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Cystatin C, a novel urinary biomarker for sensitive detection of acute kidney injury during haemorrhagic fever with renal syndrome

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Abstract

To explore the value of cystatin C for evaluating acute kidney injury (AKI) in haemorrhagic fever with renal syndrome (HFRS), the concentrations of cystatin C in serum and urine samples from HFRS patients were determined. The serum and urinary cystatin C concentrations significantly increased in HFRS patients compared with normal controls ($p < 0.001$). In the acute phase of HFRS, urinary cystatin C increased to higher levels than serum creatinine, especially in severe or critical cases in the oliguric stage. Furthermore, higher levels of urinary cystatin C in the acute phase positively correlated with increased severity of the subsequent kidney injury. In conclusion, urinary cystatin C is a more sensitive clinical marker for AKI in HFRS, which may enable us to initiate treatment measures as early as possible.

Keywords: *Cystatin C; haemorrhagic fever with renal syndrome; acute kidney injury; enzyme-linked immunosorbent assay*

Introduction

Haemorrhagic fever with renal syndrome (HFRS) is caused by Hantaan virus (HTNV) infection. More than 100 000 cases of HFRS are reported annually, with a mortality rate of 2–10% (Meyer & Schmaljohn 2000). The high mortality of HFRS is mainly caused by acute kidney injury (AKI), which is the predominant organ-specific manifestation and occasionally necessitates haemodialysis treatment (Wang et al. 2009). The typical clinical course of HFRS can be divided into five sequential phases, namely febrile, hypotensive, oliguric, diuretic and convalescent stages. A migration stage usually exists between the oliguric and diuretic phases (Muranyi et al. 2005). Our previous studies have demonstrated that the pathogenesis of HFRS is closely related to both cellular immunity and immune injury (Huang et al. 1994, Wang et al. 2009). Acute tubule necrosis (ATN) and vascular injury are the major pathological features of the kidney in HFRS. Both the renal tubules and glomeruli exhibit pathological damage, such as denaturation, necrosis, cell detachment and thrombosis (Mustonen et al. 1994). To date, serum creatinine (SCr) has been the primary laboratory indicator in clinical practice for routine estimation of the glomerular filtration rate (GFR) and determination of whether haemodialysis is required. However, the detection of the GFR by the SCr level is not very sensitive, as the SCr level does not increase until the GFR has decreased by more than 50% (Huber & Risch 2005).

Cystatin C (CysC) is considered to have many ideal features for use as a marker to estimate the GFR. This 13-kDa endogenous cysteine proteinase inhibitor, which is constantly produced by almost all nucleated cells, can be freely filtered by the glomeruli and then resorbed and fully catabolized but not secreted by the proximal renal tubules. As the kidney is the principal organ for CysC

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metabolism, after filtration from the glomeruli, the concentration of CysC in serum is dependent on the GFR, with little or no detectable CysC in urine in normal condition. Therefore, a reduction in the GFR is correlated with an elevation in the serum CysC level (Abrahamson et al. 1990, Tenstad et al. 1996, Uchida & Gotoh 2002). Recent studies of CysC have applied immunoturbidimetry or immunonephelometry assays with commercially available kits and showed remarkable elevation of the serum CysC levels in many types of kidney injury as well as a close correlation with the GFR (Finney et al. 1997, Mussap & Plebani 2004, Madero & Sarnak 2009). However, to the best of our knowledge, no previous studies have evaluated the renal function in HFRS based on changes in the CysC concentrations in serum or urine.

The aims of the present study were to measure the serum and urinary CysC levels in HFRS patients and to evaluate the renal glomerular and tubular functions based on the establishment of a highly sensitive sandwich enzyme-linked immunosorbent assay (ELISA) to quantify the concentration of CysC.

Materials and methods

Patients and samples

As this is a retrospective study working on the samples that were available from previously confirmed patients, the samples collected from each patient were not taken at the same time. Serum and urine samples were collected from 32 HFRS patients (30 male and two female subjects; age range 12–64 years) at different phases of the disease who were hospitalized in the Department of Infectious Diseases, Tangdu Hospital, the Fourth Military Medical University, Xi'an, China, from October 2008 to January 2009. All the samples were frozen at –70°C until use. The clinical diagnosis was confirmed serologically according to the presence of HTNV-specific IgM antibodies (Abs), and the clinical records were collected retrospectively. In addition, 20 normal serum samples and 16 normal urine samples were collected from healthy volunteers. Informed consent was obtained from each patient or their parents and the research protocol was approved by the Institutional Review Board of our university.

According to the diagnostic criteria for HFRS in China combined with the clinical symptoms and laboratory parameters, the degree of disease severity can be classified as mild, moderate, severe and critical.

Production of monoclonal antibodies against human cystatin C

The most commonly used method to detect the level of CysC in humans, designated the N Latex Cystatin C assay, can only detect CysC in serum or plasma. So we established a more sensitive ELISA method to detect the levels of CysC in both serum and urine. Human urinary CysC (Calbiochem, La Jolla, CA, USA) was used as an immunogen to generate murine monoclonal Abs (mAbs) against human CysC according to a conventional protocol in our laboratory (Ouyang et al. 2004). Briefly, female BALB/c mice (8 weeks of age) were immunized with 20 μg of CysC in Freund's complete adjuvant (Sigma-Aldrich, St Louis, MO, USA) by subcutaneous injection. Two subsequent immunizations were carried out with 20 μg of CysC in Freund's incomplete adjuvant (Sigma-Aldrich) by subcutaneous injection and 20 μg of CysC without adjuvant by intraperitoneal injection at 3-week intervals. Mice were bled from the caudal vein at 10 days after the third immunization, and the antiserum titres were determined by indirect ELISA. The immunized mice were then boosted with 20 μg of CysC by intraperitoneal injection. After 3 days, splenocytes isolated from the immunized mice and Sp2/0 murine myeloma cells cultured in RPMI 1640 (Hyclone, Logan, UT, USA) containing 20% fetal calf serum (GIBCO, Carlsbad, CA, USA) were fused in the presence of polyethylene glycol (MW 4000; Merck, Darmstadt, Germany). The positive hybrids were screened by indirect ELISA and subcloned four times by the limiting dilution technique. mAbs were produced from hybridoma culture supernatants or ascites isolated from BALB/c mice injected intraperitoneally with the hybridomas. The immunoglobulin isotypes were identified using an Isotype Kit (Sigma-Aldrich). The titres of the ascites were determined by indirect ELISA.

Establishment of a sandwich ELISA and detection of HFRS samples

To establish a sandwich ELISA for detecting the concentration of CysC, all the mAbs were purified from mouse ascites fluid samples using a Q Sepharose Fast Flow Anion Exchange Column (Amersham, San Francisco, CA, USA) and conjugated with horseradish peroxidase (HRP) (Sigma-Aldrich) by routine methods (Xu et al. 2008). Subsequently, a matched pair of mAbs was obtained. Briefly, anti-CysC mAbs (10 μg ml^{-1}) in coating buffer (0.05 M carbonate/bicarbonate buffer, pH 9.5) were coated at 100 μl/well on 96-well ELISA plates (Nunc, Roskilde, Denmark) and incubated overnight at 4°C. After three washes with washing buffer (0.15 M phosphate-buffered saline (PBS) containing 0.1% (v/v) Tween 20), the wells were blocked with 0.1% bovine serum albumin in PBS at room temperature for 1 h. After three further washes, a CysC standard was serially diluted in dilution buffer (0.15 M PBS containing 10% fetal calf serum and 0.05% Tween 20), added to the wells at 100 μl/well and incubated at 37°C for 2 h. After extensive washing, the wells were further incubated with 100 μl/well of HRP-conjugated anti-CysC mAb in dilution buffer at 37°C for

1 h. After washing, 100 µl of substrate buffer (5 mg of ABTS (Sigma-Aldrich) and 20 µl of 3% H_2O_2 dissolved in 10 ml of 0.1 M citrate-phosphate buffer, pH 5.0) was added to each well and incubated at room temperature for 5–10 min. The absorbances of the wells at 405 nm (OD_{405}) were determined using a microplate reader (Bio-Rad, Hercules, CA, USA). The serum and urine samples from the HFRS patients and normal controls were serially diluted and subjected to detection using this sandwich ELISA.

Statistical analysis

Statistical analyses were performed using SPSS software, version 11.5 (SPSS Inc., Chicago, IL, USA). The values are presented as the mean and 95% confidence interval (CI) or median and interquartile range (IQR) according to the results of the Kolmogorov–Smirnov normal distribution test. The Mann–Whitney rank-sum *U*-test was used to compare the differences between groups or the increasing degrees of different variables. Values of $p < 0.05$ were considered to indicate statistical significance.

Results

Characteristics of the HFRS patients

Overall, 91 serum samples and 83 urine samples were collected from the 32 HFRS patients at different phases of the disease. According to the clinical records and diagnostic criteria, two, five, 12 and 13 patients were diagnosed as mild, moderate, severe and critical HFRS, respectively. It is noteworthy that the SCr concentrations were elevated in all patients except one, and that 20 patients exhibited peak SCr levels of >707 µmol l^{-1}. Twenty-four patients underwent haemodialysis treatments, and eight of these patients accepted continuous renal replacement therapy (CRRT) (Table 1).

Properties of the sandwich ELISA

Seven positive hybridoma clones secreting mAbs against CysC were obtained and designated FMU-CysC 1, 2, 3, 4, 5, 6 and 7. To establish the CysC sandwich ELISA, FMU-CysC 4 was used as the coating Ab and HRP-conjugated FMU-CysC 6 was used as the detecting Ab. The minimum detectable concentration of CysC using this ELISA was 0.098 µg l^{-1}, and the linear dynamic range was 0.1–25 µg l^{-1} (Figure 1).

Dramatic elevations of the serum and urinary CysC levels in HFRS patients

The mean (95% CI) (Kolmogorov–Smirnov test; $p = 0.200$ in serum and $p = 0.136$ in urine) concentrations of CysC in the serum and urine samples from the normal controls were 1.918 (1.780–2.019) mg l^{-1} and 0.298 (0.250–0.317) mg l^{-1}, respectively, and consistent with previous reports (Herget-Rosenthal et al. 2004, Chew et al. 2008). Compared with the normal controls, the median (IQR) (Kolmogorov–Smirnov test; $p = 0.006$ in serum and $p < 0.001$ in urine) levels of the CysC in the samples from the HFRS patients were apparently elevated to 4.664 (3.050) mg l^{-1} in serum and 4.350 (6.637) mg l^{-1} in urine ($p < 0.001$ for both serum and urine) (Figure 2A). Regarding the different stages of HFRS, the serum CysC levels in the febrile/hypotensive phases were elevated compared with those in the normal controls ($p < 0.01$), while the urinary CysC did not increase until the oliguric stage ($p > 0.05$). The mean (95% CI) (Kolmogorov–Smirnov test; $p = 0.167$ in serum and $p = 0.200$ in urine) levels of CysC in the oliguric stage reached their peak levels of 7.376 (5.563–9.190) mg l^{-1} L in serum and 7.869 (4.240–11.499) mg l^{-1} in urine, and then gradually decreased from the diuretic phase to the convalescent phase. The concentrations of CysC in serum and urine of each stage were shown in Table 2. The elevations in the oliguric stage were significantly higher than those in the other stages ($p < 0.01$ for both serum and urine) (Figure 2B).

CysC is more sensitive than SCr in the acute-stage evaluation of renal function

We compared the levels of serum CysC, urinary CysC and SCr in each stage of HFRS with the level of normal controls, respectively. The results showed that in the febrile/hypotensive stages, the level of serum CysC elevated significantly ($p < 0.01$), while this was not the same for SCr ($p > 0.05$) (Table 2). In addition, based on the collected samples and the clinical records, 15 of the enrolled patients had acute-phase samples within about 10 days after fever onset (from the febrile stage to the early oliguric stage), among whom four patients belonged to the mild and moderate groups and 11 patients belonged to the severe and critical groups. Both the CysC and SCr levels increased to different degrees in all patients during the acute stage. However, the elevation of urinary CysC was particularly remarkable (Table 3) for these 15 patients. For the severe to critical patients, the increased ratios of the urinary CysC were significantly higher than those of SCr compared by the Mann–Whitney *U* test ($p = 0.008$). In addition, the increased ratio of urinary CysC was less than threefold in the acute stage of mild and moderate cases, but 70.5-fold for the severe and critical cases. Owning to this positive correlation, urinary CysC can be used as an additional index to grade the different severities of HFRS at the acute stage.

The increased level of urinary CysC at acute stage is a prognostic factor for kidney injury in HFRS

The 15 patients described above in Table 3 were subsequently divided into three groups according to their

Table 1. Characteristics of the patients with haemorrhagic fever with renal syndrome (HFRS).

HFRS severity, patient	Sex	Age (years)	Maximum blood urea level (mmol l^{-1})	Maximum creatinine level (μmol l^{-1})	Minimum platelet level (*1000 platelets μl^{-1})	Maximum leukocyte level (*1000 cells μl^{-1})	Haemodialysis	Urine protein
Mild								
No. 21	M	48	16.93	246.7	73	25.84	No	+++
No. 30	F	12	6.4	97.8	38	39.91	No	++
Moderate								
No. 9	M	36	9.6	192.4	36	24.53	No	+++
No. 17	M	43	35.04	941.5	53	12.31	Yes	++
No. 23	M	17	10.7	213.4	25	24.15	No	++
No. 26	M	49	11.39	148.4	35	16.62	No	+++
No. 32	M	54	7.7	137.1	136	7.86	No	+
Severe								
No. 1	M	54	30.51	817	4	37.16	Yes	+++
No. 5	M	35	23.78	1033.7	23	52.73	Yes	+++
No. 8	M	38	34.8	1139.6	25	28.94	Yes	+++
No. 11	M	60	30.15	750.5	28	18.8	Yes	+++
No. 13	M	40	22.61	914.4	10	14.06	Yes	+++
No. 14	F	57	25.04	782.3	32	13.86	Yes	+++
No. 16	M	61	34.88	943.5	20	24.17	Yes	+
No. 20	M	46	24.96	663.6	49	12.88	Yes	+++
No. 25	M	35	25.62	702.2	29	10.39	Yes	+++
No. 27	M	49	11.64	158.8	43	21.28	No	++
No. 29	M	44	11.08	145.3	48	12.24	No	+
No. 35	M	51	34.24	612.3	17	18.2	Yes	+++
Critical								
No. 2	M	31	28.59	951.2	16	31.88	Yes, crrt	+++
No. 3	M	22	24.59	1078.5	6	34.48	Yes, crrt	+++
No. 4	M	41	28.71	1098.4	7	40.66	Yes, crrt	+++
No. 6	M	60	14.92	605	173	19.72	Yes	+
No. 7	M	41	31.37	1125.7	5	31.23	Yes, crrt	+++
No. 10	M	55	31.27	960.3	6	15.62	Yes, crrt	+++
No. 12	M	62	24.34	755.7	6	43.09	Yes, crrt	+++
No. 15	M	28	32.34	1284	18	32.23	Yes	+++
No. 18	M	39	21.86	721.4	2	24.35	Yes, crrt	+++
No. 22	M	50	22.22	1007.8	32	16.84	Yes	++
No. 24	M	59	23.08	872.6	79	12.29	Yes, crrt	+++
No. 33	M	29	28.57	1070	6	23.33	Yes	+++
No. 34	M	64	43.87	1018.8	140	9.55	Yes	++

M, male; F, female; crrt, continuous renal replacement therapy.

increasing ratio of urinary CysC in the acute stage compared with that of normal controls. The time points and the stages of each sample we chose were the same as in columns 2 and 3 in Table 3, respectively. In group 1, urinary CysC increased from the normal level to 3.22-fold, the maximum SCr increases during the whole disease course were 1.03-fold to 1.85-fold, the duration of the oliguric stage was less than 1 day, and no haemodialysis was needed. In group 2, urinary CysC increased from 5.15-fold to 21.19-fold, the maximum SCr increases were 4.99-fold to 7.58-fold, the duration of the oliguric stage was 6–12 days, and haemodialysis was performed 1–4 times during the whole disease course. In group 3, urinary CysC increased from 38.78-fold to 70.5-fold, the maximum SCr increases were 5.42-fold to 8.46-fold, the duration of the oliguric stage was 7–28 days, and haemodialysis was performed more than 10 times or CRRT was required (Figure 2C). In other words, the level of the increase in urinary CysC in the acute stage reflects the severity of the subsequent renal injury. Taking patient 7 as a typical example, on the 10th day after the fever onset in the oliguric stage, the 70.5-fold increase in urinary CysC was accompanied by an 8.46-fold increase in the maximum SCr level during the whole disease course, 28 days for the duration of the oliguric stage, and 13 haemodialysis treatments as well as CRRT (Table 3). Furthermore, significant differences existed among the three groups for all the parameters ($p < 0.01$).

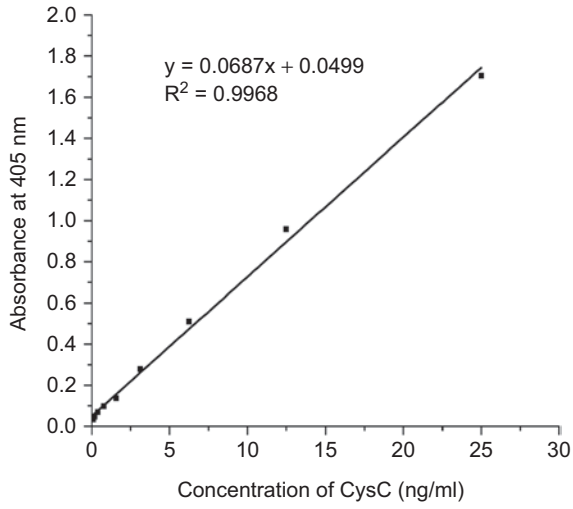

Figure 1. Standard curve for cystatin C (CysC). Human urinary CysC is used as the standard, and the linear dynamic range is between 0.1 and 25 μg l^{-1} CysC.

Discussion

Although the kidney function of HFRS patients decreases transiently, it can lead to oliguria or even anuria as well as proteinuria, and patients with severe symptoms have to be treated by haemodialysis (Schonrich et al. 2008). As the high mortality of HFRS is mainly caused by AKI, it is important to find a better marker to assess the kidney injury in HFRS patients. As a conventional biochemical indicator, SCr is widely accepted in clinical studies for evaluating the renal function, although several extrarenal factors limit its accuracy (Dharnidharka et al. 2002). The inulin and ^{51}Cr-EDTA clearances are considered to comprise the gold standard for estimating the GFR, but the methods are impractical in some conditions. Recently, CysC has generally been considered to represent an important indicator for renal function impairment. Serum CysC was first reported for its potential use as a marker for the GFR in 1985 (Simonsen et al. 1985). Furthermore, considerable evidence has proven the superiority of serum CysC over SCr or creatinine clearance as an indicator of the GFR (Herget-Rosenthal et al. 2004, Villa et al. 2005), particularly because of its better discriminatory capabilities and increased sensitivities for detecting mild kidney diseases with increased risks of mortality (Coll et al. 2000, Hoek et al. 2003, 2007, Lankisch et al. 2006, Zahran et al. 2007). Nevertheless, few reports have shown the importance of urinary CysC for evaluating kidney injury, and neither serum nor urinary CysC has previously been applied for HFRS.

In patients with AKI, urinary CysC may represent an additional tool for detecting acute injury and potentially quantifying the severity of the tubular injury (Herget-Rosenthal et al. 2004). However, CysC is not normally detected in urine. The specific CysC ELISA we established can be used to detect the CysC levels in both serum and urine and is 50-fold more sensitive than other ELISAs (Uchida & Gotoh 2002).

The most common histopathological lesions in kidney of HFRS patients were acute tubulointerstitial nephritis and interstitial oedema followed by tubular epithelial alterations, while the glomerular mesangial changes were relatively slight. Furthermore, the tubular, interstitial and glomerular histological damages are associated with the clinical severity of AKI (Muranyi et al. 2005, Kuchuloria et al. 2009). With our ELISA, we found that the serum and urinary CysC levels in the HFRS patients were increased by approximately 2.43-fold and 14.58-fold compared with normal controls, respectively, and that the peak CysC levels in the oliguric stage were 3.85-fold higher in serum and 26.37-fold higher in urine compared with normal controls. These findings indicate that CysC might be almost completely filtered in the renal glomeruli with mild injury but cannot be resorbed and catabolized in the damaged renal proximal tubules, suggesting that the renal tubules are probably the major lesion sites and more severely injured parts in the AKI. Therefore, the elevated CysC levels might help us to understand comprehensively the pathological renal injury of HFRS.

To the best of our knowledge, this study is the first to describe the role of the novel marker CysC in the prognosis of renal function in HFRS. The obvious positive relationships between the elevation of urinary CysC in the acute stage and other parameters, including the maximum SCr, duration of oliguric stage and number of haemodialysis treatments during the disease course, demonstrated that the level of the acute stage increase in urinary CysC can be attributed to the later kidney injury. As the treatment of HFRS patients is restricted to supportive procedures to control their symptoms, which can be life-threatening, evaluation of CysC may be helpful in guiding doctors to perform the correct treatment and clinical care of renal function to reduce the mortality induced by AKI.

In addition, we evaluated the renal function of renal transplantation patients with this ELISA method. Results also showed that the different elevation of CysC levels could be used to indicate the development of acute rejection or delayed graft function (data not shown).

In conclusion, we have evaluated the renal function in HFRS through changes in the CysC levels in serum and urine for the first time. The elevation of the urinary CysC level in the acute phase of HFRS was more sensitive than the SCr level for indicating the pathological injury to renal tubules, and could also be used as a prognostic factor for the degree of subsequent AKI such that treatment measures can be initiated as early as possible.

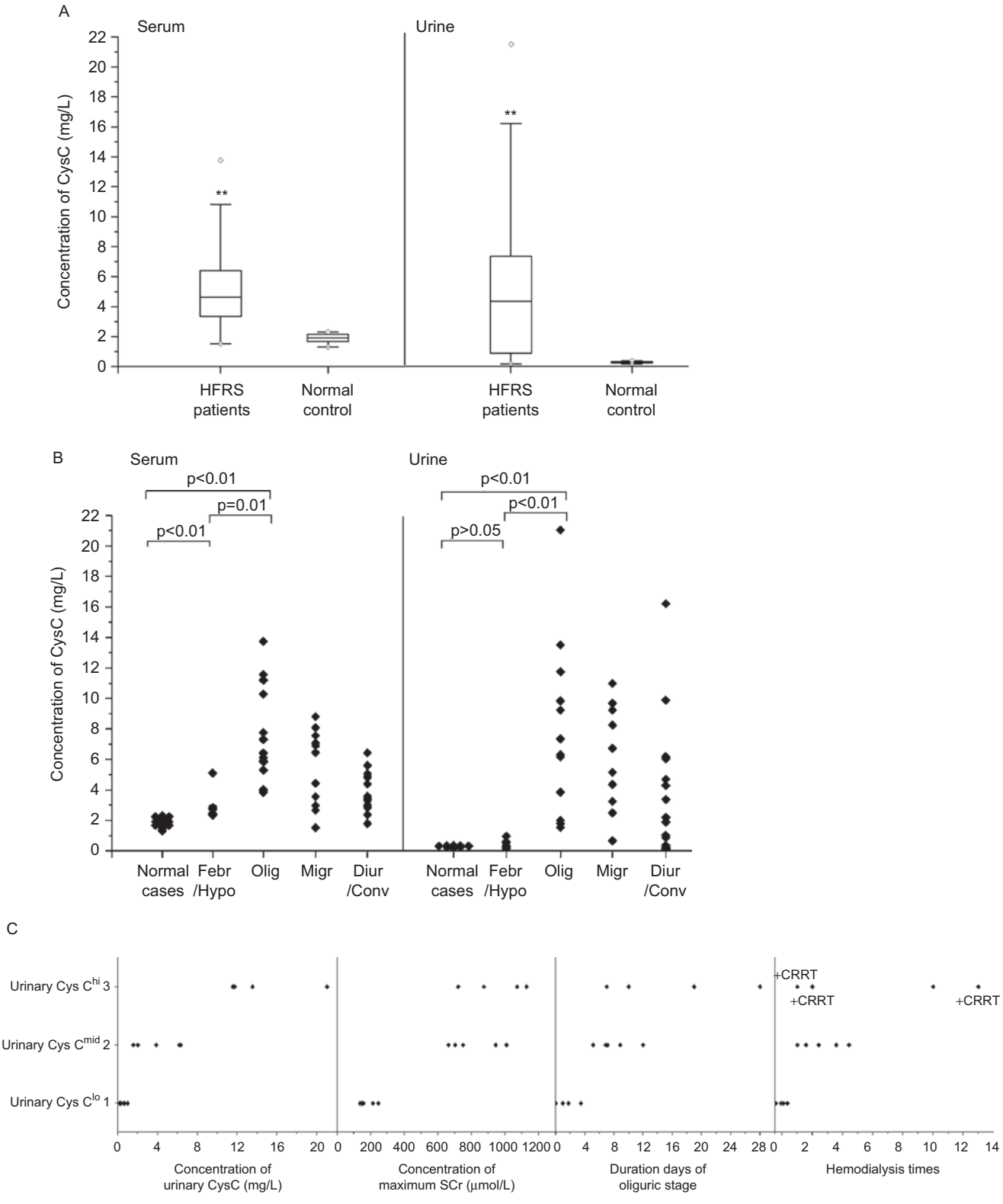

Figure 2. (A) The cystatin C (CysC) levels are obviously elevated in all samples from the haemorrhagic fever with renal syndrome (HFRS) patients, and show significant differences compared with the levels in the normal controls (**$p<0.001$). (B) The concentration of CysC increases in the febrile/hypotensive (Febr/Hypo) stages in serum ($p<0.001$) but remains almost normal in urine ($p>0.05$). Then it reaches peak levels in the oliguric (Olig) stage before gradually decreasing from the migration (Migr) phase to the diuretic/convalescent (Diur/Conv) phases. (C) From the viewpoint of the whole disease course, the maximum serum creatinine (SCr), duration (number of days) of the oliguric stage and number of haemodialysis treatments were positively correlated with the elevation ratio of urinary CysC in the acute phase. Urinary CysC^{lo}, group 1; urinary CysC^{mid}, group 2; urinary CysC^{hi}, group 3.

Table 2. The value of serum cystatin C (CysC), urinary CysC and serum creatinine (SCr) for normal controls and each phase of the disease.

	Serum CysC (mg l^{-1})	Urinary CysC (mg l^{-1})	SCr (μmol l^{-1})
Normal controls	1.918 (1.780–2.019)†	0.298 (0.250–0.317)†	53-133¶
HFRS - Febrile/hypotensive	2.602 (1.016)**,‡	0.456 (0.052– 0.860)†	118.550 (61.875)‡
HFRS – oliguric	7.376 (5.563– 9.190)**,†	7.869 (4.240– 11.499)**,†	675.300 (574.853– 775.747)**,†
HFRS – migration	5.458 (3.779–7.237)**,†	6.076 (3.617– 8.525)**,†	674.109 (523.939– 824.280)**,†
HFRS – diuretic/convalescent	3.434 (1.989)**,‡	1.454 (5.485)*,‡	425.281 (293.869–556.693)**,†

*The value was compared with their normal controls; $p<0.05$; **the value was compared with their normal controls; $p<0.01$; †according to the Kolmogorov-Smirnov normal distribution test, $p>0.05$. The values are presented as the mean (95% confidence interval); ‡according to the Kolmogorov–Smirnov normal distribution test, $p<0.05$. The values are presented as median (interquartile range); ¶the values are presented with typically accepted values. HFRS, haemorrhagic fever with renal syndrome.

Table 3. Comparisons of the increasing levels of cystatin C (CysC) in the early stage to the prognoses of the subsequent renal injury in patients with haemorrhagic fever with renal syndrome (HFRS).

HFRS severity, patient*	Days after fever onset	Phase of disease	Concentration of urinary CysC (mg l^{-1})/ increasing fold†,‡	Concentration of serum CysC (mg l^{-1})/ increasing fold†,‡	Concentration of SCr (μmol l^{-1})/ increasing fold†,‡	Concentration of maximum SCr (μmol l^{-1})/ increasing fold†,¶	Duration days of oliguric¶	Times of haemodialysis¶
Mild/moderate								
No. 21 (Mi.)	D6	Oliguric	0.66/2.20	3.55/1.85	246.70/1.85	246.10/1.85	1	No
No. 23 (Mo.)	D8	Febrile	0.15/0.52	2.84/1.48	108.30/0.81	212.80/1.60	0	No
No. 26 (Mo.)	D8	Febrile	0.59/1.99	2.34/1.22	120.80/0.91	148.90/1.12	0	No
No. 32 (Mo.)	D15	Febrile to hypotensive	0.17/0.57	3.30/1.72	126.00/0.95	137.00/1.03	0	No
Severe/critical								
No. 11 (Se.)	D7	Oliguric	3.86/12.94	10.28/5.36	492.70/3.70	750.10/5.64	8	2
No. 16 (Se.)	D6	Oliguric	6.16/20.66	4.04/2.10	775.90/5.83	943.00/7.09	12	4
No. 20 (Se.)	D8	Oliguric	2.02/6.75	6.11/3.19	575.40/4.33	663.70/4.99	8	2
No. 25 (Se.)	D10	Oliguric	1.54/5.15	7.32/3.81	670.50/5.04	702.20/5.28	6	1
No. 27 (Se.)	D5	Febrile	0.96/3.22	2.41/1.26	103.80/0.78	158.30/1.19	0	No
No. 29 (Se.)	D9	Febrile	0.30/1.01	2.47/1.29	116.30/0.87	145.00/1.09	0	No
No. 7 (Cr.)	D10	Oliguric	21.04/70.50	No sample	1018.00/7.65	1125.20/8.46	28	13+1 time CRRT
No. 18 (Cr.)	D8	Oliguric	13.52/45.31	5.31/2.77	627.00/4.71	720.90/5.42	10	1+3 times CRRT
No. 22 (Cr.)	D8	Oliguric	6.323/21.19	11.20/5.84	512.50/3.85	1008.10/7.58	6	4
No. 24 (Cr.)	D7	Oliguric	11.75/39.38	13.76/7.17	615.20/4.77	872.50/6.56	7	2+2 times CRRT
No. 33 (Cr.)	D7	Oliguric	11.57/38.78	No sample	445.90/3.68	1070.70/8.05	19	10

*These 15 subjects are cases with samples in acute phases. †The increasing fold was calculated using the reference ranges we detected or typically accepted values (serum CysC: 1.918 mg l^{-1}; urinary CysC: 0.298 mg l^{-1}; SCr: 133 μmol l^{-1}). ‡The data in these three columns refer to the values on the day mentioned in the second column. ¶The data in these three columns refer to the values during the course of disease, but not refer to the values on the day mentioned in the second column.
CRRT, continuous renal replacement therapy; SCr, serum creatinine.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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