

URINARY TISSUE FACTOR LEVELS IN NEOPLASTIC DISEASES

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Background: Abnormalities in laboratory coagulation and fibrinolysis parameters can be detected in cancer patients, and tissue factor (TF) is implicated. TF is produced by certain tumors and is increased in both tumor-associated macrophages and blood monocytes (mTF). TF is also found in urine (uTF), and its levels may be clinically important.

Materials and Methods: Using a simple and highly standardized kinetic chromogenic assay (KCA), we measured uTF levels in controls (normal, n=57; patients with renal stones, n=30), patients with benign and malignant conditions of the bladder (n=75), prostate (n=106), breast (n=94), and colorectum (n=62). Each benign disease group was subdivided into inflammatory and noninflammatory categories.

Results: The controls and benign noninflammatory groups gave similar results and were, therefore, unified for further analysis. The malignant and inflammatory groups showed higher uTF levels than the controls ($P<0.001$ for bladder, $P<0.01$ prostate, $P<0.001$ breast, and $P<0.001$ for colorectum). The difference between malignant and benign inflammatory disease was significant for the bladder group ($P<0.05$). Cancer patients showed uTF activity above the upper quartile range of the normal control group—74.4% for bladder, 68.0% for prostate, 77.3% for breast and 73.0% for colorectal disease. uTF levels were related to tumor progression, patients' survival time, serum prostate specific antigen (PSA), and static bone scan images (SBSI). Levels were also higher in patients with bladder cancer recurrence and those who subsequently died.

Conclusion: uTF levels are raised in malignant and inflammatory disease compared to controls and patients with noninflammatory conditions, and are related to tumor grade or stage, patients' survival and to markers of tumor progression.

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Key Words: Urinary tissue factor, coagulation, solid tumors.

Coagulation activation is common in human and experimental malignancy. In 1865, Trousseau made the first observation of an association between malignancy and vascular thrombosis.¹ Since then, a growing body of clinical, laboratory, pharmacological and histological evidence has emerged to support the finding that coagulation activation is frequent in a tumor-bearing host, and that the hypercoagulable state associated with malignant diseases is disadvantageous to the host.

Local or systemic initiation of blood coagulation can be triggered by a tumor or related products. Tumor cells can directly interact with platelets² and produce procoagulants such as cancer procoagulant (CP)³ and/or tissue factor

X to be accomplished. TF expression can also be induced in monocytes, macrophages and endothelial cells in response to a direct stimulation by tumor products (antigens, protease, etc.), or indirectly following activation of other components of the immune system.^{6,7}

TF apoprotein (tissue thromboplastin, CD142) is a 46 kDA, single-chain, integral plasma membrane glycoprotein with no intrinsic protease activity.^{8,9} TF serves as a receptor and essential cofactor for blood coagulation factors VII and VIIa.¹⁰ This bimolecular complex (TF-VII/VIIa) in the presence of Ca^{++} and lipid activates factors IX and X by limited proteolysis. It is considered as the primary initiator of coagulation.¹¹

Urine is known to contain a potent procoagulant,¹² whose identity has been confirmed as TF.^{13,14} Previous studies have shown that uTF is increased in patients with cancer and inflammatory bowel diseases,¹³⁻¹⁶ although unpublished studies by other workers failed to confirm this finding. Recently, we described a highly standardized assay for measuring uTF activity, with a coefficient of variation (CV) $<10\%$. The assay is reproducible and is not significantly

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Accepted for publication 4 March 2000. Received 5 October 1999. (TF).^{4,5} The CP activates factor X directly, whereas TF requires factors VII/VIIa for its proteolytic activity on factor

TABLE 1. *Sample size of the studied groups.*

Group	N	Median (age)	Age (range)	Male (n)	Female (n)
Normal	57	33	5-69	30	27
Renal stones	30	44	20-88	14	16
Bladder					
Benign, noninflammatory*	26	64	23-79	17	9
Benign, inflammatory**	9	76	40-85	4	5
Cancer	40	71	24-95	27	13
Prostate					
Benign, noninflammatory*	67	72	41-95	67	–
Benign, inflammatory**	13	65	38-78	13	–
Cancer	26	74	60-90	26	–
Breast					
Benign, noninflammatory*	24	43	20-72	–	24
Benign, inflammatory**	7	56	40-76	–	7
Cancer	63	61	26-87	–	63
Colorectal					
Benign, noninflammatory*	26	60	39-88	15	11
Benign, inflammatory**	13	65	45-92	8	5
Cancer	23	73	43-96	10	13
TCC					
No recurrence ⁺	4	74	53-89	14	–
With recurrence ⁺	16	72	63-85	14	2

*negative cystoscopy following hematuria; histologically proven BPH; known diverticular disease but not active, fibrocystic disease, fibroadenoma or intraductal papilloma; **cystitis, prostatitis, mastitis or mammary duct ectasia, ulcerative colitis or diverticulitis; ⁺previous bladder malignancy; TCC=transitional cell carcinoma.

affected by age, gender or cigarette smoking.¹⁷ In the current study, we investigated the reliability of the uTF assay, assessed uTF levels in controls and patients with solid malignancy, and examined whether uTF levels are related to malignant disease grade or stage, patients' survival time and conventional markers of tumor progression, i.e., prostate specific antigen (PSA) and static bone scan images (SBSI).

Materials and Methods

Controls and Subjects

A total of 454 subjects were studied: patients were admitted into the surgical wards of Southampton University Hospitals (Table 1). Ethical approval was obtained for the study, and informed consent was sought from each patient. Urine specimens were obtained prior to operation except for those with recurrent diseases. The control group consisted of normal volunteers and patients with renal stones (nonspecific organ and noninflammatory benign disease) who had no inflammatory symptoms and normal erythrocyte sedimentation rate (ESR). Patients with benign disease were subdivided into noninflammatory and inflammatory disease groups (specific organ noninflammatory or inflammatory benign disease). All patients had been clinically diagnosed and confirmed by biopsy. The histopathology reports were subsequently reviewed. Tumors were classified as follows: the modified classification of the World Health Organization (WHO) for the bladder,¹⁸ the Gleason's system for the

prostate,¹⁹ WHO classification for breast cancer,²⁰ and Dukes' categorization for colorectal cancer.²¹

Urinary Tissue Factor Measurements

Urine samples were collected from each subject into sterile universal containers without preservative. Samples were then sedimented, solubilized and assayed for uTF activity, using a one-stage kinetic chromogenic assay (KCA).¹⁷

Serum Prostate Specific Antigen

PSA was measured by the IMx^R PSA assay (Abbott Laboratories, USA) in patients with benign prostatic hypertrophy (BPH) and patients with prostate cancer.

Static Bone Scan Imaging

Single phase SBSI was assessed as described by McKillop and Fogelman.²²

Statistical Analysis

Data were analyzed by the STATGRAPHICSTM statistical software system. Data were not normally distributed, and summary statistics were expressed as medians and interquartile ranges (IQR). Differences between two groups were assessed by the Mann-Whitney U-test. Differences in tumor grading or staging were tested by Kruskal-Wallis One-Way Analysis by Ranks. uTF and PSA were log-transformed, thus correlations were determined using Pearson's correlation test.

Results

The Reliability of the uTF Assay

The assay showed sensitivity and specificity. The average cancer figures overall were 77.0% and 77.9%, respectively.

Assessment of uTF Activity in Hospital Patients

uTF activity was assessed in the malignant groups against controls and the relevant benign diseases groups. The median and IQR of the uTF levels for these groups are shown in Table 2. In the bladder group, there was no significant difference between the normal controls, the renal stones and the noninflammatory benign disease groups (Table 2). These three groups showed significantly lower uTF levels than the inflammatory benign disease ($P<0.001$) or the malignant group ($P<0.001$, Table 2). The difference between uTF activity in inflammatory benign disease and bladder malignancy was statistically significant ($P<0.05$).

For the prostate group, no significant difference was observed between the control groups. The benign inflammatory disease and the malignant group showed significantly higher levels than the three control groups ($P<0.01$). However, there was no significant difference between the benign inflammatory and the malignant cases (Table 2).

TABLE 2. uTF activity (ng/mL) in normal controls, renal stones, noninflammatory and inflammatory benign conditions and malignant disease, for bladder, prostate, breast and colorectal diseases.

Group	N	Median	IQR	Comparison group P-value		
				Normal controls	Benign inflammatory	Malignant
Bladder disease						
Normal controls	57	8	6-10	–	<0.001	<0.001
Renal stones	30	9	3-12	NS	<0.001	<0.001
Benign noninflammatory	26	8	3-11	NS	<0.001	<0.001
Benign inflammatory	9	27	16-39	<0.001	–	<0.05
Malignant	40	13	10-22	<0.001	<0.05	–
Prostate disease						
Normal controls	57	8	6-10	–	<0.001	<0.001
Renal stones	30	9	3-12	NS	<0.01	<0.01
Benign noninflammatory	67	9	4-13	NS	<0.01	<0.01
Benign inflammatory	13	14	13-33.5	<0.001	–	NS
Malignant	26	15	8.5-21	<0.001	NS	–
Breast disease						
Normal controls	57	8	6-10	–	<0.001	<0.001
Renal stones	30	9	3-12	NS	<0.001	<0.001
Benign noninflammatory	24	8.5	4.5-13.5	NS	<0.01	<0.001
Benign inflammatory	7	17	15-20	<0.001	–	NS
Malignant	63	17	11.5-22	<0.001	NS	–
Colorectal disease						
Normal controls	57	8	6-10	–	<0.001	<0.001
Renal stones	30	9	3-12	NS	<0.001	<0.001
Benign noninflammatory	26	8.5	5-15	NS	<0.001	<0.01
Benign inflammatory	13	21	19-33	<0.001	–	NS
Malignant	23	18	10-23	<0.001	NS	–

NS=not significant; IQR=interquartile ranges.

As in the above-mentioned groups, in the breast disease patients, there was no significant difference within the controls (Table 2). However, the control groups showed significantly lower uTF levels than the inflammatory benign disease group ($P<0.01$) and the malignant group ($P<0.001$, Table 2). No significant difference was observed between the latter two groups.

In the colorectal group, again no significant difference was seen among the control groups. The inflammatory benign disease group ($P<0.001$) and the malignant group ($P<0.01$) showed significant increase over the control groups (Table 2). However, no significant differences were observed between the benign inflammatory and the malignant disease groups.

We found 74.4%, 68.0%, 77.3%, and 73.0% of patients with bladder, prostate, breast, and colorectal cancer, respectively, had uTF activity above the upper quartile ranges of the normal control group.

Tumor Grade or Stage

There was an increase in uTF levels corresponding to a higher tumor grade or stage. This was only statistically significant (by Kruskal-Wallis) for bladder ($P<0.01$) and prostate cancer ($P<0.05$).

Patient Survival

There was a trend towards decreased uTF activity in longer-surviving patients with malignancy. However, the differences were not statistically significant (Mann-Whitney).

Patients With and Without Recurrent Bladder Cancer

uTF levels were studied in: 1) patients without recurrent tumor and 2) patients with recurrent tumor. Analysis of uTF levels in the two groups showed that in group B there was a wide range and a higher median compared to group A. The difference between the two groups was statistically significant ($P<0.01$).

Serum Prostate-Specific Antigen

Log-uTF showed a moderate and significant association with the Log-PSA in both BPH ($n=31$; $r=0.43$; $P<0.05$; Figure 1A) and malignant group ($n=26$; $r=0.35$; $P<0.05$; Figure 1B), respectively.

Static Bone Scan Imaging

Patients with positive bone scans had higher uTF levels than those with negative bone scans. The difference between these two categories was statistically significant ($P<0.05$).

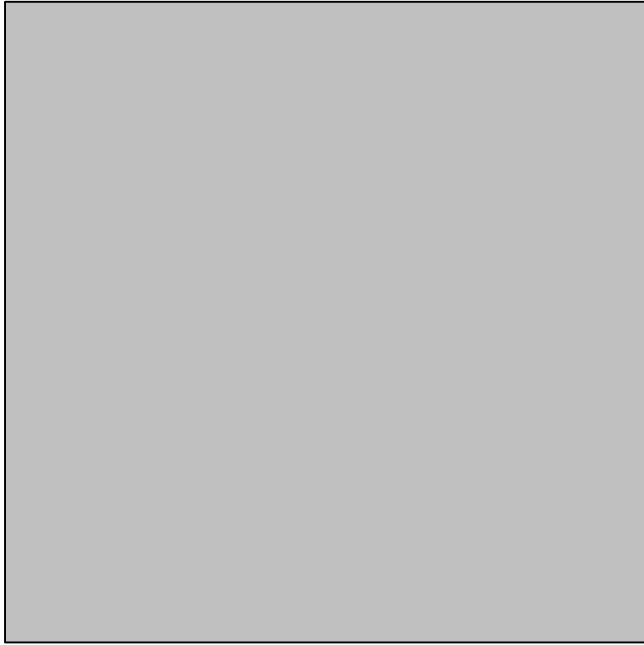


FIGURE 1A. Pearson's correlation between Log-uTF and Log-PSA for the BPH group.

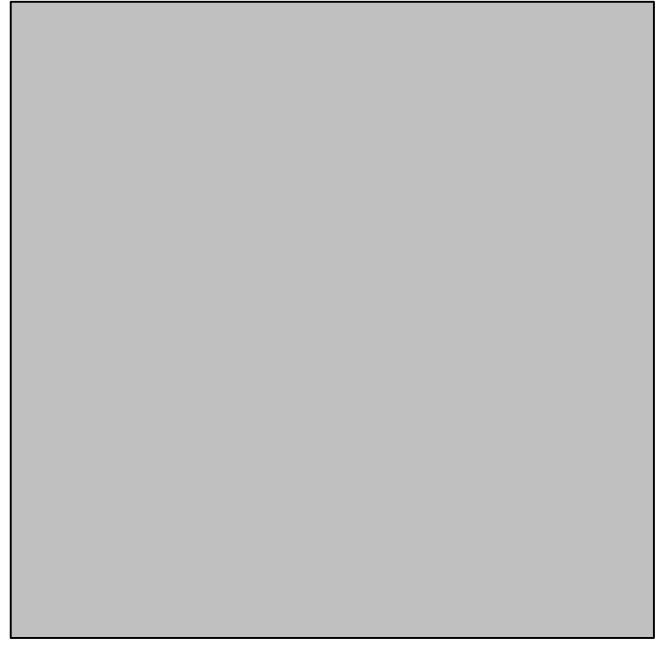


FIGURE 1B. Pearson's correlation between Log-uTF and Log-PSA for the malignant group of the prostate.

Discussion

The evaluation of urine constituents as a marker for solid tumors has attracted many investigators.²³⁻²⁵ Carty et al.^{13,14} and Adamson et al.^{15,16} demonstrated increased levels of uTF in patients with cancer and inflammatory bowel disease. However, the protocol used for measuring uTF activity in the previous studies may not have been completely reliable. Recently, we described a simple and clinically applicable KCA for uTF measurement, which is not significantly affected by age, gender or cigarette smoking.¹⁷ A major advance of the new assay is the smaller variation within, and better separation between, the study groups, particularly with the normal controls, which exhibited a bell-shaped distribution. Accordingly, the assay showed satisfactory sensitivity and specificity.

There was no significant difference between the control groups and those with benign noninflammatory conditions of the bladder, prostate, breast and colorectum, whereas a significant difference was observed between the controls and those with benign inflammatory conditions, which included cystitis, prostatitis, mastitis, mammary duct ectasia and inflammatory bowel disease, some of which are conditions known to enhance uTF expressions.¹⁴ It has been suggested that inflammatory conditions interfere with the host immune response, which leads to an increase in mTF expression,²⁶⁻²⁸ a mechanism which could explain the elevation of uTF in these conditions. Each malignant disease group showed significant differences from the control groups and the appropriate organ-specific noninflammatory diseases, but not with the relevant inflammatory benign disease. When the benign

groups were subdivided into organ-specific noninflammatory and inflammatory benign diseases, the within-group variation was significantly reduced in the noninflammatory compared to the inflammatory diseases. The renal stone group, which was included as a noninflammatory control, also showed a very low variation compared to the inflammatory groups. However, Carty et al. found that subjects with rheumatoid arthritis, all of whom had active disease, had generally normal uTF levels.^{13,14} This suggests that uTF does not behave simply as an acute phase reactant.

Not surprisingly, the malignant and the benign inflammatory groups showed wider ranges in uTF levels than the normal controls. The variations in the malignant group may be attributed to variations in tumor grades and type. Previous studies failed to demonstrate a relationship between tumor grade and uTF levels.^{15,16} In our study, there was a trend towards increasing uTF levels corresponding to those of tumor grade or stage. uTF activity also decreased in longer-surviving patients with malignancy. A great increase in uTF activity was observed for colorectal cancer as compared with other tumors.

Patients with recurrent bladder disease showed increased uTF levels compared to those with a normal cystoscopy. Such an increase has been reported by Adamson et al.¹⁵ Treatment protocols and undetected carcinoma *in situ*²⁹ are factors that may have contributed to the observed overlap, as is the possible presence of undetected urothelial tumor higher in the urothelial tract (e.g., ureter).

uTF showed a weak positive correlation with PSA. A preceding study showed an overall correlation between the two parameters when both patients with BPH and prostate

cancer were analyzed, but not when the cohort with prostate cancer were tested alone.¹⁶ However, we observed no significant difference in the correlations when it was examined for the two groups independently, although it tended to be higher numerically with the BPH. This apparent difference in those studies could be attributed to the number in each case.

uTF levels were significantly raised in patients with metastatic disease, as detected by positive bone scans compared to those with negative scans. This result confirms other reports¹⁶ and is also consistent with our finding that uTF activity is increased with tumor grade or stage.

In conclusion, the use of the new uTF protocol markedly reduced within-group variation and improved discrimination between the study groups. The test can distinguish patients with malignant tumors from normal controls and benign noninflammatory diseases, but not those with inflammatory diseases. uTF measurements may play a role in identifying patients that are at risk of a significant disease, including cancer, and who are most likely to benefit from detailed investigations. It may also play a residual role after diagnosis as levels are related to indices of tumor progression.

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