Plasma tissue factor antigen in localized prostate cancer: Distribution, clinical significance and correlation with haemostatic activation markers

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Summary

Tissue factor (TF) is involved in cancer growth and metastasis, and haemostatic abnormalities are found in most patients with advanced malignancies, including prostate cancer (PC). Because anti-haemostatic agents are increasingly screened for their potential to prolong survival in tumor patients, a detailed characterization of haemostatic markers in selected cancer subtypes and clinical stages is warranted. In this study, we measured preoperative plasma TF antigen in a large cohort of patients with localized PC and correlated its levels with markers of coagulation and platelet activation, prostate-specific antigen (PSA), and histopathological findings to explore its potential as a prognostic marker in this tumor entity. Out of 140 patients, 19% and 23% had plasma TF antigen levels of <40 pg/ml (low-TF) and >200 pg/ml (high-TF), respectively, which was substantially higher than in 42 healthy male controls. Patients also had low-grade systemic coagulation activation as evidenced by elevated D-dimer, F1+2, and PAP plasma levels. Furthermore, similar to sP-selectin and sCD40L antigen, flow cytometric analysis of platelet-derived microparticles in plasma revealed significantly increased numbers in high-TF as compared to low-TF patients and controls. Whereas elevated D-dimer was associated with larger and less differentiated tumors, preoperative plasma TF antigen levels (median [IQR]) were higher in patients with (161 pg/ml [100–236]) than in those without recurrent PC (105 pg/ml [52–182]), as indicated by a serum PSA of >0.1 ng/ml during ambulatory follow-up. In patients with localized PC, preoperative plasma TF antigen levels correlate with platelet activation in vivo and may indicate an increased risk for recurrent disease.

Keywords

Tissue factor / factor VII, cancer, microparticles

Introduction

Tissue factor (TF), an integral transmembrane glycoprotein of 47 kDa, is the cellular receptor for factor VII (FVII)/FVIIa and the physiologic initiator of blood coagulation (1). Besides its role in thrombosis and haemostasis, TF has been implicated in inflammation, vasculogenesis, and cancer. For instance, there is overwhelming evidence linking TF to various cellular processes directly involved in tumor growth and dissemination such as adhesion, migration, proliferation, angiogenesis, and haematogenous metastasis (2).

On the one hand, TF-mediated coagulation activation leads to thrombin generation and platelet activation, both of which promote cancer spread on multiple levels (3). On the other hand, upstream formation of the TF-FVIIa and TF-FVIIa-FXa complex initiates signaling via protease-activated receptors (PAR-1, PAR-2), thus affecting cancer biology in a “coagulation-independent” manner (2). Furthermore, the TF cytoplasmic domain may be directly involved in gene regulation of tumor cells (3). TF expression has been demonstrated in several types of malignancy, including prostate cancer, and its expression levels appear to correlate with various histopathological and clinical parameters such as microvessel density, histological differentiation, and patient outcome (4–8). Furthermore, systemic coagulation activation in cancer, which may ultimately lead to thromboembolic complications commonly referred to as Trousseau’s
syndrome, has long been known to negatively affect patient outcome, thus providing additional support for the concept that the haemostatic system promotes cancer progression (9–13).

Considering its plethora of functions in cancer biology, the question arises of whether TF may serve as a tumor marker with prognostic relevance. Due to their lack of specificity, tumor markers are not used for diagnostic purposes, but their measurement may be helpful in the risk stratification of patients after diagnosis, in the assessment of treatment efficacy, and during follow-up. Besides its expression on cell membranes, TF may be released into plasma as a microparticle-associated and/or a soluble variant (14, 15), potentially making its detection less cumbersome and time consuming. Although absolute concentrations differ between studies, levels of circulating TF are typically low or undetectable in healthy individuals (16), but may be significantly increased in patients with diabetes mellitus, infections, inflammatory, sickle cell or cardiovascular disease, and cancer (17–20). Most notably, in a recent study, elevated serum TF antigen levels have been suggested to be an independent predictor of poor clinical outcome in women with ovarian cancer (21).

Prostate cancer is the most common malignancy in older men and has been associated, in advanced stages, with a systemic coagulopathy characterized by disseminated intravascular coagulation (DIC) and excessive fibrinogenolysis (22–24). In localized stages, definitive cure may only be achieved by surgical means, whereas in patients with metastatic disease, anti-hormonal and/or cytotoxic strategies are used to relieve symptoms.

Because 15–20% of patients with localized prostate cancer experience recurrent local and/or distant metastatic disease within ten years after surgery (25), additional tools are needed to better predict the risk of individual patients and to provide a basis for adjuvant treatment strategies. For instance, anti-haemostatic therapy with low-molecular-weight heparin has been shown to result in a limited albeit significant survival benefit in unselected cancer patients with an a priori better clinical prognosis (26, 27).

In this prospective study, we measured preoperative plasma TF antigen in a large cohort of patients with localized prostate cancer and correlated its levels with various haemostatic, histopathological, and clinical parameters to explore its potential as a prognostic marker in this tumor entity.

**Materials and methods**

**Patients and controls**

All patients with prostate cancer admitted to the Department of Urology at the University Hospital Hamburg-Eppendorf, Germany, for radical prostatectomy between February 2002 and March 2003 were eligible for the study. Patients were to have histologically proven prostate cancer by means of fine needle biopsy with no clinical evidence for locally advanced or distant metastatic disease. Patients with any medical condition potentially affecting coagulation assessment, such as surgical procedures, including prostate biopsy, within four weeks of admission, acute or chronic infections, advanced hepatic or renal failure, inflammatory disorders, thromboembolic complications, or significant cardiovascular disease, were excluded from the study. Any anticoagulant or anti-platelet medication was an exclusion criterion. Healthy male controls were recruited from the hospital’s local blood bank.

**Patient follow-up**

In June of 2006, primary physicians or urologists were contacted by telephone to inquire on data on serum prostate-specific antigen (PSA). For each patient, the latest recorded PSA level was used to calculate duration of follow-up. A serum PSA of >0.1 ng/ml was prospectively defined as indicative for recurrent prostate cancer. This postoperative PSA cut-off is significantly lower than the PSA cut-off commonly used for prostate cancer screening in adult men before radical prostatectomy (4 ng/ml).

**Blood sampling and processing**

After obtaining informed consent from participating patients and before administration of heparin for perioperative thromboprophylaxis, venous blood was drawn into 1/9 volume of 3.2% (0.106 M) trisodium citrate. Following centrifugation of whole blood for 15 minutes (min) at 1,500 x g, plasma was aliquoted and stored at −80°C until analysis. For each assay, a previously unthawed aliquot was used.

**Measurement of plasma TF antigen**

A commercially available enzyme-linked immunosorbent assay (ELISA) kit (Imubind™, American Diagnostica, Greenwich, CT, USA) was used according to the manufacturer’s instructions to measure TF antigen in plasma. Briefly, samples were diluted 1:4 in PBS (pH 7.4) containing 0.1% Triton X-100 and 1% (w/v) bovine serum albumin and incubated overnight in the wells of a microtiter plate precoated with murine anti-human TF monoclonal antibody for antigen capture. After detection of bound antigen by biotinylated polyclonal anti-TF F(ab′)2, streptavidin-conjugated horseradish peroxidase was added to complete formation of the antibody fragment-enzyme detection complex. Following the stepwise addition of tetramethylbenzidine chromogenic substrate and sulfuric acid stopping solution, absorbances of yellowish colored solutions were read at 450 nm. Mean optical densities of duplicate incubations were referred to a standard curve obtained by serial concentrations (0–1,000 pg/ml) of non-lipidated, full-length recombinant human TF. Lyophilized standards were provided in separate vials with the lowest TF-containing calibrator having a concentration of 50 pg/ml upon reconstitution.

According to the manufacturer, the lower detection limit of the ELISA is 10 pg/ml. This limit converts to a final TF concentration of 40 pg/ml in plasma, which is diluted 1:4 before being assayed in the system. However, even plasma TF antigen concentrations of 40–200 pg/ml are read from a hypothetical standard curve connecting the 50 pg/ml calibrator with the 0 pg/ml data point. Results for plasma TF antigen were therefore grouped in the following three categories of concentrations: i) TF<40 pg/ml, which was considered below the detection limit of the ELISA; ii) 40≤TF≤200 pg/ml, for which no real TF calibrators were available; iii) TF>200 pg/ml, for which real TF calibrators were available. As explicitly stated by the manufacturer, a normal range of TF antigen in human plasma has not been established.

**Measurement of haemostatic markers**

Commercially available ELISA kits were used according to the manufacturers’ instructions to measure plasma antigen levels of prothrombin fragment F1+2 (Enzygnost™; Dade Behring,
Schwalbach, Germany), plasmin-α2-antiplasmin (PAP) complex (Technoclone, Vienna, Austria), soluble CD40 ligand (sCD40L), and soluble P-selectin (sP-selectin) (Quantikine™; R&D Systems, Minneapolis, MN, USA). Plasma levels of D-dimer were measured using a latex agglutination test for the quantitative turbidimetric determination of cross-linked fibrin split products (Organon Teknika, Brisbane, Australia).

Flow cytometric analysis of plasma microparticles
A previously described assay was used with minor modifications to enumerate platelet-derived microparticles (PMP) in plasma (28). Briefly, 10 µl of sample were incubated with 10 µl of FITC-conjugated CD41 monoclonal (clone, P2) or isotype-matched control antibody (Immunotech, Marseille, France) for 20 min at room temperature in the dark. After adding a defined number of fluorescent 1.9-µm microspheres (Molecular Probes, Eugene, OR, USA) samples were diluted to a final volume of 1 ml with 0.2-µm-filtered PBS. PMP were enumerated using an EPICS™ Elite ESP flow cytometer (Coulter Electronics, Hialeah, FL, USA) counting a predefined number of microspheres for sample flow standardization, which were separated from PMP by their fluorescence and side/forward scatter characteristics. Only events positive for CD41 and <1.1 µm were included in the analysis. After subtraction of non-specific background events, total numbers of PMP per 1 ml of plasma were calculated according to the following formula: N=C x (TM/CM) x 100 (N, total numbers of PMP/ml; C, PMP counted during analysis; TM, total number of microspheres added; CM, microspheres counted during analysis). The inter-assay coefficient of variation for this methodology was 8.6% (n=15). For the enumeration of monocyte- and leukocyte-derived microparticles, FITC-conjugated monoclonal antibodies against CD14 (clone, RM052) and CD11b (clone, Bear-1; Immunotech), respectively, were used. TF was labeled on CD41+ microparticles by a PE-conjugated monoclonal (clone, HTF-1; BD Pharmingen, San Jose, CA, USA) in comparison to an isotype-matched control antibody (Immunotech).

High-speed centrifugation and filtration of plasma
To separate cellular microparticles from plasma, 200-µl aliquots of individual patients were centrifuged for 30 min at 16,000 x g. For ultracentrifugation (1:45 h at 300,000 x g) or 0.2-µm filtration, plasma samples of 11 patients with TF antigen levels of >100 pg/ml were pooled to achieve a sufficiently high sample volume.

Statistical analysis
Distribution of data was assessed by normal probability plots (Statistica™ for Windows; StatSoft, Tulsa, OK, USA). Normally distributed data were presented as mean ± SD and differences between groups were analyzed by two-sided, non-paired Student’s t-test. Non-normally distributed data were presented as median and range or inter-quartile range (IQR) and analyzed by Wilcoxon rank sum (Mann-Whitney U) test. Correlation coefficients (r) between normally and non-normally distributed data were calculated according to the methods of Pearson and Spearman, respectively. Throughout the study, statistical significance was assumed when p<0.05.

Results
Plasma TF antigen and markers of coagulation activation in patients with localized prostate cancer
A total of 140 patients were included in the study (Table 1). Out of these, 19% had plasma TF antigen levels below the detection limit of the ELISA (<40 pg/ml), whereas 23% had plasma TF antigen levels of >200 pg/ml (Fig. 1A). The remaining patients (58%) had plasma TF antigen levels of 40–200 pg/ml. The median plasma TF antigen concentration in the patient population was 107 pg/ml (IQR, 51–188 pg/ml). In contrast, 41 out of 42 healthy male controls (98%) had plasma TF antigen levels below 40 pg/ml, whereas only one individual had a plasma TF antigen level of 44 pg/ml. Thus, preoperative plasma TF antigen levels were significantly increased in patients with localized prostate cancer (p<0.001).

Additional haemostatic markers were measured in a representative subgroup of patients (n=98) to assess coagulation activation in vivo. Compared to controls (n=42), patients had significantly elevated D-dimer, F1+2, and PAP plasma levels (Fig. 1B), although the absolute differences were only minor. The findings may therefore be best interpreted as low-grade DIC in patients with localized prostate cancer.

Table 1: Clinical and histopathological characteristics of patients with localized prostate cancer (n=140).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (mean ± SD)</td>
<td>62 ± 6</td>
</tr>
<tr>
<td>Preoperative PSA, ng/ml</td>
<td></td>
</tr>
<tr>
<td>● median (range)</td>
<td>5.9 (0.5–60.9)</td>
</tr>
<tr>
<td>Pathological tumor stage, n (%)</td>
<td></td>
</tr>
<tr>
<td>● pT2a</td>
<td>10 (7)</td>
</tr>
<tr>
<td>● pT2b</td>
<td>92 (66)</td>
</tr>
<tr>
<td>● pT3a</td>
<td>25 (18)</td>
</tr>
<tr>
<td>● pT3b</td>
<td>13 (9)</td>
</tr>
<tr>
<td>Resection margins, n (%)</td>
<td></td>
</tr>
<tr>
<td>● positive</td>
<td>28 (20)</td>
</tr>
<tr>
<td>● negative</td>
<td>112 (80)</td>
</tr>
<tr>
<td>Gleason score</td>
<td></td>
</tr>
<tr>
<td>● median (range)</td>
<td>6 (5–7)</td>
</tr>
<tr>
<td>Lymph node metastases, n (%)</td>
<td></td>
</tr>
<tr>
<td>● yes</td>
<td>5 (4)</td>
</tr>
<tr>
<td>● no</td>
<td>20 (14)</td>
</tr>
<tr>
<td>● no lymphadenectomy performed</td>
<td>109 (78)</td>
</tr>
<tr>
<td>● unknown</td>
<td>6 (4)</td>
</tr>
<tr>
<td>Preoperative hormone therapy, n (%)</td>
<td></td>
</tr>
<tr>
<td>● yes</td>
<td>20 (14)</td>
</tr>
<tr>
<td>● no</td>
<td>120 (86)</td>
</tr>
</tbody>
</table>
Correlation of haemostatic markers with clinicopathological findings

Patients with more differentiated tumors, as indicated by a Gleason score of ≤6 (n=58), had significantly lower plasma D-dimer levels than patients with less differentiated tumors, as indicated by a Gleason score of ≥6 (n=40) (0.25 ± 0.13 vs. 0.33 ± 0.16 mg/l; p<0.02). Furthermore, there was a trend towards lower D-dimer levels in patients with tumors confined to the organ capsule (pT2, n=74) compared to patients whose tumors already showed extracapsular extension (pT3, n=24) (0.27 ± 0.13 vs. 0.33 ± 0.19 mg/l; p=0.06). These findings suggested that larger (pT3) and histologically more aggressive tumors (Gleason score, >6) were associated with increased plasma levels of D-dimer. There was no other correlation between any of the haemostatic parameters, including TF, and clinical or histopathological patient characteristics. Specifically, preoperative hormone therapy did not affect markers of coagulation activation (not shown).

PMP are increased in patients with localized prostate cancer

To further elucidate the origin of plasma TF antigen in patients with localized prostate cancer, we measured platelet- (PMP) and monocyte-derived microparticles (MoMP) in plasmas of randomly selected patients with low (<40 pg/ml) or high (≥200 pg/ml) TF-antigen levels (n=18 per group). Compared to controls (n=18), plasma PMP were significantly increased in both low-TF and high-TF patients (Fig. 2A, B). However, there was also a significant difference in PMP counts between the two patient groups: median PMP numbers were 7 x 10^9/ml in low-TF and 19 x 10^9/ml in high-TF patients (p<0.05). Furthermore, in high-TF patients, there was a significant correlation between plasma PMP and TF antigen levels (Fig. 2C).

Because these findings suggested a structural association of plasma TF antigen with PMP, we aimed to detect TF antigen on PMP from five high-TF patients using two-color flow cytometry. In none of the patients, TF antigen was unambiguously detectable on the surface of CD41+ events (Fig. 2D). To further explore the possibility that plasma TF antigen was directly associated with PMP, we subjected 200-µl plasma aliquots of 12 high-TF patients to high-speed centrifugation for 30 min at 16,000 x g, which was sufficient to reduce plasma PMP, as enumerated by flow cytometry, by >90%. In two patients (17%), there was a >80% reduction of plasma TF antigen in supernatants of centrifuged as compared to non-centrifuged samples, whereas TF antigen levels remained essentially unchanged in the other ten individuals. Similarly, while 0.2-µm filtration had no effect, ultracentrifugation (1:45 h at 300,000 x g) reduced TF antigen in pooled patient plasmas (n=11) by only 28% (Fig. 2E). Overall, these findings suggested that, in patients with localized prostate cancer, a substantial fraction of the plasma TF antigen was not directly associated with PMP or other cellular microparticles.

To rule out the possibility that cross-reactivity of the TF ELISA with platelet antigen(s) was responsible for increased signals in plasmas of high-TF patients, we measured TF antigen in matched plasma and serum samples of healthy individuals (n=9) prepared from whole blood after a standardized 1-hour incubation at 37°C. There was no difference in TF antigen levels between plasma and serum samples (not shown), whereas antigen levels of CD40 ligand, a platelet-derived protein released in soluble (sCD40L) and microparticle-associated form during the physiological blood clotting reaction, was 23-fold higher in serum as compared to plasma. Importantly, there was no significant difference in whole blood platelet counts between low-TF (274 ± 50/µl) and high TF patients (262 ± 80/µl), suggesting that plasma PMP numbers were not just a pure reflection of whole blood platelet counts.

MoMP, defined as <1.1 µm and positive for CD14 by flow cytometry, were below the detection limit of the assay in 15 out of 18 controls (83%). In the remaining three subjects, the mean MoMP number was 0.4 ± 0.4 x 10^9/µl. MoMP were undetectable in 61% and 56% of low-TF and high-TF patients, respectively. In the remaining patients, there was no difference in MoMP numbers between those with low and those with high plasma TF-antigen levels (3.1 ± 2.0 vs. 3.3 ± 2.4 x 10^9/µl). Virtually identical results were obtained when staining was carried out with a FITC-conjugated monoclonal antibody against CD11b (Mac-1) to potentially detect all leukocyte-derived microparticles (not shown).
Further evidence of platelet activation in high-TF patients
Because PMP are generated in vivo following stimulation of platelets, we measured two additional markers in plasmas of low-TF and high-TF patients, which have previously been associated with increased platelet activation: sP-selectin and sCD40L (17, 29). Antigen levels of sP-selectin were significantly increased in high-TF as compared to low-TF patients (Fig. 3). Furthermore, sCD40L antigen levels were clearly detectable (>100 pg/ml) in 44% of high-TF as compared to only 17% of low-TF patients (Fig. 3), suggesting that elevated PMP numbers in high-TF patients indeed reflected increased platelet activation in vivo.

PSA follow-up of patients
Serum TF antigen levels of ≥190 pg/ml have been associated with a poor clinical outcome in women with ovarian cancer (21). We therefore sought to investigate whether there was also an association between preoperative plasma TF antigen levels and the incidence of recurrent prostate cancer during ambulatory follow-up. PSA data were available for 132 of the 140 patients (94%) with a median follow-up of 37 months (range, 2–50 months). In 17 of the 132 patients (13%), a serum PSA of >0.1 ng/ml was recorded, fulfilling the formal criterion for recurrent disease. In these 17 patients, the median preoperative plasma TF antigen level was 161 pg/ml (IQR, 100–236 pg/ml) as compared to 105 pg/ml (IQR, 52–182 pg/ml) in patients with no evidence for recurrent disease (p=0.11). Accordingly, 41% and 21% of patients with and without recurrent prostate cancer, respectively, had preoperative plasma TF antigen levels of >200 pg/ml. The relatively short follow-up period and the low number of recurrences may partially explain why the difference in plasma TF antigen levels did not reach statistical significance.

Figure 2: PMP are increased in patients with localized prostate cancer. A) Representative dot plots from a control subject and a low-TF and high-TF patient are shown. All events inside the 1.1-µm gate are positive for CD41a and therefore defined as PMP. B) Quantitative analysis of plasma PMP in controls, low-TF, and high-TF patients (n=18 per group). P values are according to Wilcoxon rank sum test. C) In high-TF patients, a significant correlation was found between plasma PMP and TF antigen levels. Correlation coefficient (r) is according to Spearman's. D) Using two-color flow cytometry, TF antigen was not reliably detectable on PMP from patients with high plasma TF antigen levels. Dot plots are representative of five patients randomly selected from this group. E) Plasma pooled from 11 patients was either filtered (0.2 µm) or ultracentrifuged (1:45 h at 300,000 x g) and then subjected to the TF Imubind™ ELISA. In addition, TF antigen was measured in the resuspended microparticle pellet following ultracentrifugation. Results are presented as mean ± SD of quadruplicate measurements.
Discussion

In this study, we measured preoperative plasma TF antigen in a large cohort of patients with localized prostate cancer to elucidate its potential as a prognostic marker in this tumor entity. Patients with locally advanced and/or metastatic disease were not included to eliminate gross differences in tumor burden as a potentially confounding factor for systemic coagulation activation (11, 13, 22).

While D-dimer, F1+2, and PAP levels were only slightly increased in patients compared to controls (Fig. 1B), plasma TF antigen allowed a clearer separation of the two groups: 23% of patients had plasma TF antigen levels of >200 pg/ml, which is in the same magnitude of patients expected to experience recurrent disease after surgery (25). In contrast, most controls (98%) had plasma TF antigen levels below the detection limit of the ELISA (<40 pg/ml). The latter finding is somewhat surprising considering previous studies reporting mean or median plasma TF antigen concentrations of 50–150 pg/ml in healthy individuals (for a summary of these studies see Table 2 in reference 16). Two groups, however, have recently measured median TF antigen levels of 14–31 pg/ml in their respective control cohorts, which is more consistent with our finding (21, 30). Still, the stringent criteria according to which healthy blood donors were screened for underlying diseases in our study may explain some of the overall discrepancy. In addition, a recent study has emphasized the importance of exercising caution when interpreting results obtained with the Imubind™ TF ELISA, which may measure falsely elevated plasma TF antigen levels in normal subjects (16). Overall, the presence of intravascular TF in healthy individuals remains controversial with respect to its concentration and functionality (16, 31), especially, when considering the different TF variants potentially present in blood: full-length TF maybe be present on blood cells and microparticles, whereas truncated TF and alternatively spliced TF are proteins soluble in aqueous solutions. Neither the functional nor the biological relevance of the latter two TF variants is conclusively resolved. In plasma, however, both microparticle-associated and soluble TF may be detected by ELISA.

While there was no association of preoperative TF antigen levels with any of the clinical or histopathological patient characteristics, we observed a significant correlation between plasma TF antigen and PMP in high-TF patients (Fig. 2C). Although de-novo synthesis of TF by resting platelets is still a matter of debate (32, 33), platelets have been implicated in the uptake, transport, and release of TF antigen (15, 34, 35). Specifically, activated platelets may play a critical role in the decryption of latent monocyte TF procoagulant activity (36), and fusion of TF-bearing monocyte-derived microparticles (MoMP) with activated platelets has been demonstrated (37). Therefore, an association of platelet activation markers with plasma TF antigen levels in cancer patients is plausible, especially, when considering that platelet activation and stimulation of circulating monocytes by cytokines are common phenomena in malignancy, including prostate cancer (18, 30, 38). Indeed, we found increased numbers of CD14+ MoMP in patient plasmas, although there was no difference in MoMP counts between low-TF and high-TF patients.

A most recent study has demonstrated the splicing of endogenous TF pre-mRNA and the subsequent production of surface and microparticle-associated TF protein by activated platelets (39), potentially providing a mechanistic explanation for our findings. However, our experiments suggested that a substantial fraction of the plasma TF antigen was present as a soluble, i.e., non-microparticle-associated variant (Fig. 2D, E), arguing against a direct structural association of plasma TF antigen with PMP. Thus, alternative cellular sources have to be taken into consideration, including leukocytes, malignant epithelial cells, and cells of the tumor microenvironment such as angiogenic endothelial cells, fibroblasts, and macrophages (2, 3, 15, 40). Up-regulation of TF by these cells with subsequent coagulation and platelet activation may therefore explain the numerical correlation between plasma TF antigen and PMP in high-TF patients. In this regard it is noteworthy, however, that we did not find a significant difference in TF antigen staining pattern between primary tumors of low-TF and high-TF patients by immunohistochemistry (not shown).

This study has several limitations. First, we cannot rule out the possibility that, at least in some individuals, increased plasma TF antigen levels were not directly related to malignant prostate tumors. For instance, vascular damage due to clinically unapparent atherosclerosis may have accounted for some of our findings. Second, no follow-up plasma samples were available. Therefore, we cannot comment on intra-individual changes in plasma TF antigen levels after surgery or at diagnosis of recur-
rent prostate cancer. Third, patients with benign prostate hyperplasia were not included. Therefore, we cannot comment on whether increased plasma TF antigen levels were specific for a subset of malignant prostate tumors. Last, during a rather short follow-up period, the diagnosis of recurrent prostate cancer was entirely based on a serum PSA of >0.1 ng/ml, leaving the possibility of false positive and false negative results.

Nevertheless, this is the first report on preoperative plasma TF antigen levels in a large cohort of patients with localized prostate cancer, demonstrating a significant correlation between plasma TF antigen levels and markers of in vivo platelet activation. In addition, further follow-up will show whether plasma TF antigen levels can indeed identify those individuals at high risk for recurrent local and/or metastatic disease, similar to what has been suggested for patients with ovarian cancer. Based on the findings presented here, future studies in tumor patients should carefully consider the different variants and sources of intravascular TF when evaluating its potential as a prognostic marker in malignant disease.

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