Effect of kidney function and disease status on urinary tissue factor measurements

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Abstract
Aim—To investigate factors that influence urinary tissue factor (uTF) measurements: glomerular permeability and filtration, tubular function, haematuria, and urine bacterial growth.
Methods—uTF, protein creatinine index, glomerular filtration rate, retinol binding protein, N-acetyl-β-D-glucosaminidase (NAG) and urinary haemoglobin (uHb) were measured in patients with hypertension, diabetes mellitus and nephrotic syndrome (n = 342), tubulo-interstitial disease (n = 50), and haematuria of uncertain cause (n = 50); measurements were also made in urine samples from healthy subjects for "simulated" haematuria (n = 6) and bacterial growth (n = 4) studies.
Results—There was a weak correlation of uTF with glomerular permeability and filtration (protein creatinine index and glomerular filtration rate) and with markers of tubular function (retinol binding protein and NAG). uTF concentrations were not affected by the presence of blood or bacteria in the urine sample.
Conclusion—uTF concentrations are relatively stable. This is an important finding if the assay is to be used in clinical practice.
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Keywords: urinary tissue factor; glomerular permeability and filtration; tubular function; haematuria; bacterial growth

Urine has long been known to contain procoagulant activity[1] that normalises the clotting time of haemophilic patients in vivo.[2] Initially, the procoagulant activity was thought to be due to a tissue thromboplastin related substance.[3] Further studies showed that urinary procoagulant activity catalysed the conversion of prothrombin to thrombin only in the presence of platelet factor 3, factor V, and calcium ions—a prothrombinase type of activity, suggesting that it was a "platelet cofactor" rather than tissue factor (TF).[4 5] Subsequently, Kurosawa *et al* purified urinary procoagulant activity and showed that it promoted clot formation in a factor VII dependent manner.[6] Wiggins *et al* showed that urinary procoagulant activity was raised in lipid associated vesicles and was mainly factor VII dependent, as assessed by clotting assays in human factor VII deficient plasma.[7] Thus, procoagulant activity can activate factor X in the presence of factor VII and calcium and, like TF, it is inhibited by conconavalin A and its activity is restored by the addition of α-methyl-glucoside.[6 8] Carty *et al* confirmed that

the procoagulant activity was TF by demonstrating almost total inhibition of the activity by a specific antibody to human TF.[9]

Tissue factor apoprotein is a 46 kDa, single chain, integral plasma membrane glycoprotein with no intrinsic protease activity.[10 11] It is a receptor and essential cofactor for the serine protease blood coagulation factors VII and VIIa in the activation of factors X and IX.[12 13] As the principal biological initiator of blood coagulation, TF is believed to play a critical role in thrombosis and thrombogenesis[12 14 15] and concentrations in several biological fluids correlate with different pathological conditions.[16] In addition, TF factor is involved in the effector phase of the cellular immune response and in the pathogenesis of certain infections,[17 18] and may also be involved in tumour neovascularisation and angiogenesis.[19 20] Thus, TF measurements in a variety of conditions may be clinically important.

Changes in urinary tissue factor (uTF) concentrations have been demonstrated in many diseases and have been proposed as a marker for inflammatory conditions and malignancy.[9 21 22] Previously, we have reported that uTF concentrations are increased in patients with glomerular disease and suggested that concentrations may reflect the aetiopathogenesis of glomerular injury.[22] In the present study, different factors that might influence uTF measurements such as glomerular permeability and filtration, tubular function, haematuria, and urine bacterial growth were examined.

Methods and materials
CONTROLS AND PATIENTS
Ethical committee approval was obtained for the study and informed consent was sought from each patient attending the Renal Medical Outpatient Clinic at the Royal South Hants Hospital, Southampton. Urine was collected from 452 subjects; patients with hypertension, diabetes mellitus and nephrotic syndrome (n = 342), tubulo-interstitial disease (n = 50), and haematuria of uncertain cause (n = 50); urine was also collected from healthy subjects for "simulated" haematuria (n = 6) and bacterial growth (n = 4) studies.

UTF MEASUREMENTS
uTF concentrations were measured in random midstream urine samples using a one stage kinetic chromogenic assay as described by Lwaleed and colleagues.[22]

URINARY PROTEIN MEASUREMENTS
Urinary protein excretion was studied using the protein creatinine index in patients with renal impairment (n = 123). Total urinary protein
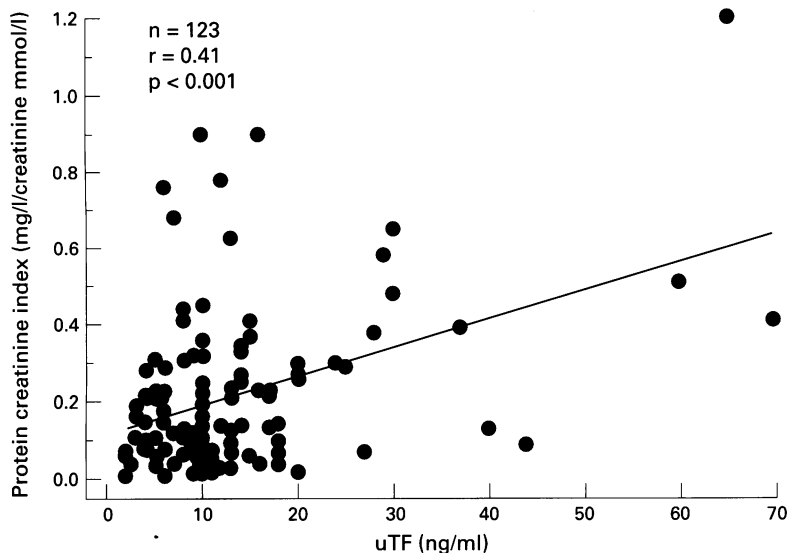
Figure 1   The relation between urinary tissue factor (uTF) activity and protein creatinine index.

and creatinine were measured according to the standard CX7 protocol automated analyser (Beckman, Brea, California, USA). The protein creatinine index was then calculated as: total protein (g/l)/creatinine (g/mmol).[23] [24]

GLOMERULAR FILTRATION RATE MEASUREMENTS
Estimated glomerular filtration rate was calculated in patients with a wide range of renal function and glomerular filtration rates (n = 149) using predicted creatinine clearance derived from the plasma creatinine level via the formula of Cockcroft and Gault.[25] A subgroup of patients (n = 70) had undergone isotopic glomerular filtration rate measurement by determining the rate of technetium ^{99m}Tc DTPA clearance according to the protocol of Waller and colleagues.[26]

TUBULAR FUNCTION MEASUREMENTS
Tubular function was assessed by urinary retinol binding protein measurements as described by Rowe *et al*, and urinary N-acetyl-β-D-glucosaminidase (NAG) based on the method of Yuen *et al*, in 50 urine samples obtained from patients with abnormalities of renal tubular function.[27] [28]

EFFECTS OF HAEMATURIA
"Genuine" haematuria
Urine samples were collected from patients with haematuria (n = 50). Urinary haemoglobin (uHb) was measured by electron-osmosis using the Glytrac system (Ciba-Corning Ltd, Halstead, UK) as described by Twyman and Rowe.[29]

"Simulated" haematuria
Urine samples were collected from six healthy volunteers and "spiked" with different amounts of normal whole blood. Each 1 ml sample was spiked with 10 µl of blood. Each spiked sample was then serially diluted 1/2, 1/4, 1/8, 1/16, and 1/32 with additional urine from the same sample to give a total of seven different levels of "haematuria". A control sample containing 25 µl of assay buffer was also proc-

essed for each sample to determine baseline activity. All samples were thoroughly mixed and then incubated for one hour at 37°C. Each sample was then assayed for uTF activity.

EFFECTS OF BACTERIAL GROWTH
Urine samples were collected from four healthy volunteers. The samples were then divided aseptically into two universal containers, test and control. Baseline uTF activity was measured for both the test and control at zero time. An estimate of the number of bacteria in each tube (test and control) was made by taking a loopful (10 µl calibrated loop) of each sample and spreading on a nutrient agar plate. The plate was incubated at 37°C in the presence of 5% CO_2 overnight. The number of bacteria was calculated by colony counting (Manostate Colony Counter 81-520-000; Manostate Corporation, New York, USA) and multiplied by 1000. None of the samples were positive. Subsequently, *Escherichia coli* was inoculated into the test samples with a 10 µl calibrated loop. The uTF activity was then measured for the test and control at two, four, six, 12, and 24 hours and then for eight consecutive days. The bacterial population was also estimated over these time points.

STATISTICAL ANALYSIS
Data were included in a database and analysed by the STATGRAPHICS statistical software system. Correlations were determined using Spearman's rank correlation test. The effect of simulated haematuria was tested by one way analysis of variance (ANOVA) whereas the effect of bacterial growth was analysed by the paired *t* test.

Results
URINARY PROTEIN EXCRETION
The protein creatinine index displayed a weak association with the uTF activity (fig 1), which was statistically significant ($r = 0.41$; $p < 0.001$).

GLOMERULAR FILTRATION RATE
Using both the estimated and isotopically determined glomerular filtration rate, there was a very weak correlation between glomerular filtration rate and uTF activity. However, these correlations were significant in both cases (table 1).

TUBULAR FUNCTION
Retinol binding protein and NAG excretion showed no correlation with uTF activity (table 1).

Table 1   Spearman's rank correlation between urinary tissue factor activity and estimated glomerular filtration rate (GFR), isotopically determined GFR, retinol binding protein (RBP), N-acetyl-β-D-glucosaminidase (NAG), and urinary haemoglobin (uHb)

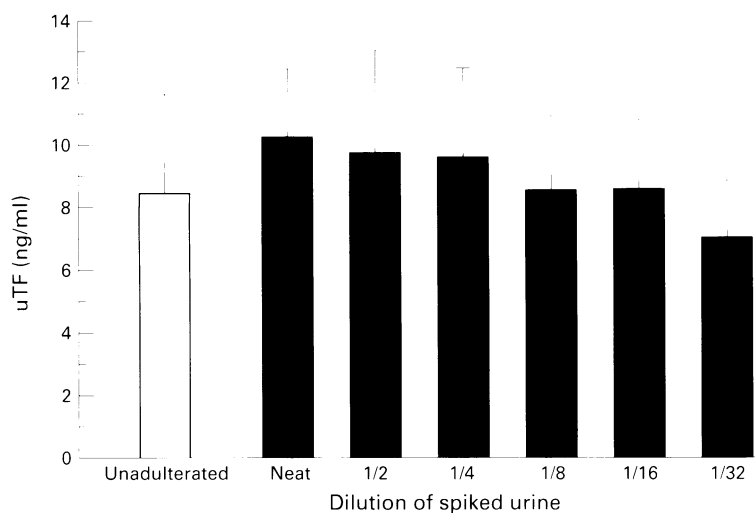| Group	n	Correlation coefficient (r)	p value
Estimated GFR	149	0.29	< 0.05
Isotopic GFR	70	0.32	< 0.001
RBP	50	0.10	NS
NAG	50	0.13	NS
uHb	50	0.10	NS

Figure 2   Effect of blood on urinary tissue factor (uTF) activity. The bars represent the mean (SD) for six urine samples.
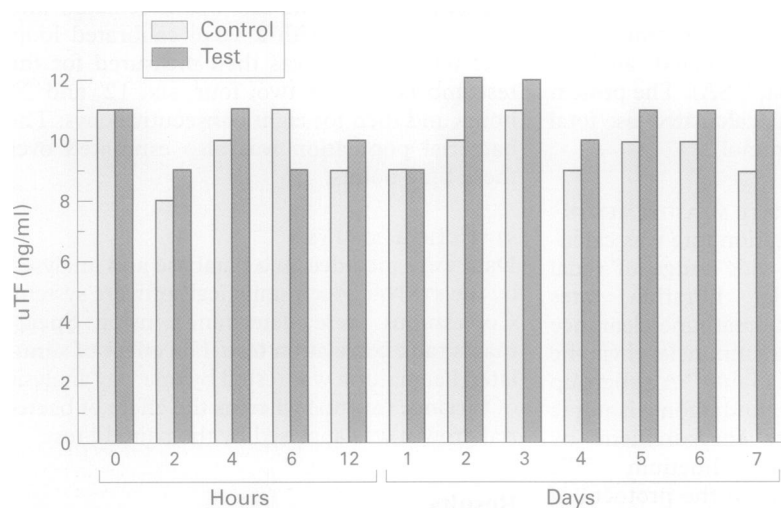

Figure 3   Effect of bacterial growth on urinary tissue factor (uTF) activity.
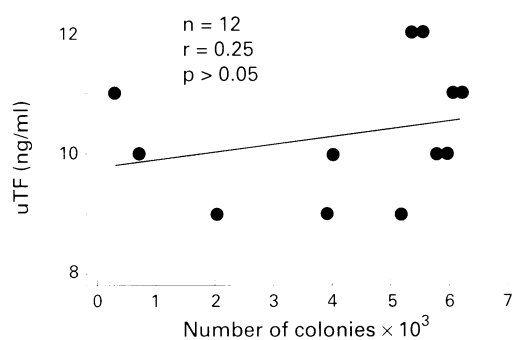

Figure 4   The relation between bacterial colonies and urinary tissue factor (uTF) activity.

HAEMATURIA

Genuine clinical haematuria

uHb did not correlate with uTF activity (table 1).

Simulated haematuria

Comparison of the results from the control unspiked sample and the samples spiked with increasing amounts of blood showed that haematuria has a negligible and statistically insignificant effect on uTF activity (fig 2).

BACTERIAL GROWTH

The time of bacterial incubation in the urine samples had no significant effect on the measurements of uTF activity in vitro (fig 3). Similarly, the number of bacterial colonies in the urine samples did not correlate with uTF activity (fig 4) (n = 12; $r = 0.25$; $p > 0.05$).

Discussion

Previously, we have shown that uTF concentrations are raised in patients with different forms of glomerulonephritis and suggested that this may reflect the aetiopathogenesis of the disease.[22] The aim of the present study was to evaluate the effect of glomerular filtration rate, tubular function, haematuria, and bacterial growth on uTF measurements. uTF showed a weak association with measures of protein excretion and glomerular filtration rate (protein creatinine index, predicted glomerular filtration rate or isotopically determined glomerular filtration rate). In addition to the limitations of the glomerular filtration rate prediction methods reviewed by Smith,[30] the Cockcroft and Gault formula tends to overestimate the glomerular filtration rate at a low creatinine clearance and underestimate it at a higher clearance.[31] Indeed, in the present study the predicted and isotopical glomerular filtration rates were only moderately related. Poor association between the two has also been reported by others.[32] Thus, the limitations and the inaccuracy of the predicted glomerular filtration rate measurements may have contributed to the poor relations with uTF concentrations. However, this would not have been the case when the absolute glomerular filtration rate is used, such as when using the isotopic clearance method.[31 33]

Tubular function has been shown to affect uTF excretion.[4 31 35] In our laboratory (unpublished data, 1997) and in other studies,[35 36] the TF antigen has been localised to renal tubules. In the present study, uTF concentrations were not significantly altered in patients with tubular malfunction. There was no correlation between uTF concentrations and retinol binding protein or NAG excretion (markers of tubular pathology). Even though uTF is localised to the tubules, it is not known whether this reflects synthesis or reabsorption. Retinol binding protein is filtered and reabsorbed by the tubules. The apparent lack of association between the concentrations of the two moieties could indicate differences in their origin or processing. The low concentrations of uTF observed in patients with tubular pathology and the lack of association between uTF and both retinol binding protein and NAG suggest that the uTF concentrations are independent of tubular impairment.

Haematuria is a common finding in nephrological and urological practice and there is a possibility that blood components may interfere with the colorimetric assay of uTF. The presence of blood may introduce factors that could influence the assay (such as small amounts of factors VII and X). In addition, haemoglobin may artefactually affect chromogenic assays because it absorbs light at the

same wavelength as the indicator chromophore. However, in the present study, readings from blank wells containing urine but not factor X were subtracted from the test well readings. Neither genuine nor simulated haematuria had a significant effect on uTF activity. This agrees with the findings of others.[37] The finding that haematuria has no significant effect on uTF activity is important if the assay is to be used in clinical practice.

Monocyte TF concentrations are raised in response to infections[38] and it has been suggested that uTF may represent a "spillover" from monocytes.[9] Therefore, we decided to assess the effect of bacterial growth on uTF measurements. Ideally, urine samples should be free from contaminants. Bacterial contamination may cause false negative results in the measurement of proteins or enzyme activity either as a result of direct inhibition through bacterial protease synthesis or via changes in the pH of the medium.[39 40] In vitro inoculation of normal human urine samples with *E coli* had no significant effect on uTF measurements. The methods and results presented here exclude the possible effect of gross contamination of urine in vitro, but they do not rule out possible interference in the case of in vivo bacterial infection. Indeed, a few patients (n = 8) with urinary tract infection did show increased uTF activity (data not shown). The finding that bacterial contamination in urinary samples had negligible effects on uTF measurements has important clinical implications. In addition, as preservatives were not used, the possibility of preservatives affecting uTF activity is eliminated, and the effective cost of the assay is reduced.

In conclusion, we have shown previously that uTF is raised in various forms of renal disease. We have now shown that there is only a weak correlation of uTF with protein creatinine index and glomerular filtration rate and no correlation with markers of tubular damage or the presence of blood and bacteria in the urine.

1 Grunke W. Studien über die Bluterinnung mit besonderer Berücksichtigung der Haemophilie. I. Mitteilung: Einfluss des Harnes auf die Gerinnung haemophilen Blutes. *Blutes Z Ges Exp Med* 1835;**96**:512–16.
2 Tocantins LM, Lindquist JN. Thromboplastic activity of the urine. *Proc Soc Exp Biol NY* 1947;**65**:44–9.
3 von Kaulla KN. Extraction and concentration of thromboplastic material from human urine. *Proc Soc Exp Biol Med NY* 1956;**91**:543–5.
4 Matsumura T, Hutt MP, von Kulla KN. Urinary procoagulant excretion and its relation to kidney function. *Nephron* 1970;**7**:165–77.
5 Caldwell J, von Kulla KN, von Kulla E, et al. Procoagulant material from human urine in prothrombin activation. *Thromb Diath Haemorrh* 1963;**61**:53–61.
6 Kurosawa S, Matsuda M, Aoki N. Urinary procoagulant behaves as tissue factor by promoting factor VIIa-catalyzed activation of factor X. *Thromb Res* 1984;**33**:595–606.
7 Wiggins R, Glatfelter A, Kshirsagar B, et al. Lipid vesicles and their association with procoagulant activity and glomeruli of rabbits with nephrotoxic nephritis. *Lab Invest* 1987;**56**:264–72.
8 Zacharski LR, Rosenstein R, Phillips PG. Concanavalin A inhibition of tissue factor (thromboplastin) activity. *Blood* 1974;**44**:783–7.
9 Carty N, Taylor I, Roath OS, et al. Urinary tissue factor activity in malignancy. *Thromb Res* 1990;**57**:473–8.

10 Bach RR, Nemrson Y, Konigsberg WK. Purification and characterization of bovine tissue factor. *J Biol Chem* 1981;**256**:8324–31.
11 Guha A, Bach RR, Konigsberg W, et al. Affinity purification of human tissue factor. Interaction of factor VII and tissue factor in detergents micelles. *Proc Natl Acad Sci USA* 1986;**83**:299–302.
12 Nemerson Y, Bach R. Tissue factor revisited. *Prog Hemost Thromb* 1982;**6**:237–61.
13 Nemerson Y. The tissue factor pathway of blood coagulation. *Semin Haemost* 1992;**29**:170–6.
14 Osterud B, Rapaport S. Activation of factor IX by the reaction product of tissue factor and factor VII: additional pathway for initiating blood coagulation. *Proc Natl Acad Sci USA* 1977;**74**:5260–4.
15 Wada H, Wakita Y, Shiku H. Tissue factor expression in endothelial cells in health and disease. *Blood Coag Fibrinol* 1995;**6**:26–31.
16 Fareed J, Callas DD, Hoppensteadt D, et al. Tissue factor antigen levels in various biological fluids. *Blood Coag Fibrinol* 1995;**6**:32–6.
17 Drake TA, Rogers GM, Sande MA. Tissue factor is a major stimulus for vegetation formation in enterococcal endocarditis in rabbits. *J Clin Invest* 1984;**73**:1750–3.
18 Prydz H. Synthesis and surface availability of thromboplastin and plasminogen activator in mononuclear phagocytes. In: *Mononuclear phagocytes: physiology and pathology*. Dean, RT, Jessup, W, eds. New York: Elsevier, 1985:115–37.
19 Francis JL. Haemostasis and cancer. *Med Lab Sci* 1989;**46**: 331–46.
20 Folkman J. Tumour angiogenesis and tissue factor. *Nat Med* 1996;**2**:167–8.
21 Lwaleed BA, Francis JL, Chisholm M. Urinary tissue factor level in neoplastic diseases [abstract]. *Br J Haematol* 1996; **27**:103.
22 Lwaleed BA, Bass PS, Chisholm M, et al. Urinary tissue factor levels in glomerulonephritis: a potential marker of glomerular injury? *J Clin Pathol* 1997;**50**:336–40.
23 Watanabe N, Kamei S, Ohkubo A, et al. Urinary protein as measured with a pyrogallo red-molybdate complex. Manually and in a Hitachi 726 automated analyzer. *Clin Chem* 1986;**32**:1551–4.
24 Orsonneau J, Douet P, Massoubre C, et al. An improved pyrogallol red-molybdate method of determining total urinary protein. *Clin Chem* 1989;**35**:2233–6.
25 Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 1976;**16**:31–41.
26 Waller DG, Keast CM, Fleming SJ, et al. Measurement of glomerular filtration rate with technetium-99m DTPA: comparison of plasma clearance techniques. *J Nucl Med* 1987;**28**:372–7.
27 Rowe DJF, Anthony F, Polak A, et al. Retinol binding protein as a small molecular weight marker of renal tubular function in diabetes mellitus. *Ann Clin Biochem* 1987;**24**: 477–82.
28 Yuen CT, Price RG, Chattagoon L, et al. Colorimetric assays for N-acetyl-β-D-glucosaminidase and β-D-galactosidase in human urine using newly-developed w-nitrostyryl substrates. *Clin Chem Acta* 1982;**124**:195–204.
29 Twyman SJ, Rowe DJ. A sensitive radio-immunoassay for the determination of haemoglobin in urine. *Ann Clin Biochem* 1995;**32**:506–8.
30 Smith SA. Estimation of glomerular filtration rate from the serum creatinine. *Postgrad Med J* 1988;**64**:204–8.
31 Waller DG, Fleming JS, Ramsay B, et al. The accuracy of creatinine clearance with and without urine collection as a measure of glomerular filtration rate. *Postgrad Med J* 1991; **67**:42–6.
32 Daugaard G, Rossing N, Rorth M. Effects of cisplatin on different measures of glomerular function in the human kidney with special emphasis on high-dose. *Cancer Chemother Pharmacol* 1988;**21**:163–7.
33 Brochner-Mortensen J, Rodbro P. Selection of routine method for determination of glomerular filtration rate in adults. *Scand J Clin Lab Invest* 1976;**36**:35–43.
34 Matsumura T, von Kaulla KN. Procoagulant content of human urine in health and disease. *Am J Clin Pathol* 1968; **50**:198–210.
35 Matsumura T, Hutt MP, von Kulla KN. Urinary procoagulant excretion in experimental renal disease. *Proc Soc Exp Biol Med* 1968;**127**:147–53.
36 Matsuda M, Aoki N, Kawaoi A. Localization of urinary procoagulant in human kidney. *Kidney Int* 1979;**15**:612–17.
37 Adamson AS, Francis JL, Roath OS, et al. Urinary tissue factor levels in transitional cell carcinoma of the bladder. *J Urol* 1992;**148**:449–52.
38 Osterud B, Flaegstad T. Increased thromboplastin activity in monocytes of patients with meningococcal infection: related to an unfavourable prognosis. *Thromb Haemost* 1983;**49**:5–7.
39 Wellwood JM, Price RG, Ellis BG, et al. A note on the practical aspects of the assay of N-acetyl-β-glucosaminidase in human urine. *Clin Chim Acta* 1976;**69**:85–91.
40 Bernard AM, Moreau D, Lauwerys R. Comparison of retinol-binding protein and β₂-microglobulin determination in urine for the early detection of tubular proteinuria. *Clin Chim Acta* 1982;**126**:1–7.