Tissue factor-bearing microparticles and CA19.9: two players in pancreatic cancer-associated thrombosis?

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Background: Cancer-related venous thromboembolism (VTE) heralds a poor prognosis, especially in pancreatic adenocarcinoma (PAC). Tissue factor (TF) is implicated as one of the main culprits in PAC-associated VTE and disease progression.

Methods: In a prospective cohort study of 79 PAC patients, we measured plasma CA19–9 and microparticle-associated TF activity (MP-TF activity). In addition, we enumerated TF+ MPs and MUC1+ MPs in plasma (n = 55), and studied the expression of TF, MUC1, CD31 and CD68 in tumour tissue (n = 44).

Results: Plasma MP-TF activity was markedly elevated in PAC patients with VTE compared with those without (median: 1925 vs 113 fM Xa min/C0; P < 0.001) and correlated with the extent of thromboembolic events, metastatic disease and short survival. Similar results were found for CA19–9. Patients with massively progressing thrombosis and cerebral embolisms despite anticoagulant therapy (n = 3) had the highest MP-TF activities (12 118–40 188 fM Xa min/C0) and CA19–9 (40 730–197 000 kU l/C0). All tumours expressed MUC1 and TF. MP-TF activity did not correlate with intensity of TF expression in adenocarcinoma cells, but corresponded with numbers of TF+ macrophages in the surrounding stroma.

Conclusions: Circulating TF+ MPs and mucins may concertedly aggravate coagulopathy in PAC. Understanding of underlying mechanisms may result in new treatment strategies for VTE prevention and improvement of survival.

Pancreatic adenocarcinoma (PAC) is the most lethal among adenocarcinomas with a mortality rate that equals the incidence (Torre et al, 2015). Less than 20% of patients are candidates for curative surgery and more than 75% of patients die within 1 year (Bilimoria et al, 2007). After ‘curative’ resection, PAC invariably recurs and rapidly disseminates (Michl and Gress, 2013). Up to 5 years ago, median survival time was 7 months for patients with locally advanced PAC and 4.5 months for patients with metastatic disease (Bilimoria et al, 2007). Since then, treatment of advanced stage PAC patients with FOLFIRINOX (i.e., 5-FU/LV, irinotecan and oxaliplatin) has resulted in a median survival of 11 months for only those fit enough to undergo this toxic regimen (Conroy et al, 2011).

Cancer patients have an increased risk of venous thromboembolism (VTE) and the risk is especially high in PAC (58-fold increased) (Blom et al, 2006). Thus, in addition to a short lifespan, quality of life is often hampered by thromboembolic complications such as deep vein thrombosis (DVT), pulmonary embolism (PE).
and occasionally marantic endocarditis or phlegmasia cerulea dolens, that is, massive venous thrombosis resulting in ischaemia (Howard, 1960; Smeglin et al, 2008).

Tissue Factor (TF) is expressed in exocrine pancreatic cells upon malignant transformation and may be one of the key proteins involved in cancer-related thrombosis and rapid metastatic spread in PAC (Kakkhar et al, 1995). Complex formation with coagulation factor VII (FVII) not only initiates the coagulation cascade, but also activates intracellular protease-activated receptor 2 signalling after β1-integrin ligation, thus contributing to angiogenesis, tumour cell proliferation and migration (Raf et al, 2010). In a cohort of 113 PAC patients with a median follow-up of 16 months, overall survival was significantly shorter in case of high-tumour-TF expression (Nitioti et al, 2005). A study of 122 PAC patients with a similar follow-up period did not confirm these findings, but showed a higher prevalence of VTE in patients with high tumour-TF expression (26.3%) compared with patients with low tumour-TF expression (4.5%) in a subgroup analysis of 41 patients (Khorana et al, 2007).

As procoagulant TF on tumour cells does not explain why thrombosis is typically found at sites distant from the primary tumour and its metastases, it is hypothesised that circulating TF attached to submicron cellular membrane vesicles – so-called microparticles (MPs) – may be one of the factors contributing to thrombosis. MPs are assumed to originate from tumour cells, but may very well be released by macrophages or endothelial cells upon activation (Ahamed et al, 2007; Østerud, 2010). To contribute to the observed thromboembolic events.

In order to gain more insight into the role of TF in PAC with respect to coagulopathy and survival, we measured numbers of TF⁺ MPs, plasma MP-TF activity and tumour-TF expression within a cohort of 79 patients. In addition, we assessed whether mucins in the tumour and in the circulation could have contributed to the observed thromboembolic events.

**PATIENTS AND METHODS**

**Study design.** From December 2000 to June 2009, pancreatic cancer patients who were referred to the Departments of Surgery and Clinical Oncology of the Leiden University Medical Centre were screened for eligibility for study participation. Inclusion criteria were age ≥18 years and any stage of PAC, including ampullary cancer. Exclusion criteria were revised histological classification other than adenocarcinoma, duodenal carcinoma or ‘adenocarcinoma of unknown primary’. Patients were also excluded when blood sample processing within 1 h after venepuncture was not possible. All patients gave their informed consent.

Seventy-nine patients were included in the study. We collected blood from all patients at inclusion. Two patients were unintentionally included twice and thus a second plasma sample was obtained during follow-up. In 65 of 79 patients, PAC was confirmed by surgical biopsy (n = 32), core needle biopsy (n = 23) or fine needle aspiration (n = 10). Fourteen patients (failed biopsy: n = 2) were diagnosed on typical radiological findings and elevated CA19–9 levels. In 44 patients, tumour tissue specimens were suitable for immunohistochemical analysis. In a representative subset of 37 patients with similar sex and age distributions as the original cohort of 79 patients, tissue samples were obtained within a median interval of 0 days after study entry (IQR 0–3; range 70 to 30 days), enabling assessment of the relation between plasma MP-TF activity, CA19–9 levels and antigen expression in the tumour.

**Procedures and definitions.** Demographic and clinical data were obtained by reviewing the medical records and subsequently cross-referenced with the Leiden Cancer Registry. Tumour stage (i.e., local, locally advanced and metastatic disease) was established based on findings at surgery and thoraco-abdominal computed tomography. Radiologic evidence of VTE was recorded and patients were followed until death or 1 October 2014, whichever came first. The extent of thrombosis was semi-quantitatively scored as: no thrombosis, small VT, DVT and/or PE, massive VTE. ‘Small VT’ was defined as any coincidental radiological diagnosis of VT visible over a very short distance in the absence of clinical symptoms (e.g., splenic vein thrombosis), whereas ‘massive VTE’ was defined as extensive VT involving a long trajectory of veins. ‘Cancer-related thrombosis’ was defined as unprovoked thrombosis in cancer patients in the absence of chemotherapeutic treatment, venous catheters or recent surgery. To assess the relation between plasma MP-TF activity, CA19–9 and the severity of VTE, the maximum interval between blood sampling and the occurrence of VTE was arbitrarily set at ±16 days.

**Isolation of microparticles, measurement of plasma MP-TF activity and CA19–9.** Upon inclusion into the study, venous blood samples were collected in citrated BD Vacutainer tubes (Beckton Dickinson, Franklin Lakes, NJ, USA) using minimal venostasis and discarding the first tube. Within 1 h, platelet poor plasma was prepared (20 min, 1550 g, room temperature), snap frozen in liquid nitrogen and stored in aliquots at −80 °C until analysis. After thawing of deep-frozen platelet poor plasma samples, blood MPs were pelleted and washed twice with filtered 0.32% citrate/PBS buffer, pH 7.45 (30 min at 17 570 g with minimum brake, 20 °C) (Van Wijk et al, 2002). Isolated MPs were immediately tested for MP-associated TF (MP-TF) activity, as previously described (Tessaal et al, 2007; Woei-A-Jin et al, 2014). Results are reported as FVII/TF-dependent FXa formation (FM Xa min⁻¹) in plasma. Mean and median plasma MP-TF activity in 66 healthy volunteers without medical history or current illness were 70 and 69 FM Xa min⁻¹, respectively (range 3–238). Plasma MP-TF activities higher than the mean plus 2 s.d. (i.e., >155 FM Xa min⁻¹) were considered to be elevated. All samples were analysed batchwise within 1 year after blood collection. No difference in MP-TF activity was found following prolonged frozen storage (>15 months; n = 16).

CA19–9, the sialylated lacto-N-fucopentaose II (Lewis blood group) antigen, was measured in plasma using an immunoenzymatic assay on an IMX Automated Immunoassay Analyzer (Abbott Diagnostics, Lake Forest, IL, USA). CA19–9 levels >37 kU L⁻¹ were considered to be elevated (Galli et al, 2013).

**Flowcytometric analysis of microparticles.** From 55 of 79 patients, frozen plasma samples were available for further analysis. Microparticles were isolated from plasma as described above, resuspended in 2.5 mM CaCl₂/PBS buffer, pH 7.4 and incubated in the dark for 15 min at room temperature with Annexin V (AnnV) labelled with Allophycocyanin (APC) (Caltag Laboratories, Burlingame, CA, USA) and one of the following monoclonal antibodies labelled with fluorescein isothiocyanate (FITC): anti-epithelial membrane antigen (EMA/MUC1)-IgG1-FITC (clone B24.1; Biomedia, Foster City, CA, USA), anti-TF-IgG1-FITC (No. 4508C), American Diagnostica, Stamford, CT, USA) and mouse IgG1-FITC (clone X40; BD Biosciences, San Jose, CA, USA). The MP suspension was washed with excess 2.5 mM CaCl₂/PBS buffer, pH 7.4, then diluting the antibody concentration 52×. Following centrifugation (30 min at 17 570 g with minimum brake, 20 °C), removal of the supernatant and resuspension in 2.5 mM CaCl₂/PBS buffer, pH 7.4, samples were analysed on a FACSCalibur using the
Cell Quest software (BD Biosciences). Forward and sideward scatter of light were set at logarithmic gain. Microparticles were identified on the basis of their size, density and capacity to bind Ann V (Nieuwland et al, 1997). Fluorescent colour profiles of the selected population were subsequently recorded for 1 min. The number of MP per litre plasma was calculated as previously described (VanWijk et al, 2002). In seven patients, measurement of AnnV+/TF+/MPs was not possible due to insufficient plasma.

For our experiments, we used one single batch of FITC-labelled anti-TF-IgG1 antibody (clone 4508C) from American Diagnostica, currently Sekisui Diagnostics). This is an inhibitory antibody directed against the first 25 amino acids of TF. Previously, the same batch of antibody was used to demonstrate the presence of CD14+/TF+ MPs in plasma from a patient with fulminant meningococcal sepsis and disseminated intravascular coagulopathy (Nieuwland et al, 2000), and to detect a TF expressing subset of MPs derived from activated HUVEC cells (Abid Hussein et al, 2003). We confirmed the specificity and sensitivity of the anti-TF antibody using MPs generated from TF-expressing breast cancer cell lines MDA-231 (high TF) and MCF-7 (low TF) after stimulation with Ca 2+ ionophore A23187. In addition, we used MPs derived from cells that do not express TF. Simultaneous staining of MPs with AnnV-APC, anti-TF-IgG1-FITC and either anti-CD235a-IgG1-PE (clone JC159; Dako, Glostrup, Denmark) or anti-CD66e-IgG1-PE (clone CLB-gran/10; HH4Fc; Sanquin, Amsterdam, Netherlands) showed that neither erythrocyte nor granulocyte-derived MPs expressed TF.

Immunohistochemistry. Formalin-fixed 4 μm sections were deparaffinised with xylene and rehydrated through graded ethanol and demineralised water. Antigens were retrieved by boiling in 10 mm citrate buffer, pH 6.0 in a pressure cooker. Non-specific binding was blocked using Antibody Diluent S2022 (Dako). Consecutive tissue sections were incubated with the following primary monoclonal mouse anti-human antibodies diluted in Antibody Diluent S2022 (Dako): anti-TF (No. 4509, American Diagnostica), anti-Epithelial Membrane Antigen (MUC1) (clone E29), anti-CD68 (clone KP1) and anti-CD31 (clone JC70A) from Dako. After blocking endogenous peroxidases, staining was visualised using a peroxidase-labelled polymer conjugated to goat anti-mouse immunoglobulins and 3,3′-diaminobenzidine (K4007, Mouse EnVision + kit, Dako). Sections were decolourised, counterstained with haematoxylin (1:1 dilution with demineralised water; Merck, Darmstadt, Germany), decolourised again, dehydrated and finally permanently mounted.

Mouse IgG1 (BD Biosciences) and omission of the primary antibody served as specificity controls. Normal pancreatic islet cells, tonsils and ductal breast cancer cells served as positive control for TF, CD68 and MUC1 staining, respectively. No separate control was prepared for CD31 staining.

Tumour grading and scoring of antigen expression. TF, MUC1, CD31 and CD68 antigen expression was assessed at ×100 magnification. The intensity of TF staining on tumour cells was qualitatively scored as weak, intermediate or strong. All tissue sections were reviewed independently by two investigators blinded for clinical data. Discrepancies were resolved by consensus. To prevent observer bias, tumour grade was determined by one pathologist not involved in the study. The presence of TF+CD68+ macrophages and vascular TF expression was established by examining serial sections at ×200 magnification. Numbers of macrophages expressing TF were semi-quantitatively scored as absent (no TF+CD68+ macrophages), sporadic (solitary dispersed TF+CD68+ macrophages) or in clusters (increased number of TF+CD68+ macrophages in groups).

Statistical analysis. All data were recorded and analysed using SPSS software (version 20.0; SPSS, Inc., Chicago, IL, USA). Survival time was defined as the interval from enrolment to death and compared between groups using the log-rank test. The Mann–Whitney U-test was applied for univariate analysis and the differences between independent categories were determined with the Kruskal–Wallis test (reported as H(d), P-value). Correlations were assessed using the Spearman’s rho (r) test. Finally, the association between TF expression on tumour cells and differentiation grade was investigated using the χ2-test. P-values < 0.05 were considered significant.
Categorisation of patients according to the magnitude of thrombosis at inclusion (i.e., no thrombosis, small thrombosis, DVT and/or PE, massive VTE) demonstrated an increase in plasma MP-TF activity with increasing extent of thrombosis (Figure 2A) (Kruskal–Wallis test: H(3) = 15.77, P = 0.001). All patients with VTE had metastatic disease and elevated plasma MP-TF activities, except for one patient with local disease who developed PE following surgery (plasma MP-TF activity 91 fM Xa min⁻¹). Median plasma CA19–9 was higher in patients with VTE than in patients without VTE and increased with the extent of thrombosis (Figure 2B). Similarly, serial measurements in one metastatic patient with a plasma MP-TF activity of 151 fM Xa min⁻¹ demonstrated an increase to 2567 fM Xa min⁻¹ at the time DVT was diagnosed, whereas CA19–9 levels increased from 20 to 37 751 kU l⁻¹.

The highest MP-TF activities (12 118–40 188 fM Xa min⁻¹) were detected in three metastatic cancer patients with striking fulminant venous thrombosis and even cerebral arterial thromboembolic events in two of the three cases. One patient, who presented with intraocular lesions, was diagnosed with extensive DVT reaching from the popliteal into the common femoral vein. Despite low-molecular-weight heparin (LMWH) treatment, surgical closure of a patent foramen ovale and insertion of a caval vein filter, he persistently suffered multiple bilateral ischaemic cerebral vascular attacks and died 22 days later. This patient had the highest MP-TF activity (40 188 fM Xa min⁻¹; CA19–9 of 40 730 kU l⁻¹; no enumeration of MPs).

Another patient presented with a popliteal vein thrombosis for which vitamin K antagonists and low-dose LMWH were started. Nevertheless, the thrombosis progressed into the iliac vein within 2 weeks, resulting in phlegmasia cerulia dolens and venous gangrene. He died within 44 days. Both MP-TF activity and CA19–9 were very high (13 887 fM Xa min⁻¹ and 145 110 kU l⁻¹, respectively). Numbers of AnnV⁺TF⁺MPs and AnnV⁺MUC1⁺MPs were 353 (>75th percentile) and 194 (>50th percentile), respectively.

### Table 1. Patient characteristics according to tumour stage

|                          | Local (n = 26) | Locally advanced (n = 19) | Metastasis (n = 34) |
|--------------------------|---------------|-------------------------|---------------------|
| Male, n (%)              | 12 (46.2)     | 8 (42.1)                | 20 (58.8)           |
| Age (years), median (IQR)| 65 (59–70)    | 58 (52–64)              | 64 (57–68)          |
| Localisation of primary tumour, n (%) |               |                         |                     |
| Caput                    | 20 (76.9)     | 15 (78.9)               | 18 (52.9)           |
| Corpus                   | 0 (0)         | 2 (10.5)                | 4 (11.8)            |
| Cauda                    | 1 (3.8)       | 2 (10.5)                | 10 (29.4)           |
| Vater’s papilla          | 5 (19.2)      | 0 (0)                   | 2 (5.9)             |
| MP-TF activity (fM Xa min⁻¹), median (IQR) | 46 (24–149)   | 123 (41–181)            | 328 (111–1184)      |
| CA19–9 (kU l⁻¹), median (IQR) | 39 (9–374)    | 1820 (72–3000)          | 2139 (268–39 241)   |
| Venous thromboembolism at inclusion, n (%) | 1 (3.8)       | 0 (0)                   | 13 (38.2)           |
| Overall survival (months), median (IQR) | 21.5 (11.9–41.6) | 6.9 (5.2–9.6) | 2.1 (1.1–4.7) |
| Death during follow-up, n (%) | 20 (76.9)     | 19 (100)                | 34 (100)            |
| Flowcytometric subset analysis (n = 55) |             |                         |                     |
| Total AnnV⁺MP (x 10⁹l⁻¹), median (IQR) | 3204 (1677–5873) | 4114 (2600–6106) | 4249 (3118–6864) |
| AnnV⁺TF⁺MP (x 10⁹l⁻¹), median (IQR) | 0 (0–40)      | 0 (0–0)                 | 83 (30–307)         |
| AnnV⁺MUC1⁺MP (x 10⁹l⁻¹), median (IQR) | 0 (0–0)       | 0 (0–282)               | 152 (0–314)         |

**Abbreviations:** IQR = interquartile range; MP = microparticle; TF = tissue factor; AnnV = Annexin V.
*CA19–9, 6 missing values.

Figure 1. Plasma MP-TF activity (A) and CA19–9 levels (B) show a moderate negative correlation with overall survival (r = -0.471 and -0.499, respectively; P<0.001) and are related to tumour stage.
The third patient presented with massive thrombosis ranging from the popliteal to iliac vein, bilateral PEs and a cerebral infarction despite anticoagulant treatment with a vitamin K antagonist and INR > 6. The VTE-related symptoms improved after switching to LMWH, but he succumbed 25 days later. This patient had the highest CA19-9 levels of 197 000 kU L⁻¹ and the highest numbers of circulating AnnV⁺ MUC1⁺ MPs (1 700 × 10⁹ L⁻¹), Plasma MP-TF activity was 12 118 fM Xa min⁻¹ and the number of AnnV⁺ TF⁺ MPs was 252 × 10⁹ L⁻¹.

Expression of TF in pancreatic adenocarcinoma and circulating microparticles. In all 44 tumour specimens, adenocarcinoma cells expressed TF and MUC1 (representative specimens shown in Figure 3A–C). The intensity of tumour-TF expression (low: n = 21, intermediate: n = 18, strong: n = 5) increased with histological tumour grade (χ² = 32.62, df = 4, P < 0.0001). No significant correlation between tumour-TF expression and tumour stage was found. Nevertheless, local disease patients with intermediate/strong tumour-TF expression (n = 16) had a shorter overall survival than patients with low tumour-TF expression (n = 12) (median 12.7 vs 25.8 months, log-rank: P = 0.008). The difference in survival was not significant for patients with (locally) advanced disease. During follow-up, 6 of 44 patients developed DVT or PE, two of which were within the immediate post-surgery period of 6 weeks. The unprovoked VTE rate was similar in patients with intermediate/high tumour-TF expression (2 of 21; 10%) and patients with low tumour-TF expression (2 of 23; 9%).

Endothelial cells stained positive for CD31 (Figure 3D), but were predominantly TF-negative. CD68⁺ macrophages were unevenly distributed throughout the stroma and adipose tissue. In 16 of 43 tumours (one biopsy specimen too small for reliable assessment of macrophages), TF⁺ macrophages were detected. In eight of these, moderate to large clusters of TF⁺ macrophages were found in the tumour environment (Figure 3E–F). All TF⁺ macrophages showed very strong TF expression compared with tumour cells.

In the 37 patients in whom tumour tissue was collected in a pre-specified time frame of 70 to 30 days following blood sampling, we found no correlation between the intensity of TF expression in adenocarcinoma cells and plasma MP-TF activity. Subgroup analysis of 19 patients also showed no correlation between the intensity of TF expression in adenocarcinoma cells and numbers of circulating AnnV⁺ TF⁺ MPs. In contrast, in patients with large clusters of TF⁺ macrophages infiltrating the tumour-surrounding stroma, median plasma MP-TF activity was higher (743 fM Xa min⁻¹ (IQR 267–5468); n = 6) than in patients with sporadic infiltration of TF⁺ macrophages (57 fM Xa min⁻¹ (IQR 33–123); n = 7) or without TF⁺ macrophages (44 fM Xa min⁻¹ (IQR 24–124); n = 24; P < 0.001).

DISCUSSION

This prospective cohort study in 79 patients reflects the natural disease course in PAC as it was conducted in the era before introduction of the FOLFIRINOX chemotherapeutic regimen (Conroy et al, 2011). Survival rates were similar as those reported in the literature (Bilimoria et al, 2007). We confirmed that high plasma MP-TF activity correlates with short survival (Tesselaar et al, 2007; Thaler et al, 2012, 2013; Bharthuar et al, 2013), but additionally demonstrated that this is mostly related to its association with tumour stage. PAC cells expressed MUC1 and TF. In accordance with the published data, tumour-TF expression increased with histological grade (Kakkar et al, 1995; Nitori et al, 2005; Khorana et al, 2007). We confirmed in a homogeneous group of local disease patients (n = 28) that high tumour-TF expression corresponds with poor survival (Nitori et al, 2005).

Patients with cancer-related thrombosis had higher MP-TF activities than patients without thrombosis, which is in agreement with previous observations (Tesselaar et al, 2007; Bharthuar et al, 2013). Although all cancer-related VTE patients had metastatic disease, we demonstrated that the magnitude of VTE increased significantly with plasma MP-TF activity, indicating that the release of TF⁺ MPs is not merely an epiphenomenon, but may contribute to the development of thrombosis.
The intensity of TF expression by adenocarcinoma cells did not correlate with plasma MP-TF activity. In some patients with AnnV⁺ TF⁺ MPs, no AnnV⁺ MUC1⁺ MPs were detected. These findings are consistent with a study of three pancreatic cancer patients, which showed that 50% of TF⁺ MPs were MUC1-negative (Zwicker et al, 2009) and may be related to an additional source of TF⁺ MPs other than tumour cells. Interestingly, tissue specimens of patients with high plasma MP-TF activity demonstrated large clusters of very strongly stained TF⁺ macrophages invading the vast tumour-surrounding stroma. Thus, in addition to PAC cells, macrophages may also form a significant source of procoagulant MP-TF activity. We hypothesise that the pro-inflammatory state in advanced stage pancreatic cancer induces the activation of monocytes and macrophages expressing large amounts of TF and that both TF⁺ tumour cells and activated TF⁺ macrophages in the tumour environment are the source of TF⁺ MPs in the circulation.

Alternative pathways may contribute to the thrombotic phenomena, early dissemination and strikingly poor prognosis of pancreas adenocarcinoma patients. Adenocarcinomas and in particular PAC express aberrantly glycosylated structures on their cell surface and shed large quantities of mucins into the circulation. These glycan structures may mediate crosstalk between the tumour and its microenvironment. Their presence seems to correlate with cancer progression and may affect tumour cell migration and dissemination (Tei et al, 2002). In the absence of a method to quantify mucins in blood, CA19-9 may serve as a surrogate marker as it binds to apomucins, including MUC1, MUC5AC and MUC16 (Yue et al, 2011). In our study cohort, plasma CA19-9 levels correlated with stage, short survival and – albeit to a lesser extent than MP-TF activity – with the severity of VTE.

Experimental studies in mice provided evidence that intravenous injection of carcinoma mucins carrying selectin ligands resulted in the formation of platelet-rich microthrombi, whereas thrombosis was markedly diminished in P-selectin or L-selectin-deficient mice (Wahrenbrock et al, 2003).

Three patients with fulminant massive thromboembolisms despite anticoagulant therapy had extremely high MP-TF activity and CA19-9 levels. Devastating warfarin-refractory arterial and venous thromboembolisms have been described previously and heparins may be more effective (Bell et al, 1985; Walsh-McMonagle and Green, 1997). In contrast to vitamin K antagonists, which decrease thrombin production, heparin additionally blocks binding of mucins to selectins thus hampering platelet aggregation. In our study, the patient with the highest levels of AnnV⁺ MUC1⁺ MPs and extremely high CA19-9 (which may reflect levels of circulating mucins) developed overwhelming progressive thrombosis while receiving vitamin K antagonist treatment. Strikingly, this patient improved clinically with heparin treatment in accordance with the hypothesis that circulating tumour-derived mucins also have a role in cancer-related thrombosis.

In conclusion, our current findings support the notion that circulating procoagulant TF⁺ MPs are mechanistically related to the severe coagulopathy observed in PAC patients. Until now,
studies focussed on the role of TF in pancreatic cancer-associated VTE, but TF may not be the only player. Circulating MUC1
attached to MPs and other soluble mucins may also initiate and
aggregate clotting. We hypothesise that PAC cells, as well as
stromal macrophages, release procoagulant TF + MPs. These TF +
MPs may, in a concerted action with soluble- or MP-bound
mucins, potentiate coagulopathy and the aggressive behaviour
of PAC. Improved insight into these mechanisms are relevant to
develop optimal treatment strategies targeting TF/EVII on tumour
cells and macrophages, as well as blocking circulating CA19-9 and
mucins released by tumour cells.

ACKNOWLEDGEMENTS

This work was supported by the Dutch Cancer Society (KWF UL
2006–3618).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Abid Hussein MN, Meesters EW, Osmanovic N, Romijn FP, Nieuwland R, Sturk
A (2003) Antigenic characterization of endothelial cell-derived microparticles
and their detection ex vivo. J Thromb Haemost 1(11): 2434–2443.
Agham J, Niessen F, Kurokawa T, Lee YK, Bhattacharjee G, Morrissey JH,
Romijn FP, Westendorp R, Hack CE, Sturk A (2007) Cell-derived microparticles generated in patients during
cardiopulmonary bypass are highly procoagulant. Circulation 96(10):
3534–3541.
Nieuwland R, Berckmans RJ, McGregor S, Böing AN, Romijn FP, Westendorp R,
Hack CE, Sturk A (2000) Cellular origin and procoagulant properties of
microparticles in meningococcal sepsis. Blood 95(3): 930–935.
Nitori N, Ino Y, Nakanishi Y, Yamada T, Honda K, Yanagihara K, Kosuge T,
Kanai Y, Kitajima M, Hirohashi S (2005) Prognostic significance of tissue
factor factor in pancreatic ductal adenocarcinoma. Clin Cancer Res
11(7): 2531–2539.
Österud B (2010) Tissue factor expression in blood cells. Thromb Res
125(Suppl 1): S31–S34.
Ruf W, Yokota N, Schaffner F (2010) Tissue factor in cancer progression
and angiogenesis. Thromb Res 125(Suppl 2): S36–S58.
Smeingin A, Ansari M, Skali H, Oo TH, Maysky M (2008) Marantico endo-
carditis and disseminated intravascular coagulation with systemic emboli
in presentation of pancreatic cancer. J Clin Oncol 26(8): 1383–1385.
Soft GA (2014) Commentary on ‘microparticle-associated tissue factor activity
in patients with metastatic pancreatic cancer and its effect on fibrin clot
formation’. Transl Res 163(2): 136–140.
Tei K, Kawakami-Kimura N, Taguchi O, Kumamoto K, Higashiya S,
Taniguchi N, Toda K, Kawata R, Hisa Y, Kannagi R (2002) Roles of cell
adhesion molecules in tumor angiogenesis induced by cotransplantation
of cancer and endothelial cells to nude rats. Cancer Res 62(21):
6289–6296.
Tesselaar ME, Romijn FP, van der Linden JK, Prins FA, Bertina RM, Osanto S
(2007) Microparticle-associated tissue factor activity: a link between
cancer and thrombosis? J Thromb Haemost 5(3): 520–527.
Thaler J, Ay C, Mackman N, Bertina RM, Kaider A, Marosi C, Key NS,
Barcel DA, Scheithauer W, Kornek G, Zielinski C, Pabinger I (2012)
Microparticle-associated tissue factor activity, venous thromboembolism
and mortality in pancreatic, gastric, colorectal and brain cancer patients.
J Thromb Haemost 10(7): 1363–1370.
Thaler J, Ay C, Mackman N, Metz-Schimmerl S, Stift J, Kaider A, Mullauer L,
Grant M, Scheithauer W, Pabinger I (2013) Microparticle-associated
tissue factor activity in patients with pancreatic cancer: correlation with
clinopathological features. Eur J Clin Invest 43(3): 277–285.
Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A (2015)
Global cancer statistics, 2012. CA Cancer J Clin 65(2): 87–108.
VanVijk MJ, Nieuwland R, Boer K, van der Post JA, VanBavel E, Sturk
A (2002) Microparticle subpopulations are increased in preeclampsia:
possible involvement in vascular dysfunction? Am J Obstet Gynecol
187(2): 452–456.
Wahrenbrock M, Borsig L, Le D, Varki N, Varki A (2003) Selectin-mucin
interactions as a probable molecular explanation for the association of
Trousseau syndrome with mucinous adenocarcinomas. J Clin Invest
112(6): 853–862.
Walsh-Moncaleg D, Green D (1997) Low-molecular-weight heparin in the
management of Trousseau’s syndrome. Cancer 80(4): 649–655.
Woei-A-Jin FJ, van der Starre WE, Tesselaar ME, Garcia Rodriguez P,
van Nieuwkoop C, Bertina RM, van Dissel JT, Osanto S (2014)
Procoagulant tissue factor activity on microparticles is associated with
disease severity and bacteremia in febrile urinary tract infections.
Thromb Res 133(5): 799–803.
Yue T, Maupin KA, Fallon B, Li L, Partyka K, Anderson MA, Brenner DE,
Kaul K, Zeh H, Moser AJ, Simeone DM, Feng Z, Brand RE, Haab BB
(2011) Enhanced discrimination of malignant from benign pancreatic
masses using microparticle-associated tissue factor expression.
PLoS One 6(12): e29180.
Zwicker JJ, Liebman HA, Neuberg D, Lacroix R, Bauer KA, Furie BC, Furie
B (2009) Tumor-derived tissue factor-bearing microparticles are associated
with venous thromboembolic events in malignancy. Clin Cancer Res
15(22): 6830–6840.

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