Clinical Evidence that Coagulation Activation Drives Cancer Progression – a Report of 2 Cases

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Established Facts
- Tissue factor (TF) is over-expressed by primary tumors and can be released into the bloodstream on plasma microparticles (MPs).
- Experimental studies indicate that TF may facilitate hematogenous metastasis by promoting tumor cell-induced microvascular thrombosis.

Novel Insights
- Further clinical evidence is provided that TF directly links coagulation activation to cancer cell dissemination.
- TF can cause thrombotic microangiopathy (TMA) and disseminated intravascular coagulation (DIC) in urothelial carcinoma.

Keywords
Tissue factor · Cancer · Thrombotic microangiopathy · Disseminated intravascular coagulation · Pulmonary hypertension

Summary
Background: Tissue factor (TF), the principal initiator of the extrinsic coagulation pathway, is expressed by many tumors and can be released into the bloodstream on plasma microparticles (MPs). Experimental studies indicate that TF may facilitate hematogenous metastasis by promoting tumor cell-induced microvascular thrombosis, but clinical data supporting this hypothesis is sparse. Case Reports: Here, we report 2 unusual cases of rapidly progressive solid malignancies (gastric and urothelial carcinoma). In both patients, cancer cell dissemination with diffuse bone marrow involvement was either strongly suggested by leukoerythroblastic changes on peripheral blood smear or directly proven by positive findings on aspiration cytology. Furthermore, laboratory evidence of thrombotic microangiopathy (TMA) and disseminated intravascular coagulation was accompanied by new-onset severe pulmonary hypertension and a hemolytic uremic syndrome-like disorder in the gastric and the urothelial carcinoma patient, respectively. TF-specific procoagulant activity of isolated plasma MPs, as assessed by single-stage clotting assay, was dramatically increased in both patients compared to healthy controls (21- and 55-fold), and primary tumor samples stained strongly positive for TF by immunohistochemistry. Conclusion: TMA was likely caused by TF-triggered tumor cell embolization in both patients. Further clinical evidence is thus provided that TF directly links coagulation activation to cancer cell dissemination.

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Fig. 1. Laboratory and microscopic signs of thrombotic microangiopathy (TMA) and disseminated intravascular coagulation (DIC). A Laboratory work-up at the time of diagnosis of TMA and DIC. B, C Increased numbers of fragmentocytes (→) on peripheral blood smear (May-Grünwald-Giemsa stain) indicated TMA in patient 1. The presence of immature precursor cells such as polychromatic erythroblasts (*) and myelocytes (#) further suggested bone marrow carcinomatosis. D In patient 2, increased numbers of fragmentocytes (→) and presence of polychromatic erythroblasts (*) on peripheral blood smear were indicative of TMA and bone marrow carcinomatosis, respectively. E Bone marrow carcinomatosis was confirmed by the detection of scattered tumor cells in a bone marrow aspirate (May-Grünwald-Giemsa stain). Photo was taken through a 40× objective.

Introduction

In patients with cancer, tumor growth and coagulation activation are closely interrelated [1]. In this regard, malignancy may result in both macrovascular thrombosis (e.g. venous thromboembolism) and more complex clotting abnormalities such as thrombotic microangiopathy (TMA) or disseminated intravascular coagulation (DIC) [2].

Preclinical studies further indicate that formation of tumor cell-induced microvascular thrombosis participates in hematogenous metastasis by shielding adherent cancer cells from the innate immune system, thus conferring a survival benefit during the blood-borne phase of the metastatic cascade [3]. Importantly, tissue factor (TF), the principal initiator of the extrinsic coagulation pathway that is over-expressed by highly aggressive tumors and that can be released into the circulation in association with plasma microparticles (MPs), is a key player in this process [4]. However, direct clinical evidence that TF indeed links coagulation activation to tumor cell dissemination is still sparse.

Case Reports

Case 1

A 70-year-old woman presented to an outside hospital with a 3-month history of progressive shortness of breath. An increased mean pulmonary artery pressure of 44 mm Hg (normal, < 25 mm Hg), as assessed by transthoracic echocardiography (TTE), and an elevated NT-proBNP serum level of 338 ng/l (normal, < 285 ng/l) indicated mild-to-moderate pulmonary hypertension, but further diagnostic work-up for an underlying heart or lung disease was unremarkable. A complete blood count and global coagulation tests were normal at this time. Because the patient also reported rather mild epigastric discomfort, an endoscopy of the upper gastrointestinal tract was performed that showed a fairly large gastric ulcer surrounded by reddish infiltrated mucosa. Microscopic examination of the biopsy revealed a poorly differentiated adenocarcinoma of the mixed type surrounding by reddish infiltrated mucosa. Microscopic examination of the biopsy specimen revealed a poorly differentiated adenocarcinoma of the mixed type according to the classification by Lauren. Computed tomography (CT) scanning of the chest and abdomen showed marginally enlarged mediastinal lymph nodes, but no convincing evidence for regional or distant metastases. The patient was discharged home for the Christmas holidays, and multimodal therapy of the clinically localized gastric carcinoma was scheduled for the next year. On admission to our hospital about 1.5 weeks later, the patient complained of progressive dyspnea and fatigue. The heart rate and blood pressure were 95/ min and 135/85 mm Hg, respectively, and the peripheral oxygen saturation was 94% while the patient was breathing ambient air. There was pitting edema at the lower extremities, but no other findings suggestive of a cardiac or pulmonary disease. Initial laboratory tests revealed a prothrombin time (PT) of 55% (normal, 80–130%) and a decreased plasma fibrinogen level of 0.76 g/l (normal, 1.8–4.0 g/l). The activated partial thromboplastin time (aPTT) and the platelet count were normal as was the hemoglobin level.

To further assess local and regional tumor extension, an endobronchial and endoscopic ultrasound were scheduled, but within 3 days, the patient's clinical condition deteriorated and the peripheral oxygen saturation repeatedly declined to 75% despite inflation of 100% oxygen via a face mask. CT scanning of the chest excluded gross pulmonary embolism, but findings of dilated pulmonary arteries and interstitial opacities with both linear and reticular ground-glass appearance were consistent with pulmonary arterial hypertension. A repeated TTE revealed severe right ventricular dysfunction due to pressure overload with a right basal ventricular diameter of 4.3 cm (normal, < 2.9 cm) and paradoxical septal motion. The NT-proBNP serum level had increased to 7,034 ng/l. The patient was transferred to our intensive care unit, where endotracheal intubation became necessary due to cardiorespiratory distress. At this time, laboratory tests showed moderate-to-severe thrombocytopenia, normocytic (mean corpuscular volume (MCV), 85.9 fl) normochromic (mean corpuscular hemoglobin (MCH), 30.5 pg) anemia with 28‰ fragmentocytes on peripheral blood smear indirectly suggested bone marrow carcinomatosis (fig. 1 A–C). Collectively, these findings were consistent with pulmonary tumor thrombotic microangiopathy (PTTM) and DIC due to disseminated gastric carcinoma. Despite maximal supportive care, the patient died from cardiac arrest 6 days after hospital admission.

MPs were isolated from the patient’s plasma that was left over from routine coagulation testing by sequential high-speed centrifugation and analyzed for TF-specific procoagulant activity (TF PCA) using a previously described single-stage clotting assay [5]. Compared to the mean level of 34 healthy controls, MP TF PCA was increased 21-fold (fig. 2 A). In addition, we performed TF immunochemistry on the gastric biopsy specimen using a monoclonal IgG antibody (no. 4509; Sekisui Diagnostics, LLC, Lexington, MA, USA), which showed strong TF expression by the adenocarcinoma cells (fig. 2 B, C).
Fig. 2. Analysis of tissue factor (TF) on plasma microparticles and in primary tumor specimens. A Plasma microparticles (MPs) were isolated by high-speed centrifugation and analyzed for TF-specific procoagulant activity (PCA) by single-stage clotting assay, essentially as described [5]. Results are expressed as arbitrary TF activity units (U) per ml of plasma. B, C Immunohistochemistry on the gastric biopsy specimen from patient 1 revealed strong TF expression by adenocarcinoma cells. Photos were taken through a 10× objective. D TF immunohistochemistry on the bladder biopsy specimen from patient 2 revealed strong staining of the urothelial carcinoma cells, but not of the adjacent transitional epithelium (20× objective).

Case 2
An 81-year-old man was admitted to an outside hospital because of symptomatic right-sided hydronephrosis, most likely resulting from a bladder diverticulum. A nephrostomy tube was placed, and a transurethral bladder biopsy was unremarkable. 3 months later, the nephrostomy tube was replaced by a double J stent. Another 2 months later, premature replacement of the ureteric stent became necessary due to recurrent ureteric obstruction. A repeated transurethral biopsy now revealed a poorly differentiated muscle-invasive bladder carcinoma. Shortly after the intervention, the serum creatinine level increased to 4.0 mg/dl (normal, 0.7–1.2 mg/dl). Bilateral nephrostomy tubes were placed for suspected postrenal acute kidney injury, but there was no substantial improvement in kidney function (serum creatinine, 3.1 mg/dl). Progressive thrombocytopenia, normocytic (MCV, 90.5 fl) normochromic (MCH, 30.0 pg) anemia with 10‰ fragmentocytes on peripheral blood smear, an increased indirect bilirubin serum level of 1.4 mg/dl, and a maximally decreased hemoglobin of < 0.2 g/l (normal, 0.3–2 g/l) suggested TMA with renal involvement (fig. 1 A, D).

On referral to our hospital, the patient was in moderate distress with multiple cutaneous hematomas. Further laboratory work-up revealed a prolonged PT, severe hypofibrinogenemia, and excessively increased plasma D-dimer consistent with decompensated DIC (fig. 1 A). Right-sided peripheral pulmonary embolism and left-sided iliofemoral hematóma were detected by ventilation/perfusion scintigraphy and native CT scanning, respectively. There was no evidence for gross metastatic disease, but scattered tumor cells were found in a bone marrow aspirate (fig. 1 E). The patient received best supportive care and died within 2 weeks after onset of renal failure. TF PCA of MPs that had been isolated from residual patient plasma was increased 55-fold (fig. 2 A). Furthermore, urothelial cancer cells of the bladder biopsy stained strongly positive for TF by immunohistochemistry (fig. 2 D).

Discussion
TMAs are a heterogeneous group of disorders characterized by microvascular occlusion, resulting in thrombocytopenia, microangiopathic hemolytic anemia, and ischemic organ dysfunction [6]. The pathophysiology of cancer-associated TMA remains incompletely understood, but may involve the release of ultra-large von Willebrand factor multimers and/or an acquired deficiency of its cleaving metalloproteinase, ADAMTS13 [7].

Although no direct histological evidence is provided to support this hypothesis, both clinical and laboratory findings in our patients suggested that TMA was at least partially caused by embolization of tumor cells into organ microvessels. In this regard, diffuse pulmonary involvement has been described in a small case series [8]. Histopathological studies have further shown that such tumor microemboli not only cause acute small-vessel occlusion, but also induce obliterator fibrinointimal hyperplasia within pulmonary arteries through activation of the coagulation protease cascade and release of inflammatory and angiogenic mediators [9]. This clinicopathological condition is referred to as PTTM [10]. In some of these patients, strong tumor cell TF expression has been demonstrated by in-situ immunohistochemistry [9, 11]. Interestingly, these findings of PTTM resemble those obtained in mice after intravenous injection of TF-bearing tumor cells [12].

In both of our patients, TF-specific PCA of isolated plasma MPs was dramatically increased. Furthermore, strong TF expression in primary tumor samples suggested that this MP TF PCA was indeed cancer cell-derived. It is therefore tempting to speculate that, in addition to TF expressed by primary tumor cells, recruitment of TF-bearing MPs into the developing thrombus contributed to the formation of tumor cell microemboli by propagating further thrombin generation and fibrin deposition following separation of the TF-rich tumor cell surface from the flowing blood [1]. However, larger studies with more practical and better standardized assays for the measurement of MP-associated TF are needed to confirm these findings.

In mice, TF promotes hematogenous metastasis [1, 3]. Specifically, coating of circulating tumor cells by fibrin(ogen)-platelet aggregates facilitates their firm adhesion to, and subsequent spreading on, the vascular endothelium and confers protection from natural killer cell-mediated cytotoxicity [4, 13]. Both of our patients initially presented with clinically localized solid malignancies. However, systemic spread of cancer cells with diffuse bone marrow involvement was strongly suggested in patient 1 and cytologically proven in patient 2, which may be regarded as further clinical evidence that TF is indeed a critical determinant of a tumor’s metastatic potential.

In patient 1, rapid progression of respiratory and right-sided heart failure as well as findings on imaging studies were consistent with pulmonary hypertension due to diffuse obstruction of arterioles by embolized tumor cells [14], especially, since PTTM has previously been reported in patients with gastric adenocarcinoma.
Nevertheless, a lung biopsy is usually required to establish a definitive diagnosis [15], but the clinical condition of patients presenting with signs of PTM may preclude such an invasive procedure. Therefore, PTM often remains an elusive antemortem diagnosis. In this regard, the diagnostic process is further complicated by the fact that significantly elevated serum levels of NT-proBNP, as measured in our patient, are not clearly correlated with heart failure or volume overload in patients with malignant diseases [16]. Rapid evolution of a consumptive coagulopathy in our patients was in line with massive intravascular activation of the TF-dependent coagulation pathway. Formation of fibrin-rich microthrombi not directly related to embolized tumor cells, but secondary to DIC may have contributed to clinical symptoms in our patients, with pulmonary hypertension and a hemolytic uremic syndrome-like disorder as the predominant types of organ dysfunction in patient 1 and 2, respectively. DIC is quite common in malignancy [17] and may occur simultaneously with cancer-associated TMA [18]. Because both disorders share certain clinical and laboratory characteristics, a clear distinction may not be possible without pathological examination. Yet, microangiopathic changes in DIC patients are generally mild with only scattered fragmented cells visible on peripheral blood smears [19], while severe derangement of global coagulation tests is not a typical feature of primary TMA [20].

Only a few cases of DIC complicating urothelial carcinoma have been reported in the literature [21–23]. Although in 1 of these cases, direct contact of the flowing blood with an intracardiac metastasis was thought to be the causative event, the molecular mechanisms underlying this interaction remained speculative [22]. Here, we provide both functional and immunohistochemical evidence that TF plays an important thrombogenic role not only in (mucin-producing) adenocarcinomas, but also in urothelial bladder carcinoma.

Cancer-associated DIC is frequently associated with excessive fibrinogenolysis, which, in addition to the consumptive coagulopathy, significantly contributes to the bleeding tendency of affected patients [5, 24]. The rather modestly decreased plasmin inhibitor levels in our patients, however, may argue against relevant exhaustion of the antifibrinolytic system and could explain why, despite evolution of a thrombohemorrhagic syndrome in patient 2, no life-threatening bleeding complications occurred (fig. 1A). In addition, this finding may also be interpreted in favor of our hypothesis that TMA was mainly due to tumor cell embolization and not DIC-related fibrin strand formation.

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Disclosure Statement

All authors have read and approved this manuscript and declared no conflict of interest.

References