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Acute kidney injury and tools for risk-stratification in 456 patients with hantavirus-induced nephropathia epidemica

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ABSTRACT

Background. Puumala virus (PUUV) is the most common species of hantavirus in Central Europe. Nephropathia epidemica (NE), caused by PUUV, is characterized by acute kidney injury (AKI) and thrombocytopenia. The major goals of this study were to provide a clear clinical phenotyping of AKI in patients with NE and to develop an easy prediction rule to identify patients, who are at lower risk to develop severe AKI.
Methods. A cross-sectional prospective survey of 456 adult patients with serologically confirmed NE was performed. Data were collected from medical records and prospectively at follow-up visit. Severe AKI was defined by standard criteria according to the RIFLE (Risk, Injury, Failure, Loss, End-stage kidney disease) classification. Fuller statistical models were developed and validated to estimate the probability for severe AKI.
Results. During acute NE, 88% of the patients had AKI according to the RILFE criteria during acute NE. A risk index score for severe AKI was derived by using three independent risk factors in patients with normal kidney function at time of diagnosis: thrombocytopenia [two points; odds ratios (OR): 3.77; 95% confidence intervals (CI): 1.82, 8.03], elevated C-reactive protein levels (one point; OR: 3.02; 95% CI: 1.42, 6.58) and proteinuria (one point; OR: 3.92; 95% CI: 1.33, 13.35). On the basis of a point score of one or two, the probability of severe AKI was 0.18 and 0.28 with an area under the curve of 0.71.
Conclusion. This clinical prediction rule provides a novel and diagnostically accurate strategy for the potential prevention and improved management of kidney complications in patients with NE and, ultimately, for a possible decrease in unnecessary hospitalization in a high number of patients.

Keywords: acute kidney injury, hanta virus, nephropathia epidemica, predictors for severe course, PUUV

INTRODUCTION

Hantavirus disease, along with rotavirus, norovirus, flu and hepatitis C, is one of the five most common notifiable viral diseases in Germany [1]. The viruses are single-stranded, enveloped RNA viruses of the Bunyaviridae family for which rodents are the natural reservoir [2] and can lead to haemorrhagic fever with renal syndrome (HFRS) in Asia and Europe, and hantavirus cardiopulmonary syndrome in the Americas with reported case fatality rates of up to 35% [3–6]. Several thousand cases of hantavirus infection occur annually throughout Europe [7]. Although a number of different hantavirus species (e.g. Dobrava-Belgrade virus, Tula virus) are circulating in Europe, Puumala virus (PUUV) is by far the most frequent cause of disease [1], being responsible for a milder form of HFRS [8], called nephropathia epidemica (NE).

The clinical picture of NE is characterized by acute kidney injury (AKI) associated with thrombocytopenia and often proteinuria [9]. A small number of patients with severe AKI are at risk to develop severe electrolyte disorders, anuria with fluid overload and uraemia with the requirement for renal replacement therapy or intensive supportive therapy [8], whereas a high proportion of patients could be treated as outpatients by a general practitioner or a nephrologist. Previous studies [10–21], mainly due to the study design's biases (graduation of the severity of the disease was based on maximum levels of, e.g. serum creatinine instead of peak levels), were not able to define patients at higher or lower risk to develop severe AKI at time of onset of the disease. Because only a proportion of patients have to be treated in hospital, risk prediction tools at time of diagnosis in the management of patients with NE are warranted.

The aims of this study were to give a clear clinical phenotyping in a representative cohort of patients and to establish a simple prediction score for patients with NE to allow physicians (especially general practitioner or nephrologists) to identify, at time of onset of disease, patients at low risk for severe AKI and subsequent complications. The availability of this information would allow physicians to streamline hospitalization in a high proportion of patients.

MATERIALS AND METHODS

Study population

Since 2001, German laboratories have the obligation by law (Section 7 of the German Law on the Prevention and Control of Infectious Diseases) to report confirmed cases of HFRS to the local health authorities. Subsequently, all HFRS cases are reported to the Robert Koch Institute in Berlin, Germany, the central federal institution responsible for disease control and prevention.

A total of 7476 patients with serologically and clinically confirmed NE were reported to the Robert Koch Institute in Berlin (Robert Koch Institute, SurvStat, www3.rki.de/SurvStat) from 2001 to 2012 [21]. In cooperation with four selected local health authorities in southern Germany (Stuttgart, Boeblingen/Sindelfingen, Esslingen and Reutlingen), we identified 1570 serologically confirmed patients with NE infected between 2001 and 2012. These patients were contacted by mail and asked to attend the outpatient clinic between September 2012 and April 2013 for follow-up examination. All patients gave written consent before participating in the study, which was approved by the Ethics Committee of the Ethics Commission of the State Chamber of Medicine in Baden-Wuerttemberg (Stuttgart) (F-2012-046). Studies were conducted in concordance with the Declaration of Helsinki.

Data acquisition during acute course of NE

Clinical and laboratory data at the time of diagnosis and during the acute course of the disease were obtained from medical reports and files from each patient at the time of follow-up in our outpatient clinic.

AKI was classified on the basis of the RIFLE (Risk, Injury, Failure, Loss, End-stage kidney disease) criteria [22]. Mild/moderate AKI was defined in patients with no AKI and AKI Risk (R) and severe AKI was classified as RIFLE Injury (I) and RIFLE Failure (F). Oliguria was classified as temporary loss of kidney function and refers to a 24-h urine output of <500 mL. Anuria was classified as a 24-h urine output of <50 mL. Leucocytosis was defined as leucocytes $>10 \times 10^9$/L, and thrombocytopenia was defined as thrombocytes $<90 \times 10^9$/L. Haematuria was defined as a positive dipstick test for erythrocytes and over two erythrocytes per high-power field. Proteinuria was defined by an albumin/creatinine ratio (ACR) >0.25 g/g creatinine in spot urine sample. Abdominal pain was defined as acute onset of pain in the lower quadrants and back pain/flank pain was scored positive in patients with acute onset of pain felt in the low or upper back. Peak or nadir levels were defined in patients where an increase or a decrease to a peak level or nadir followed by a decline [serum creatinine levels, C-reactive protein (CrP) and lactate dehydrogenase (LDH)] or increment (thrombocytes) was available. Patients with normal kidney function at the time of diagnosis were identified and a risk score for development of severe AKI was calculated in this patient cohort.

Statistical analysis

Continuous data are expressed as median and IR. Comparisons between different groups were made using analysis of variances (ANOVA) and the Fisher test. Univariate logistic-regression models were considered with the severity of the disease as binomial response variables increasing from levels low to high to ascertain the effect of demographic, clinical and laboratory variables. We had a quasi-complete separation for the response variable severity of AKI [RIFLE (0, R versus I, F)] and an exact logistic regression was performed. To predict severe AKI, a fuller multivariate logistic-regression model was constructed by considering factors that were significant in the univariate model ($P < 0.05$). Finally, to develop a risk score for clinical practice predicting severe AKI at the time of diagnosis, we assigned risk factors used in the multivariate analysis and weighted points proportional to the β regression coefficient (Supplementary data, Appendix). Odds ratios (OR) are given with corresponding 95% confidence intervals (CI) and two-sided P-values. A P-value of <0.05 was considered to be statistically significant. Statistical analysis was performed using R (version 3.0) [23] together with libraries elrm (version 1.2.1) [24], ROCR (version 1.0) [25], pROC (version 1.5.4), MASS (version 7.3) [26] and rms (version 4.0) [27]. Validation of the model was conducted using 5-fold cross-validation [28, 29] (Supplementary data, Appendix and Figure 3).

RESULTS

Clinical findings and course of AKI in NE

Between September 2012 and April 2013, 1570 patients with serologically confirmed NE (laboratory diagnosis was

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confirmed in all patients by detection of circulating anti-hantavirus IgG- and IgM-antibodies) diagnosed between 2001 and 2012 in Baden-Wuerttemberg were contacted by email. Overall, 456 patients (29%) were included in the study. This sample represents 6.1% of ever-reported cases of NE in Germany. Three patients were excluded by age <18 years at the time of diagnosis.

The median age at diagnosis was 48 years (IR, 40–59); male was the predominant gender (290 male and 166 female). Seventy per cent of patients had AKI according to RIFLE criteria at the time of admission to hospital or to the ambulatory care physician. During the acute course of the disease, serum creatinine increased in 31% of these patients, whereas in the remaining patients a continuous decrease of serum creatinine could be observed. Overall, 88% of the patients had AKI according to the RILFE criteria during acute course of NE. Serum creatinine peak levels were available in 52% of patients and duration of onset of symptoms associated with NE to peak serum creatinine was 8 (7–9) days. At the time of diagnosis, 137 of the 456 patients had normal kidney function. During acute NE, 61% of patients developed mild/moderate AKI (no AKI and RIFLE R), whereas 39% of patients developed severe AKI (RIFLE I and F) based on peak serum creatinine levels. Eleven patients (3%) required haemodialysis for 4 days (3–4.5) and three of these four patients had normal kidney function at time of diagnosis. The following parameters were statistically significant in difference between patients with impaired kidney function and patients with serum creatinine levels in the normal range at time of diagnosis: thrombocytes were lower, percentage of female gender was higher, CrP peak levels were higher, onset of symptoms to diagnosis was shorter and AKI (peak creatinine levels and AKI according to RIFLE criteria) was less severe in the normal kidney function group compared with the patient group presenting with impaired kidney function at the time of diagnosis of acute NE (Table 1).

During acute course of the disease, 32% of the patients were treated with antibiotics due to suspected bacterial infection. The classes of antibiotics used were mainly cephalosporines, different penicillin subgroups and gyrase inhibitors. Dose adaption based on renal function was done in all patients, and none of the patients received typical antibiotics with renal toxicity.

Risk score for predicting AKI in patients with normal kidney function at time of diagnosis

Patients with normal kidney function at time of diagnosis were included in the calculation of the risk score. Classification of stage of AKI during acute NE was based on peak serum creatinine levels in these patients. Severe renal involvement was defined as AKI RIFLE Stage I and F. On univariate analysis, four variables were statistically significant risk factors for severe AKI and were included in the logistic model (see Figure 1). A risk index score was derived by using the following three independent risk factors associated with severe AKI at logistic-regression analysis: thrombocytopenia (two points; OR: 3.77; 95% CI: 1.82, 8.03), 12-fold increase of CrP levels (one point; OR: 3.02; 95% CI: 1.42, 6.58) and proteinuria (one point; OR: 3.92; 95% CI: 1.33, 13.35) at time of presentation in

Table 1. Baseline characteristics of study population during acute hantavirus infection

Variable	Patients with impaired kidney function at time of diagnosis	Patients with normal kidney function at time of diagnosis
n	319	137
Age at diagnosis (years)	48 (40–59)	48 (40–59)
Female/male*	101/218	65/72
Inpatients/outpatients	241/78	94/43
Onset of symptoms prior to admission to hospital (days)**	6 (4–7)	4 (3–6)
Duration of hospital stay (days)	6 (4–9)	7 (5–10)
Laboratory findings		
Thrombocytes ($\times 10^9$/L)**	141 (93–212)	95 (72–135)
Creatinine at admission [mg/dL (0.5–1.4)]**	2.7 (1.8–4.3)	1.0 (0.8–1.2)
Creatinine peak levels [mg/dL (0.5–1.4)]**	4.8 (3.2–6.7)	2.5 (1.6–4.1)
Onset of symptoms to creatinine peak levels (days)	8 (7–9)	8 (7–10)
Renal replacement therapy	8/319	3/137
CrP at admission [mg/dL (0.1–0.4)]	3.9 (2.4–6.4)	4.3 (2.4–8.6)
CrP peak levels [mg/dL (0.1–0.4)]**	4.1 (2.6–6.8)	6.9 (3.7–10.1)
AKI at time of diagnosis**		
AKI	319/319	0/137
Risk	102	
Injury	71	
Failure	146	
AKI during course of disease**		
AKI	319/319	81/137
Risk	68	27
Injury	62	24
Failure	189	30

CrP, C-reactive protein; RRT, renal replacement therapy; AKI, acute kidney injury.
*P < 0.01.
**P < 0.001.

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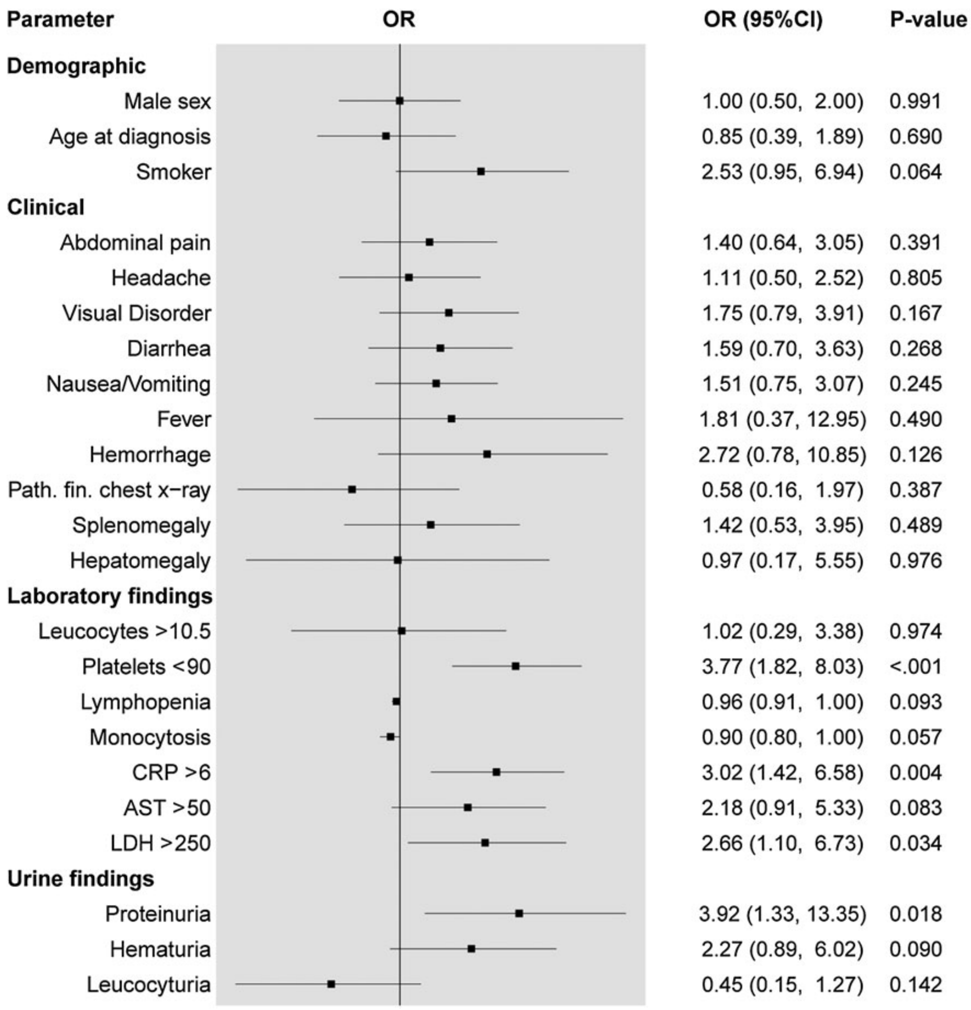

FIGURE 1: Univariate logistic-regression analysis for severe AKI (AKI I and AKI F) in study subjects with NE; OR for developing severe AKI compared with mild/moderate AKI. Severe AKI was defined as RIFLE I and RIFLE F compared with baseline values. The parameter age at time of diagnosis was defined as age >39 years. 95% CI for the OR. Normal ranges: leucocytes (3.5–10.5) × 10^9/L. AST, aspartate aminotransferase (<50) U/L; LDH, lactate dehydrogenase (<250) U/L; CrP, C-reactive protein (0.1–0.4) mg/dL; thrombocytes (>150) × 10^9/L.

the emergency department or in the outpatient clinic. In patients with no risk factors or only one point using the risk score, the probability of severe AKI was 0.18 (SEM ± 0.06) and 0.28 (SEM ± 0.07), respectively (see Table 2). Our model showed satisfactory discrimination with an area under the curve (AUC) of 0.71 (see Figure 2 and Supplementary data, Appendix). Internal 5-fold cross-validation of our model revealed an AUC of 0.67 (Figure 3 and Supplementary data, Appendix). The sensitivity and specificity of the calculated probability using the risk score for severe AKI could be deduced from this figure by taking into account the calculated probability for severe AKI, which is mapped on the right *Y*-axis in Figure 2. This allows the physician to determine the sensitivity and specificity individually after calculating the risk score (Figure 2).

Table 2. The risk score allows a calculation of the risk for severe AKI individually depending on the absence or presence of the described parameters for each patient at time of diagnosis

Risk factor	Points
Thrombocytopenia	2
Elevated CrP levels (12-fold increase)	1
Proteinuria	1
Risk score	Probability of severe AKI (%)
0 Point	18
1 Point	28
2 Points	38
3 Points	50
4 Points	64

Total points were calculated by adding together the points for each parameter and the predicted probability for severe AKI could be determined by using the pocket scoring system. Normal ranges: CrP (0.1–0.4) mg/dL, thrombocytes (>150) × 10^9/L.

DISCUSSION

This study comprises, to the best of our knowledge, the largest cohort of patients with NE reported to date and provides, for the first time, a prediction score to define patients at low risk to develop severe kidney disease.

The clinical prediction rule developed in this cross-sectional study provides a very specific test that identifies patients with NE at hospital admission with low risk of development of

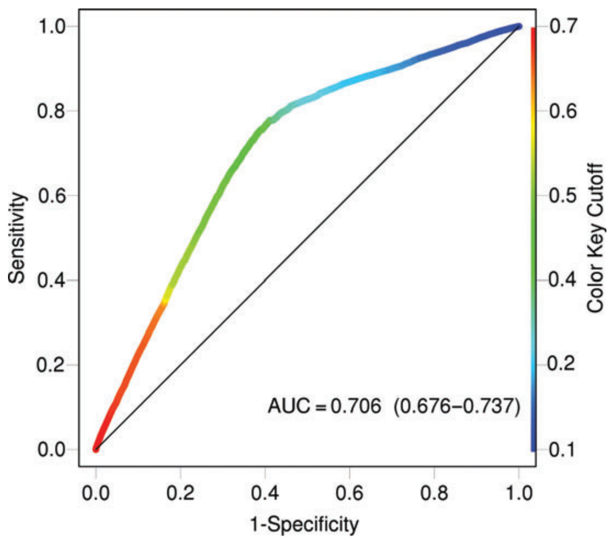

FIGURE 2: Receiver-operating-characteristic (ROC) curve for the prediction model for severe AKI with AUC of 0.71. The sensitivity and specificity of the calculated probability using the risk score for severe AKI (Table 2) could be deduced from this figure by taking into account the calculated probability for severe AKI, which is mapped on the right *Y*-axis. This allows the physician to determine the sensitivity and specificity individually after calculating the risk score.

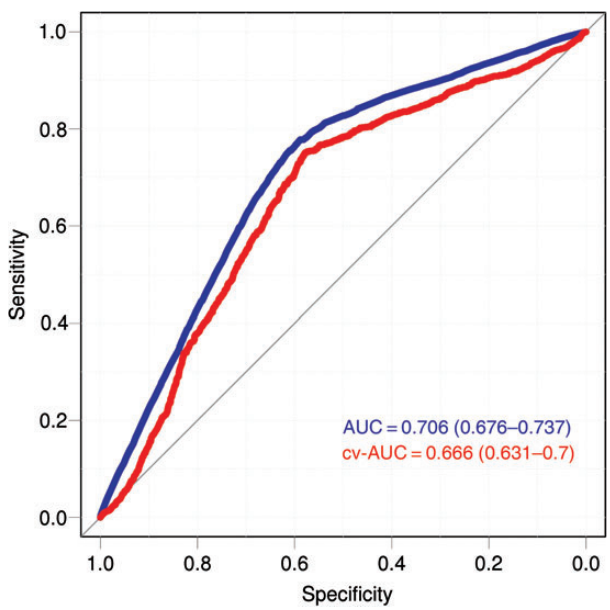

FIGURE 3: ROC curve for the prediction model for severe AKI with AUC of 0.71 (black line). Internal 5-fold cross-validation of the model revealed an AUC of 0.67 (red line).

severe kidney injury. Three variables were identified, for which data are easily (even in the outpatient setting) obtained during assessment of patients, and weighted scores were applied: the variables were thrombocytopenia, elevated CrP levels and proteinuria. When a value of one is used for the point score, the risk to develop severe AKI is 1.8. Given an OR of this magnitude, physicians have to decide whether the patient should be admitted to hospital or discharged at home with a low risk for severe AKI. These results suggest that the use of this prediction rule for patients would correctly identify a large proportion of patients with low risk of severe kidney injury. In clinical practice, stated OR in terms of an event (severe AKI in our study) are difficult to calculate for physicians and act upon on a patient-by-patient basis. It is easier to use declared probabilities for the occurrence of a given event. Our model is coupled with a pocket scoring system, which enables an easy and rapid calculation of risk for severe AKI at time of diagnosis in each patient using only three clinical variables. Due to the increasing number of patients treated in an ambulatory setting, we focused on medical history and routine laboratory workup to develop a simple risk score for the prediction of a severe AKI in patients with normal kidney function at time of diagnosis.

In contrast to smaller previously published studies, no single clinical symptom or sign predicted risk for severe AKI in our large study population [10, 11, 30–35]. The association of thrombocytopenia and severe AKI in patients with NE is widely discussed. Previous smaller studies suggested that severe thrombocytopenia during acute hantavirus infection is associated with severity of AKI [9, 20], whereas other studies found no association. Many of these studies raise methodological concern regarding grouping of more severe or less severe AKI, because the maximum measured creatinine value, not the peak creatinine value, was taken for further calculation [14, 36, 37].

Regarding laboratory findings at time of diagnosis, the elevated CrP level (12-fold increase) at time of diagnosis, but not leucocytosis, was a predictor for severe AKI in our study population. Previously, Libraty *et al.* showed in 36 patients with NE that leucocyte counts, but not CrP levels during acute NE, were associated with severe course of the disease [17]. Furthermore, Outinen *et al.* reported that high plasma IL-6 levels were associated with severe course of NE and could be used as a marker of the severity of the disease, whereas high CrP levels did not indicate severe acute NE [19].

Proteinuria at time of diagnosis was a significant predictor for developing severe AKI. Krautkramer *et al.* [38] showed that both tubular and glomerular cells were affected during hantavirus infection. It is known, too, that damage to tubular, interstitial and glomerular cells, detected by histologic changes, is associated with the clinical severity of renal failure in hantavirus infection [39].

It is noteworthy that this is the first study, that has identified risk factors for development of severe AKI in a large cohort of patients with no impairment of kidney function at time of diagnosis. Additionally, severity of AKI was classified using creatinine peak levels instead of maximum levels, which ensures correct graduation of the study population regarding severity of AKI. Thrombocytes were lower and time of onset of symptoms to diagnosis was shorter in the normal kidney function compared with the impaired kidney function group, which reflects earlier stages of the disease. Due to the highly dynamic process of NE [20], transient thrombocytopenia is present within the first days after the start of symptoms [9].

Within the population of hantavirus patients, age and comorbidities of the patients differ widely. In younger patients without significant comorbidities, the treating physician might accept a lower sensitivity of the predicted probability of AKI whereas in older patients higher sensitivity of the predicted probability is mandatory. Therefore, we did not define a cutoff value. From a statistical point of view, it would have been possible to further increase sensitivity and specificity including more variables and/or more not everyday practice parameters. We decided to keep the model simple and feasible to treat patients in an outpatient setting and accepted the loss in sensitivity and specificity. The final statistical model showed satisfactory discrimination with an AUC of 0.71. To ensure that the model assessment was not conducted on precisely the same data as used for model development, the AUC of the final model was also examined using predicted probabilities from 5-fold cross-validation. The AUC values of the proposed classifier and of one which was based on a 5-fold cross-validation procedure differed only slightly (0.70 and 0.67).

Our study design has several limitations that have to be addressed. First, regarding acute course of the disease, we did a retrospective study of medical case reports, associated with all known limitations (e.g. time of onset of symptoms associated with NE to presentation at hospital or to the ambulatory care physician). Due to a high proportion of patients already presenting with AKI at the time of diagnosis, the number of patients that could be included in the risk score calculation was about one-third of the studied patients. Furthermore, more females were included in the calculation of the risk score compared with the overall study population, but there were no gender-related differences regarding severity of AKI in our study population and in the study from Krautkramer *et al.* [21]. The risk score could not be used to predict severe AKI (probability of AKI was 0.64 in patients with four points), but is a useful tool in everyday clinical practice to minimize the number of patients who have to be admitted to hospital. Furthermore, our risk score could not be used to predict the diagnosis of acute hantavirus infection in, for example, patients with AKI, abdominal pain and thrombocytopenia. First, differential diagnosis, e.g. leptospirosis, sepsis, autoimmune disease or thrombotic microangiopathy, must be excluded and laboratory diagnosis of acute hantavirus infection must be confirmed in all patients by detection of circulating anti-hantavirus IgG- and IgM-antibodies. Sera from PUUV-infected patients cross-react strongly with Sin Nombre virus and weakly with Hantaan virus, Seoul virus and Dobrava virus [40, 41]. Although Dobrava-Belgrade virus and Tula virus circulate in rodent hosts in Germany and might cause an infection in humans [42–44], almost all hantavirus infections (especially southern Germany) are caused by PUUV [1, 45]. Recently, intestinal biopsies from 13 patients out of this study population were investigated using immunohistochemistry (IHC). IHC revealed PUUV nucleocapsid antigen in 11 biopsies from eight patients during the acute phase of NE [46]. These findings minimize the risk of misdiagnosed HFRS caused by other hantavirus than PUUV in our study.

In summary, NE is responsible for severe AKI in a high proportion of patients. Thrombocytopenia, elevated CrP levels and proteinuria at disease onset are likely to be associated with severe AKI during the acute course of the disease. The clinical prediction rule developed in this large cross-sectional study provides a novel and diagnostically accurate strategy for the potential prevention and improved management of kidney complications in patients with NE and, ultimately, to prevent unnecessary hospitalization in a high number of patients.

SUPPLEMENTARY DATA

Supplementary data are available online at http://ndt.oxfordjournals.org.

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CONFLICT OF INTERESTS STATEMENT

None declared.

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