Venous thromboembolism, coagulation and cancer
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Microparticle-associated tissue factor activity in cancer patients predicts the risk for developing venous thromboembolism


Submitted for publication
ABSTRACT
Procoagulant microparticles (MP) have been observed in cancer patients with venous thromboembolism (VTE). This study evaluates the predictive value of the MP-associated procoagulant activity for development of VTE in cancer patients. The procoagulant activity was measured by (i) a phospholipid-dependent coagulation test (PPLT), (ii) a factor Xa-generation assay and (iii) a fibrin generation test (FGT). The last two tests were also performed in the presence of anti-tissue factor (TF; factor Xa-generation assay) or anti-factor VII(a) (FGT). Plasma was collected of 43 unselected cancer patients. Five patients (12%) developed VTE within six months. No difference was observed in the PPLT at baseline between patients with and without VTE (p=0.519). Marked differences, however, were present in the FGT in the absence of antibodies (p=0.014), and in TF-dependent factor Xa generation and fibrin generation (p=0.016 and p=0.036, respectively). Receiver operating characteristic analyses showed that the FGT had the highest area under the curve (0.83; 95% CI 0.68-0.98; p=0.017). A cut off level of 909 seconds resulted in a sensitivity of 80% and a specificity of 84%. We conclude that the MP-associated procoagulant activity may identify those cancer patients at high risk for development of VTE.
INTRODUCTION
Venous thromboembolism (VTE) often complicates the clinical course of cancer [1]. It is estimated that almost 15% of all cancer patients develop VTE in the course of their disease [2]. In addition, the risk of recurrent thrombotic events after the first episode of venous thrombosis is significantly higher in patients with cancer compared to patients without cancer [3]. Despite the fact that VTE is the second cause of death in cancer patients [4], it is still difficult to identify those patients who have such a high risk that primary thromboprophylaxis is warranted [5].

Several factors including surgery, central lines, infection, immobilization, chemotherapy, hormonal agents and cancer cells themselves contribute to the hypercoagulable state in cancer patients [6]. In 1995, a strong association was noticed between malignant disease and elevated levels of circulating non-cell bound tissue factor (TF), activated factor VII, thrombin-antithrombin complexes (TAT) and prothrombin fragment F1+2 compared to healthy subjects [7]. At least part of this “blood-borne” TF is associated with cell-derived microparticles (MP). Although the cellular origin of circulating TF-bearing MP in cancer patients is still debated, a recent study by Davila and co-workers showed that at least a part of the circulating MP-exposed TF may originate directly from the cancer cells [8].

There is increasing evidence that circulating MP in cancer patients are associated with VTE. Plasma from cancer patients with VTE contains elevated levels of MP-associated TF compared to those without VTE, and patients with a low likelihood of survival had a high MP-associated TF activity [9;10]. More recently, an association was shown between development of VTE and the plasma levels of non-cell bound TF and MP-associated TF activity in eleven pancreatic cancer patients [11]. Based on these observations, we hypothesized that circulating TF-bearing MP in cancer patients may be a predictive biomarker for the development of VTE.

The aim of this pilot study was to assess the ability of three new rapid coagulation assays to identify patients at risk for developing VTE, i.e. by measuring the procoagulant- and/or TF-activity associated with circulating MP in cancer patients before the onset of VTE.
MATERIAL AND METHODS

Population
Blood samples were collected from 43 consecutive cancer patients seen at the Department of Medical Oncology of the Academic Medical Center in Amsterdam. Inclusion criteria were age above 18 years and active cancer, and in all patients the diagnosis of cancer was confirmed by pathology. Patients using anticoagulant treatment or with a VTE in the medical history were excluded. The incidence of venous thrombosis was assessed after six months. Furthermore, blood samples were collected from 22 healthy individuals. All patients and healthy individuals signed an informed consent and the protocol was approved by the institutional review board.

Collection of blood samples
Patient blood was collected via a venous catheter (20-gauge). All blood samples were directly taken from the catheter without the use of a connecting-piece. After discarding the first tube of blood, blood (13.5 mL) was collected into three tubes each containing 0.5 mL 105 mmol/L buffered sodium citrate (BD, Franklin Lakes, NJ). Citrate-anticoagulated blood samples from healthy individuals (n=22) were taken from the antecubital vein without tourniquet through a 21-gauge needle using a vacutainer system. Within 15 minutes after blood collection, cells were removed by centrifugation for 20 minutes at 1550 x g and 20 °C. Part of the plasma was immediately used for experiments. The remainder was immediately frozen as 0.25 mL aliquots in liquid nitrogen and stored at -80 °C.

Isolation of microparticles for flow cytometry
A sample of 250 µL frozen plasma was thawed on melting ice for one hour and centrifuged for 30 minutes at 18.890 x g and 20 °C to pellet MP. After centrifugation, 225 µL of the supernatant was removed. The pellet and remaining supernatant were resuspended in 225 µL phosphate-buffered saline (PBS) containing citrate (154 mmol/L NaCl, 1.4 mmol/L phosphate, 10.9 mmol/L trisodium citrate; pH 7.4). After centrifugation for 30 minutes at 18.890 x g and 20 °C, 225 µL of the supernatant was removed. The MP pellet was then resuspended with 75 µL PBS-citrate. Five µL of the MP suspension was diluted in 35 µL CaCl₂ (2.5 mmol/L)-containing PBS. Then 5 µL APC-labelled annexin V was added to all tubes plus 5 µL of the cell-specific monoclonal antibody or isotype-matched control antibodies. The samples were incubated in the dark for 15 minutes at room temperature.
After incubation, 900 μL of calcium-containing PBS was added to all tubes (except to the annexin V control, to which 900 μL citrate-containing PBS was added). Samples were analyzed for one minute in a fluorescence automated cell sorter (FACS Calibur) with CellQuest software (Becton Dickenson Biosciences, San Jose, CA, USA). Both forward scatter (FSC) and sideward scatter (SSC) were set at logarithmic gain. MP were identified on basis of their size and density and on their ability to bind cell-type specific antibodies and annexin V [12]. The gate settings were confirmed using beads with a maximum diameter of 1.0 μm. Background signal in a typical experiment accounted for 3-5% of the total signal. The within-run coefficient of variation is 8% and the day-to-day coefficient of variation, based on analysis of MP in a standardized normal pool sample, 13%. Annexin V measurements were corrected for auto-fluorescence. Labelling with cell-specific monoclonal antibodies was corrected for identical concentrations of isotype-matched control antibodies by subtracting the amount of isotype-matched positive events from the total positive events [13]. The antibodies against fluorescein isothiocyanate (FITC)-labelled IgG1 and phycoerythrin (PE)-labelled IgG1 and anti-TF-PE were derived from BD. Anti-CD61-FITC (anti-GP-IIIa) was obtained from Dako (Glostrup, Denmark). Allophycocyanin (APC)-conjugated annexin V was purchased from Caltag (Burlingame, CA, USA).

Fibrin generation test (FGT)
The ability of MP to generate fibrin was measured directly in plasma in the absence or presence of anti-human FVIIa (Sanquin; Amsterdam, The Netherlands). After pre-incubation for 5 minutes at 37 °C, clotting was initiated by addition of CaCl2. Fibrin formation was determined by measuring the optical density (λ = 405 nm) in duplicate on a spectrophotometer (SPECTRAmx microplate reader; Molecular Devices Corp., Sunnyvale, CA) at 37 °C.

STA Procoag PLL assay
MP-containing plasma (25 μL) was mixed with phospholipid-free plasma (25 μL) and pre-heated for 2 minutes at 37 °C in a cuvette. Then, pre-warmed XACT reagent (100 μL; containing activated factor X and calcium) was added and the clotting time was measured on an ACL Top (Instrumentation Laboratory Company; Lexington, MA, USA). The assay was a gift from Dr. B. Woodhams (Stago; Gennevilliers Cedex, France).
The total procoagulant activity and TF activity assay

TF was measured using a procoagulant activity (PCA) assay exactly as described earlier [11]. In brief, MP were pelleted from plasma (200 μL) by centrifugation (20,000 x g at 15 minutes and 4 °C), washed twice with HBSA buffer (137 mmol/L NaCl, 5.38 mmol/L KCl, 5.55 mmol/L glucose, 10 mmol/L HEPES, 0.1% bovine serum albumin; pH 7.5), and re-suspended in HBSA (200 μL). Samples were incubated with either anti-human TF-antibody called HTF-1 (4 μg/mL) or a control antibody (mouse IgG: 4 μg/mL) for 15 minutes at 25 °C, and then aliquots (50 μL) were added to duplicate wells of a 96-well plate. Next, 50 μL of HBSA containing FVIIa (10 nmol/L), FX (300 nmol/L) and CaCl₂ (10 mmol/L) was added to each sample and the mixture was incubated for 2 hours at 37 °C. FXa generation was stopped by addition 25 mmol/L EDTA buffer (25 μL) and chromogenic substrate S2765 (25 μL; 4 mmol/L), and incubated at 37 °C for 15 minutes. Finally, absorbance was measured at 405 nm using a VERSAmax microplate reader (Molecular Devices Corp.; Sunnyvale, CA, USA). TF activity was calculated by reference to a standard curve generated using relipidated recombinant human TF (0-55 pg/mL). The TF-dependent FXa generation (pg/mL) was determined by subtracting the amount of FXa generated in the presence of HTF-1 from the amount of FXa generated in the presence of the control antibody. Mean TF PCA in healthy controls was 0.21 pg/mL (SD 0.11; interassay CV of 21%).

Other assays

All other assays were performed as described by the manufacturer. ELISA kits to measure human non-cell bound (soluble) TF were obtained from American Diagnostica (Greenwich, Conn, USA), F₁⁺₂ and TAT from Enzygnost (Dade Behring; Marburg, Germany) and D-dimer from Innovance (Dade-Behring). The kits to determine factor VIIa-antithrombin complexes (FVII-AT) were a gift from Dr. B. Woodhams (Stago; Gennevilliers Cedex, France).

Statistics

Continuous data were expressed as medians with corresponding inter-quartile ranges (IQR). Between group differences were tested with the Mann-Whitney U test. Categorical data are presented as percentages or numbers. All data shown are presented as medians (IQR), unless stated otherwise. Receiver operating curve (ROC) analyses were performed to determine- the best predictive value of the different assays for development of VTE.
in cancer patients. P-values ≤0.05 were considered statistically significant. All statistical analyses were performed by using SPSS 15.0.1 (SPSS Inc, Chicago, IL).

RESULTS
Patients
A total of 43 cancer patients were included. Their mean age was 59 ± 12 years and 58% were male. The patients suffered from pancreatic carcinoma (n=13), gastrointestinal carcinoma (n=11), breast carcinoma (n=8), oesophagus carcinoma (n=5), biliary tract carcinoma (n=2) and other types of cancer (n=4). Seven patients had local disease and came for neo-adjuvant therapy, the other patients had locally advanced or metastatic disease.

Within six months after blood collection, five patients (12%; 95% CI 4-25), three men and two women, developed an objectively confirmed VTE: deep vein thromboses of the leg (n=2), pulmonary embolism (n=2) and vena lienalis thrombosis (n=1). The median time to develop VTE was 3.1 months (range 1.3-4.4) after blood collection. Three of these patients had pancreatic cancer, of whom two patients had locally advanced disease and one patient had metastasized disease. One patient had metastasized gastrointestinal cancer and the remaining patient had locally advanced oesophagus cancer. Their mean age was 61 ± 7 years. Five of 43 study patients died within six months, of whom one patient had developed a VTE before dying.

In vivo coagulation activation status of cancer patients developing VTE
The concentrations of F$_{1+2}$ and TAT tended to be higher in cancer patients who developed VTE (n=5) compared to those who did not develop VTE within six months after blood collection, although none reached statistical significance (n=38; Table 1). Also plasma concentrations of non-cell bound (soluble) TF and FVIIa-AT appeared to be elevated in patients with VTE. Levels of D-dimer, however, were comparable. These data suggest that at the moment of blood collection the patients destined to develop VTE showed modest signs of increased coagulation activation (F$_{1+2}$, TAT, soluble TF and FVIIa-AT) compared to those who did not develop VTE.
Table 1 - Coagulation in cancer patients developing VTE

<table>
<thead>
<tr>
<th></th>
<th>Cancer patients + VTE</th>
<th>Cancer patients - VTE</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1+2 (pmol/L)</td>
<td>319 (187-558)</td>
<td>241 (179-312)</td>
<td>0.427</td>
</tr>
<tr>
<td>TAT (mg/L)</td>
<td>5.7 (3.5-13.7)</td>
<td>4.1 (3.2-6.2)</td>
<td>0.290</td>
</tr>
<tr>
<td>D-dimer (µg/L)</td>
<td>0.66 (0.55-7.5)</td>
<td>0.77 (0.38-2.0)</td>
<td>0.326</td>
</tr>
<tr>
<td>sTF (ng/mL) ¹</td>
<td>145.5 (49.0-369.0)</td>
<td>86.8 (50.7-153.6)</td>
<td>0.445</td>
</tr>
<tr>
<td>FVIIa-AT (pmol/L)²</td>
<td>9.2 (6.2-12.3)</td>
<td>6.6 (5.4-9.2)</td>
<td>0.159</td>
</tr>
</tbody>
</table>

Data are presented as median (IQR); ¹sTF: soluble (non-cell bound) TF; ²FVIIa-AT: activated factor VII-antithrombin complexes.

MP and MP-initiated coagulation activation in cancer patients developing VTE

The numbers of circulating MP were estimated by flow cytometry. As shown in Table 2, comparable numbers of circulating MP binding annexin V, i.e. MP exposing negatively charged phospholipids, as well as MP exposing TF were present in plasma samples from cancer patients who developed VTE and patients who did not develop VTE.

Subsequently, we tested the procoagulant activity of the MP by using three different MP-based coagulation assays. First, the ability of MP to provide a phospholipid surface to propagate coagulation and clot formation was determined in the newly developed STA Procoag PPL assay. The clotting time in this assay, which is entirely independent on the presence of TF activity (R.J. Berckmans, pers. commun.), was similar between the two groups of cancer patients (p=0.519; Table 2), which is in line with the observation that a comparable number of annexin V-binding MP were present.

In contrast, there were marked differences in the ability of MP to promote coagulation in the other coagulation assays. First, the “total procoagulant activity” of the MP, i.e. the ability of isolated MP to promote factor Xa generation, in patients who developed VTE tended to be elevated compared to patients without VTE (2.16 (0.74-9.4) versus 0.91 (0.64-1.37); p=0.091). However, a marked difference in TF-dependent factor Xa generation was observed between isolated MP from patients who developed VTE and isolated MP fractions from patients who did not develop VTE (p=0.016). Similarly, the MP-mediated clotting time observed in the FGT was markedly faster in plasma samples from patients developing VTE compared to plasma samples from patients not developing VTE (801 s versus 1568 s; p=0.014). Again, the TF dependent MP-initiated
clotting time was significantly different between patients developing VTE compared with those without VTE (13.3% versus 0%, respectively; p=0.036).

**Table 2 - Microparticles (MP) and MP-mediated coagulation in cancer patients developing VTE**

<table>
<thead>
<tr>
<th></th>
<th>Cancer patients + VTE</th>
<th>Cancer patients - VTE</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>MP (Annexin V⁺)</td>
<td>5.53 x 10⁶</td>
<td>5.51 x 10⁶</td>
<td>0.629</td>
</tr>
<tr>
<td></td>
<td>(5.06-9.84)</td>
<td>(3.27-8.92)</td>
<td></td>
</tr>
<tr>
<td>MP (TF⁺)</td>
<td>34.8 x 10³</td>
<td>10.9 x 10³</td>
<td>0.672</td>
</tr>
<tr>
<td></td>
<td>(1.33-104.0)</td>
<td>(2.67-36.8)</td>
<td></td>
</tr>
<tr>
<td>PPLT (s)</td>
<td>73</td>
<td>79</td>
<td>0.519</td>
</tr>
<tr>
<td></td>
<td>(68-81)</td>
<td>(69-82)</td>
<td></td>
</tr>
<tr>
<td>Total PCA (pg/mL)</td>
<td>2.16</td>
<td>0.91</td>
<td>0.091</td>
</tr>
<tr>
<td></td>
<td>(0.74-9.4)</td>
<td>(0.64-1.37)</td>
<td></td>
</tr>
<tr>
<td>TF-dependent PCA</td>
<td>0.82</td>
<td>0.21</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>(0.25-6.9)</td>
<td>(0.04-0.35)</td>
<td></td>
</tr>
<tr>
<td>FGT (s)</td>
<td>801</td>
<td>1568</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td>(769-1121)</td>
<td>(1036-3008)</td>
<td></td>
</tr>
<tr>
<td>TF-dependent FGT (%)</td>
<td>13</td>
<td>0</td>
<td>0.036</td>
</tr>
<tr>
<td></td>
<td>(6 - 31)</td>
<td>(-12-11)</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as median (IQR); ¹Number/mL; ²PPLT: phospholipid dependent clotting time; ³Total PCA: total procoagulant activity; ⁴FGT: Fibrin Generation Time

**Predictive value for development of VTE in cancer patients**

ROC analyses showed that the TF activity assay and the FGT could significantly discriminate between cancer patients developing or not developing VTE during a six month follow up period (Figure 1). Both tests were discriminatory also in the presence of antibodies. The area under the curve was 0.82 (95% CI 0.61-1.04) for the TF activity assay, 0.83 (95% CI 0.68-0.98) for the FGT and 0.79 (95% CI 0.61-0.97) for the FGT in the presence of anti-FVII(a). With regard to the TF activity assay, the ROC curve analysis showed that a cut-off of 0.37 pg/ml was the best trade off between false positive and false negative results, with a sensitivity of 80% and specificity of 84%. For the FGT a cut off level of 909 seconds resulted in a sensitivity of 80% and specificity of 84%. In the presence of the anti-FVII(a), the best cut off level of prolongation of the clotting time in the FGT was 13%, resulting in a sensitivity of 80% and a specificity of 82%.
Fig. 1. Receiver operating characteristic curve for procoagulant activity assays

**A - D-dimer**
AUC 0.62 (95% CI 0.38-0.90); p=0.315

**B - PPLT**
AUC 0.41 (95% CI 0.19-0.63); p=0.507
C- Total Xa generation
AUC 0.74 (95% CI 0.42-1.05); p=0.091

D- Total Xa generation + anti TF
AUC 0.82 (95% CI 0.61-1.04); p=0.020
E- Fibrin generation
AUC 0.83 (95% CI 0.68-0.98); p=0.017

F- Fibrin generation + anti-TF
AUC 0.79 (95% CI 0.61-0.97); p=0.039
Coagulation in cancer patients and healthy individuals: a comparison

For comparison, we also collected blood from healthy individuals (n=22; mean age 38 ± 10 years 32% men). Compared to healthy individuals, cancer patients had higher plasma concentrations of F$_{1+2}$, TAT as well as D-dimer (Table 3; p<0.001 for all). There was no evidence for increased levels of TF antigen or activity in cancer patients, since the levels of non-cell bound TF and FVIIa-AT were comparable to controls. With regard to the MP, the numbers of annexin V-binding MP were increased in patients compared to controls (p=0.001), but the numbers of TF-exposing MP were comparable (p=0.186). The MP-associated ability to initiate and/or propagate coagulation tested in the three coagulation assays was similar compared to cancer patients (Table 3).

Table 3 - Coagulation in cancer patients and healthy individuals

<table>
<thead>
<tr>
<th></th>
<th>Cancer patients</th>
<th>Healthy individuals</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>F$_{1+2}$ (pmol/L)</td>
<td>246 (181-319)</td>
<td>156 (139-198)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TAT (mg/L)</td>
<td>4.1 (3.2-7.8)</td>
<td>3.0 (2.5-3.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>D-dimer (μg/L)</td>
<td>0.76 (0.40-2.26)</td>
<td>0.22 (0.17-0.54)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>sTF (ng/mL)$^1$</td>
<td>92.6 (20.7-170.9)</td>
<td>66.0 (30.4-124.9)</td>
<td>0.163</td>
</tr>
<tr>
<td>FVIIa-AT (pmol/L)$^2$</td>
<td>6.7 (5.4-9.2)</td>
<td>6.6 (5.9-8.6)</td>
<td>0.960</td>
</tr>
<tr>
<td>MP (Annexin V$^+$)$^3$</td>
<td>5.53 x 10$^6$</td>
<td>3.37 x 10$^6$</td>
<td>0.001</td>
</tr>
<tr>
<td>MP (TF$^+$)$^3$</td>
<td>11.0 x 10$^3$</td>
<td>39.8 x 10$^3$</td>
<td>0.186</td>
</tr>
<tr>
<td>PPLT (s)$^4$</td>
<td>78 (69-82)</td>
<td>78 (73-85)</td>
<td>0.411</td>
</tr>
<tr>
<td>Total PCA (pg/mL)$^5$</td>
<td>0.98 (0.64-1.43)</td>
<td>0.75 (0.54-0.95)</td>
<td>0.066</td>
</tr>
<tr>
<td>TF-dependent PCA</td>
<td>0.21 (0.05-0.36)</td>
<td>0.26 (0.06-0.41)</td>
<td>0.899</td>
</tr>
<tr>
<td>FGT (s)$^6$</td>
<td>1386 (968-2852)</td>
<td>1299 (930-1776)</td>
<td>0.417</td>
</tr>
<tr>
<td>TF-dependent FGT (%)</td>
<td>0 (-13-9)</td>
<td>-6 (-12-1)</td>
<td>0.349</td>
</tr>
</tbody>
</table>

Data are presented as median (IQR); $^1$sTF: soluble (non-cell bound) TF; $^2$FVIIa-AT: activated factor VII-antithrombin complexes; $^3$Number/mL; $^4$PPLT: phospholipid dependent clotting time; $^5$Total PCA: total procoagulant activity; $^6$FGT: Fibrin Generation Time
Chapter 11

DISCUSSION

Our present study shows that the ability of MP to promote TF-dependent coagulation is higher in plasma samples from cancer patients weeks to months before the onset of VTE compared to cancer patients who did not develop VTE. Both the FGT and a Xa generation assay identified cancer patients at high risk for developing VTE with a sensitivity and specificity of approximately 80% at baseline. Thus, these data suggest that the presence of TF-dependent procoagulant activity of MP in plasma samples from cancer patients may predict the development of VTE.

Two earlier studies showed that higher levels of MP-associated TF activity could be demonstrated in plasma samples of cancer patients with VTE compared to patients without VTE, but the blood samples in these studies were collected after the diagnosis of VTE [9;10]. This is illustrated by a recent case report, in which Del Conde and coworkers showed that a 55 year old patient with giant-cell lung carcinoma developed eleven major arterial and venous thrombotic events despite antithrombotic therapy [14]. This patient, who suffered from a severe form of “Trousseau’s syndrome”, had an extremely elevated plasma level of TF, which was entirely associated with MP. Recently, the MP-associated TF activity in plasma samples of 11 patients with pancreatic cancer was suggested to be associated with development of VTE [11]. Our present results extend these findings. Taken together it is tempting to speculate that the detection of MP-associated procoagulant TF activity in plasma samples of cancer patients enables the identification of those patients at risk to develop VTE.

Although our findings did reach statistical significance and are confirmed using different coagulation assays, the relatively small number of patients and events limits the robustness of our findings and therefore we consider this to be a pilot study. The predictive value of the various coagulation assays will be studied in a future prospective study. If this study confirms the present findings, more firm conclusions can be drawn.

Strikingly, the levels of non-cell bound (soluble) TF and FVIIa-AT were similar in cancer patients and healthy controls. Compared to the cancer patients, controls were not matched for age or gender, and their blood was collected by venapuncture and not via a venous catheter, which may contribute to the observed differences in concentrations of F1+2, TAT and D-dimer. Despite these differences, however, comparable levels of non-cell bound (soluble) TF, FVII-AT complexes and the numbers of TF-exposing MP were present. Thus, there seems not to be a general shift in this heterogeneous group of cancer patients towards increased TF-dependent coagulation. Our findings are confirmed by
earlier data of Tesselaar et al., who also reported comparable numbers of TF-exposing MP in cancer patients and controls [10]. Furthermore, they showed that the extent of the MP-associated “TF activity”, as determined in a factor Xa generation assay, was also comparable for most cancer patients and controls. Although Hron et al. reported two-fold higher levels of TF-exposing MP in patients with advanced colon carcinoma compared to controls, they performed no coagulation activity assays [15]. Antigen assays, however, do not distinguish between encrypted TF and non-encrypted (procoagulant) TF.

Taken together, the present data confirm the hypothesis that circulating TF, present in blood and associated with cell-derived MP, is associated with a hypercoagulable state of cancer patients prone to develop VTE.

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AUTHORS CONTRIBUTION
The authors FFvD, RJB, PWK, HRB and RN contributed to the design of the study. FFvD, RJB, AK, NM, and DM were responsible for data collection. The analyses were performed by FFvD. The interpretation of the results and the writing of the manuscript were performed by all authors.

DISCLOSURE OF CONFLICT OF INTEREST
None of the authors do have a conflict of interest.
REFERENCES

