Usefulness of blood biomarkers for predicting venous thromboembolism in Japanese patients with cancer

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Abstract. Prospective studies on risk factors for the occurrence of venous thromboembolism (VTE) in Asian patients with cancer are limited. Therefore, the present study assessed risk factors for VTE, including multiple blood biomarkers and risk scores consisting of several risk factors, in Japanese patients receiving anticancer drug therapy. In this single-center, prospective, observational study, 200 patients with six types of cancer were enrolled and followed for 1 year to observe the occurrence of symptomatic or asymptomatic VTE. The present study evaluated risk factors, Khorana and Vienna cancer and thrombosis study (CATS) scores at enrollment, and longitudinal data on various blood biomarkers. A Vienna CATS score of ≥3 was significantly associated with VTE occurrence (HR, 2.8; 95% CI, 0.9-8.7; P=0.045). In multivariable analysis, there was a significant association between VTE and the presence of pancreatic cancer (HR, 3.2; 95% CI, 1.1-8.8; P=0.028) and high soluble fibrin (HR, 3.7; 95% CI, 1.1-7.8; P=0.036). Covariate analysis using the propensity score also showed a significant association with hemoglobin dichotomized at <100 g/l (HR, 3.9; 95% CI, 1.1-14.0; P=0.034). Longitudinal data indicated that VTE was associated with soluble fibrin baseline values and an increase in D-dimer levels over time. The present results suggested that blood biomarkers are beneficial for predicting the risk of VTE in Japanese patients with cancer. The present study also provided novel evidence for the importance of measuring soluble fibrin in patients with cancer.

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Abbreviations: VTE, venous thromboembolism; CAT, cancer-associated thrombosis; RPM, risk prediction models; WBC, white blood cell count; Plt, platelet; Hb, hemoglobin; BMI, body mass index; sP-selectin, soluble P-selectin; SF, soluble fibrin; tPA/PAI-1, tissue plasminogen activator/plasminogen activator inhibitor type 1 antigens complex; F 1 + 2, prothrombin fragment 1 + 2; FDP, fibrin/fibrinogen degradation products; CATS, cancer and thrombosis study; PS, performance status; FEU, fibrinogen equivalent units; DDU, D-dimer units; ROC, receiver operating characteristic; PPV, positive predictive value; NPV, negative predictive value; OS, overall survival

Key words: neoplasms, VTE, risk factors, biomarkers, prospective studies

Introduction

Venous thromboembolism (VTE) is one of the most common non-tumor related causes of death in patients with cancer (1), so its early diagnosis and prevention are important for prolonging survival. Patients with cancer have a 4.1-fold higher risk of VTE than other patients, and the risk increases up to 6.5-fold with chemotherapy (2,3). Studies on risk factors for cancer-associated thrombosis (CAT) have been conducted mainly in Europe and North America, and risk prediction models (RPM) based on that evidence have been developed for stratifying patients with cancer according to their risk of VTE (4-8). CAT risk studies can be broadly divided into studies on occurrence risk and recurrence risk. The most well-known RPM for CAT occurrence is the Khorana score, which is based on site of cancer, white blood cell (WBC) count, platelet (Plt) count, hemoglobin (Hb) and/or use of erythropoiesis-stimulating agents, and body mass index (BMI) (4). Since publication of the original Khorana score, many studies have been conducted to improve it (9-11). Ay *et al* (7) developed an RPM incorporating the hemostasis biomarkers D-dimer and soluble P-selectin (sP-selectin) into the Khorana score.

In Asian patients with cancer, there are few prospective studies on risk factors for, and an RPM of, CAT occurrence (12-14). Although the risk of CAT is lower in Asian people than in other ethnic groups (15-17), VTE risk is much higher than usual in patients with metastatic cancer who are receiving chemotherapy, and VTE is one of the major causes of death among them (14,18). The occurrence of VTE can also interrupt or delay essential treatment, worsen quality of life, and increase the use of health care resources, including

hospitalization (19). Therefore, predicting the risk of VTE is an important issue in Asian cancer patients. In addition, measurement of blood biomarkers for prediction of CAT has been limited to a single point in time (often before treatment initiation) in most previous reports, and to our knowledge there is only one study with data measured repeatedly over time in cancer patients (20). Examining the relationship between longitudinal changes in blood biomarkers and the occurrence of CAT would allow us to understand the characteristics of the biomarkers and to consider the optimal timing for measuring them in clinical practice.

We therefore conducted a prospective observational study in Japanese patients undergoing anticancer drug therapy for advanced cancer. The purpose was to identify clinical characteristics and blood biomarkers that are risk factors for predicting CAT, and to propose an RPM based on these risk factors. The blood biomarkers included soluble fibrin (SF) and tissue plasminogen activator/plasminogen activator inhibitor type 1 antigens complex (tPA/PAI-1), for which there is still no evidence of an association with CAT. In addition, we investigated the association between longitudinal data on blood biomarkers and the occurrence of CAT.

Materials and methods

Patients and study design. This was a single-center, prospective, observational study (UMIN000026826) conducted at Saga University Hospital in accordance with the Declaration of Helsinki and with the approval of the Saga University Hospital Institutional Review Board (2016-12-05). From all study participants the informed written consent was obtained. The patient enrollment period was from March 2017 to March 2020, and the study population consisted of patients with unresectable cancer for whom anticancer drug therapy was planned. Inclusion criteria were as follows: patients with cancers of the pancreas, biliary tract, stomach, esophagus, colorectum, or lung; UICC classification stage III-IV or postoperative recurrence of cancer, and cancer not curatively resectable; scheduled by the attending physician to begin anticancer drug therapies, including chemotherapy, molecular targeted agents and immune checkpoint inhibitors; expected survival at least 3 months; written consent obtained; 20 years of age or older; and contrast-enhanced whole-body CT scan planned before and after the start of anticancer drug therapy (to ensure that the conditions for finding asymptomatic VTE, which is included among the current study endpoints, are as similar as possible). Exclusion criteria were as follows: history of VTE; treatment within 4 weeks prior to enrollment, where treatment could be any of radiation therapy (with the exception of palliative radiation therapies), anticancer drug therapy including adjuvant anticancer therapy, and surgery (with the exception of minor surgeries); ongoing anticoagulant use; active infection; pregnancy; history of lower extremity amputation; inability to perform contrast-enhanced CT due to impaired renal function or allergy to contrast media; or judged by the principal investigator to be unsuitable for this study.

At the time of enrollment, bilateral lower extremity venous vascular echocardiography was performed to assess the presence of pre-existing deep vein thrombosis (Fig. 1). If the D-dimer value measured at the time of registration was less than 1.2 μg/ml, the echo examination could be omitted (21). The presence of deep venous thrombus in the trunk was also assessed by contrast-enhanced CT for the evaluation of cancer disease at the time of registration.

As for blood biomarkers, WBC, Hb, and Plt were measured and included in the Khorana score, and sP-selectin and D-dimer were measured and included in the Vienna CATS score (Fig. 1). In addition to the above, we measured prothrombin fragment 1 + 2 (F 1 + 2) and SF as coagulation system markers, and fibrin/fibrinogen degradation products (FDP) and tPA/PAI-1 as fibrinolytic system markers. All biomarkers were measured at enrollment, and WBC, Hb, Plt, D-dimer, FDP, SF, and tPA/PAI-1 were measured additionally every month during the observation period.

For detecting asymptomatic VTE during the observation period, bilateral lower extremity venous echocardiography was performed to evaluate the presence of deep vein thrombosis when the physician judged that there was clinical progression of cancer. The presence of VTE in the trunk, including asymptomatic PE, was confirmed by using the results of whole body CT, which was performed to assess the state of the cancer. If the physician suspected VTE, imaging studies could be performed at any time. The occurrence of VTE was defined as an objective diagnosis using either contrast-enhanced CT or lower extremity venous echocardiography.

Considering the prognosis of patients with advanced cancer and the fact that VTE occurs most frequently during the first year after diagnosis (22), patients were followed until the end of a 1-year observation period, the occurrence of VTE, death, or difficulties in follow-up associated with best supportive care, whichever occurred first.

Outcome measures. The primary endpoint was the occurrence of VTE. The definition of VTE occurrence in this study was the objective confirmation of the diagnosis of symptomatic or asymptomatic VTE using the detection methods described above from the time of enrollment to the end of the observation period. WBC, Hb, and Plt values were taken from the earliest day of the month in which they were clinically examined. Plasma levels of D-dimer, FDP, SF, and tPA/PAI-1 were measured by latex immunoagglutination with the LPIA GENESIS D-dimer (cat. no. 756818; LSI Medience Corporation, Tokyo, Japan), LPIA FDP-P (cat. no. 753725; LSI Medience Corporation, Tokyo, Japan), IATRO SF II (cat. no. 757419; LSI Medience Corporation, Tokyo, Japan), and LPIA tPAI test (cat. no. 757211; LSI Medience Corporation, Tokyo, Japan). F 1 + 2 levels were measured by enzyme-linked immunosorbent assay using Enzygnost® F 1 + 2 (cat. no. 10445978; Siemens Healthcare Diagnostics, Marburg, Germany), according to the manufacturer's protocol. sP-Selectin levels were measured with the human sP-selectin Immunoassay (cat. no. DPSE00; R&D Systems, Minneapolis, MN) according to previous reports (23).

Data gathering and monitoring. To ensure data quality, data collection and regular monitoring for protocol compliance were performed at an independent clinical research center at Saga University Hospital.

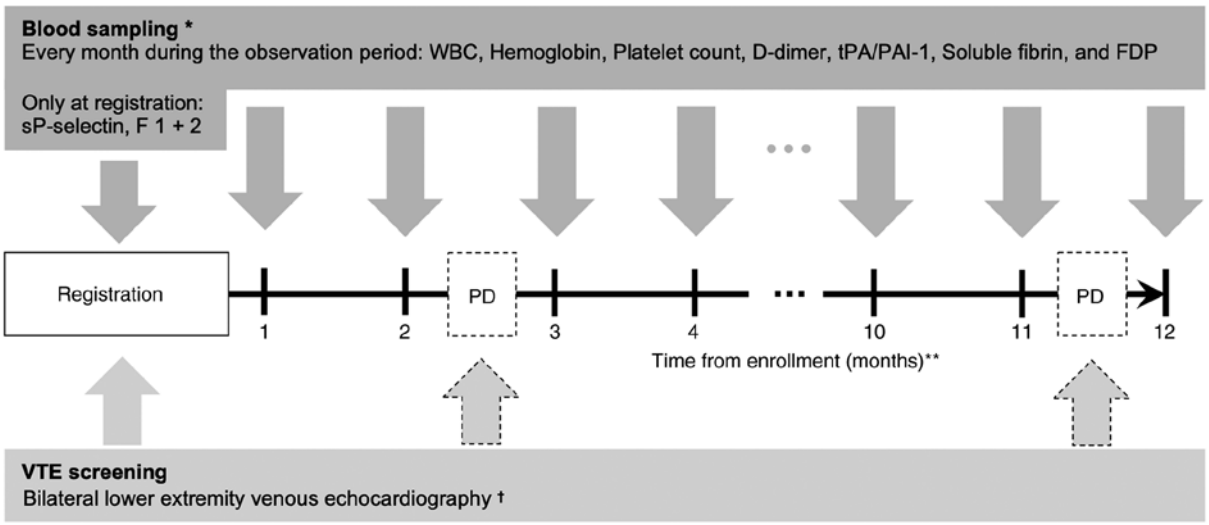


Figure 1. Outline of the study flow. *All blood tests for the present study were performed on blood samples collected along with blood collection for clinical use. **The patients were followed for up to 1 year. †To detect asymptomatic VTE, bilateral lower extremity venous echocardiography was performed at enrollment and at the time of cancer progression (time depending on the individual patient; examples shown in boxes with 'PD' and dashed-line boundaries). WBC, white blood cell count; tPA/PAI-1, tissue plasminogen activator/plasminogen-activator inhibitor complex; FDP, fibrin/fibrinogen degradation products; sP-selectin, soluble P-selectin; F 1 + 2, prothrombin fragment 1 + 2; PD, clinical progression of cancer judged by the attending physicians; VTE, venous thromboembolism.

Proposed RPM. Risk factors that were statistically significant in multivariable Cox regression analysis and in covariate analysis adjusted for the propensity score were used for our proposed RPM. Numerical values of the elements constituting the RPM were assigned by considering the hazard ratios in the above analyses. The RPMs were evaluated by generating receiver operating characteristic (ROC) curves and calculating sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for the internal cohort only.

Sample size. Since the difference in Khorana score between the high risk and low risk groups is smaller than the difference between groups defined by Vienna CATS score ≥3 and <3, detecting a significant difference in Khorana score between the high risk and low risk groups requires a larger sample size (7). To estimate the necessary sample size, we assumed that the occurrence proportion of VTE in the high risk and low risk groups based on the Khorana score is 17.7 and 1.5%, respectively [on the basis of previous studies (7)]. The number of cases required to detect this difference by a chi-square test or a Fisher's exact test with 80% power and 5% two-sided significance level is 220. Further allowing for dropouts, we considered that 240 patients would need to be enrolled.

Data analysis and statistical methods. As the principal analyses in this study, assessment of the relationship between the occurrence of VTE and the two risk scores, Khorana and Vienna cancer and thrombosis study (CATS), as well as the relationships of individual risk factors to the occurrence of VTE, were planned. For the Khorana score, occurrence proportion of VTE in the high risk group (score ≥3) was compared to that in the low risk group (score 0) with a Fisher's exact test. A secondary comparison was made between the high risk group (score ≥3) and the low + intermediate risk group (score 0-2) as well. For the Vienna CATS score, occurrence proportion of VTE in the group with a score ≥3 was compared to that in the group with a score <3 with a Fisher's exact test. In addition, to take into account censoring during the observation period, we constructed Kaplan-Meier plots that express the cumulative probability of VTE and preformed log-rank tests to compare the same groups as above. Single-variable and multivariable analyses by Cox proportional hazards model were used for calculating the VTE risk of individual risk factors as follows: the factors used in Cox proportional hazards model included clinical characteristics and multiple blood biomarkers, and some clinical factors that might have been confounders at the time of study enrollment: age, gender, BMI, ECOG performance status (PS), and Charlson comorbidity index (24). If previous studies had established a cutoff value, that value was used; otherwise, the 75th percentile in the current population was used. In the previous literature, the cutoff value of D-dimer is ≥1.44 μg/ml, which is reported in fibrinogen equivalent units (FEU) (6,7), and the D-dimer measurement method used in Japan is reported in D-dimer units (DDU). Therefore, because of the approximate relationship 2 x FEU=DDU (25,26), we used ≥2.88 μg/ml as the cutoff value for D-dimer.

As secondary analyses, considering that the number of variables that can be included in a Cox proportional hazards model in a conventional multivariable analysis is limited by the expected number of events, covariate analysis using propensity scores was performed to adjust for confounding factors and to calculate the VTE risk for individual factors. Moreover, longitudinal data analyses were performed by using the enrollment point values, final time-point values and average rate of change. To address missing values in longitudinal data, we used the average rate of change reflecting the values at all time points during the observation period, which was calculated as the slope of a linear trend with a mixed effects model for repeated measures. These parameters were compared between the VTE and non-VTE groups using Wilcoxon's rank-sum test. The level of statistical significance for all analyses was defined as $P<0.05$. Statistical analyses were performed with JMP Pro 15.2.0 software.

Table I. Baseline characteristics of patients (n=190).

Characteristics	Value
Median age, years (IQR)	69 (62-74)
Sex, n (%)	
Male	139 (73)
Female	51 (27)
Primary site of cancer, n (%)	
Lung	61 (32)
Stomach	44 (23)
Colorectal	34 (18)
Pancreas	26 (14)
Esophagus	15 (8)
Biliary tract	10 (5)
CCI, n (%)	
6	140 (74)
≥7	50 (27)
ECOG PS, n (%)	
0	105 (55)
1	71 (37)
Median BMI, kg/m^2 (IQR)	21.6 (18.7-23.7)
Anti-cancer therapy, n (%)	
Chemotherapy	170 (89)
Platinum-based Chx	122 (64)
MTA	94 (49)
Anti-VEGF mAb	69 (36)
ICI	33 (17)
Median laboratory values (IQR)	
WBC, x10^9/l	6.8 (5.3-8.6)
Hemoglobin, g/l	127 (110-138)
Platelet count, x10^9/l	254 (197-334)
D-Dimer, μg/ml	1.46 (0.79-3.14)
Soluble P-selectin, ng/ml	33.5 (26.9-43.4)
FDP, μg/ml	1.95 (1.20-3.63)
Soluble fibrin, μg/ml	3.2 (1.6-6.2)
tPA/PAI-1, ng/ml	18.0 (12.0-25.3)
F 1 + 2, pmol/l	261 (190-371)
Khorana score, n (%)	
Low: 0	18 (10)
Intermediate: 1-2	137 (72)
High: ≥3	35 (18)
Vienna CATS score, n (%)	
0	15 (8)
1	58 (31)
2	60 (32)
3	33 (17)
4	14 (7)
≥5	10 (5)

IQR, interquartile range; CATS, cancer and thrombosis study; CCI, Charlson comorbidity index; ECOG PS, Eastern Cooperative Oncology Group Performance Status; BMI, body mass index; Chx, chemotherapy; MTA, molecular targeted agents; mAb, monoclonal antibody; ICI, immune check point inhibitors; WBC, white blood cell count; FDP, fibrin/fibrinogen degradation products; tPA/PAI-1, tissue plasminogen activator/plasminogen-activator inhibitor complex; F 1 + 2, prothrombin fragment 1 + 2.

Table II. Overall occurrence of venous thromboembolism events (n=17).

Classification	No. of patients (%)
Types	
DVT alone	15 (88)
DVT + PE	2 (12)
Subtypes	
Distal lower extremity	10 (59)
Proximal lower and distal lower extremity	2 (12)
Internal jugular vein	2 (12)
Superior vena cava	1 (6)
Inferior vena cava	1 (6)
Superior mesenteric vein	1 (6)
Symptomatic or asymptomatic	
Symptomatic	5 (29)
Asymptomatic	12 (71)
Anticoagulant therapy	
Received	13 (77)
Edoxaban	11 (65)
Apixaban	1 (1)
Unfractionated heparin	1 (1)
Not Received	4 (24)

DVT, deep vein thrombosis; PE, pulmonary embolism.

Results

Patient characteristics and VTE events. In total, 200 patients were enrolled during the recruitment period. Among these patients, 10 were excluded from the analysis: two were judged unsuitable for assessing the risk of developing VTE due to loss of baseline blood samples (n=2); seven had VTE detected by the screening test at enrollment; and one had a change in pathological diagnosis from lung cancer to mesothelioma, a type of cancer that was not included in this study. Baseline characteristics and blood biomarker values at enrollment (n=190) are shown in Table I. The proportion of males was high (73%), as is typical with lung or esophagus cancer. Charlson comorbidity index was very high, ≥6 in all patients. With this index, metastatic solid cancer is counted as 6, and 1 or 2 represents complications (27% of patients in this study had Charlson index 7 or 8). Distributions of WBC, Hb, and Plt values were similar to those in the Vienna CATS score cohort in Austria (7), but D-dimer and sP-selectin tended to be higher and lower, respectively. BMI was much lower in our cohort. Ninety percent of the patients received chemotherapy as anti-cancer therapies, and 50% were treated with other anti-cancer drugs in addition to chemotherapy. During the observation period, 31% (n=58) and 9% (n=17) of patients were censored because active treatment was discontinued due to deterioration of the patient's general condition or death, respectively. Two patients were censored because anticoagulants were administered due to portal vein thrombosis. The maximum observation period was one year of planned observation; mean observation period was 265 days with median 365 days and 25th percentile 165 days.

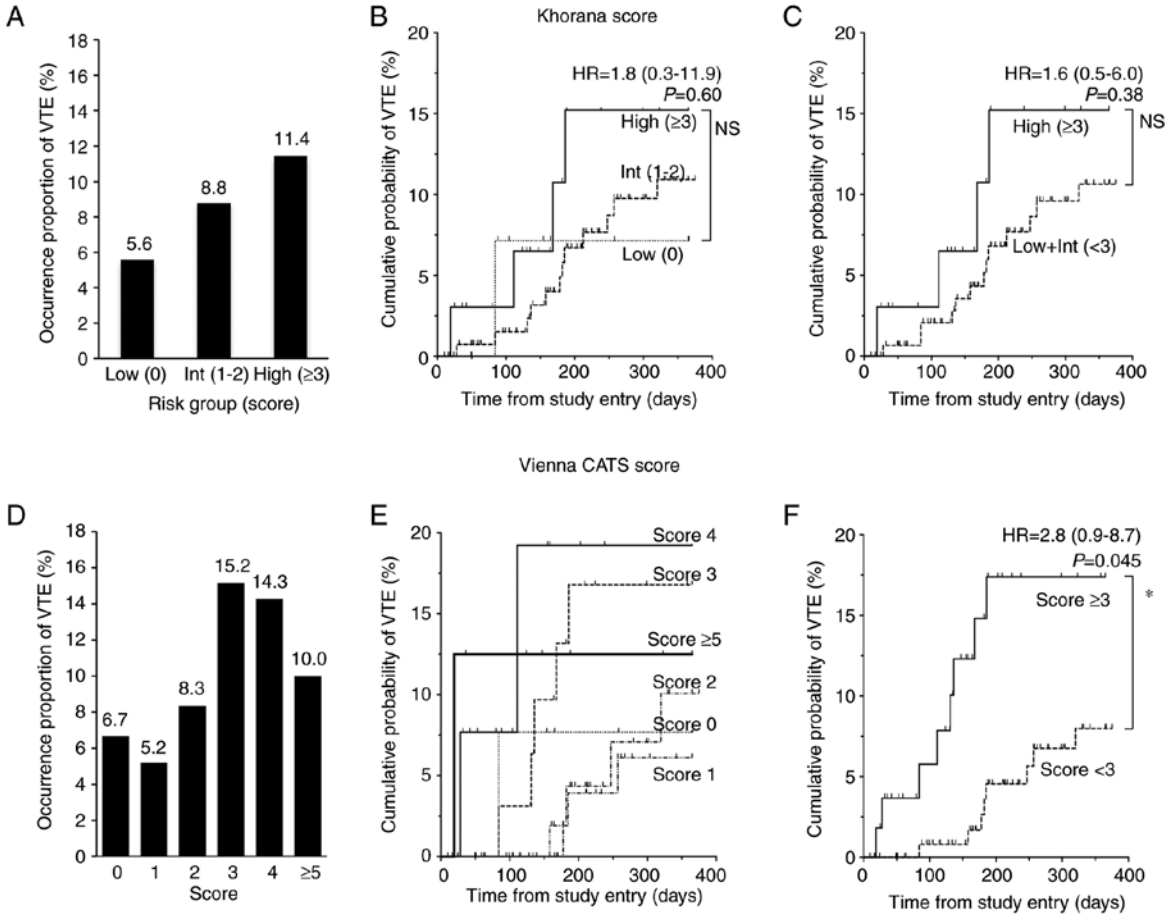


Figure 2. Occurrence proportion and cumulative probabilities of VTE for Khorana and Vienna CATS scores. Comparisons among risk groups based on the Khorana score are shown for (A) occurrence proportion of VTE, and (B and C) cumulative probability of VTE. Comparisons in (A and B) are among all three risk groups: Low, Int and High; the comparison in (C) is between High and Low + Int groups. (D-F) Analogous comparisons based on the Vienna CATS score. Comparisons in (D and E) are among all score levels and the comparison in (F) is between the group with a score <3 and the group with a score ≥3, the intermediate point of the scores. Data in (B, C and F) were analyzed using the log-rank test. No statistical comparisons were performed for (E). The Khorana score assigns 2 points for very high-risk cancer sites (pancreas, stomach) and 1 point for high-risk cancer sites (lung, ovary, bladder). One point is also assigned for each of the following: White blood cell count ≥11x10^9/l, hemoglobin <100 g/l or use of erythropoiesis-stimulating agents, platelet count ≥350x10^9/l, and body mass index 35 kg/m^2. The Vienna CATS score adds one point to the Khorana score if D-dimer ≥2.88 μg/ml (when using the fibrinogen equivalent unit test, the cutoff value is ≥1.44 μg/ml) or soluble P-selectin ≥53.1 ng/ml, respectively. *P<0.05. CATS, cancer and thrombosis study; HR, hazard ratio; Int, intermediate; Low + Int, combined low plus intermediate groups; NS, not significant; VTE, venous thromboembolism.

During the follow-up period, 17 (9%) of the 190 patients eligible for analysis developed VTE. Site of VTE and detailed information are shown in Table II. PE was complicated in 2 of these patients (12% of VTE events). Among the VTE events, the most common site of deep vein thrombosis was a distal lower extremity vein in 10 patients, and symptomatic VTE was observed in 5 patients. Anticoagulant therapy was administered to 13 of these patients.

Assessment of VTE risk using Khorana and Vienna CATS risk scores. At first, we evaluated whether the Khorana and Vienna CATS risk scores could predict VTE in our cohort (Fig. 2). The higher the Khorana score, the more frequently VTE occurred; occurrence proportion of VTE was about twice in the High risk group what it was in the Low risk group. However, a Fisher's exact test using a 2x2 contingency table with Low risk vs. High risk and with VTE vs. without VTE showed no statistically significant differences (P=0.44) (Table SI). A secondary analysis of low + intermediate risk (score 0-2) vs. High risk (score ≥3) was performed similarly but did not show a significant difference (P=0.38) (Table SI). A log-rank test (based on the Kaplan-Meier curve to account for data censoring) showed HR 1.8 (95% CI, 0.3-11.9; P=0.60) for High vs Low (Fig. 2B), and HR 1.6 (95% CI, 0.5-6.0; P=0.38) for High vs. Low + Int (Fig. 2C). Because the number of patients in the analysis did not reach the target number, it is possible that power was insufficient. With the Vienna CATS risk score, there was a trend toward higher occurrence proportion of VTE in patients with a score of ≥3 than in those with a score of <3, but the Fisher's exact test using a 2x2 contingency table with score ≥3 vs. score <3 and with VTE vs. without VTE was not significant (P=0.09) (Table SI). On the other hand, the log-rank test showed a statistically significant difference in cumulative probability of VTE between patients with a score of ≥3 and those with a score of <3, with an HR of 2.8 (95% CI, 0.9-8.7; P=0.045) (Fig. 2F).

Association between VTE occurrence and each clinical factor at baseline using single-variable and multivariable

Table III. Association between VTE occurrence and baseline characteristics of patients.

			Single-variable			Multivariable[b]			Propensity score adjustment		
Variable	No.	VTE%[a]	HR	95% CI	P-value	HR	95% CI	P-value	HR	95% CI	P-value
Age ≥65 years											
Yes	130	10	1.5	0.5-4.7	0.448				1.4	0.4-4.3	0.597
No	60	7									
Sex, female											
Yes	51	12	1.5	0.6-4.2	0.400				1.5	0.5-4.1	0.492
No	139	8									
BMI ≥25[c] kg/m^2											
Yes	31	13	1.4	0.4-4.2	0.593				1.7	0.5-5.9	0.416
No	159	8									
ECOG PS ≥1											
Yes	85	7	0.8	0.3-2.1	0.634				0.9	0.3-2.6	0.834
No	105	11									
CCI ≥7											
Yes	50	10	1.2	0.4-3.4	0.742				1.0	0.3-3.1	0.966
No	140	9									
Primary site: Lung											
Yes	61	5	0.4	0.1-1.3	0.118				0.4	0.1-1.3	0.117
No	129	11									
Primary site: Stomach											
Yes	44	7	0.8	0.2-2.6	0.647				0.7	0.2-2.6	0.634
No	146	10									
Primary site: Colorectal											
Yes	34	12	1.4	0.5-4.4	0.530				1.3	0.4-4.2	0.630
No	156	8									
Primary site: Pancreas											
Yes	26	23	4.0	1.5-10.8	0.007	3.2	1.1-8.8	0.028	4.4	1.5-12.3	0.006
No	164	7									
Primary site: Esophagus											
Yes	15	0	NE						NE		0.999
No	175	10									

Table III. Continued.

Variable	No.	VTE%[a]	Single-variable			Multivariable[b]			Propensity score adjustment		
			HR	95% CI	P-value	HR	95% CI	P-value	HR	95% CI	P-value
Primary site: Biliary tract											
Yes	10	10	1.3	0.2-6.0	0.818				1.2	0.2-9.1	0.865
No	180	9									

[a]The rate of VTE occurrence among patients in each category. [b]Enrolled for multivariable analysis if P<0.05. [c]Since there were no cases >35 kg/m^2, a cutoff of ≥25 kg/m^2 was used according to the definition of obesity in the classification of BMI in Asians. BMI, body mass index; CCI, Charlson comorbidity index; HR; hazard ratio; ECOG PS, ECOG Performance Status; NE, could not be estimated; VTE, venous thromboembolism.

analyses. Next, we examined the relationship between risk factors at the time of enrollment, including blood biomarkers, and subsequent VTE occurrence. Single-variable Cox regression analysis showed that pancreatic cancer (HR, 4.0; 95% CI 1.5-10.8; P=0.007), F 1 + 2 (HR, 2.8; 95% CI 1.0-7.2; P=0.041), and SF (HR, 3.6; 95% CI 1.4-9.4; P=0.008) were associated with increased risk of VTE during chemotherapy (Tables III and IV). With multivariable Cox regression analysis using the above three risk factors, statistically significant differences remained with pancreatic cancer (HR, 3.2; 95% CI, 1.1-8.8; P=0.028) and SF (HR, 3.7; 95% CI, 1.1-7.8; P=0.036) (Tables III and IV). To confirm the above results in analyses adjusted for confounding factors, covariate analyses were performed as secondary analyses using a propensity score calculated from six patients' background factors: age, gender, BMI, PS, Charlson comorbidity index, and site of primary lesion. The analysis with propensity score showed that pancreatic cancer (HR, 4.4; 95% CI 1.5-12.3; P=0.006) and SF (HR, 3.9; 95% CI 1.4-10.5; P=0.008) were associated with an increased risk of VTE during chemotherapy, with statistical significance (Tables III and IV). This analysis also showed a statistically significant increase in VTE risk with a binary indicator of Hb <100 g/l (HR, 3.9; 95% CI 1.1-14.0; P=0.034).

Longitudinal changes of biomarkers and VTE occurrence. In addition, we investigated the relationship between VTE occurrence and changes in blood biomarkers during the observation period. Among the seven biomarkers examined in this study, longitudinal patterns of SF, D-dimer, and FDP differed between the VTE and non-VTE groups (Figs. 3A and C, and S1). FDP and D-dimer showed similar patterns in the two groups. Therefore, SF and D-dimer values were compared between the two groups by using three parameters: value at the time of enrollment, value at the final time point, and average rate of change during the observation period (Fig. 3). SF showed a significant difference only at the time of enrollment (Fig. 3B). D-dimer showed significant differences at the final time-point and in average rate of change, but was not different at the time of enrollment (Fig. 3D).

Proposed RPM of VTE. On the basis of the results with individual risk factors, we propose an RPM including existence of pancreatic cancer, SF (>13.6 ng/ml), and Hb (<100 g/l) (Fig. 4A), with SF and Hb dichotomized at their respective cutoff values. We calculated the optimal cutoff value of SF for predicting development of VTE by using the ROC curve, and found that the sum of specificity and sensitivity was greatest when SF >13.6 ng/ml. In our RPM, each of the elements is assigned the same dichotomous value-'1' if present, '0' if absent, on the basis of their estimated hazard ratios being similar. The area under the curve (AUC) of the ROC curve for assessing performance of our RPM was 0.72 (95% CI 0.57-0.86), better for predicting VTE than the Khorana score (AUC=0.60; 95% CI 0.47-0.73) and Vienna CATS score (AUC=0.60; 95% CI 0.46-0.75) (Fig. 4B). When a score ≥2 was used as the cutoff value, the sensitivity was 35%, specificity was 97%, PPV was 50%, and NPV was 94% (Fig. 4C). A Kaplan-Meier plot showed that the group with score ≥2 had a much higher incidence of VTE than the other groups (Fig. 4D).

Table IV. Association between VTE occurrence and laboratory values at baseline.

			Single-variable			Multivariable[b]			Propensity score adjustment		
Variable	No.	VTE%[a]	HR	95% CI	P-value	HR	95% CI	P-value	HR	95% CI	P-value
WBC ≥11x10^9/l											
Yes	19	11	1.7	0.4-7.5	0.471				2.5	0.5-11.4	0.247
No	171	9									
Hemoglobin <100 g/l											
Yes	23	17	2.8	0.9-8.6	0.071				3.9	1.1-14.0	0.034
No	167	8									
Platelet count ≥350x10^9/l											
Yes	38	5	0.6	0.1-2.7	0.506				0.7	0.2-3.5	0.689
No	152	10									
D-dimer ≥2.88[c] μg/ml											
Yes	52	14	2.4	0.9-6.4	0.071				2.4	0.9-6.6	0.085
No	138	7									
sP-selectin ≥53.1 ng/ml											
Yes	20	10	1.9	0.4-8.2	0.402				1.9	0.4-8.6	0.433
No	170	9									
F 1 + 2 ≥358 pmol/l											
Yes	49	14	2.8	1.0-7.2	0.041	1.8	0.7-5.1	0.255	2.3	0.8-6.6	0.110
No	141	7									
Soluble fibrin ≥6.3[d] μg/ml											
Yes	47	17	3.6	1.4-9.4	0.008	3.7	1.1-7.8	0.036	3.9	1.4-10.5	0.008
No	143	6									
tPA/PAI-1 ≥26[d] ng/ml											
Yes	47	9	1.1	0.4-3.4	0.841				1.2	0.4-3.7	0.793
No	143	9									
FDP ≥3.7[d] μg/ml											
Yes	47	13	2.3	0.8-6.1	0.109				2.3	0.8-6.6	0.111
No	143	8									

[a]The rate of VTE occurrence among patients in each category. [b]Enrolled for multivariable analysis if P<0.05. [c]In the previous literature (6,7), the cutoff value of D-dimer is ≥1.44 μg/ml, which is reported in FEU, and the D-dimer measurement method used in Japan is reported in DDU. Therefore, because of the approximate relationship 2 x FEU=DDU (24,25), ≥2.88 μg/ml was used as the cutoff value for D-dimer. [d]≥75th percentile. HR, hazard ratio; WBC, white blood cell count; sP-selectin, soluble P-selectin; F 1 + 2, prothrombin fragment 1 + 2; tPA/PAI-1, tissue plasminogen activator/plasminogen-activator inhibitor complex; FDP, fibrin/fibrinogen degradation products; VTE, venous thromboembolism; FEU, fibrinogen equivalent units; DDU, D-dimer units.

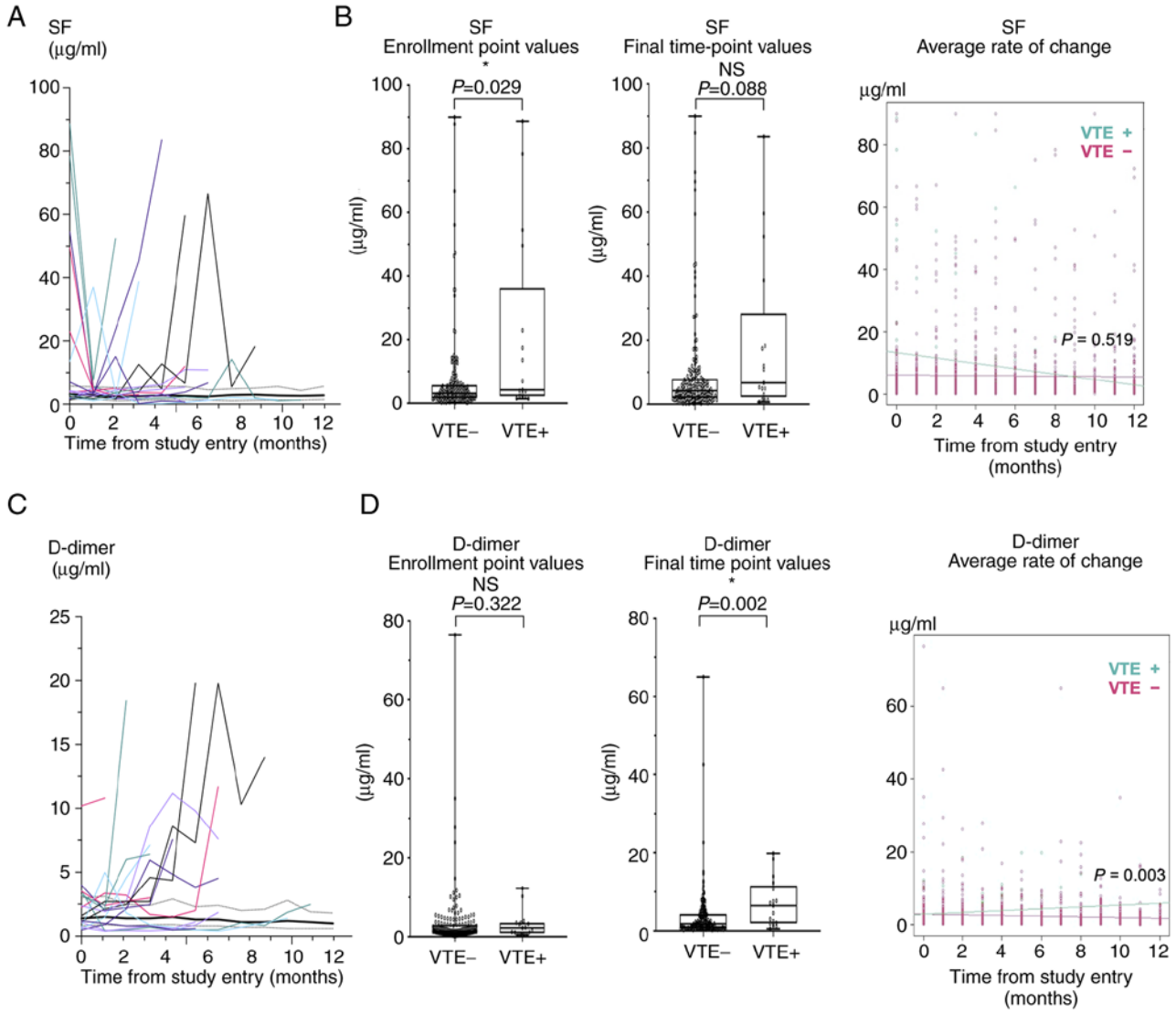


Figure 3. Longitudinal analysis of SF and D-dimer values. (A) SF levels and (C) D-dimer levels during the observation period. Colored lines represent data from patients who developed VTE. Data for patients who did not develop VTE are summarized by the median (bold lines) and quartiles (dashed lines). Comparison between patients who developed VTE (VTE+) and patients who did not (VTE-) using three parameters (enrollment point values, final time-point values and average rate of change) are shown based on the longitudinal values of (B) SF and (D) D-dimer. The final time-point value was defined as the value one point before the time when VTE was confirmed (if VTE occurred) or the last recorded value if VTE did not occur. The average rate of change was calculated using a mixed effects model for repeated measures. Dots represent the values of the longitudinal data for each group, and the gradient of the line represents the average rate of change calculated from those data. *P<0.05. NS, not significant; SF, soluble fibrin; VTE, venous thromboembolism.

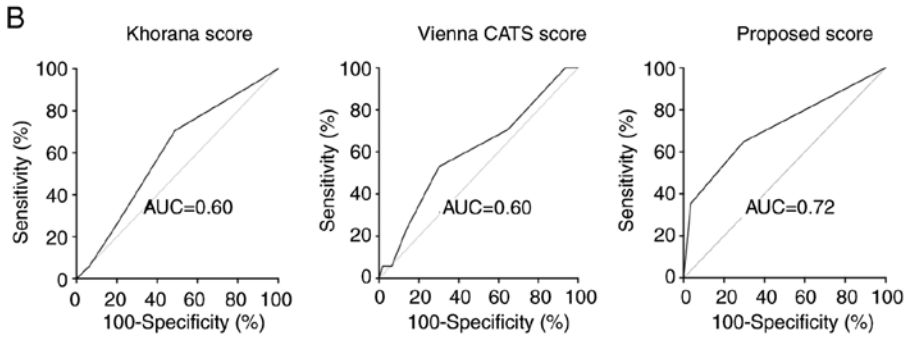
Discussion

There are few prospective studies in Asian patients on the risk factors for CAT, especially on blood biomarkers of CAT occurrence and RPM. In this prospective study with examination of seven blood biomarkers, we showed that Vienna CATS risk score ≥3 was associated with VTE occurrence in Japanese patients receiving chemotherapy for treatment of advanced cancer. Furthermore, pancreatic cancer, low Hb, and high SF were associated with CAT risk, suggesting that the inclusion of blood biomarkers in RPM may help improve the accuracy of predicting VTE occurrence even in persons of Asian ethnicity.

Asians have a lower risk of developing VTE than persons of Caucasian or Black ethnicity (15,16) and several studies have been conducted on the biological mechanisms that may be involved in this difference. Studies on coagulation factors related to VTE risk across races have reported that factor VIII levels are significantly higher in persons of Black ethnicity than in other ethnic groups (27) and that the factor V Leiden polymorphism and the factor II G20210A variant, which both increase VTE risk, are more common in Caucasians from northern and southern Europe (28). Recently, an increasing trend in VTE occurrence even in Asians has been reported (18,29), and cancer is the most critical risk factor for VTE, as in other races (30,31). However, studies of risk factors for CAT are particularly limited in Asians, with few prospective studies, apart from a study in China on the risk of CAT occurrence in the perioperative period among patients with cancer (n=262) (12) and a study in Korea on the risk of CAT occurrence in hospitalized patients with cancer (n=140) (13). There was also a retrospective study in Taiwan on CAT occurrence risk and RPM in patients with cancer, which used

A

Proposed score	
Element	Value
Pancreatic cancer	1
Hemoglobin <100 g/l	1
Soluble fibrin >13.6 μg/ml	1



C

	Khorana score		Vienna CATS score			Proposed score
Cut-off value	≥2	≥3	≥3	≥4	≥5	≥2
PPV	12.5%	11.4%	14.0%	12.5%	10.0%	50.0%
NPV	94.7%	91.6%	93.2%	91.6%	91.1%	93.8%
Sensitivity	70.6%	23.5%	47%	17.6%	5.9%	35.3%
Specificity	51.4%	82.1%	71.7%	87.9%	94.8%	96.5%

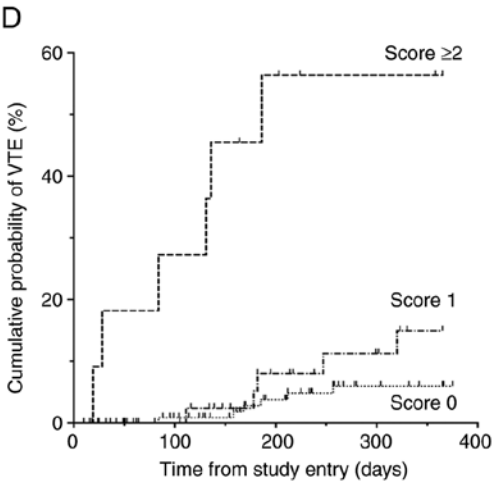


Figure 4. Proposed risk score for predicting VTE and its performance. (A) Parameters used for calculating our proposed score. (B) Receiver operating characteristic curves reflecting the VTE prediction performance of Khorana score, Vienna CATS score and our proposed score. (C) PPV, NPV, sensitivity and specificity for the occurrence of VTE with each of the three risk scores. (D) Cumulative probability of VTE based on the Kaplan-Meier curve for our proposed score in the present study population. A score of 0 was assigned if the patient had none of the elements of the score shown in (A); a score of 1 or 2 was assigned if the patient had one or two of the elements, respectively. AUC, area under the curve; CATS, cancer and thrombosis study; NPV, negative predictive value; PPV, positive predictive value; VTE, venous thromboembolism.

Taiwan's National Health Insurance Research Database (29), but only clinical parameters were used in those studies, not blood biomarkers. In prospective studies performed in Europe and North America, blood biomarkers including WBC count, Hb level, and Plt count (4,32), as well as hemostasis biomarkers such as D-dimer (6,7), F 1 + 2 (6), and sP-selectin (23,33), were evaluated. In the present study, having pancreatic cancer, low Hb levels, and high SF levels were associated with risk of CAT occurrence. The Khorana score incorporates Hb <100 g/l or the use of erythropoiesis-stimulating agents as risk factors. However, in Japan, erythropoiesis-stimulating agents are not used to treat anemia in patients with cancer, and there were no instances of their use in the present study. Therefore, our results indicate that Hb <100 g/l alone is a risk factor for CAT occurrence. SF, which is a hemostasis biomarker reflecting thrombin activation, as well as F 1 + 2 (Fig. 5), have been reported to be useful in diagnosis of VTE (34,35) and disseminated intravascular coagulation (36,37), as well as in predicting VTE after orthopedic surgery (38,39). The present study is the first to demonstrate the usefulness of SF in predicting CAT occurrence. In this study, patients received a variety of anti-cancer drug therapies (Table SII). Among these treatments, platinum-based chemotherapy and anti-angiogenesis agents in particular have been reported to confer a risk of VTE (40). We have not been able to eliminate the influence of all confounding

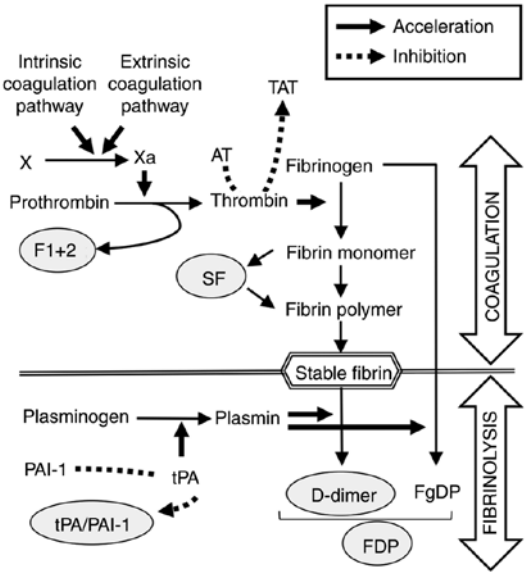


Figure 5. Coagulation and fibrinolysis systems. Coagulation and fibrinolysis biomarkers are illustrated in relation to other factors of the coagulation and fibrinolysis system. The circled biomarkers are those measured in the present study. SF, soluble fibrin; TAT, thrombin antithrombin III complex; F 1 + 2, prothrombin fragment 1 + 2; tPA/PAI-1, tissue plasminogen activator/plasminogen activator inhibitor type 1 antigens complex; PAI-1, plasminogen activator inhibitor type 1 antigens; FDP, fibrin/fibrinogen degradation products; FgDP, fibrinogen degradation product.

factors due to our sample size. However, at least there was no significant relationship between these drug types and either SF or Hb (Table SIII), the biomarkers that we identified as useful in the present study. On the other hand, the present study did not show a significant association of CAT occurrence with D-dimer, F 1 + 2, or sP-selectin. However, the hazard ratios for those markers were relatively large, and the lower bounds of their confidence intervals with both single-variable and multivariable analyses, shown in Tables III and IV, were close to, if not greater than, 1.0. That they are not statistically significant is considered to be due to the small number of cases. Moreover, the present study showed that the Vienna CATS score, which includes hemostasis biomarkers, was better at predicting CAT than the Khorana score, although the small number of events precluded sufficient validation. These results support a conclusion that including hemostasis biomarkers improves the accuracy of RPM in patients of Asian ethnicity who have cancer.

Data on longitudinal changes in biomarkers of hemostasis in patients with cancer is only available from an Austrian study that examined 112 patients with any of four cancer types (20). In that study, D-dimer values at the last blood-sampling time point before VTE onset in 14 patients who developed VTE tended to be higher than D-dimer values in patients without VTE. Similarly, in the present study we found that D-dimer was significantly higher at the final time point before VTE onset in patients who developed VTE than in patients without VTE. In addition, an increase in D-dimer during the observation period was associated with occurrence of VTE. In contrast, with SF the value of the point at enrollment, not at the time point before the onset of VTE, was significantly associated with occurrence of VTE. One reason for the difference between SF and D-dimer is that SF reflects the early phase of VTE whereas D-dimer reflects secondary fibrinolysis after thrombus formation (Fig. 5) (34,41). Another reason could be different lengths of times that these two biomarkers persist: SF decreases relatively quickly after thrombus formation, whereas D-dimer values remain high even for 7 days (35). In fact, SF quickly decreased after the point of estimated VTE occurrence in some patients (Fig. 3A). Therefore, we speculate that SF is more advantageous for revealing the hypercoagulable state before VTE formation, whereas D-dimer is more suitable for confirming the VTE state after thrombus formation has started. From this perspective, SF may be a better biomarker than D-dimer for predicting VTE. Based on these data, we proposed an RPM to estimate the risk of VTE among Japanese patients undergoing anticancer drug therapy. In some countries, prophylactic anticoagulation therapy is being considered for populations at high risk of VTE (42-44). However, cancer patients are known to be at higher risk of bleeding than the general population (45), so prophylactic use of anticoagulants should be made very cautiously. RPM should be easy to calculate in clinical practice and so should contribute effectively to clinical decision making. The present RPM is a simple score consisting of only three factors with high NPV and specificity, and may be clinically useful in identifying populations that do not require prophylactic anticoagulation, but it needs to be validated in other cohorts in the future. In post-hoc analyses, median overall survival (OS) of all patients was 747 days (Fig. S2A), and there was no statistical difference in prognosis between patients with vs. without VTE (Fig. S2B). This may be because we performed detection of asymptomatic VTE and therefore treatment for VTE was initiated early. Similar to previous reports that predictive scores for VTE are associated with OS in cancer patients (46,47), our proposed score was also associated with OS (Fig. S2C).

We acknowledge several limitations of this study. First, it was conducted at a single center with a relatively small number of patients, resulting in insufficient power to assess the Khorana scores and the Vienna CATS score adequately. However, a main objective of this study was to examine multiple blood biomarkers to predict the risk of VTE, and we obtained longitudinal data of blood biomarkers in each individual patient. We restricted our study to a single center to maintain quality control over the measurements of hemostasis biomarkers, as the time and conditions between blood collection and plasma separation can affect the results. A second limitation is that only six cancer types (lung, colorectum, pancreas, stomach, biliary tract, and esophagus) were included in the study; patients with other types of cancer that are considered to have a high risk of thrombosis, such as gynecological cancer and brain tumors, were not enrolled. In addition, it was difficult to adequately assess differences in usefulness of the biomarkers for each individual type of tumor given our sample size. Therefore, the usefulness of SF should be validated in further studies with more patients, including those with other types of cancer. The strengths of the present study are that we prospectively evaluated multiple blood biomarkers of risk for VTE, which is still scarce in Asian cancer patients, and we collected longitudinal data.

In conclusion, we showed that having pancreatic cancer, high SF, and Hb <100 g/l were significantly associated with VTE occurrence among Japanese patients undergoing anticancer drug therapy for cancer. Our study supports a conclusion that blood biomarkers can improve RPM performance in Asian cancer patients. In particular, our study suggests that SF could be a promising predictive factor for VTE in cancer patients, and further evaluation in other cohorts of Asians and other ethnic groups is expected in the future. Also, other biomarkers reflecting inflammation, such as CRP, may help predict thrombosis, and we would like to consider them in further studies.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YH, NSA, AS, AN, SO, MM, ES and SK were responsible for the conception and design of the study. YH, AS, AN and SO collected the data. YH, AK and NSA analyzed the data. YH and NSA drafted the manuscript. YH and NSA confirm

the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by Institutional Review Board of the Saga University Hospital (Saga, Japan), and all patients provided written informed consent. This observational study was registered at UMIN Clinical Trials Registry System, using identifier 000026826.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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