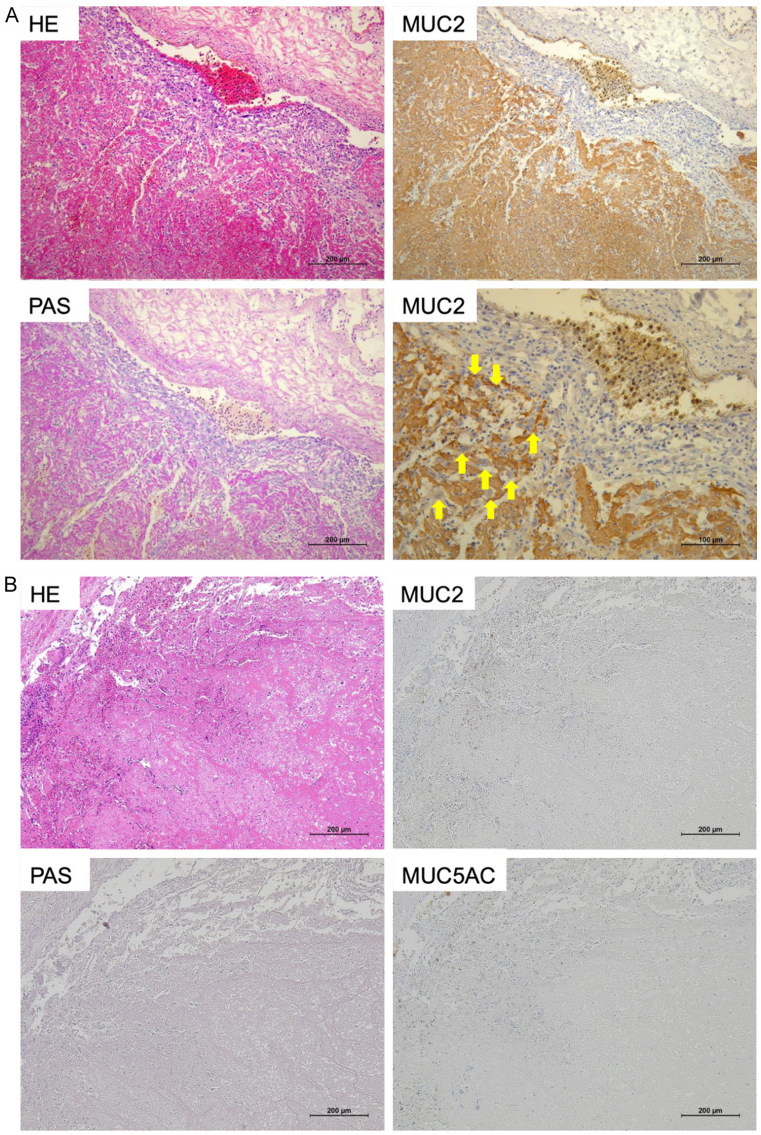

Figure 3. Representative histopathological images and immunohistochemical staining results of thrombi. (A) Cancer-associated thrombosis (CAT) and (B) a control specimen (no tumor). (A) Thrombus in a CAT case (same as case 2A). This thrombus shows positive staining for mucin (MUC) 2 immunostaining and periodic acid-schiff (PAS) staining (magnification ×100) in line with the tumor area of this case showing positive staining for MUC2. Tumor cells in the thrombus are also MUC2$^+$ (yellow arrows, magnification ×200). (B) Portal vein thrombus in a control case. The formation of Zahn lines is shown. MUC2, MUC5AC, and PAS are all negative (magnification ×100). HE: Hematoxylin and eosin staining.

fferentiated components with TF$^-$ thrombi (P=0.034), intra-tumoral thrombi with MUC2$^-$ thrombi (*P*=0.003), thrombin$^+$ thrombi with TF$^+$ thrombi (*P*=0.004), thrombin$^+$ thrombi with MUC5AC$^+$ tumors (*P*=0.004), TF$^+$ thrombi with MUC2$^-$ thrombi (*P*=0.035) and MUC5AC$^+$ tumors (*P*=0.034), MUC2$^+$ thrombi with MUC6$^-$ tumors (*P*=0.002), and male sex with MUC5AC$^-$ thrombi (*P*=0.002).

Discussion

The aim of this study was to investigate whether tumor-derived substances were involved in thrombus formation in CAT and to determine how the coagulation system was associated *in vivo*. Immunohistochemical analysis was used to determine the expressions of antibodies related to the coagulation cascade and MUC in cases including non-CAT tumors and non-cancerous thrombi as control examples. To the best of our knowledge, this is the first study comparing MUC and coagulation factor expression in tumor and thrombus tissues from the same patient.

Regarding clinicopathological features, no significant differences were found for age or sex between CAT and controls, with the appropriate selection of cases. In the comparison between three groups, a significant difference in age was observed between the two controls (Groups 2 and 3), but this was expected, given that non-neoplastic thrombosis can occur in younger individuals compared to the typical onset age of tumors. Although cases of Trousseau syndrome in the narrow sense with CI were limited, we attributed this finding to advances in treatment, reflecting an increasing incidence of venous thromboses such as deep vein thrombosis (DVT) rather than arterial thrombosis in cancer patients [16, 17]. Indeed, non-bacterial thrombotic endocarditis, a complication of malignant tumors, was not observed. Although it is difficult to differentiate pulmonary arterial thrombosis from thromboembolism *in situ*, pulmonary embolism originating from DVT is generally more common. The focus of this study was on existing thrombi as specimens, which may not necessarily corre-

Table 3. Expression of MUC in tumor and thrombus areas in Group 1

MUC2 (cancer)	MUC2 (thrombus)	Number of cases
+	+	12
+	-	0
-	+	4
-	-	2
MUC5AC (cancer)	MUC5AC (thrombus)	Number of cases
+	+	2
+	-	3
-	+	0
-	-	13

MUC: mucin.

spond to the largest thrombus observed macroscopically, but rather thrombi extending from the pulmonary artery to the lobar artery. The CIs in this study were observed clinically, but there was no evidence of thrombi in cerebral arteries. Furthermore, cerebral thrombi might have dissolved due to treatment or were not found due to their small size.

Concerning tumor histology, adenocarcinomas, particularly MUC-producing adenocarcinomas of the pancreas and digestive tract, have been reported as common histological types with CAT [8, 9], but their degree of differentiation has not been elucidated clearly. In this study, many cases of CAT contained poorly differentiated components. This is consistent with a previous report of six cases of gastric cancer presenting with pulmonary tumor thrombotic microangiopathy with poorly differentiated histology [18], suggesting that poorly differentiated adenocarcinomas are prone to thrombosis. Furthermore, male sex was significantly correlated with poorly differentiated components and intra-thrombotic cancers, but inversely correlated with TF and thrombin expression in thrombi; TF- and thrombin-independent pathways were assumed to induce thrombi, especially in males with poorly differentiated cancers, which is discussed below in conjunction with MUC2.

Immunohistochemical analysis indicated a significant increase in TF expression in the tumor areas of CAT cases compared to non-CAT cases, suggesting the involvement of tumor-derived TF production in thrombus formation. Previous reports also suggested that TF^+ tumors were an important factor in the pathogenesis of CAT in some cancer patients [2, 14, 19, 20]. Gi et al. recently reported the direct infiltration of cancer or cancer clusters into DVT and pulmonary emboli in autopsy cases of CAT, along with the expression of TF and podoplanin [15]. However, in the thrombus region in the present study, TF and thrombin expressions were lower in Group 1 compared with Group 3. This suggests that although TF derived from cancer is involved in CAT, other factors may have a greater role compared to normal thrombi [21]. Notably, a significantly higher expression of MUC2 was found in CAT thrombi compared with non-CAT thrombi. Furthermore, in the thrombus region, TF positivity was significantly and inversely correlated with MUC2 positivity. Therefore, MUC2 is likely to contribute to thrombus formation in CAT via pathways other than TF.

Regarding MUC, differences in the expression of MUC type according to the primary cancer site are well known. Gastric-intestinal type MUC such as MUC2, MUC5AC, and MUC6 are not secreted from normal pulmonary alveolar epithelium but may be positive in lung cancer, suggesting their production during cancer development [22]. It was also reported that cases with positive MUC traits had a poor prognosis [23-25]. In this study, MUC2 positivity rates were significantly higher in Group 1 than in Group 2. This increased occurrence of $MUC2^+$ tumors especially lung cancer in CAT is considered interesting as mentioned below.

To the best of our knowledge, this is the first study to reveal MUC^+ thrombi in CAT cases. Of note, 89% of CAT thrombi were MUC2 positive, whereas most control thrombi were negative, which was significantly different. Additionally, when intra-thrombotic tumor cells were present, there was a tendency for $MUC2^+$ thrombi, although this was not statistically significant. These findings suggest that MUC2 produced by tumors may be involved in the tendency towards thrombus formation in CAT cases, especially at the local level. This is supported by the high concordance rate of MUC type between tumors and thrombus in a single case (MUC2: n=12, MUC5AC: n=2, one of which was double positive). Indeed, within Group 1, a significant cor-

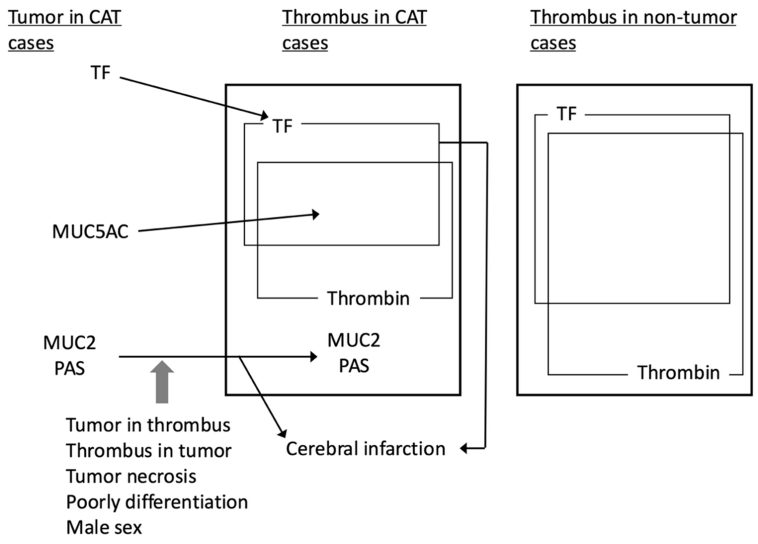

Figure 4. Presumed mechanisms of thrombus formation in cancer-associated thrombosis (CAT). It is assumed that tumor-derived TF and mucin (MUC) 2 form thrombi independently. TF: tissue factor, PAS: Periodic acid-Schiff.

relation was observed between tumor and thrombus positivity for MUC2 and MUC5AC, respectively. In cancer patients, the risk of developing VTE increased in those who underwent chemotherapy compared to those who received no treatment [3, 24, 26] suggesting that the production of MUC associated with tumor lysis during treatment may be involved in thrombus formation. This may explain the poor prognosis of MUC2$^+$ lung cancer reported in previous studies [25, 27]. Taken together, our immunohistochemical results suggest the involvement of tumor-produced MUC in thrombus formation.

In the thrombus region, MUC2 expression matched the staining characteristics of PAS, and these were negatively correlated with TF/thrombin expression. Additionally, the presence of intra-thrombotic tumor cells inversely correlated with thrombin$^+$ thrombi, whereas it tended to correlate with MUC2$^+$/PAS$^+$ thrombi. These results suggest that in CAT thrombi, tumor-derived TF is involved, but components indicating tumor-derived MUC2 and PAS positivity are not directly associated with TF; they are associated with male sex and local thrombus formation.

Regarding other significant correlations within CAT (**Table 2C**), CI correlated with aging, a well-known risk factor for CI, as well as TF$^+$ thrombi, suggesting that local TF affects the whole body. Interestingly, TF$^+$ thrombi were inversely correlated with the presence of poorly differentiated tumor components. This indicates that a poorly differentiated tumor causes thrombus formation through a pathway unrelated to TF. Furthermore, intra-tumoral thrombus presence correlated with MUC2$^+$ thrombi. In comparison, thrombin$^+$ thrombi correlated with TF$^+$ thrombi, as well as with MUC5AC$^+$ tumors. This suggests that even if a tumor expresses MUC phenotypes, the process of thrombus formation still involves thrombin. Furthermore, male sex was significantly inversely correlated with TF, thrombin, and MUC5AC positivity within the thrombi. A summary presenting the results of this study is provided in **Figure 4**.

This study had some limitations. First, the relatively small number of cases might have resulted in a lack of statistical significance for some findings. Second, due to the diversity of tumor and thrombus locations, organ-specific differences remain unclear. Third, the heterogeneity of anticancer and antithrombotic treatments may have influenced the results. Nevertheless, the study is notable for including intra-individual comparisons in autopsy cases and for being the first to demonstrate MUC staining in thrombi.

In the future, it may be possible to predict subsequent thrombosis formation (potential occurrence of CAT) by the immunohistochemical analysis of TF during cancer biopsy. This procedure may be particularly useful because many newer drugs including direct oral anticoagulants have become available for the prevention of VTE in patients with cancer [4, 24]. Additionally, in some cases, thrombosis may be detected before cancer is found [28], and immunostaining of MUC on thrombus specimens could help identify hidden cancers.

In conclusion, immunohistochemical evidence suggests that TF production in tumors is involved in thrombosis formation in CAT. Furthermore, it is conceivable that MUC, particu-

larly MUC2, secreted from tumor cells, has a significant role in thrombosis formation in CAT. Accumulating more cases and conducting further investigations are needed to elucidate the nature of CAT.

Acknowledgements

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Disclosure of conflict of interest

None.

Abbreviations

CAT, cancer-associated thrombosis; MUC, mucin; TF, tissue factor; VTE, venous thromboembolism; CI, cerebral infarction; PAS, periodic acid-Schiff; PBS, phosphate-buffered saline; RT, room temperature; DVT, deep vein thrombosis.

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