Urinary tissue factor in glomerulonephritis: a potential marker of glomerular injury?

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Abstract

**Aim**—To investigate the significance of urinary tissue factor (uTF) concentrations in patients with glomerulonephritis. Methods—Urine samples were collected from normal subjects (n = 57), patients with uncomplicated renal stones (n = 30), and patients with glomerulonephritis (n = 150). Samples were then centrifuged and the pellets solubilised in n-octyl-β-glucopyranoside. uTF concentrations were determined using a one stage kinetic chromogenic assay. **Results**—The uTF concentration was higher in patients with glomerulonephritis than in normal controls (p < 0.01) or in patients with renal stones (p < 0.05). uTF activity correlated with the protein creatinine index (PCI, r = 0.41, p < 0.001) and seven patients with glomerulonephritis and a PCI ≤ 0.1 g/mmol had raised uTF. Glomerulonephritis patients were subdivided into two groups depending on the PCI: < 0.2 g/mmol creatinine (mild to moderate proteinuria, group I) and ≥ 0.2 g/mmol creatinine (heavy proteinuria, group II). In group I, uTF concentrations were higher in patients with either immune complex (IC) glomerulonephritis (p < 0.01) or non-IC glomerulonephritis than in normal controls. In group II, the IC glomerulonephritis group had higher uTF concentrations than normal controls (p < 0.001) or patients with renal stones (p < 0.01); and non-IC glomerulonephritis patients had higher uTF than normal controls (p < 0.01). When the glomerulonephritis groups were divided into broad WHO subtypes, the significance level varied with the type of glomerulonephritis. **Conclusions**—uTF is increased in patients with glomerulonephritis, and its concentration may reflect the aetio-pathogenesis of glomerulonephritis.

Keywords: urinary tissue factor; monocyte/macrophage tissue factor; coagulopathy; glomerulonephritis

Activation of blood coagulation and fibrin deposition are associated with various forms of inflammatory glomerular diseases in humans and laboratory animals. Fibrin deposition within and around the glomerulus has been observed by several investigators and is thought to play a major role in the development and the progression of some forms of glomerular disease. The underlying biological mechanism of such phenomena remains poorly understood. However, it has been suggested that the source of glomerular procoagulant activity could either be bloodborne or generated by resident glomerular cells. Glomerular fibrin deposition is dependent on leucocyte accumulation, and rabbits treated with mustard hydrochloride develop severe leucopenia, which prevents glomerular macrophage accumulation and glomerular fibrin deposition without any functional alteration of the host coagulation factors. Mononuclear cells are known to be a potent source of procoagulant activity. Increased expression of procoagulant activity by monocytes/macrophages in several inflammatory conditions, particularly after stimulation with Escherichia coli endotoxin, is well documented. Similarly, isolated human and animal glomeruli with different forms of glomerular disease express high levels of procoagulant activity.

Conclusions—uTF is increased in patients with glomerulonephritis, and its concentration may reflect the aetio-pathogenesis of glomerulonephritis. (J Clin Pathol 1997; 50:336–340)

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recently been limited to animal models, with rather conflicting results.\textsuperscript{17} Recently, we have
developed a highly standardised assay for uTF measurement (to be published). In this pre-
liminary study, we applied this assay to patients with glomerulonephritis and examined the
hypothesis that uTF concentrations may reflect the pathogenesis of glomerular damage or its
degree.

**Methods**

**PATIENTS**

Ethics committee approval was obtained for the study and informed consent was sought from
each patient on arrival at the outpatient clinic at the Royal South Hants Hospital,
Southampton, during 1990 to 1994. All patients included in the study had biopsy
proven renal disease (biopsies performed between 1990 and 1994). The biopsy reports
were retrieved and classification of glomeru-
lonephritis was made according to the criteria of the Collaborating Centre for the Histologi-
cal Classification of Renal Diseases of the
WHO.\textsuperscript{36} Urine was collected from 237 sub-
jects: controls (healthy volunteers (n = 57) and patients with “uncomplicated” renal stones
and a normal erythrocyte sedimentation rate (n = 30)); and patients with glomerulonephri-
itis (n = 150). The glomerulonephritis group was made up of minor glomerular abnormali-
ties (n = 4); diffuse glomerulopathy (type I
membrano-proliferative glomerulonephritis
(n = 11); mesangio-proliferative glomerulone-
phritis (n = 33); and membranous glomeru-
lonephritis (n = 19)); focal segmental prolif-
erative glomerulonephritis (n = 26); focal
segmental glomerulosclerosis (n = 20); and
miscellaneous diseases (n = 37). The latter
group included patients with diabetes, amy-
l oid, and ischaemic changes. All patients on
steroids at the time of urine collection were
excluded from the study, as were patients with
crescentic glomerulonephritis.

**uTF MEASUREMENT**

Urine samples were collected from each
subject into sterile universal containers with
out preservative. Samples were then sedimented, solubilised, and assayed. Briefly, the extracted
uTF, in the presence of recombinant factor
VIIa (rVIIa) and Ca\textsuperscript{2+}, forms a complex (tissue
factor/rVIIa/Ca\textsuperscript{2+}) which then directly activates
factor X to factor Xa. Generation of factor Xa,
which is proportional to the amount of tissue
factor in the urine sample, was determined by
measuring its action on a factor Xa specific
cromogenic substrate. The rate of the reaction
at 405 nM was determined in a Biokinetics
EL-312e microplate reader (Bio Tek Instru-
mements, Winooski, Vermont, USA), pro-
gammed to read the absorbance at 30 second
intervals over a period of 35 minutes. Absorb-
ance values were converted to tissue factor (ng/
ml) using a calibration curve constructed from
serial dilutions of recombinant relipidated
tissue factor (rTF, 0.4-83 ng/ml) prepared
according to Carson and Konigsberg\textsuperscript{37} in BOG
(β-octyl-glucopyranoside, Sigma, Poole, Dor-
set). For maximum accuracy the calibration
standards were measured in each plate.

**EXPRESSION OF UTF RESULTS**

The results (ng/ml) were corrected for the
method of the urine using the creatinine
concentration of the sample. Final results were
expressed as uTF ng/ml per mg creatinine:
uTF (ng/ml)/creatinine (mg/dl) x 100.

**PROTEIN CREATININE INDEX MEASUREMENTS**

Total protein and creatinine were measured
according to the standard CX7 protocol auto-
mated analyser (Beckman, Brea, California,
USA). The protein creatinine index (PCI) was
then calculated as: total protein (g/l)/creatinine
(g/mmol).\textsuperscript{38 39}

**STATISTICAL ANALYSIS**

Data were included in a database and analysed
by the Statgraphics statistical software system.
Data were not normally distributed, and sum-
mary statistics were expressed as medians and
interquartile ranges (IQR). Differences be-
tween two groups were assessed by the Mann-
Whitney U test.

**Results**

There was a significant difference in uTF con-
centrations between patients with glomerulo-
ephritis and normal controls (p < 0.01) and
patients with renal stones (p < 0.05, fig 1); 54.9% of patients with glomerulonephritis
showed a uTF level above the upper quartile
of the normal controls, in contrast to 36.7% in
the renal stones group. There was a weak correla-
tion between uTF and PCI values (r = 0.41,
p < 0.001). The glomerulonephritis group was
then subdivided according to the PCI level at
the time of the biopsy. Group I had a PCI of
< 0.2 g/mmol (that is, less than about 2 g/day,
mild to moderate proteinuria), and group II
\geq 0.2 g/mmol (heavy proteinuria). In addition,
two broad groups of glomerulonephritis pa-
tients were distinguished—namely immune
complex (IC) glomerulonephritis (type I
membrano-proliferative glomerulonephritis,
mesangio-proliferative glomerulonephritis,
and membranous glomerulonephritis) and non-IC
glomerulonephritis (minor glomerular abnor-
malities and focal segmental glomerulosclero-
sis). In group I (low PCI), the IC group
(p < 0.01) and the non-IC group (p < 0.05)
both had higher uTF values than the normal
controls, but not than the renal stones group.
In group II (high PCI), the IC glomerulone-
phritis group (p < 0.001) and the non-IC
glomerulonephritis group (p < 0.01) also had
higher uTF values than the normal controls,
and a significant difference was observed
between the renal stones group and the IC
glomerulonephritis group as well (p < 0.01, fig
2A and 2B).

In group I, no significant difference was
found between the IC and the non-IC groups;
in group II, the IC glomerulonephritis group
had a higher, but wider, range of uTF values (8
to 29) compared with the non-IC glomeru-
lonephritis group (7 to 16), but the difference
was not significant (fig 3A and 3B). There was

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no correlation between the stage of the membranous glomerulonephritis or the degree of tubulo-interstitial damage and the uTF concentrations in all three forms of IC glomerulonephritis studied \( r = 0.1, p > 0.05 \).

Analysis of the uTF concentrations in other forms of glomerulonephritis showed that for low PCI there was a significant difference between the focal segmental proliferative group and normal controls only \( p < 0.05 \), and

Discussion

The fact that several human and experimental models of glomerulonephritis show alterations in kidney tissue factor levels highlights the importance of this glycoprotein in glomerular pathology. Tissue factor, the normal route to coagulation activation, triggers glomerular fibrin deposition, but which has been observed in many forms of clinical and experimental glomerulonephritis. Increased concentrations of tissue factor in glomerular diseases are found not only in the kidney itself, but also in the urine, where it is associated with lipid. Assessment of uTF in renal diseases has until now been mainly restricted to experimental models, with conflicting results. Thus urinary procoagulant activity in rabbits with nephrotoxic nephritis is reported to have disappeared during the onset of glomerulonephritis, while in a subsequent study using the same animal model a marked increase in the urinary procoagulant activity was observed during the development of glomerular injury. Recently, how-
havior, the relation between the urinary procoagulant activity and fibrinolytic activity in human glomerulonephritis has been evaluated and a negative association found. The assay system employed in this latter study, however, may not have been completely reliable. In addition, the samples were dialysed, though uTF is believed to remain in microvesicles, which provide the lipid for its functional activity. The structure and the orientation of these vesicles in relation to uTF is still poorly understood. This is important since the orientation of the lipid vesicles has been shown to affect the accessibility of tissue factor to factor VII/VIIa.

Recently we have developed and validated a simple, clinically applicable kinetic chromogenic assay for measuring uTF activity which does not use dialysis (to be published).

The clinical applications of this new assay to patients with glomerulonephritis have not been explored before, although the assay has been successfully used to distinguish normal subjects from patients with malignant disease in the absence of inflammation.

Using this assay system, uTF was found to be increased in patients with glomerulonephritis compared to normal controls and to patients with uncomplicated renal stones: 54.9% of the glomerulonephritis group had results above the upper quartile of the healthy control group, while only 36.7% of the renal stones group had abnormal values. Although the glomerulonephritis group had increased uTF concentrations, there was an overlap with the normal and renal stones groups (Fig 1). This may have been due to the heterogeneity of the various groups, including the stage of evolution of the disease or treatment schedules (some patients were on treatment at the time of sample collection, though all patients on steroids were excluded from the study).

Although it could be argued that circulating tissue factor may enter the urinary filtrate in glomerulonephritis, tissue factor is constitutively expressed only by cells outside the vasculature, though its expression could be induced in endothelial cells and monocytes by endotoxin or cytokines. However, the procoagulant activity of blood monocytes in different forms of glomerulonephritis was not significantly different from that in a normal control group, neither were plasma tissue factor concentrations in patients with chronic glomerulonephritis.

In our study, we found that uTF showed only a weak correlation with urinary protein excretion (expressed as protein creatinine index). We then divided the glomerulonephritis study groups into group I, with a PCI of < 0.2 g/mmol, and group II, with PCI values ≥ 0.2 g/mmol. When the types of glomerulonephritis were divided into immune complex and non-immune complex subtypes, both groups showed a significant increase in the uTF concentrations compared with the controls, even when the PCI was only moderately raised.

Although the immune complex subtypes in group II showed a wider range of uTF (8 to 29) compared with the non-immune complex group (7 to 16), the differences between the groups failed to reach statistical significance. The high levels of uTF observed in the immune complex subgroup could be related to various factors, including immunoglobulin and complement deposition, mononuclear cell infiltration, fibrin formation, and glomerular cell proliferation. This may cause direct or indirect activation of the resident glomerular cells or infiltrating inflammatory cells to express increased amounts of tissue factor. The presence of fibrin or fibrin related products acts as a macrophage aggregating agent and stimulates glomerular mesangial cells directly through cytotoxic effects. In addition, changes in membrane structure cause tissue factor de-encryption which would result in enhanced tissue factor procoagulant activity. An obvious example of this is cell apoptosis, which has been shown to be associated with an increased cell surface tissue factor procoagulant activity. Whatever the mechanisms involved, activation of tissue factor may lead to local activation of blood coagulation or of the fibrinolytic system. The extent of glomerular damage may therefore be reflected in the amount of uTF produced. This is supported by the finding of Wiggins et al, who showed, using an animal model, that procoagulant activity in rabbits with nephrotoxic nephritis not only increased in glomeruli but also appeared in urine during the progression of glomerular injury. This suggests that haemostatic alterations may represent changes of pathological importance in the development and progression of the disease. However, we have not been able to find any significant association between uTF activity and either tuft morphological changes or interstitial cell infiltration. The apparent lack of correlation found in this study could be a time factor, since the period between sample collection and biopsy exceeded three years in some cases. However, uTF may be a useful tool in assessing glomerular damage, particularly in immune complex glomerulonephritis, and perhaps in monitoring response to treatment. Further studies are required to evaluate its use as a marker of glomerular injury.

In conclusion, the uTF concentration is increased in patients with glomerulonephritis compared with controls, and is increased even when the protein creatinine index is only moderately raised. Our immune complex glomerulonephritis group showed wider ranges than the non-immune complex glomerulonephritis group and the results suggested that uTF production may be more important in immune complex glomerulonephritis. Further studies to evaluate uTF level in patients with glomerular abnormalities are required, especially to evaluate the influence of disease activity, stage, and the influence of treatment.

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