Tissue factor pathway inhibitor is associated with risk of venous thromboembolism and all-cause mortality in patients with cancer

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Abstract

Venous thromboembolism (VTE) is a common complication in patients with cancer. Data on the role of natural inhibitors of coagulation for occurrence of cancer-associated VTE are limited, thus, we investigated the association of tissue factor pathway inhibitor (TFPI) with risk of VTE and all-cause mortality in patients with cancer. Total TFPI antigen levels were measured with a commercially available enzyme-linked immunosorbant assay in patients included in the Vienna Cancer and Thrombosis Study, a prospective observational cohort study with the primary outcome VTE. Competing risk analysis and Cox regression analysis were performed to explore the association of TFPI levels with VTE and all-cause mortality. TFPI was analyzed in 898 patients (median age 62 years; interquartile range [IQR], 53-68; 407 (45%) women). Sixty-seven patients developed VTE and 387 died (24-month cumulative risk 7.5% and 42.1%, respectively). Patients had median TFPI levels at study inclusion of 56.4 ng/mL (IQR, 45.7-70.0), with highest levels in tumor types known to have a high risk of VTE (gastroesophageal, pancreatic and brain cancer: 62.0 ng/mL; IQR, 52.0-75.0). In multivariable analysis adjusting for age, sex, cancer type and stage, TFPI levels were associated with VTE risk (subdistribution hazard ratio per doubling =1.63, 95% confidence interval [CI]: 1.03-2.57). When patients with high and intermediate/low VTE risk were analyzed separately, the association remained independently associated in the high risk group only (subdistribution hazard ratio =2.63, 95% CI: 1.40-4.94). TFPI levels were independently associated with all-cause mortality (hazard ratio =2.36, 95% CI: 1.85-3.00). In cancer patients increased TFPI levels are associated with VTE risk, specifically in patients with high-risk tumor types, and with all-cause mortality.

Introduction

Venous thromboembolism (VTE) is a common complication in patients with cancer. The risk for VTE in this patient population is about 7-9-fold higher compared to the general population.[1-3] VTE is not only a major cause of morbidity but also a negative prognostic factor in patients with cancer.[4-7] The mechanisms of cancer-associated thrombosis are not completely understood. However, several hemostatic biomarkers have been found to predict risk of VTE in patients with cancer, for instance, procoagulant factors, such as tissue factor (TF), neutrophil extracellular traps (NETS), and podoplanin, have been implicated in the development of cancer-associated thrombosis.[8-11] In contrast, the association of natural inhibitors of hemostasis with cancer-associated thrombosis is currently unclear.

Tissue factor pathway inhibitor (TFPI) is a natural anticoagulant that inhibits complexes of tissue factor (TF) and factor VIIa via its Kunitz-1 domain and factor Xa via its Kunitz-2 domain. The majority (about 80%) of TFPI is bound to the endothelium, with the remaining 20% circulating freely (about 2%) or bound to low-density lipoproteins.[12] Furthermore, humans have a heparin-releasable and a platelet pool of TFPI.[13]

Conflicting results on the association of TFPI with VTE risk have been reported in the general population. Several case-control studies found an association between low levels of TFPI and risk of VTE.[14-17] However, these results were not replicated in a longitudinal study.[15] Therefore, it is unclear, if low TFPI levels in the case-control studies were the cause or the consequence of a VTE event. Further, it has been speculated that TFPI levels may be increased due

to binding to TF^+ extracellular vesicles (EV).[18,19] It is known that TF is expressed by cancer cells of various types and is thought to play a significant role in tumor growth and metastasis formation.[20,21] Thus, high TF levels are associated with poor overall survival in patients with various tumor types.[5,20,22]

In comparison to the non-cancer population, higher plasma TFPI levels were reported in patients with cancer and especially in those with metastatic disease.[23,24] It has been suggested that TFPI also plays a role in cancer progression in different tumor types.[25] Low expression of TFPI on tumor cells was found to be associated with cancer progression and poor overall survival.[26-28] However, the association of TFPI with risk of developing VTE and prognosis of the disease has not been studied in a broad population of patients with cancer. Therefore, we measured TFPI levels in a prospective cohort study of patients with cancer for prediction of VTE and risk of all-cause mortality.

Methods

Study population

This analysis was performed within the framework of the Vienna Cancer and Thrombosis Study (CATS). The study is a single-center, prospective observational cohort study including patients with newly diagnosed or recurrent cancer after full or partial remission. The study has been approved by the local ethics committee (EC no.: 126/2003, ethik-kom@meduniwien.ac.at) and was performed according to the Declaration of Helsinki and its later amendments. Detailed information about the study, its inclusion and exclusion criteria and the study procedures has been published in previous publications[29,30] and can be found in the *Online Supplementary Appendix*.

Laboratory analysis

Blood was drawn at study inclusion and collected in Vacutainer citrate tubes (Vacuette; Greiner-Bio One) by sterile venipuncture. Blood preparation is explained in the *Online Supplementary Appendix*.

Total TFPI antigen levels were measured with a commercial assay in citrate plasma samples according to the manufacturer´s instructions (Imubind total TFPI ELISA kit; American Diagnostica Inc., Stamford, CT, USA). EV-TF activity was measured according to standardized protocols for a chromogenic endpoint assay as reported in detail previously.[22,23] D-dimer levels were measured by a quantitative latex assay (STA-LIAtest D-DI; Diagnostica-Stago, Asnieres, France) on an STA-R analyzer (Diagnostica-Stago).[32] sP-selectin levels were measured using a human sP-selectin Immunoassay (R&D Systems; Minneapolis, MN) following the manufacturer's instructions as described previously.[30] Fibrinogen was determined according to Clauss (STA Fibrinogen; Diagnostica Stago, Asnieres, France; normal range: 180-390 mg/dL).[33] C-reactive protein (CRP) serum levels were determined by an immuno-nephelometric assay (Olympus Diagnostics; Southall, UK) on an AU 2700 chemistry analyzer.[34]

Statistical analysis

Statistical analyses were performed with STATA 17 (Stata Corp., Houston, TX, USA), SPSS 28.0 (IBM SPSS Statistics; Chicago, IL, USA) and R (Version 4.1.0; R Core Team). Standard summary statistics were used to report patient baseline characteristics (absolute frequencies, percentages, median, interquartile range [IQR, indicating the 25^{th} to 75^{th} percentile of the distribution of metric variables]). Medians between two groups were compared with a Mann-Whitney U test. Correlations were analyzed with a Spearman correlation coefficient. As death was considered as a competing event during follow-up time, VTE outcomes were studied in a competing risk framework.[35] In order to compare the cumulative VTE incidence between groups, a proportional sub-hazard regression model according to Fine and Gray was conducted. The association of TFPI levels with all-cause mortality was assessed in Cox regression analysis. A multivariable model including age, sex, cancer type and stage (stage 4 *vs*. stage 1, 2, 3) was conducted as well. For cancer type adjustment patients were stratified according to tumor type VTE risk category. Patients with pancreatic, gastroesophageal and brain tumors were considered to be at very high risk, those with breast and prostate cancer at low risk and all others to be at intermediate risk.[36] Furthermore, a model adjusting for EV-TF activity, D-dimer or sP-selectin levels was used for competing-risk and Cox regression analysis.

For a subgroup analysis patients were divided according to the baseline VTE risk according to the tumor type into high, intermediate, and low risk.[36] A ROC curve analysis with AUC computing was performed for the different VTE multivariable models to obtain the AUC values.

Results

Patient characteristics

TFPI was analyzed in 898 patients with available samples. The median age was 62 years (IQR, 53-68) and 407 (45%) patients were female. Patients were followed for a median follow-up of 22 months (IQR, 7-25). Overall, 67 patients (7.5%) were diagnosed with VTE (6-, 12-, and 24-month cumulative risk: 5.5%, 6.7%, 7.5%) and 387 patients (43.1%) died (6-, 12-, and 24-month cumulative risk: 15.1%, 27%, 42.1%) (Table 1). In detail, 27 patients were diagnosed with PE only, 31 with a DVT only, four with both PE and DVT, one with DVT and portal vein thrombosis, one with portal vein thrombosis, one with sinus vein thrombosis and two with catheter-related thrombosis as index event.

Distribution of tissue factor pathway inhibitor levels

Patients had median TFPI levels of 56.4 ng/mL (IQR, 45.7-70.0). The distribution of TFPI levels did not significantly differ between cancer types (Figure 1). Patients with metastatic disease had higher levels compared to those with non-metastatic disease (median 61.0 ng/mL; IQR, 50.0-74.0 *vs*. 50.4 ng/mL, IQR, 42.0-62.0; *P*<0.001). TFPI levels showed a weak positive correlation with CRP, fibrinogen, D-dimer, sP-selectin, and platelet counts, but not with EV-TF activity (Table 2).

Association between tissue factor pathway inhibitor levels and venous thromboembolism

Baseline levels of TFPI were associated with risk of VTE (subdistribution hazard ratio [SHR] per doubling of TFPI levels: 1.92, 95% confidence interval [CI]: 1.33-2.79). This association remained significant in multivariable analysis adjusting for age, sex, cancer type and stage (adjusted subdistribution hazard ratio [SHR] per doubling =1.62, 95% CI: 1.03-2.57). When comparing the 1-year and 2-year cumulative incidence of patients with TFPI levels above the 75th percentile (>70 ng/mL) to those with levels equal to or below the 75th percentile (≤70 ng/mL) a significant difference was observed (9.7% *vs*. 5.8%; 10.7% *vs*. 6.6%; *P*=0.04 for 12-month cumulative incidence; Figure 2). In a separate analysis adjusting for EV-TF activity, D-dimer, or sP-selectin levels, the association remained significant (EV-TF activity adjusted SHR per doubling of TFPI =2.00, 95% CI: 1.25-3.21; D-dimer adjusted SHR per doubling of TFPI =1.92, 95% CI: 1.29-2.85; sP-selectin adjusted SHR per doubling of TFPI =1.60, 95% CI: 1.08-2.38). When D-dimer (adjusted SHR per doubling of TFPI =1.62, 95% CI: 0.99-2.63) or sP-selectin (adjusted SHR per doubling of TFPI =1.42, 95% CI: 0.87-2.23) levels were added to the clinical covariates model the association was weakened significantly (Table 3).

In a smaller subgroup (n=291) EV-TF activity was available and the addition of this biomarker to the clinical multivariate model did not lead to a significant weakening of the association (adjusted SHR per doubling of TFPI =2.12, 95% CI: 1.20-3.43; Table 3).

In a subgroup analysis we stratified according to tumor type based VTE risk categories in high risk (brain, pancreas, gastroesophageal) and low/intermediate risk.[36] Patients with high VTE risk tumor types had the highest TFPI levels (62.0, IQR, 52.0-75.0). In the high-risk group we found an association between TFPI and risk of VTE (sex, age, and stage adjusted SHR per doubling of TFPI =2.63, 95% CI: 1.40-4.94), also after adding D-dimer to the clinical covariable model (adjusted SHR per doubling of TFPI =2.49, 95% CI: 1.34-4.62). This association was not observed when analyzing patients with low/intermediate-risk tumor types (sex and age adjusted SHR per doubling of TFPI =1.34, 95% CI: 0.68-2.63).

Association between tissue factor pathway inhibitor levels and all-cause mortality

The baseline level of TFPI was associated with risk of all-

Table 1. Details on cohort characteristics at study inclusion.

Characteristics	Available data N	Median (IQR) or N (%)
Age in years	898	62 (53-68)
Female	898	407 (45)
VTE	898	67 (7.5)
Died	898	387 (43)
Metastatic disease (solid tumor patients)	663	370 (56)
Non-classifiable into metastatic *vs.* not-metastatic (brain, hematological)	235	235 (26)
High-risk tumor type (brain, pancreas, gastroesophageal)	898	202 (22.5)
Intermediate-risk tumor type (all others)	898	439 (48.9)
Low-risk tumor type (breast, prostate)	898	257 (28.6)
TFPI levels, ng/mL	898	56.4 (45-70)
EV-TF activity, pg/mL	291	0.05 (0.01-0.10)
D-dimer, μg/mL	893	0.71 (0.35-1.38)
CRP, mg/dL	840	0.55 (0.17-1.87)
sP-selectin, ng/mL	898	43.5 (34-54.1)
Fibrinogen, mg/dL	894	394 (323-474.25)

IQR: interquartile range; VTE: venous thromboembolism; TFPI: tissue factor pathway inhibitor; EV-TF: extracellular vesicle-associated tissue factor; CRP: C-reactive protein.

cause mortality (hazard ratio [HR] =2.70, 95% CI: 2.21-3.03). In multivariable analysis (adjusting for age, sex, cancer type and stage), the association was weakened in magnitude but remained statistically significant (adjusted HR per doubling of TFPI =2.36, 95% CI: 1. 85-3.00). The 1-year and 2-year cumulative risk of all-cause mortality in patients with TFPI levels above the 75th percentile (>70 ng/mL) and those equal to or below the 75th percentile (≤70 ng/mL) was significantly higher (47.2% *vs*. 21.7%; 54.4% *vs*. 34.7%; *P*<0.001 for 12-month cumulative incidence; Figure 3). Further, upon adding D-dimer or sP-selectin levels to the clinical multivariable analysis (including sex, age, stage and cancer type) the association was weakened but remained significant (D-dimer adjusted HR per doubling of TFPI =1.56, 95% CI: 1.21-2.01; sP-selectin adjusted HR per doubling of TFPI =2.15, 95% CI: 1.67-2.76, respectively). In the subgroup with EV-TF activity levels available, the addition of this biomarker to the clinical multivariable model led to a weakened but significant association (EV-TF activity adjusted HR per doubling of TFPI =2.08, 95% CI: 1.42-3.04).

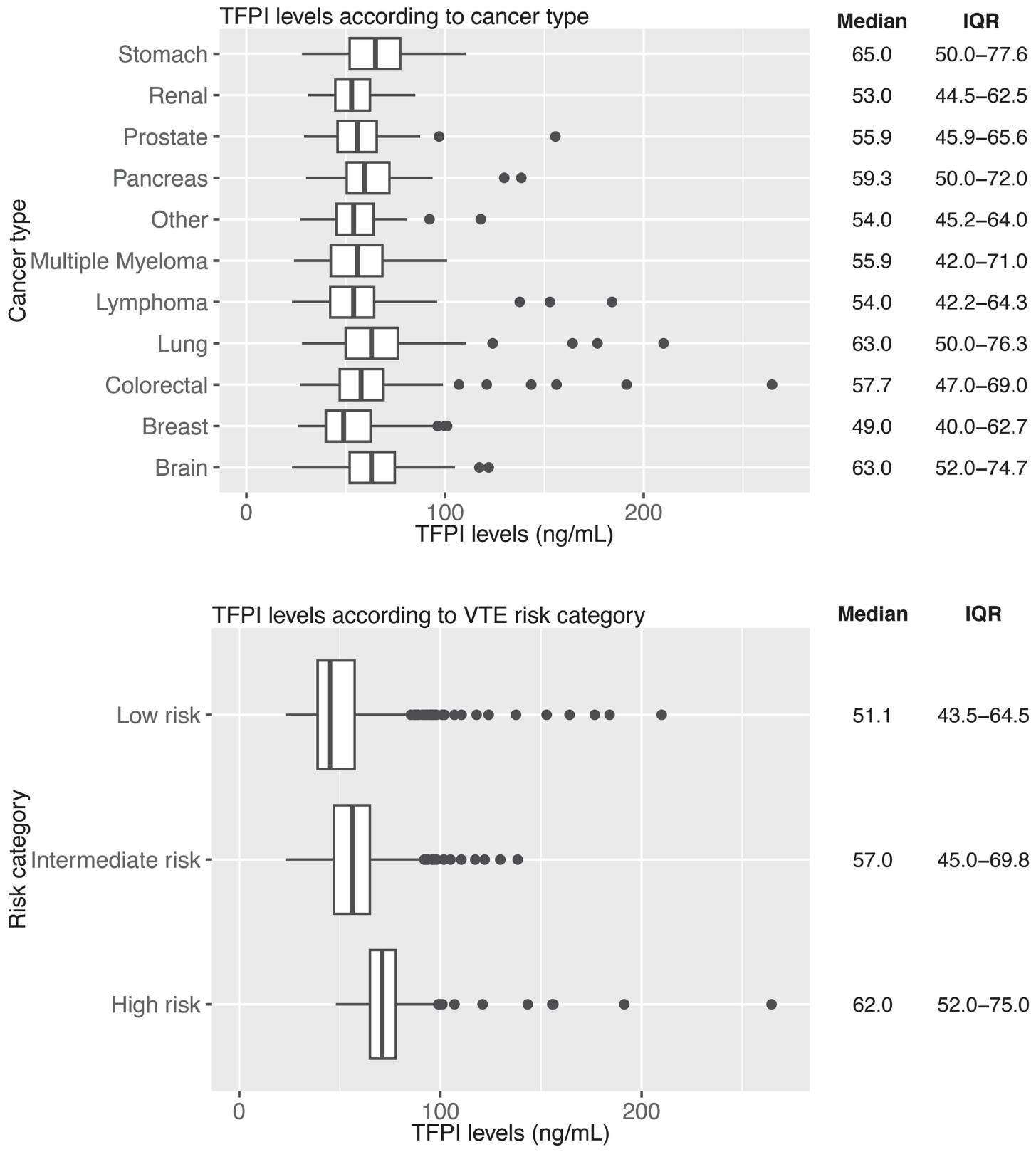

Figure 1. Tissue factor pathway inhibitor total antigen levels according to different cancer types and venous thromboembolism risk categories. (A) Cancer types. (B) Venous thromboembolism risk (VTE) categories. Category brain includes only primary brain tumors: glioma, medulloblastoma, meningioma. Category other includes esophageal, sarcoma, testis, hepatocellular, thymus, genitourinary, thyroidal, mesothelioma. High risk includes patients with brain, pancreatic and gastroesophageal cancer, low risk breast and prostate cancer and intermediate risk all others. Bold line represents median; upper and lower hinge represent third and first quartile, respectively; points indicate outliers. TFPI: tissue factor pathway inhibitor; IQR: interquartile range.

Discussion

In the present prospective cohort study of patients with cancer, we found an association between TFPI levels and cancer-associated VTE and all-cause mortality. In a subgroup analysis, we identified the association between TFPI levels and risk of VTE to be refined to the population of patients with tumor types at a high risk of VTE, but not in the intermediate/low-risk population.

It is counter intuitive that higher levels of an anticoagulant are associated with cancer-associated thrombosis. However, this may be, in part, due to a change in distribution of TFPI from the endothelium into the circulation during cancer progression. Indeed, we found higher levels of circulating TFPI in patients with metastatic disease compared to patients with non-metastatic disease. This is consistent with two other studies.[23,24] Patients with metastatic disease have a higher risk of VTE compared with patients with non-metastatic disease.[7,37] Similar findings, i.e., an association between higher levels and adverse outcomes, were reported in the setting of arterial thrombosis and atherosclerosis studies.[38]

The association remained beyond clinical co-variates such as age, sex, cancer stage, and type. However, upon adding D-dimer to this clinical co-variable analysis, an established predictive biomarker for VTE in patients with cancer,[32] the association between total TFPI levels and VTE did not remain statistically significant. A similar observation has been reported in a previous study of patients with acute deep vein thrombosis (DVT).[39] It has been suggested that the close interrelation between TFPI and D-dimer could be due to release of fibrin-bound TFPI and D-dimer from a fibrin clot, which could explain why TFPI is higher in patients with VTE.[39] However, in our study TFPI levels were not measured during an acute VTE event. Therefore, the prediction of future VTE events with higher levels cannot be completely explained by this hypothesis. D-dimer levels are elevated in patients with cancer even in the absence of a thrombotic event and indicate a general systemic hypercoagulable state. Higher D-dimer levels have been associated with poor survival and higher risk of all-cause mortality beyond its association with increased risk of VTE in patients with cancer.[6,32,40] One possible explanation for our observation could be that TFPI levels measured in our study are reflecting fibrin-bound TFPI that is co-released with D-dimer after activation of hemostasis and fibrinolysis, also in the absence of overt thrombotic manifestations. Interestingly, TFPI levels in our study correlated positively with D-dimer levels, albeit only weakly.

When adding sP-selectin to the clinical covariables, the association between TFPI levels and VTE was significantly weakened. sP-selectin was previously shown to be associated with an increased risk for cancer-associated VTE.[30] Thus, some of the circulating TFPI levels might be derived from activated platelets in patients with cancer.[41,42] This

Table 2. Correlation between tissue factor pathway inhibitor levels and markers of hemostasis and inflammation. Statistical analysis was performed with a Spearman correlation coefficient.

	r	95% CI	*P*
CRP	0.20	0.13-0.27	<0.001
Fibrinogen	0.15	0.08-0.22	<0.001
D-dimer	0.25	0.19-0.31	<0.001
EV-TF activity	0.09	-0.03-0.21	0.13
sP-selectin	0.23	0.17-0.30	<0.001
Platelet counts	0.26	0.01-0.14	0.03

CRP: C-reactive protein; EV-TF: extracellular vesicle-associated tissue factor.

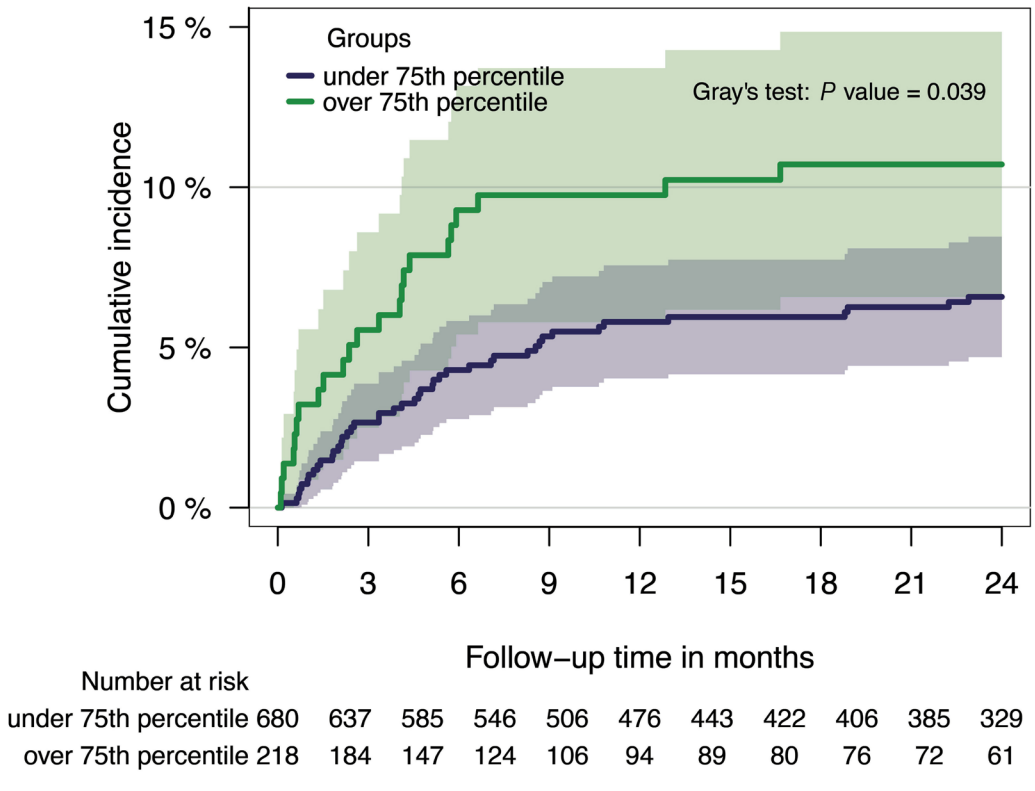

Figure 2. Cumulative venous thromboembolism incidence of patients (N=898) with tissue factor pathway inhibitor levels ≤75th (N=680) (≤70 ng/mL) *versus* >75th percentile (N=218) (>70 ng/mL). Patients were divided according to their tissue factor pathway inhibitor (TFPI) level and the group with levels equal to or under 70 ng/mL (≤75th percentile) was compared to the group with levels over 70 ng/mL (>75th percentile) within a Fine and Gray subdistribution hazard model, *P*=0.04.

hypothesis is supported by the fact that we observed a positive correlation between TFPI and sP-selectin levels. The endothelium is another potential source of the increased levels of circulating sP-selectin and TFPI.

Interestingly, the results differed when stratifying the association of TFPI levels with risk of VTE according to tumor site risk categories (i.e., high VTE risk *vs*. intermediate/low VTE risk tumor type groups). An independent association in the high-risk subgroup was seen, whereas this was not the case in the intermediate/low-risk group. This subgroup also had the highest TFPI levels. One explanation for this finding could be that patients with pancreatic cancer were allocated in the high-risk group. It is known that TF plays a role in VTE development in this tumor type.[5,20,43] Thus, it could be speculated that its inhibitor, TPFI, might be of importance in pancreatic cancer patients as well.

Based on the known systemic hypercoagulability and subclinical hemostatic activation in patients with cancer, our study provides a suitable framework to evaluate the interrelation of TFPI in the context of other biomarkers of hemostasis and inflammation. We found a positive and weak correlation between TFPI and CRP, fibrinogen, and sP-selectin. While some studies have reported positive correlations with various coagulation factors and hemostatic biomarkers (including e.g., FVIII and D-dimer), this was not replicated by others.[15,23,39,44] A weak positive correlation between TF and TFPI was described previously.[45] However, it is important to note that this study used a commercial enzyme-linked immunosorbant assay to measure TF antigen levels that was shown to not reliably detect TF antigen in plasma.[46] We did

Table 3. Association between tissue factor pathway inhibitor antigen levels and venous thromboembolism risk in different multivariable models.

	SHR (95% CI)	*P*	AUC (95% CI)
Model 1, N=872			71.5 (64.6-78.4)
TFPI (per double)	1.62 (81.03-2.579	0.04	
Age	0.99 (0.98-1.0)	0.59	
Sex	1.18 (0.71-1.96)	0.53	
Cancer stage	1.34 (0.79-2.26)	0.28	
Cancer type	2.12 (1.50-2.98)	<0.001	
Model 2, N=291			68.5 (59.9-77.1)
TFPI (per double)	2.00 (1.25-3.21)	0.004	
EV-TF activity	0.66 (0.16-2.74)	0.56	
Model 3, N=893			62.1 (55.6-68.6)
TFPI (per double)	1.92 (1.29-2.85)	0.001	
D-dimer	1.01 (0.97-1.04)	0.67	
Model 4, N=898			66.6 (59.6-73.6)
TFPI (per double)	1.60 (1.08-2.38)	0.019	
sP-selectin	1.01 (1.00-1.02)	0.014	
Model 5, N=291			68.5 (59.9-77.1)
TFPI (per double)	2.12 (1.20-3.73)	0.009	
Age	0.98 (0.96-1.00)	0.09	
Sex	1.46 (0.74-2.88)	0.25	
Cancer type	1.15 (0.57-2.29)	0.7	
Cancer stage	1.35 (0.91-2.00)	0.14	
EV-TF activity	0.64 (0.14-2.83)	0.55	
Model 6, N=867			71.5 (64.7-78.4)
TFPI (per double)	1.62 (0.99-2.63)	0.051	
Age	0.99 (0.98-1.01)	0.60	
Sex	1.17 (0.70-1.97)	0.54	
Cancer type	1.34 (0.79-2.27)	0.28	
Cancer stage	2.11 (1.50-2.98)	0.21	
D-dimer	1.00 (0.96-1.04)	0.93	
Model 7, N=872			72.3 (65.3-79.2)
TFPI (per double)	1.42 (0.90-2.23)	0.14	
Age	0.99 (0.98-1.01)	0.64	
Sex	1.190 (0.71-2.00)	0.51	
Cancer type	1.33 (0.79-2.25)	0.28	
Cancer stage	2.11 (1.50-2.96)	<0.001	
sP-selectin	1.01 (1.00-1.02)	0.061	

SHR: subdistribution hazard ratio; CI: confidence interval; AUC: area under the curve; TFPI: tissue factor pathway inhibitor; EV-TF: extracellular vesicle-associated tissue factor.

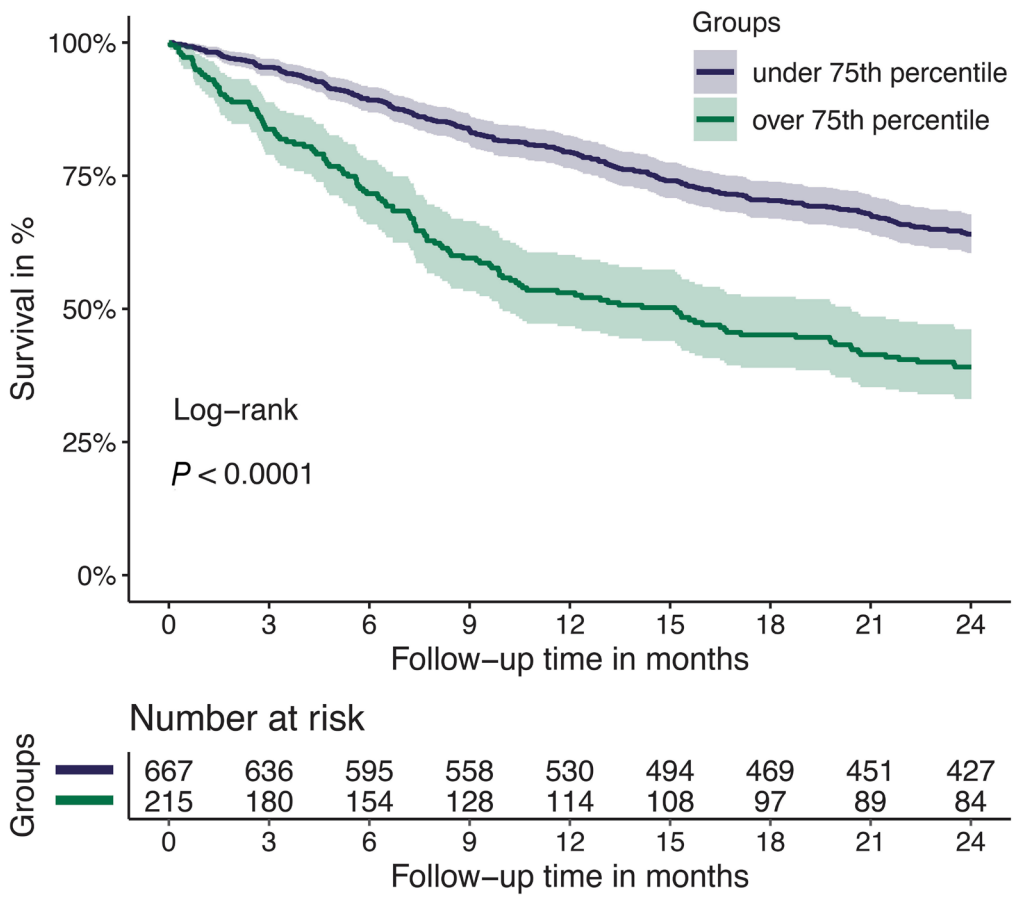

Figure 3. Overall survival of patients (N=898) with tissue factor pathway inhibitor levels ≤75th (N=680) (≤70 ng/mL) *versus* >75th percentile (N=218) (>70 ng/mL). Patients were divided according to their tissue factor pathway inhibitor level and the group with levels equal to or under 70 ng/mL (≤75th percentile) was compared to the group with levels over 70 ng/mL (>75th percentile) within a Kaplan-Meier analysis and with a log-rank test, *P*<0.001.

not find an association between TFPI and EV-TF activity. However, our power for this analysis was low as EV-TF was not measured in all patients, leaving us with 34 VTE events in this subgroup. Overall, our results may indicate that TFPI is a marker of disease severity and this could explain the opposing results reported previously due to different time points of measurements and patient populations.

TFPI was reported to play a role in the cancer environment as well.[25] High levels were described to have a favorable effect and absence of TFPI may propagate cancer progression in experimental studies.[26-28,45] Important to note is that previously no correlation between tumor TFPI and plasma TFPI levels could be established.[45] This could explain why divergent results are reported, namely that high plasma TFPI levels were associated with poor overall survival in our cohort of cancer patients.

With respect to the association between TFPI and risk of mortality, we only observed a slightly weakened association when adding D-dimer, EV-TF activity or sP-selectin levels to the clinical co-variable model in multivariate analysis. The association of TFPI and mortality risk might be explained by increased plasma levels of TFPI mediated through pathways involving activation of the hemostatic system, endothelium and/or inflammation, which in return might be involved in cancer-promoting mechanisms or adverse clinical outcomes. Interestingly, we previously found an association between higher levels of antithrombin, another natural anticoagulant, and all-cause mortality in patients with cancer, specifically in those with brain tumors.[47]

Elevated EV-TF activity levels were reported to be associated with increased VTE risk and in specific cancer types with poor overall survival.[5,20,48] Interestingly, in our cohort, TFPI levels were higher in patients with metastatic disease, similarly to higher TF levels that were previously reported.[20] Our observation that adding EV-TF activity to the multivariate analysis did not influence the association of TFPI and VTE risk, could indicate that TFPI might have a better predictive capacity than TF in a heterogeneous cohort of cancer patients. Important to note is that this was only a subgroup analysis as EV-TF activity was available in a small group of patients. Furthermore, these patients were mostly the ones with a high VTE risk tumor type, of which especially patients with pancreatic tumors are known to have elevated EV-TF activity.[22] Thus, TFPI could act as a surrogate marker, as the physiological response to elevated TF levels could be an increase in TFPI levels to maintain the hemostatic balance as proposed previously.[49] In addition, EV captured from plasma using an anti-TF antibody were shown to contain TFPI.[18] Further, it was reported that TF activity of EV isolated from plasma is increased in the presence of an anti-TFPI antibody, thus, this data suggests that TFPI is bound to TF^+ EV.[19]

TF is a biomarker that was found to have predictive and prognostic capacity in patients with cancer, most pronounced in patients with pancreatic cancer.[5] However, most of the methods purported to measure TF in plasma are not reliable or are highly variable and, therefore, cannot be used in the clinic.[50] Thus, TFPI might be more reliable and reproducible to measure in the clinical setting. Generally, hemostatic biomarkers that have a predictive or prognostic potential are of interest in oncological research. Various markers have shown to have this potential and might aid in personalized clinical decision making in oncology.[6]

The study had some limitations. Firstly, there is no stan-

dardized TFPI measurement. However, we decided to measure total TFPI antigen levels with a widely used commercial enzyme-linked immunsorbant assay in plasma samples. Second, EV-TF activity levels were not available in all patients and there was some missing data. Third, we could not investigate endothelial activation due to the lack of an adequate marker. Furthermore, prophylactic heparin injections were not an exclusion criterion in our study, and heparins were shown to lead to a transient but short increase of TFPI plasma levels. However, only a very small proportion (<5%) of patients received prophylactic heparin injections 12-24 hours before blood sampling. Lastly, our analysis was exploratory in nature. We used a predefined cutoff (75th percentile of TFPI levels of the whole cohort) for elevated levels and thus, in the future validation and further investigations of the causality of our findings are needed.

To conclude, TFPI levels are associated with risk of VTE and all-cause mortality in a prospective cohort of patients with cancer. TFPI levels might represent a surrogate marker for hemostatic activation and thus should be further investigated in independent studies.

Disclosures

MP has received honoraria for lectures, consultation or advisory board participation from the following companies: Bayer, Bristol-Myers Squibb, Novartis, Gerson Lehrman Group (GLG), CMC Contrast, GlaxoSmithKline, Mundipharma, Roche, BMJ Journals, MedMedia, Astra Zeneca, AbbVie, Lilly, Medahead, Daiichi Sankyo, Sanofi, Merck Sharp & Dome, Tocagen, Adastra, Gan & Lee Pharmaceuticals and Servier.

Contributions

CE and CA developed the concept of the study. SK and JT performed measurements. CE and FM performed data analysis. NM and IP supervised the project. MP, IP and CP recruited patients. All authors drafted and revised the manuscript.

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Data-sharing statement

The data presented in this study are available on request from the corresponding author.

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