Urol Res (1994) 22:131–136

ORIGINAL PAPER

J. Kaden · J. Groth · G. May · B. Liedvogel

Urinary Tamm-Horsfall protein as a marker of renal transplant function

Received: 15 November 1993 / Accepted: 10 February 1994

Abstract In a total of 428 urine samples collected from 15 patients aged between 23 and 60 years after cadaveric kidney transplantation during a postoperative hospital stay, Tamm-Horsfall protein (THP) was quantitatively determined using the *ELIAS SYNELISA-THP* immunoassay. All patients were treated with azathioprine, cyclosporine, prednisolone, given an intraoperative high-dose single antilymphocyte globulin bolus and discharged with functioning grafts. In clinically uncomplicated courses, even after immediate transplant function, the recovery of graft function took on average 7 days. Thereafter the urinary THP excretion was relatively stable and amounted, on average, to 14.5 ± 4.9 mg/24 h (i.e. was at the lower limit of normal urinary THP excretion). In cases of delayed onset of graft function of undetermined origin accompanied by extremely reduced urinary THP excretion, the functional recovery, whether spontaneous or brought about by treatment, was characterized by a continuous increase in urinary THP excretion. In connection with interstitial rejections urinary THP excretion seems not to be a recommendable diagnostic parameter. Daily measurement of urinary THP is, however, suitable for monitoring the functional state of transplanted kidneys.

Key words Kidney transplantation · Tamm-Horsfall protein · Acute tubular necrosis · Rejection episodes · Cytomegalovirus infection

J. Kaden (✉) · J. Groth
Department of Laboratory Medicine,
Friedrichshain Hospital,
Landsberger Allee 49, D-10249 Berlin, Germany

G. May
Kidney Transplant Center, Friedrichshain Hospital,
Landsberger Allee 49, D-10249 Berlin, Germany

B. Liedvogel
ELIAS Medizintechnik,
Obere Hardtstrasse 18, D-79114 Freiburg, Germany

In 1950 Tamm and Horsfall [23] isolated, by a salt precipitation method, a urinary glycoprotein functionally characterized by its ability to inhibit myxovirus-induced hemagglutination. In 1985, using lectin affinity columns, Muchmore and Decker [15] purified another glycoprotein from urine of pregnant women and called it uromodulin. This protein binds to recombinant murine interleukin 1 alpha (rIL-1α), human rIL-1α, rIL-1β and recombinant tumor necrosis factor (TNF) with high affinity [15, 22]. By means of amino acid sequencing of clones for uromodulin, Hession et al. [9] and Pennica et al. [16] demonstrated that the protein portion of uromodulin is identical to that of Tamm-Horsfall glycoprotein (THP). Because of its binding activity to cytokines it could be possible that THP plays a role in the regulation of circulating cytokine levels as well as their intrarenal bioactivity [14].

THP is localized to the epithelial cells of the thick ascending limbs of Henle's loop and the most proximal part of the distal convoluted tubule [1, 13]. This organ-limited distribution as well as the fact that THP is the most abundant protein in normal human urine make this glycoprotein very interesting for kidney transplantation (KTx). Howie and Brewer [10] described deposits of THP in kidneys that had tubular damage from acute rejection reactions. Cohen et al. [4], in a study of renal allografts with acute cellular rejections, found extratubular THP in 63.6% of the specimens, representing 76.1% of the patients whose tissues were examined. This free communication between lumina and interstitium was possible by lymphocytes and monocytes infiltrating the walls of tubules causing disruption of basement membranes. Hartmann et al. [8] studied the 24-h urinary excretion of THP in renal transplant patients with favorable and unfavorable courses. The results obtained were in accordance with that of the blood creatinine assay and the *N*-acetyl-beta-D-glucosaminidase (NAG)/creatinine ratio in urine. Avis et al. [2], while measuring serum THP, observed significantly lower THP levels in patients on cyclosporine than in both controls and patients on azathioprine and prednisolone and pointed out that serum THP may be a valuable tool in assessing tubular damage produced by cyclosporine or

Table 1 Urinary Tamm-Horsfall protein (THP) excretion, serum creatinine concentration and diuresis (mean and standard deviation, SD) in eight kidney graft recipients during post-transplant days 7–21 without any signs of complication. Functional characterization of the efficiency of kidney grafts after postoperative recovery of tubular regeneration

Parameter	Mean	SD	*n*
Diuresis/24 h (ml)	2347.1	553.4	106
Serum creatinine (μmol/l)	122.5	34.0	52
THP excretion (μg/ml)	6.3	2.4	103
THP excretion (mg/24 h)	14.5	4.9	103

(mean and standard deviation, SD)

any other nephrotoxic agent. The availability of a high-quality synchronous enzyme-linked immunosorbent assay (SYNELISA-THP, ELIAS, Freiburg, Germany) offered the opportunity to monitor the daily THP excretion in patients of KTx. The present study clearly shows the suitability of urinary THP as a marker of renal transplant function.

Materials and methods

Study population

This study includes 15 patients who consecutively received their cadaveric renal transplant (13 first and 2 second transplants) at the Kidney Transplant Center Berlin-Friedrichshain between 25 March 1992 and 4 April 1992. There were 8 women and 7 men, with ages ranging from 23 to 60 years (mean ± 1 SD: 41.7 ± 13.2 years).

Immunosuppressive protocol

All patients received 4 mg/kg azathioprine (AZA) in their dialysis unit immediately before being called to the transplant center. Intraoperatively, all patients received 500 mg methylprednisolone (MP) intravenously 30–60 min prior to a high-dose single antilymphocyte globulin bolus [11, 12] [9 mg/kg body weight (bw) rabbit anti-human-T-lymphocyte globulin (ATG, Fresenius, Oberursel, Germany) in 14 patients; 1.5 ml/bw equine anti-human thymocyte globulin (Lymphoglobulin, Institute Merieux, Lyon, France) in 1 patient]. Post-KTx the recipients received 40 mg MP for 7 days, subsequently switching to 35 mg/kg prednisolone for 14 days. After further reduction the maintenance dose was 10–15 mg/day. AZA was restarted after surgery at a dose of 1 mg/kg orally. Oral cyclosporine (CyA) was started within 24 h of surgery. A maintenance CyA level of 100 ng/ml (radioimmunoassay, Incstar, Stillwater, Minnesota, USA) during the first postoperative week and 200 ng/ml thereafter was aimed for.

Monitoring for rejection and infection

For the diagnosis of rejection both clinical and laboratory signs were decisive. Rejection was treated with MP 5 mg/kg for 5 days. In two cases of humoral rejection crises proven by the detection of donor-reactive antibodies using cryopreserved (liquid nitrogen) donor spleen lymphocytes as target cells the successful treatment consisted of high-dose MP-based OKT 3 infusions (10 × 2.5 mg combined with four plasmaphereses in one patient).

Diagnosis of cytomegalovirus (CMV) infections was done using fluorescence microscopic detection of CMV antigen-carrying peripheral blood cells or fine needle aspirated kidney graft cells by means of monoclonal antibodies (Clonab-CMV pp 65, Biotest, Dreieich, Germany, and DAKO-CMV, Dakopatts, Denmark) and the detection of CMV-specific IgM and/or IgG antibodies (CMV ELISA Enzygnost, Behring, Germany) [5]. The treatment of CMV disease depended on the severity of clinical symptoms and included the application of human immunoglobulins with a high content of CMV-specific antibodies (Cytotect, Biotest) and/or ganciclovir (Syntex, Aachen, Germany).

THP determination

The urine samples ($n = 428$) were collected daily from 24-h urine of 15 kidney transplant recipients. Because of the high-molecular weight of THP (85 kDa for the monomeric form) and the ability to form high molecular aggregates it was very important to stir up the urine before collecting samples. The THP quantification was done using the SYNELISA-THP as recommended by the manufacturer. The sensitivity (0.2 mg/ml) and reproducibility (coefficient of variation for repeated analysis < 10%) of this assay are excellent.

Results

Urinary THP excretion in patients with immediate post-transplant graft function and favorable courses

Eight of 15 recipients included in this study showed totally uncomplicated post-transplant courses. Therefore, the data obtained from these patients served to describe the kidney regeneration after grafting.

The cold ischemia time ranged from 5 to 18 ¼ h ($x \pm SD = 11 \pm 4\frac{3}{4}$ h), the serum creatinine at discharge from 65 to 167 μmol (117 ± 32 μmol/l) and the postoperative hospital stay from 16 to 34 days (23 ± 7 days). The development of serum creatinine concentrations as well as urinary THP excretions in these eight patients is shown in Fig. 1. This figure clearly demonstrates that transplants functioning immediately postoperatively also need time for regeneration. On post-transplant day 5 the mean serum creatine level was 152 ± 50 μmol/l. The urinary THP excretion continuously increased from day 1 to day 7 (from 0.6 ± 0.75 to 13.3 ± 4.7 mg/24 h). Therefore, to characterize the functional activity of highly successful grafts we chose the 7–21 day post-transplant interval. The data obtained are shown in Table 1.

Taking all interval data together we found a "normal" urinary THP excretion of 14.5 ± 4.9 mg/24 h. The daily mean values ranged from 12.8 ± 4.9 to 16.5 ± 6.6 mg/24 h during this time. After reaching stable graft function the daily urinary THP excretion was relatively constant. The mean urinary THP concentration ranged from 4.0 ± 1.9 to 8.2 ± 5.0 mg/ml during this interval.

Urinary THP excretion in patients with complicated post-transplant courses

The seven patients with complicated postoperative courses showed the following complications: delayed graft func-

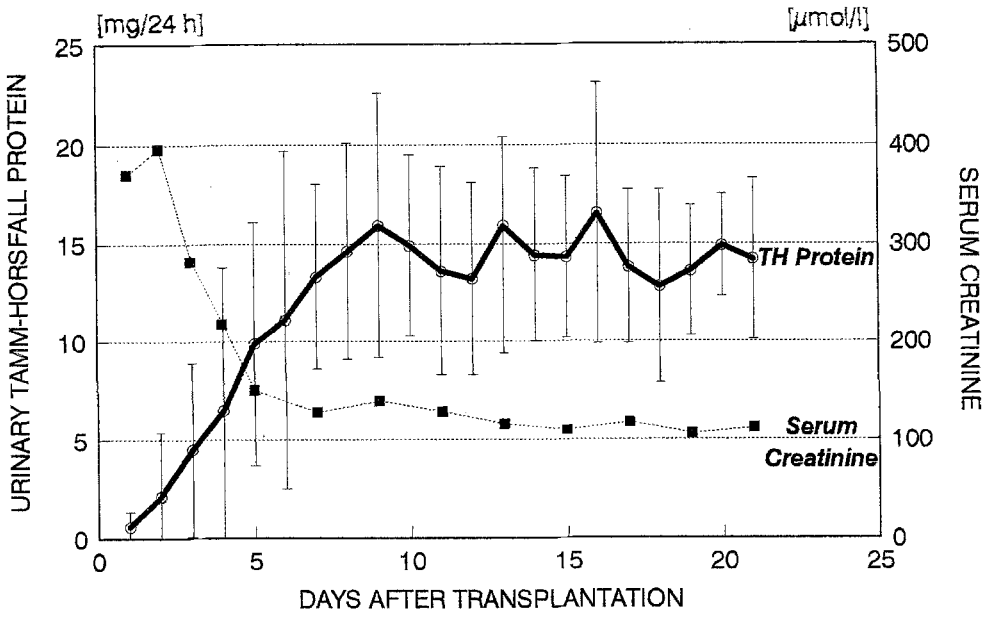

Fig. 1 The development of urinary Tamm-Horsfall (TH) protein excretion and serum creatinine concentration (mean and SD) in eight patients with totally uncomplicated post-transplant courses

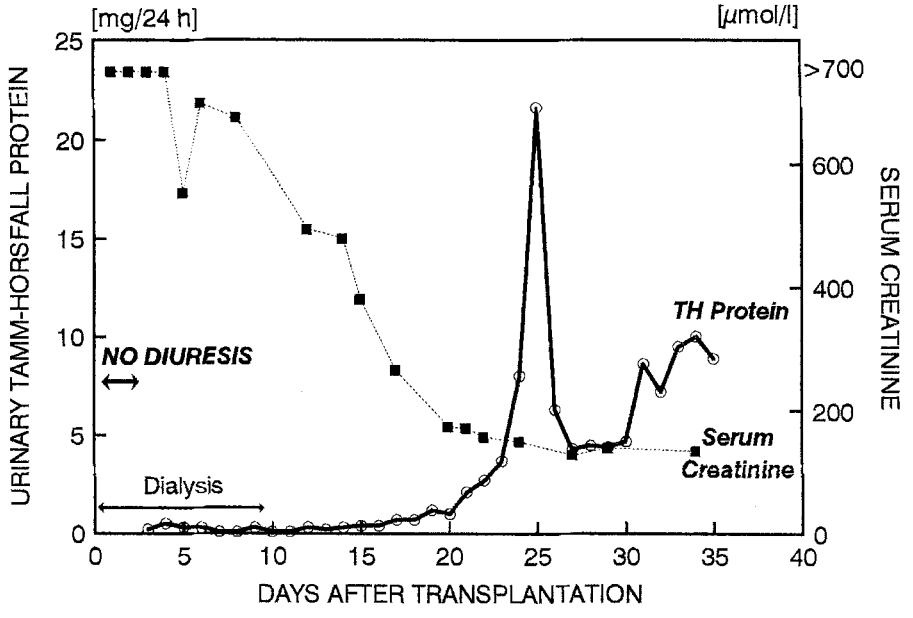

Fig. 2 Tamm-Horsfall protein excretion and serum creatinine concentration during prolonged kidney graft insufficiency. In spite of good diuresis, four postoperative dialyses were needed (on days 4, 6, 8 and 10). The serum creatinine concentration sank to 158 µmol/l for the first time on day 22. The decrease in serum creatinine concentration preceded the increase in THP excretion

tion ($n = 2$); acute interstitial rejection ($n = 3$); accelerated humoral rejection ($n = 2$); CMV infection ($n = 6$); CMV disease ($n = 2$).

The most impressive urinary THP changes were seen in patients with delayed graft function without any other complication (Fig. 2) or in association with humoral rejection diagnosed by the detection of donor-reactive antibodies (Fig. 3). In both cases the recovery of graft function, whether spontaneous or due to successful humoral rejection treatment with OKT 3, was accompanied by a continuous increase of urinary THP excretion. Clearly less pronounced were changes of urinary THP excretions in connection with acute interstitial rejections.

Figure 4 demonstrates the daily THP excretion in three acute graft-rejecting patients in comparison to the serum creatinine concentration. Before, and at the onset of cellular rejection a decrease of urinary THP excretion can be observed in connection with an increase of serum creatinine concentration but comparable changes of THP excretion can be found also during quiescent graft function. Therefore, it seems that urinary THP excretion is of relatively little value in diagnosing acute interstitial rejection, especially if the patient is MP sensitive. No association was found between CMV infection and THP excretion. In two patients with mild CMV disease no obvious changes of urinary THP excretion were seen.

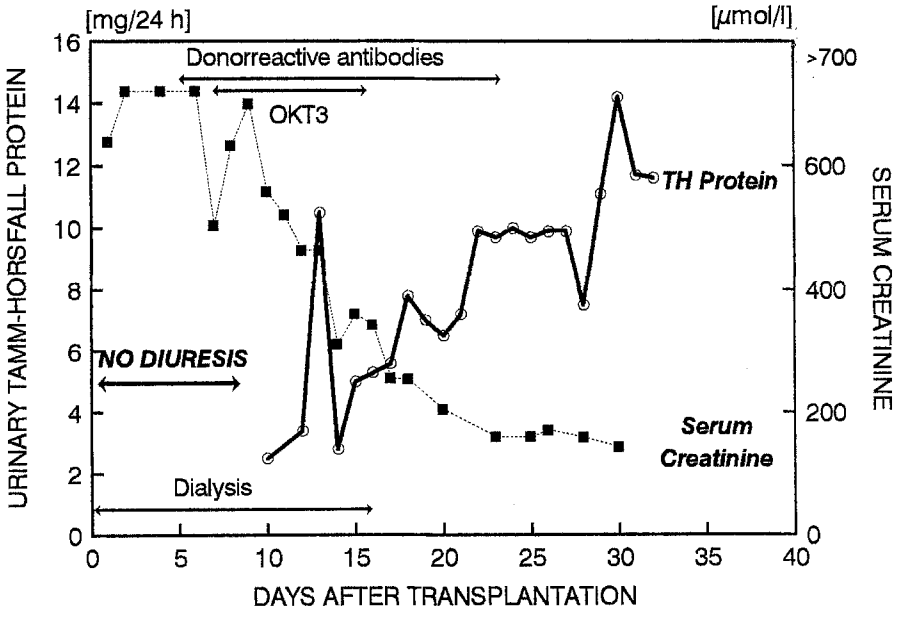

Fig. 3 Urinary Tamm-Horsfall protein excretion and serum creatinine concentration in patient CD 1508 with acute tubular necrosis after KTx, no diuresis up to the 11th day, seven postoperative dialyses in connection with accelerated humoral rejection proven by the detection of donor-reactive antibodies for the first time on post-KTx day 5. After the methylprednisolone-supported OKT 3 therapy (2.5 mg OKT 3 daily for 10 days) the transplant started to function on day 18 (diuresis 1150 ml/24 h), and on day 23 the serum creatinine concentration sank to 160 µmol/l. Starting on day 10 THP excretion steadily increased indicating the recovery of graft function

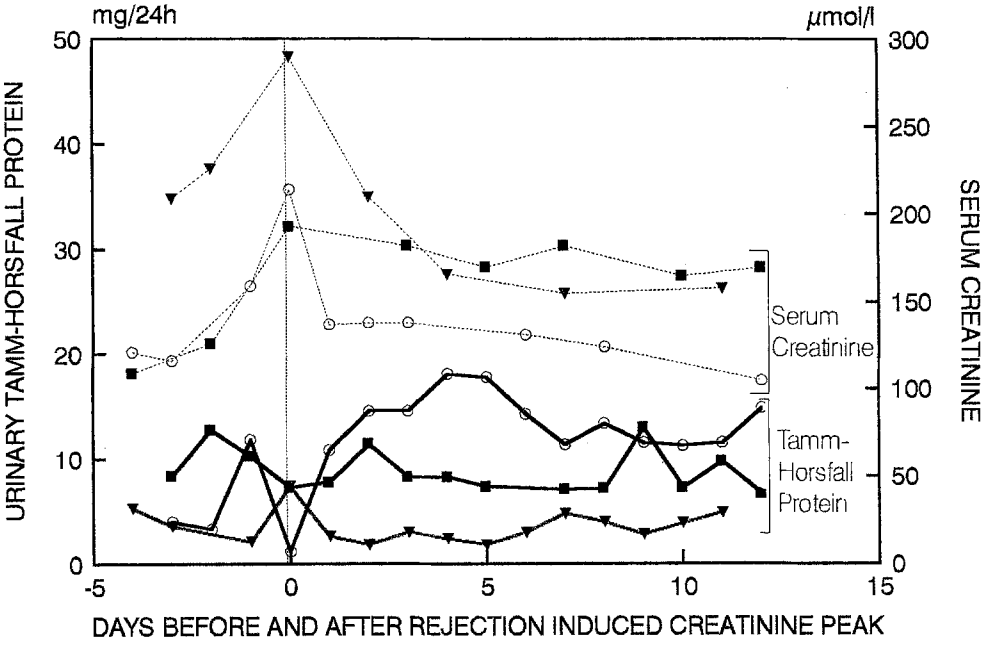

Fig. 4 Behavior of urinary THP excretion in connection with acute interstitial methylprednisolone-sensitive rejection crises in three patients. In order to compare the individual courses the curves are aligned according to the peak serum creatinine concentration. In connection with the deterioration of graft function indicated by increasing serum creatinine concentrations there were obvious reductions of THP excretion, though these changes were within the limits seen in non-rejecting kidneys

Discussion

THP is normally excreted in relatively large amounts in urine. In urine samples from 24 young women (mean age 33 years) Reinhart et al. [19] found 64.22 mg/l and in 47 women from nursing homes (mean age 84 years) 35.07 mg/l disaggregated THP and concluded that THP concentration decreases in the elderly. The SYNELISA-THP producer, ELIAS, found in urine of 36 healthy volunteers on average 41 mg/24 h and fixed the lower limit of normal THP content in 24 h urine at 15 mg. Decrease of urinary THP excretion was described in diabetics [3, 25]. Rath et al. [17] found a marked reduction in intensity or complete disappearance, of the 105-kDa protein band (identified as THP by silver staining and western blot) by means of SDS-polyacrylamide gel electrophoresis in 71 of 88 hypertensive pregnant women. In this connection these authors discuss a transitory tubular dysfunction in cases of preeclampsia. Furthermore, THP is a major constituent of casts and has been found to be present in the core of renal stones [6]; the role of THP in stone formation is, however, controversial [7, 20]. THP seems to have a much stronger physiological role as a natural defense mechanism against urinary tract infection. Reinhart et al. [18] found that type 1 fimbriae-bearing *E. coli* bound 50 times more THP than did non-type 1-fimbriated strains and that this binding was achieved via mannose side-chains.

Recent studies by Muchmore and Decker [15] and Sherblom et al. [22] suggested that THP may play an important role in the biological activity of several cytokines, including IL-1, IL-2 and TNF. Besides these studies on the physiological role of THP, the observations of Howie and Brewer [10] and Cohnen et al. [4] on interstitial deposits of THP in rejected renal transplants and those of Zimmerhackl et al. [24] and Schumann et al. [21] on the association of regeneration of tubular cell function and THP excretion stimulated us to determine the daily THP excretion after kidney transplantation and to look into its diagnostic relevance. The specific nephronal localization of THP, as well as the variety of harmful influences on tubular cells in connection with pretransplant perfusion and storage and acute tubular necroses occurring after transplantation, acute rejection processes and treatment with nephrotoxic immunosuppressants recommended THP as a marker. Therefore, in 15 renal transplant recipients we determined the daily urinary THP excretion from the time of KTx to the day of discharge from the transplant center (a total of 428 urine samples). The most important step in the preanalytic phase was to stir the collected urine, because of the ability of THP to form high-molecular aggregates which are sedimented. Eight of the 15 recipients showed totally uncomplicated courses, and the post-KTx development of the urinary THP excretion pattern in these patients served to describe the normal functional recovery of transplanted kidneys. It was clearly shown that even kidneys that function immediately after KTx need 6–7 days for full recovery. The earliest normalization of urinary THP excretion was observed on post-KTx day 4. The regeneration of kidney grafts therefore takes about a week. Thereafter, there was no great variation in THP excretion. To characterize the functional activity after full regeneration, the urinary THP excretion between post-KTx days 7 and 21 was chosen. The THP excretion of regenerated kidneys during a stable phase was ascertained to be 14.5 ± 4.9 mg/24 h. The daily mean THP excretion ranged from 12.8 ± 4.9 to 16.5 ± 6.6 mg/24 h during this time. Thus, the THP excretion of successful kidney grafts is at the lower border of those of healthy kidneys, at least after functional recovery and stabilization before discharge from the transplant centre.

In cases of post-KTx complications the greatest changes in THP excretion were seen in patients with delayed graft function, regardless of its origin. In patients with uncomplicated delayed onset of graft function accompanied or induced by donor-reactive antibodies, the functional recovery, whether spontaneous or brought about by treatment with OKT 3, was characterized by a continuous increase in THP excretion. In these cases the urinary THP excretion reflects the functional status of the kidney graft. The relevance of urinary THP excretion to the diagnosis of acute cellular rejections is rather low. In connection with the deterioration of graft function there were obvious changes in proteinuria, but these were to some extent also seen in non-rejecting kidneys. Thus, THP excretion seems not to be a good parameter of detect acute interstitial methylprednisolone-sensitive rejection crises.

The daily measurement of urinary THP excretion is, however, suitable for monitoring the functional state of transplanted kidneys.

References

1. Allen F, Tisher CC (1976) Morphology of the ascending thick limb of Henle. Kidney Int 9:8
2. Avis PJG, Williams JD, Salaman JR, Asscher AW, Newcombe N (1985) Serum Tamm-Horsfall protein (THP) concentration in cyclosporin treated renal transplant recipients. Lancet II:154
3. Bernard AM, Ouled AA, Lauwerys RR, Lambert A, Vandeleene B (1987) Pronounced decrease of Tamm-Horsfall proteinuria in diabetics. Clin Chem 33:1264
4. Cohen AH, Border WA, Rajfer J, Glassock RJ (1984) Interstitial Tamm-Horsfall protein in rejecting renal allografts. Lab Invest 50:519
5. Eichler C, Kaden J, Strobelt V, Oesterwitz H, Scholz D (1989) Cytomegalievirusnachweis mittels monoklonaler Antikörper an Aspirationsmaterial von Nierentransplantaten. Z Urol Nephrol 82:13
6. Grant AMS, Baker LRI, Neuberger A (1973) Urinary Tamm-Horsfall glycoprotein in certain kidney diseases and its content in renal and bladder calculi. Clin Sci 44:377
7. Grover PK, Ryall RL, Marshall VR (1990) Does Tamm-Horsfall mucoprotein inhibit or promote calcium oxalate crystallization in human urine? Clin Chim Acta 190:223
8. Hartmann L, Bringuier AF, Chopin N, Kechrid C, Fries D (1985) Role of serum and urinary Tamm-Horsfall protein in the surveillance of kidney transplants. CR Soc Biol Paris 179:452
9. Hession C, Decker JM, Sherblom AP, Kumar S, Yue CC, Mattaliano RJ, Tizard R, Kawashima E, Schmeissner U, Heletky S, Chow EP, Burne CA, Shaw A, Muchmore AV (1987) Uromodulin (Tamm-Horsfall glycoprotein): a renal ligand for lymphokines. Science 237:1479
10. Howie AJ, Brewer DB (1983) Extra-tubular deposits of Tamm-Horsfall protein in renal allografts. J Pathol 139:193
11. Kaden J, May G, Schönemann C, Müller P, Groth J, Seeger W, Seibt F, Henkert M, Lippert J (1992) Effect of ATG prophylaxis in sensitized and non-sensitized kidney graft recipients. Transplant Int 5 [Suppl 1]:75
12. Kaden J, May G, Weßlau C, Müller P, Henkert M, Groth J (1992) Reduktion der OKT 3-induzierten Zytokinfreisetzung in Abhängigkeit von der Prednisolondosis. TxMed 4:130
13. Kaissling B, Peters S, Kriz W (1977) The transition of the thick ascending limb of Henle's loop into the distal convoluted tubule in the nephron of the rat kidney. Cell Tissue Res 182:111
14. Kumar S, Muchmore A (1990) Tamm-Horsfall protein–uromodulin (1950–1990). Kidney Int 37:1395
15. Muchmore A, Decker JM (1985) Uromodulin: a unique 85 kilodalton immunosuppressive glycoprotein isolated from urine of pregnant women. Science 229:479
16. Pennica D, Kohr WJ, Kuang WJ, Glaister D, Aggarwal BB, Chen EY, Goeddel DV (1987) Identification of human uromodulin as the Tamm Horsfall urinary glycoprotein. Science 236:83
17. Rath W, Neßelhut T, Grospietsch G, Stadler H, Weber MH, Otto V, Kuhn W (1988) Veränderungen des Urinproteinmusters in der SDS-polyacrylamidgelelektrophorese (SDS-PAGE) bei normalen und hypertensiven Schwangerschaften. Geburtshilfe Frauenheilkd 48:724
18. Reinhart HH, Obedeanu N, Sobel JD (1990) Quantitation of Tamm-Horsfall protein binding to uropathogenic *Escherichia coli* and lectins. J Infect Dis 162:1335
19. Reinhart HH, Obedeanu N, Robinson R, Korzeniowski O, Kaye D, Sobel JD (1991) Urinary excretion of Tamm-Horsfall protein in elderly women. J Urol 146:806

20. Ryall RL, Harnett RM, Hibberd CM, Edyvane KA, Marshall VR (1991) Effects of chondroitin sulphate, human serum albumin and Tamm-Horsfall mucoprotein on calcium oxalate crystallization in undiluted human urine. Urol Res 19:181
21. Schumann V, Claus M, Fritschka E, Wagner K, Phillipp T (1992) Tamm-Horsfall-Protein als Funktionsparameter bei Nierentransplantationen. (abstract) 23rd Congress of the Gesellschaft für Nephrologie, Hanover
22. Sherblom A, Decker J, Muchmore A (1988) The lectin-like interaction between recombinant tumor necrosis factor and uromodulin. J Biol Chem 263:5418
23. Tamm I, Horsfall FL (1950) Characterisation and separation of an inhibitor of viral hemagglutination present in urine. Proc Soc Exp Biol Med 74:108
24. Zimmerhackl LB, Weber G, Höfer D, Fabricius T, Drenckhahn D, Scott DM, Kinne R, Brandis M (1989) Excretion of the renal antigens villin and Tamm-Horsfall protein as indicator of regional tubular cell regeneration after renal transplantation. (abstract 32) Eur J Ped 148:484
25. Zimmerhackl LB, Pfleiderer S, Kinne R, Manz F, Schuler G, Brandis M (1991) Tamm-Horsfall protein excretion as a marker of ascending limb transport indicates early renal tubular damage in diabetes mellitus type I. J Diabetes Complications 5:112