Glomerular proteinuria predicts the severity of acute kidney injury

in Puumala hantavirus induced tubulointerstitial nephritis

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Abstract

Background. Puumala virus (PUUV) induced hemorrhagic fever with renal syndrome is common in many European countries. The typical renal histologic lesion is acute tubulointerstitial nephritis. We examined the type and kinetics of urine protein excretion, and prognostic significance of proteinuria for the severity of acute kidney injury (AKI) in acute PUUV infection.

Methods. The amount of dipstick albuminuria at hospital admission was analyzed in 205 patients with acute PUUV infection. Dipstick albuminuria at admission was graded into three categories: 0-1+, 2+, 3+. In 70 patients also 24-hour urinary excretion of protein, and overnight urinary excretion of albumin, immunoglobulin(Ig)G, and α1-microglobulin were measured over three consecutive days during the hospital stay.

Results. Maximum median daily proteinuria, overnight albuminuria, and IgG excretion was observed five days, while that of creatinine values was observed nine days after the onset of the disease. The medians of maximum plasma creatinine levels during hospital stay were different in the 3 categories of dipstick albuminuria: 0-1+ 98 µmol/L (58-1499), 2+ 139 µmol/L (71-829), and 3+ 363 µmol/L (51-1285) (p<0.001). Dipstick albuminuria ≥2+ at admission could detect 89% of the patients who subsequently developed severe AKI. Glomerular proteinuria, but not tubular proteinuria (α1-microglobulin), correlated with the severity of the emerging AKI.

Conclusion. In acute PUUV infection, maximum median proteinuria values preceded the most severe phase of AKI by a few days. A highly useful finding for clinical work was that a quick and simple albuminuria dipstick test at hospital admission predicted the severity of the upcoming AKI.

Introduction

Hemorrhagic fever with renal syndrome (HFRS) and hantavirus cardiopulmonary syndrome (HCPS) are the manifestations of hantavirus infections in humans [1-3]. HFRS is caused by Eurasian hantaviruses, whereas HCPS occurs in the America [1-3]. These syndromes have also been referred to as "hantavirus fever", as there are similarities in the clinical picture of the two syndromes [4,5]. *Hantaan virus* (HTNV) causes HFRS in Asia, whereas *Puumala virus* (PUUV) and *Dobrava virus* (DOBV) are distributed in Europe [1-3]. PUUV, spread by bank voles (*Myodes glareolus*), causes most of the HFRS cases in Europe [1-3]. PUUV infections are most common in Northern Europe, and high numbers of these infections are reported in Finland with 1000-3000 serologically diagnosed cases annually. It is also common in Sweden, Belgium, Germany and Western Russia, but in many countries of Southern Europe, fewer cases are met [6,7].

The severity of hantavirus infections varies. HCPS can have a mortality of 35-50%, whereas HFRS caused by PUUV has a low mortality of 0.1% [2,8]. The three typical features in all HFRS cases are renal involvement, thrombocytopenia and increased capillary leakage [9-13]. In PUUV infection, the common clinical symptoms include sudden high fever with headache, abdominal and back pains, nausea, and visual disturbances, while serious hemorrhagic manifestations are rare [9-13]. The disease often leads to hospitalization and sometimes to intensive care unit (ICU) treatment, including renal replacement therapy (RRT) and mechanical ventilation [14].

Renal involvement of PUUV infection causes transient proteinuria, microscopic hematuria, and frequently acute kidney injury (AKI). Oliguria or anuria is followed by polyuria, and thereafter spontaneous recovery takes place [9-13,15]. RRT is needed in up to 6% of the hospitalized patients [10-13]. There is a genetic predisposition to the severity of PUUV infection [16,17], and smokers develop a more severe AKI than non-smokers [18,19]. The outcome of the AKI in PUUV infection is favorable, i.e. without persisting sequelae [14].

The typical renal histopathologic finding in PUUV-infection is acute tubulointerstitial nephritis, predominated by infiltrating lymphocytes, and also including plasma cells, monocytes, macrophages, and eosinophils [20,21]. Proteinuria is detected in almost all patients, which is of the nephrotic range in up to one-third of them [9-11,13,22]. The pathogenesis of this massive proteinuria is unclear. In a recent retrospective study from Germany, urinary neutrophil gelatinase-associated lipocalin (NGAL) and urine albumin level predicted the severity of AKI in acute PUUV infection [23]. In this cohort, albuminuria was determined by urine albumin-creatinine ratio in 24 patients and by dipstick in 59 patients, while tubular proteinuria was not addressed [23].

In the present study, our aim was to evaluate the prognostic significance of proteinuria on the severity of subsequent AKI in acute PUUV infection. Furthermore, we assessed the type and kinetics of proteinuria in relation to the course of PUUV infection.

Subjects and Methods

The cohort of this study consisted of 217 consecutive PUUV-infected patients treated at the Tampere University Hospital, Finland, during 1997-2014. The patients also participated in our previous studies [17,22,24-33]. All patients were examined during the acute phase of the disease, and the diagnosis of acute PUUV infection was serologically confirmed [34-37]. A detailed medical history was obtained and physical examination was performed. The median age was 41 (range 15-77) years, 12 patients (7%) were older than 65 years, and 138 patients (67%) were males. Patients <15 years of age were excluded. Also, 12 patients were excluded because of only one recorded creatinine value (5 patients) or missing urine dipstick test (7 patients). Altogether 205 patients were included in the analyses.

The following diagnoses before acute PUUV infection had been made in 56 (27%) patients: hypertension (n=17), coronary artery disease (n=7), rheumatoid arthritis (n=5), diabetes (n=4),

bronchial asthma (n=4), hypothyroidism (n=4), celiac disease (n=3), inflammatory bowel disease (n=2), psychiatric disorder (n=2), operated malignant disease (n=2), cardiac conduction disorder (n=2), aortic/mitral valve disease (n=2), epilepsy (n=2), osteoporosis (n=2), sequelae of renal tuberculosis (n=1), polycystic kidney disease (n=1), operated meningioma (n=1), chronic lymphatic leukemia in remission (n=1), psoriasis (n=1), prostate hyperplasia (n=1), sleep apnea (n=1), and TIA (n=1). Some patients had more than one disease, but none had known prior kidney insufficiency. Although two patients had a history of a kidney disease, both of them had normal kidney function before PUUV infection. None of the patients were given non-steroidal anti-inflammatory drugs in the hospital, but possible use before hospitalization was not recorded. All patients provided a written informed consent and the study was approved by the Ethics Committee of Tampere University Hospital.

Laboratory determinations

In all 205 patients, urine albumin dipstick test was performed at hospital admission, and daily plasma creatinine values during the hospital stay were determined (median number of determinations 5, range 2-15). The amount of albuminuria detected by dipstick test was graded into 3 categories: 0-1+ (n=54), 2+ (n=73), and 3+ (n=78). In 70 consecutive patients treated during 1997-1999, analyses of 24-hour urinary protein excretion, and overnight excretion of albumin, immunoglobulin (Ig)G, and α1-microglobulin were performed during 3 consecutive days. The highest and lowest values of the variables measured during hospitalization for each patient were designated as the maximum and minimum values, respectively.

The diagnosis of PUUV infection in 1982-1989 was based on duplicate samples with 4-fold or greater rise in IgG-titer by the immunofluorescence assay (IFA) [34]. Since 1989, recent PUUV infection was confirmed from a single serum sample by detecting the typical granular staining

pattern in IFA [35], and/or low avidity of IgG antibodies to PUUV [36], and/or by detecting PUUV IgM antibodies by an 'in-house' enzyme-linked immunosorbent assay (ELISA) based on a recombinant antigen [37]. The development and use of the above and other diagnostic methods has been described by Vaheri et al [38].

Plasma creatinine was determined until 1999 by Vitros (Johnson & Johnson, Rochester, N.Y., USA) and thereafter by Cobas Integra (F. Hoffman- La Roche Ltd., Basel, Switzerland). Urine dipstick analysis was made by automated tests based on refractometry: From 1997 using Miditron M (Roche), from 2004 Urisys 2400 or 1900 (Roche), and from 2009 until 2014 Siemens Clinitec Atlas or Advantus. The sensitivity of these tests to urine albumin (1+) ranges 0.15-0.3 g/l. The dipstick result 2+ stands for ≥1 g/l albumin and 3+ ≥3 g/l albumin. The albumin dipstick tests do not react with urinary globulins or immunoglobulin light chains. Urine for the dipstick test was sampled on admission already at the emergency room.

In 70 patients, urine collection was started on the first evening of hospital care and continued for 3 days. The nightly collection period was from the time of the last voiding at bedtime until the last voiding on rising. The 24-hour collection commenced immediately thereafter. The 24-hour urinary protein excretion was measured by the pyrogallol red molybdate method (Olli C.; Kone Instruments, Helsinki, Finland) until 1998, then by using Cobas Integra (Roche diagnostics) until 2008, and thereafter using Cobas 8000 analyzer (Roche diagnostics). Timed overnight urinary excretions of albumin and α1-microglobubulin were measured using nephelometry (Behring Nephelometer II Analyzer, Behringwerke AG, Marburg, Germany).

Blood cell count was determined by hematological cell counters (Bayer Diagnostics, Elkhart, IN, USA), and sodium, potassium, urea, and albumin concentrations using routine automated chemistry analyzers. All laboratory determinations were performed by the laboratory Centre of the Pirkanmaa Hospital District (later named Fimlab Laboratories), Tampere, Finland.

Statistical analyses

Medians and ranges were given for skewed continuous variables and numbers and percentages for categorical variables. Spearman's rank correlations were calculated. Categorical data were analysed by the x^2 test or the Fisher's exact test, and groups were compared using the Mann-Whitney U-test or the Kruskal-Wallis test, as appropriate. All tests were two-sided, and the analyses were performed using SPSS (version 20) statistical software (IBM, Chicago, IL).

Results

The clinical and laboratory data of the 205 patients are shown in Table 1. The median interval between the onset of symptoms (i.e. fever) and admission to hospital was 4 (range 1-15) days. Median duration of the hospital stay was 6 (range 2-25) days. Plasma creatinine was elevated (>100 µmol/L) in 153 (75%) patients. Six patients (3%) were in clinical shock at admission. Nine patients (4%) needed RRT and among them the median creatinine concentration was 706 µmol/L (range 265-1285). Among those treated with RRT, three subjects were in clinical shock and they had the lowest creatinine values (265-473 µmol/L) in this group of patients. After hospitalization two of these subjects were immediately transferred to the ICU for RRT due to oliguria or anuria, fluid retention and hypotension. None of the 205 patients had a urinary tract infection and all recovered.

In 70 consecutive patients, 24-hour urinary protein excretion and overnight excretion of albumin, α1-microglobulin and IgG were measured over three consecutive days during the hospital stay. The maximum 24-hour urinary protein excretion ranged from 0.14 to 17.78 g/24h and was of nephrotic range (>3.5 g/24h) in 34% of patients. The highest 24-hour urinary protein excretion (peak median 1.67 g/24h, range 0.26-17.78) was detected on the fifth day after the onset of fever (Figure 1A). Glomerular proteinuria, i.e. overnight excretion of albumin (peak median 734 µg/min, range 12-7026) (Figure 1B) and overnight excretion of IgG (peak median 183 µg/min, range 6-

1565) (Figure 1C) peaked on the fifth day as well. Tubular proteinuria, i.e. increased overnight urinary excretion of α1-microglobulin (>7 µg/min), was detected in 90% of the patients. The excretion was highest (peak median 38 µg/min, range 7-209) on the fourth day after the onset of fever (Figure 1D). Plasma creatinine value (peak median 229 µmol/L, range 68-725) was highest on the ninth day after the onset of fever (Figure 2).

The maximum 24-hour urinary protein excretion correlated slightly with maximum plasma creatinine level (r=0.30, p=0.012), while there was a higher correlation between glomerular proteinuria (maximum overnight excretion of albumin and IgG) and maximum plasma creatinine level (r=0.41; p<0.001 and r=0.44; p<0.001, respectively). Tubular proteinuria (maximum overnight α1-microglobulin excretion) did not correlate with maximum plasma creatinine concentration (r=0.13; p=0.291). Maximum overnight α1-microglobulin excretion correlated with maximum C-reactive protein (CRP) level (r=0.39; p=0.001), but not with other variables reflecting disease severity, i.e. length of hospital stay, weight change during hospital stay, maximum hematocrit, minimum platelet count, or maximum blood leukocyte count (data not shown).

Table 2 shows the clinical and laboratory data of 205 patients according to the three groups of urine albumin dipstick test result at hospital admission. Patients with higher albuminuria by dipstick test had greater change in body weight during hospitalization, higher maximum hematocrit, lower minimum sodium concentration and lower minimum plasma albumin than patients with less or no albuminuria. The results of these findings reflect the degree of capillary leakage. They also had more severe AKI (higher maximum plasma creatinine, urea and potassium concentrations) and longer hospital stay, reflecting the severity of the disease. Higher albuminuria by dipstick at admission was associated with higher overnight urinary excretion of albumin and IgG, but not of α1-microglobulin. All of the above associations were statistically significant (for p-values see Table 2). Platelet count was decreased (≤150 x10^9/L) in 197 patients (96%), but the minimum platelet count did not differ between the groups. The groups showed differences in the concentration of CRP, but CRP level was not associated with the amount of dipstick albuminuria. Higher category of

dipstick albuminuria was numerically related to higher 24-hour urinary protein excretion and lower plasma albumin, but these differences were not statistically significant. Among the 9 patients needing RRT, the dipstick albuminuria categories were 3+ in 6 subjects, 2+ in 1 subject, and 0-1+ in those two patients who were initially in clinical shock and treated at the ICU.

To evaluate whether the duration of symptoms before admission to the hospital influenced the ability of urine albumin dipstick test to predict the severity of AKI, we divided the patients into two subgroups: admission on days 1-4 (128 patients) or on day 5 or later (77 patients) after the onset of fever. In both groups, the amount of dipstick albuminuria at admission was associated with median maximum plasma creatinine level: (1) admission on days 1-4: U-alb 0/1+ 96 µmol/L (range 52-1499), U-alb 2+ 123 µmol/L (range 71-749), U-Alb 3+ 354 µmol/L (range 51-1285) ($p<0.001$); (2) admission on days ≥5: U-alb 0/1+ 118 µmol/L (range 58-874), U-alb 2+ 199 µmol/L (range 76-829), U-alb 3+ 376 µmol/L (range 93-1153) ($p<0.001$).

Severe stage 3 AKI (KDIGO, plasma creatinine ≥353.6 µmol/L [39]), was discovered in 63 (31%) patients during the hospitalization. Albumin dipstick test ≥2+ at admission could detect 89% of those who subsequently developed severe AKI (creatinine≥353.6 µmol/L). Furthermore, albumin dipstick test 3+ at admission showed high positive predictive value of 82% for maximum plasma creatinine >200 μmol/L (median in the study population) with a negative predictive value of 69%.

Discussion

The present study showed that in acute PUUV infection, maximum proteinuria preceded the most severe phase of AKI by some days. Glomerular proteinuria, but not tubular proteinuria, correlated with the severity of AKI. To our knowledge, this finding has not been previously reported in HFRS. The amount of albuminuria detected by dipstick test at hospital admission predicted the severity of AKI in PUUV infection. It also associated with several other disease severity markers, many of which reflect the general capillary leakage typical of HFRS.

In previous times, when reliable serological testing was not yet readily available to diagnose acute PUUV infection, kidney biopsies were performed to verify the reason of AKI in PUUV-infected patients. These biopsies have shown that the characteristic histological finding of acute PUUV-infection is acute tubulointerstitial nephritis [20,21]. Although intense proteinuria is not typical for tubulointerstitial nephritis, proteinuria in acute PUUV infection exceeds the nephrotic range in one-third of the patients [9-11,13,22], a finding that well corresponds with the present results. The contribution of tubular injury to the proteinuria in PUUV-infected patients is exemplified by the loss of low-molecular-weight proteins in the urine [40,41]. As kidney biopsies have usually been performed after the acute-phase thrombocytopenia has resolved to avoid biopsy-related bleeding complications, the timing of the biopsies has probably influenced the renal histological findings of the disease.

The glomerular alterations of acute PUUV infection in light-microscopy are minor [20], but the rapid onset of proteinuria suggests that alterations have taken place in vascular barrier function. The non-selective nature of the proteinuria indicates that the glomerular barrier is defective in acute PUUV-infection [40,41]. However, even in the case of massive transient proteinuria, the associated glomerular lesions with respect to adhesion molecules, cytokines, and cell infiltration remain negligible [21]. The hantaviruses can infect vascular endothelial cells, but also glomerular endothelial cells, tubular cells, and podocytes, disrupting the cell-to-cell contacts in all of these cell types [42]. Indeed, in electron microscopic studies, fusion of podocyte foot processes was already found in 1978 [43,44]. Recently, two reports confirmed severe ultrastructural changes in podocytes with foot-process effacement in 3 patients biopsied in the acute phase of hantavirus infection [45,46]. It seems plausible that podocyte dysfunction and disruptions of the cell-to-cell contacts impair the barrier function and result in intense glomerular proteinuria in the early phase of PUUV infection. In this study, glomerular proteinuria reached the highest values on the fifth day after the first clinical symptoms of the disease, and thereafter urine protein excretion decreased, suggesting

that proteinuria in this disease is rapidly resolving. However, we do not have data about urine protein excretion after hospitalization, and the disappearance of proteinuria in PUUV-infected patients is a subject for further studies.

In the present study, many variables reflecting capillary leakage were associated with the amount of dipstick albuminuria at hospital admission (Table 2). Thus, glomerular proteinuria may reflect the increased capillary leakage in acute PUUV infection. The main hantavirus targets are the endothelial cells of the post-capillary venules in various organs [3,12], while increased vascular permeability is a typical finding in all hantavirus infections [47]. The pathogenesis of this has not been fully elucidated, but it may involve the release of bradykinin, which increases vascular permeability in various pathological conditions. Indeed, the selective bradykinin type 2 receptor antagonist, icatibant, was successfully administered in two cases of life-threatening PUUV infection [48,49]. In imaging studies of PUUV infected patients, signs of fluid accumulation and edema have been frequently found [50]. We previously reported that during the acute phase of PUUV infection, both renal parenchymal swelling and high arterial resistive index in renal ultrasound were associated with the severity of AKI [51,52]. The amount of urinary 24-hour protein excretion, however, was not associated with quantitative or qualitative findings in the renal ultrasound study [51,52]. In addition to capillary leakage, possible explanations to kidney swelling in acute PUUV infection are tubulointerstitial inflammation and medullary hemorrhages [20,21,46].

We found that maximum CRP level was not associated with the amount of albuminuria detected by the dipstick test. Inflammatory response may nevertheless have a major role in the pathogenesis of proteinuria and plasma leakage in HFRS. Maximum urinary protein excretion was found to correlate with plasma and urine interleukin (IL)-6 concentrations but not with complement activation in the acute phase of PUUV infection [22,27,53].

Several mechanisms can lead to AKI during acute PUUV infection. In 17 French patients acute tubular necrosis (ATN) was the major histologic feature in 88% of cases, along with microvascular inflammation in peritubular capillaries [46]. Viral stimulus can trigger a major inflammatory response, causing tissue damage and ATN [54]. In kidney epithelial cell lines, apoptosis was reported in response to hantavirus infection [55,56]. Additional mechanisms of ATN in PUUV infected patients include hemodynamic alterations and intravascular hypovolemia, secondary to the capillary leakage. The use of non-steroidal anti-inflammatory drugs is another putative cause for ATN, and may contribute the severity of AKI in PUUV-infection [57]. These drugs were not given to the present patients at hospital, but possible usage before hospital admission was not recorded.

In chronic glomerular diseases, non-selective proteinuria has been associated with tubular damage and progression of renal failure [58], but the role of glomerular proteinuria in the pathogenesis of AKI is unclear. Pre-existing proteinuria is a risk factor for AKI in various clinical situations [59]. Only few studies have examined the significance of *de novo* proteinuria during AKI. New onset urinary dipstick proteinuria was associated with severe AKI in critically ill septic patients [60], and with increased risk of developing AKI in patients with severe burns [61]. In cardiac surgery patients, high amount of postoperative dipstick proteinuria was associated with the risk of AKI [62]. In our study, glomerular proteinuria correlated with the severity of upcoming AKI, but tubular proteinuria did not. Further, the peak excretion of albumin and IgG preceded the most severe phase of AKI by four days. Thus, alterations in glomerular permeability reflect the severity of upcoming AKI in acute PUUV infection. As a limitation of the present study, the type of proteinuria could only be evaluated in one-third (70/205) of the patients.

In a recent study, aimed at identifying patients who are at lower risk of developing severe AKI during acute PUUV infection, albuminuria defined by a urine albumin/creatinine ratio (ACR) >0.25g/g was one of the three risk-factors that predicted the risk of AKI [63]. The other two factors

were thrombocytopenia and the level of CRP [63]. Urinary NGAL was also found to predict the severity of AKI in PUUV infection [23]. When compared with urine ACR and NGAL determinations, albumin dipstick test is quick, inexpensive, and readily available. As albumin dipstick test result ≥2+ at hospital admission identified 89% of the patients who subsequently developed severe AKI, this simple test is a clinically applicable tool for the evaluation of severe AKI risk and the need for hospital treatment. Of note, the preceding duration of the symptoms of PUUV-infection did not influence the ability of urine albumin dipstick test to predict the severity of upcoming AKI.

In conclusion, maximum proteinuria preceded the most severe phase of AKI by some days in acute PUUV infection. Glomerular proteinuria, but not tubular proteinuria, correlated with the severity of the emerging AKI. The influence of proteinuria on the mechanisms of AKI during hantavirus infections is an interesting subject for further investigations. Finally, the determination of albuminuria by urine dipstick test at hospital admission predicted the severity of upcoming AKI in acute PUUV infection. It also associated with several variables that reflect increased capillary leakage. The predictability of this simple test should be evaluated in more severe HFRS cases caused by HTVN in Asia or DOBV in Europe, and in patients with HCPS in the Americas.

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Conflict of interest statement

The authors have no conflicts of interest to declare. We certify that the submission is original work and is not being considered for publication elsewhere, in whole or in part, except in abstract form.

References

Vaheri A, Henttonen H, Voutilainen L, Mustonen J, Sironen T, Vapalahti O: Hantavirus infections in Europe and their impact on public health. Rev Med Virol 2013;23:35-49.

Vapalahti O, Mustonen J, Lundkvist Å, Henttonen H, Plyusnin A, Vaheri A: Hantavirus infections in Europe. Lancet Infect Dis 2003;3:653-661.

Vaheri A, Strandin T, Hepojoki J, Sironen T, Henttonen H, Mäkelä S, Mustonen J: Uncovering the mysteries of hantavirus infections. Nat Rev Microbiol 2013;11:539-550.

Clement J, Maes P, Lagrou K, Van Ranst M, Lameire N: A unifying hypothesis and a single name for a complex globally emerging infection: hantavirus disease. Eur J Clin Microbiol Infect Dis 2012;31:1-5.

Rasmuson J, Andersson C, Norrman E, Haney M, Evander M, Ahlm C: Time to revise the paradigm of hantavirus syndromes? Hantavirus pulmonary syndrome caused by European hantavirus. Eur J Clin Microbiol Infect Dis 2011;30:685-690.

Heyman P, Vaheri A: Situation of hantavirus infections and haemorrhagic fever with renal syndrome in European countries as of December 2006. Euro Surveill 2008;13

Clement J, Maes P, Van Ranst M: Hemorrhagic Fever with Renal Syndrome in the New, and Hantavirus Pulmonary Syndrome in the Old World: paradi(se)gm lost or regained? Virus Res 2014;187:55-58.

Makary P, Kanerva M, Ollgren J, Virtanen MJ, Vapalahti O, Lyytikäinen O: Disease burden of Puumala virus infections, 1995-2008. Epidemiol Infect 2010;138:1484-1492.

Lähdevirta J: Nephropathia epidemica in Finland. A clinical histological and epidemiological study. Ann Clin Res 1971;3:1-54.

Settergren B, Juto P, Trollfors B, Wadell G, Norrby SR: Clinical characteristics of nephropathia epidemica in Sweden: prospective study of 74 cases. Rev Infect Dis 1989;11:921-927.

Mustonen J, Brummer-Korvenkontio M, Hedman K, Pasternack A, Pietilä K, Vaheri A: Nephropathia epidemica in Finland: a retrospective study of 126 cases. Scand J Infect Dis 1994;26:7-13.

Mustonen J, Mäkelä S, Outinen T, Laine O, Jylhävä J, Arstila PT, Hurme M, Vaheri A: The pathogenesis of nephropathia epidemica: new knowledge and unanswered questions. Antiviral Res 2013;100:589-604.

Braun N, Haap M, Overkamp D, Kimmel M, Alscher MD, Lehnert H, Haas CS: Characterization and outcome following Puumala virus infection: a retrospective analysis of 75 cases. Nephrol Dial Transplant 2010;25:2997-3003.

Outinen TK, Mäkelä S, Clement J, Paakkala A, Pörsti I, Mustonen J: Community Acquired Severe Acute Kidney Injury Caused by Hantavirus-Induced Hemorrhagic Fever with Renal Syndrome Has a Favorable Outcome. Nephron 2015;130:182-190.

Clement J: Acute kidney injury and hantavirus disease, ed Oxford Textbook of Clinical Nephrology, 4th edition. Oxford, UK, Oxford University Press, 2015, pp 2059-2066.

Mustonen J, Partanen J, Kanerva M, Pietilä K, Vapalahti O, Pasternack A, Vaheri A: Genetic susceptibility to severe course of nephropathia epidemica caused by Puumala hantavirus. Kidney Int 1996;49:217-221.

Mäkelä S, Mustonen J, Ala-Houhala I, Hurme M, Partanen J, Vapalahti O, Vaheri A, Pasternack A: Human leukocyte antigen-B8-DR3 is a more important risk factor for severe Puumala hantavirus infection than the tumor necrosis factor-alpha(-308) G/A polymorphism. J Infect Dis 2002;186:843-846.

Tervo L, Mäkelä S, Syrjänen J, Huttunen R, Rimpelä A, Huhtala H, Vapalahti O, Vaheri A, Mustonen J: Smoking is associated with aggravated kidney injury in Puumala hantavirus-induced haemorrhagic fever with renal syndrome. Nephrol Dial Transplant 2015;30:1693-1698.

Kitterer D, Segerer S, Dippon J, Alscher MD, Braun N, Latus J: Smoking Is a Risk Factor for Severe Acute Kidney Injury in Hantavirus-Induced Nephropathia Epidemica. Nephron 2016;134:89-94.

Mustonen J, Helin H, Pietilä K, Brummer-Korvenkontio M, Hedman K, Vaheri A, Pasternack A: Renal biopsy findings and clinicopathologic correlations in nephropathia epidemica. Clin Nephrol 1994;41:121-126.

Temonen M, Mustonen J, Helin H, Pasternack A, Vaheri A, Holthöfer H: Cytokines, adhesion molecules, and cellular infiltration in nephropathia epidemica kidneys: an immunohistochemical study. Clin Immunol Immunopathol 1996;78:47-55.

Mäkelä S, Mustonen J, Ala-Houhala I, Hurme M, Koivisto AM, Vaheri A, Pasternack A: Urinary excretion of interleukin-6 correlates with proteinuria in acute Puumala hantavirus-induced nephritis. Am J Kidney Dis 2004;43:809-816.

Bunz H, Weyrich P, Peter A, Baumann D, Tschritter O, Guthoff M, Beck R, Jahn G, Artunc F, Haring HU, Heyne N, Wagner R: Urinary Neutrophil Gelatinase-Associated Lipocalin (NGAL) and proteinuria predict severity of acute kidney injury in Puumala virus infection. BMC Infect Dis 2015;15:464.

Laine O, Joutsi-Korhonen L, Mäkelä S, Mikkelsson J, Pessi T, Tuomisto S, Huhtala H, Libraty D, Vaheri A, Karhunen P, Mustonen J: Polymorphisms of PAI-1 and platelet GP Ia may associate with impairment of renal function and thrombocytopenia in Puumala hantavirus infection. Thromb Res 2012;129:611-615.

Sane J, Laine O, Mäkelä S, Paakkala A, Jarva H, Mustonen J, Vapalahti O, Meri S, Vaheri A: Complement activation in Puumala hantavirus infection correlates with disease severity. Ann Med 2012;44:468-475.

Outinen TK, Tervo L, Mäkelä S, Huttunen R, Mäenpää N, Huhtala H, Vaheri A, Mustonen J, Aittoniemi J: Plasma levels of soluble urokinase-type plasminogen activator receptor associate with the clinical severity of acute Puumala hantavirus infection. PLoS One 2013;8:e71335.

Outinen TK, Mäkelä SM, Ala-Houhala IO, Huhtala HS, Hurme M, Paakkala AS, Pörsti IH, Syrjänen JT, Mustonen JT: The severity of Puumala hantavirus induced nephropathia epidemica can be better evaluated using plasma interleukin-6 than C-reactive protein determinations. BMC Infect Dis 2010;10:132.

Libraty DH, Mäkelä S, Vlk J, Hurme M, Vaheri A, Ennis FA, Mustonen J: The Degree of Leukocytosis and Urine GATA-3 mRNA Levels Are Risk Factors for Severe Acute Kidney Injury in Puumala Virus Nephropathia Epidemica. PLoS One 2012;7:e35402.

Outinen TK, Mäkelä SM, Ala-Houhala IO, Huhtala HS, Hurme M, Libraty DH, Oja SS, Pörsti IH, Syrjänen JT, Vaheri A, Mustonen JT: High activity of indoleamine 2,3-dioxygenase is associated with renal insufficiency in Puumala hantavirus induced nephropathia epidemica. J Med Virol 2011;83:731-737.

Outinen TK, Mäkelä S, Huhtala H, Hurme M, Meri S, Pörsti I, Sane J, Vaheri A, Syrjänen J, Mustonen J: High pentraxin-3 plasma levels associate with thrombocytopenia in acute Puumala hantavirus-induced nephropathia epidemica. Eur J Clin Microbiol Infect Dis 2012;31:957-963.

Outinen TK, Mäkelä S, Huttunen R, Mäenpää N, Libraty D, Vaheri A, Mustonen J, Aittoniemi J: Urine soluble urokinase-type plasminogen activator receptor levels correlate with proteinuria in Puumala hantavirus infection. J Intern Med 2014;276:387-395.

Miettinen MH, Mäkelä SM, Ala-Houhala IO, Huhtala HS, Hurme MA, Kööbi T, Partanen JA, Pasternack AI, Vaheri A, Pörsti IH, Mustonen JT: The severity of acute Puumala hantavirus infection does not predict the long-term outcome of patients. Nephron Clin Pract 2010;116:c89-94.

Miettinen MH, Mäkelä SM, Ala-Houhala IO, Huhtala HS, Kööbi T, Vaheri AI, Pasternack AI, Pörsti IH, Mustonen JT: Ten-year prognosis of Puumala hantavirus-induced acute interstitial nephritis. Kidney Int 2006;69:2043-2048.

Brummer-Korvenkontio M, Vaheri A, Hovi T, von Bonsdorff CH, Vuorimies J, Manni T, Penttinen K, Oker-Blom N, Lähdevirta J: Nephropathia epidemica: detection of antigen in bank voles and serologic diagnosis of human infection. J Infect Dis 1980;141:131-134.

Vapalahti O, Kallio-Kokko H, Narvanen A, Julkunen I, Lundkvist Å, Plyusnin A, Lehväslaiho H, Brummer-Korvenkontio M, Vaheri A, Lankinen H: Human B-cell epitopes of Puumala virus nucleocapsid protein, the major antigen in early serological response. J Med Virol 1995;46:293-303.

Hedman K, Vaheri A, Brummer-Korvenkontio M: Rapid diagnosis of hantavirus disease with an IgG-avidity assay. Lancet 1991;338:1353-1356.

Vapalahti O, Lundkvist Å, Kallio-Kokko H, Paukku K, Julkunen I, Lankinen H, Vaheri A: Antigenic properties and diagnostic potential of Puumala virus nucleocapsid protein expressed in insect cells. J Clin Microbiol 1996;34:119-125.

Vaheri A, Vapalahti O, Plyusnin A: How to diagnose hantavirus infections and detect them in rodents and insectivores. Rev Med Virol 2008;18:277-288.

KDIGO Clinical Practice Guideline for Acute Kidney Injury: Section 2: AKI Definition. Kidney Int 2011;2 Suppl:19-36.

Ala-Houhala I, Koskinen M, Ahola T, Harmoinen A, Kouri T, Laurila K, Mustonen J, Pasternack A: Increased glomerular permeability in patients with nephropathia epidemica caused by Puumala hantavirus. Nephrol Dial Transplant 2002;17:246-252.

Cosgriff TM: Mechanisms of disease in Hantavirus infection: pathophysiology of hemorrhagic fever with renal syndrome. Rev Infect Dis 1991;13:97-107.

Krautkrämer E, Grouls S, Stein N, Reiser J, Zeier M: Pathogenic old world hantaviruses infect renal glomerular and tubular cells and induce disassembling of cell-to-cell contacts. J Virol 2011;85:9811-9823.

Collan Y, Mihatsch MJ, Lähdevirta J, Jokinen EJ, Romppanen T, Jantunen E: Nephropathia epidemica: mild variant of hemorrhagic fever with renal syndrome. Kidney Int Suppl 1991;35:S62-71.

Collan Y, Lähdevirta J, Jokinen EJ: Electron Microscopy of Nephropathia Epidemica. Glomerular changes. Virchows Arch A Pathol Anat Histol 1978;377:129-144.

Boehlke C, Hartleben B, Huber TB, Hopfer H, Walz G, Neumann-Haefelin E: Hantavirus infection with severe proteinuria and podocyte foot-process effacement. Am J Kidney Dis 2014;64:452-456.

Gnemmi V, Verine J, Vrigneaud L, Glowacki F, Ratsimbazafy A, Copin MC, Dewilde A, Buob D: Microvascular inflammation and acute tubular necrosis are major histologic features of hantavirus nephropathy. Hum Pathol 2015;46:827-835.

Krautkrämer E, Zeier M, Plyusnin A: Hantavirus infection: an emerging infectious disease causing acute renal failure. Kidney Int 2013;83:23-27.

Antonen J, Leppänen I, Tenhunen J, Arvola P, Mäkelä S, Vaheri A, Mustonen J: A severe case of Puumala hantavirus infection successfully treated with bradykinin receptor antagonist icatibant. Scand J Infect Dis 2013;45:494-496.

Laine O, Leppänen I, Koskela S, Antonen J, Mäkelä S, Sinisalo M, Vaheri A, Mustonen J: Severe Puumala virus infection in a patient with a lymphoproliferative disease treated with icatibant. Infect Dis (Lond) 2015;47:107-111.

Paakkala A, Lempinen L, Paakkala T, Huhtala H, Mustonen J: Medical imaging in nephropathia epidemica and their clinical correlations. Eur J Intern Med 2004;15:284-290.

Paakkala A, Kallio T, Huhtala H, Apuli P, Paakkala T, Pasternack A, Mustonen J: Renal ultrasound findings and their clinical associations in nephropathia epidemica. Analysis of quantitative parameters. Acta Radiol 2002;43:320-325.

Paakkala A, Kallio T, Huhtala H, Apuli P, Paakkala T, Mustonen J: Value of ultrasonography in acute renal failure: analysis of qualitative features in patients with nephropathia epidemica. Acta Radiol 2004;45:785-790.

Paakkala A, Mustonen J, Viander M, Huhtala H, Pasternack A: Complement activation in nephropathia epidemica caused by Puumala hantavirus. Clin Nephrol 2000;53:424-431.

Mulay SR, Linkermann A, Anders HJ: Necroinflammation in Kidney Disease. J Am Soc Nephrol 2016;27:27-39.

Li XD, Kukkonen S, Vapalahti O, Plyusnin A, Lankinen H, Vaheri A: Tula hantavirus infection of Vero E6 cells induces apoptosis involving caspase 8 activation. J Gen Virol 2004;85:3261-3268.

Markotic A, Hensley L, Geisbert T, Spik K, Schmaljohn C: Hantaviruses induce cytopathic effects and apoptosis in continuous human embryonic kidney cells. J Gen Virol 2003;84:2197-2202.

Wagner R, Leicht-Biener U, Mucsi I, Seitz K: Ibuprofen or diclofenac is associated with more severe acute kidney injury in nephropathia epidemica. Scand J Urol Nephrol 2012;46:65-69.

D'Amico G, Bazzi C: Pathophysiology of proteinuria. Kidney Int 2003;63:809-825.

Grams ME, Sang Y, Ballew SH, Gansevoort RT, Kimm H, Kovesdy CP, Naimark D, Oien C, Smith DH, Coresh J, Sarnak MJ, Stengel B, Tonelli M, Consortium CKDP: A Meta-analysis of the Association of Estimated GFR, Albuminuria, Age, Race, and Sex With Acute Kidney Injury. Am J Kidney Dis 2015;66:591-601.

Neyra JA, Manllo J, Li X, Jacobsen G, Yee J, Yessayan L, Group AS: Association of de novo dipstick albuminuria with severe acute kidney injury in critically ill septic patients. Nephron Clin Pract 2014;128:373-380.

Hu JY, Meng XC, Han J, Xiang F, Fang YD, Wu J, Peng YZ, Wu YZ, Huang YS, Luo QZ: Relation between proteinuria and acute kidney injury in patients with severe burns. Crit Care 2012;16:R172.

Molnar AO, Parikh CR, Sint K, Coca SG, Koyner J, Patel UD, Butrymowicz I, Shlipak M, Garg AX: Association of postoperative proteinuria with AKI after cardiac surgery among patients at high risk. Clin J Am Soc Nephrol 2012;7:1749-1760.

Latus J, Schwab M, Tacconelli E, Pieper FM, Wegener D, Rettenmaier B, Schwab A, Hoffmann L, Dippon J, Muller S, Fritz P, Zakim D, Segerer S, Kitterer D, Kimmel M, Gussmann K, Priwitzer M, Mezger B, Walter-Frank B, Corea A, Wiedenmann A, Brockmann S, Pohlmann C, Alscher MD, Braun N: Acute kidney injury and tools for risk-stratification in 456 patients with hantavirus-induced nephropathia epidemica. Nephrol Dial Transplant 2015;30:245-251.

Table 1. The clinical and laboratory characteristics of 205 patients with acute Puumala hantavirus infection.

Finding	Median	Range
Age years	41	15-77
Duration of fever, days	6	2-19
Length of hospital stay, days	6	2-25
Body weight change during hospital stay, kg*	2.4	0-12
Hematocrit max	0.44	0.33-0.60
Platelets min, $\times 10^9$/L	61	3-249
Leukocytes max, $\times 10^9$/L	10.4	4.2-45.0
Plasma CRP max, mg/L	79	16-269
Plasma creatinine max, μmol/L	200	51-1448
Plasma urea max, mmol/L (n=110)	17.7	2.1-52.8
Sodium min, mmol/L	132	109-142
Potassium max, mmol/L	4.3	3.3-5.5
Plasma albumin min, g/L (n=113)	27	11-39
Urinary protein excretion max, g/24h (n=70)	1.80	0.14-17.78
Overnight urinary albumin excretion max, μg/min (n=70)	760	4-7026
Overnight urinary IgG excretion max, μg/min (n=70)	173	3-1565
Overnight urinary α1-microglobulin excretion max, μg/min (n=70)	28	2-209

4   *Difference between the highest and lowest weight during hospital stay; min=minimum,

5   max=maximum, CRP=C-reactive protein, IgG=immunoglobulin G.

Table 2. Clinical and laboratory data of 205 patients divided into three groups according to urine dipstick protein category at hospital admission.

	U-alb 0/1+	U-alb 2+	U-alb 3+	p-value
Number of patients	54	73	78	
Age, years	41 (22-77)	43 (22-74)	40 (15-65)	0.294
Length of hospital stay, days	5 (3-22)	6 (3-15)	7 (2-14)	0.026
Body weight change during hospital stay, kg*	1.6 (0-10.8)	2.2 (0-10.0)	3.7 (0-12.0)	<0.001
Hematocrit max	0.42 (0.33-0.59)	0.44 (0.34-0.59)	0.46 (0.34-0.60)	0.001
Platelets min, $\times 10^9$/L	68 (3-249)	61 (15-198)	56 (5-187)	0.232
Leukocytes max, $\times 10^9$/L	9.2 (5.1-38.6)	9.0 (4.2-31.2)	13.0 (5.7-45.0)	<0.001
Plasma CRP max, mg/L	74 (16-236)	92 (20-269)	68 (21-214)	0.012
Plasma creatinine max, μmol/L	98 (52-1447)	139 (71-829)	363 (51-1285)	<0.001
Plasma urea max, mmol/L (n=110)	9.9 (2.0-52.4)	13.3 (3.9-39.7)	25.7 (2.7-52.8)	<0.001
Sodium min, mmol/L	135 (109-141)	133 (120-141)	130 (113-142)	0.001
Potassium max, mmol/L	4.1 (3.3-5.2)	4.2 (3.3-5.3)	4.4 (3.3-5.5)	0.001
Plasma albumin min, g/L (n=113)	30 (11-39)	28 (21-37)	27 (18-37)	0.161
Urinary protein excretion max, g/24h (n=70)	0.57 (0.14-9.50)	1.74 (0.18-17.78)	2.22 (0.30-10.00)	0.076
Overnight urinary albumin excretion max, μg/min (n=70)	104 (4-4617)	756 (12-6246)	1016 (136-7026)	0.007
Overnight urinary IgG excretion max, μg/min (n=70)	16 (7-1267)	211 (3-1565)	226 (25-1542)	0.005
Overnight urinary α1-micro-globulin excretion max, μg/min (n=70)	20 (10-89)	38 (9-209)	30 (2-130)	0.693

Values are expressed as medians (range), * Difference between the highest and lowest weight during hospital stay; min=minimum, max=maximum, CRP=C-reactive protein.

Legends to the figures

Figure 1. Scatter plots show 24-hour urinary excretion of protein (A), overnight urinary excretion of albumin (B), immunoglobulin (Ig) G (C), and α1-microglobulin (D) in relation to the onset of fever in 70 PUUV infected patients; panels A-D depict values on three consecutive days in each patient. Every circle represents one measurement, the line depicts median values, outliers and extremes are omitted.
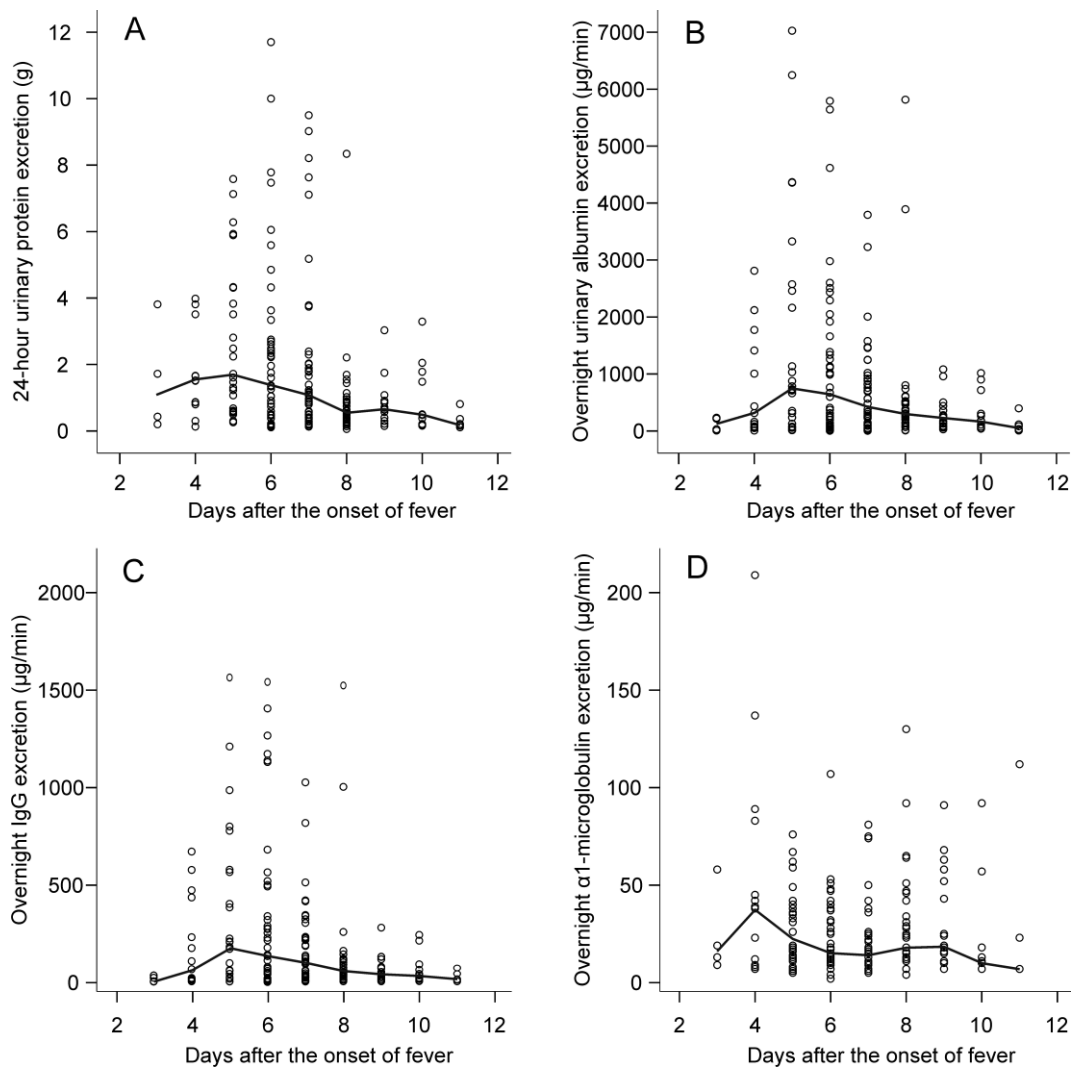
Figure 2. Scatter plot shows plasma creatinine values in relation to the onset of fever in 70 PUUV infected patients during hospital stay. Every circle represents one measurement, the line depicts median values.