Research Article

For reprint orders, please contact: reprints@futuremedicine.com

Biomarkers in Medicine

Inflammatory cytokines kinetics define the severity and phase of nephropathia epidemica

Aims: Nephropathia epidemica (NE) is a form of hemorrhagic fever with renal syndrome associated with the Puumala virus species of *Hantavirus*. The pathogenesis of NE is not well understood; therefore, investigating the inflammatory cytokine response to infection may provide useful knowledge in deciphering the pathophysiology of NE. **Materials & methods:** Using Luminex and ELISA, we analyzed the serum of 137 NE cases and 44 controls to investigate if serum cytokines associate with different clinical presentations. **Results:** Serum levels of TNF-α and IL-1β are associated with disease severity while upregulation of IL-6, CXCL10, CCL2 and CCL3 are associated with clinical presentation. **Conclusion:** Inflammatory cytokine kinetics associate with the severity and phase of NE. Our data support a role for inflammatory cytokines in the pathophysiology of NE.

Keywords: cytokine • *Hantavirus* • HRFS • nephropathia epidemica • Puumala virus

Asia A Baigildina[‡,1], Svetlana F Khaiboullina[‡,2,3], Ekaterina V Martynova[3,4], Vladimir A Anokhin[4], Vincent C Lombardi[‡,2,3] & Albert A Rizvanov[*,‡,3]

[1]Bashkir State Medical University, Ufa, Republic of Bashkortostan, Russian Federation

[2]Department of Biochemistry & Molecular Biology, University of Nevada, Reno, School of Medicine, Reno, NV, USA

[3]Institute of Fundamental Medicine & Biology, Kazan Federal University, Kazan, Republic of Tatarstan, Russian Federation

[4]Kazan State Medical University, Kazan, Republic of Tatarstan, Russian Federation

*Author for correspondence:
Tel.: +7 843 2337843
Fax: +7 843 2337814
albert.rizvanov@kpfu.ru

[‡]Authors contributed equally

Hemorrhagic fever with renal syndrome (HFRS) is a zoonotic disease associated with members of the *Hantavirus* genus, which includes Puumala virus [1]. Puumala virus-associated HFRS is often referred as nephropathia epidemica (NE), and is endemic in Bashkortostan, Ural Region of Russia, with 1000 cases diagnosed annually [1,2]. NE is characterized by acute flu-like onset with high fever, malaise and muscle pain [3]. Thrombocytopenia, lymphocytosis with left shift, proteinuria, increased serum levels of creatinine and urea are frequently observed in HFRS cases [3]. Clinically, HFRS manifests with bleeding disorders and kidney dysfunction [4,5]. Various degrees of vascular leakage are common and can manifest as petechiae, mucosal bleeding and, in severe cases, disseminated intravascular coagulation (DIC) [6]. Necrosis, ischemia and hemorrhages in the medulla of the kidney of HFRS cases are typically associated with inflammatory cell infiltration [7].

HFRS generally manifests as three main forms: mild, moderate and severe. Headache, nausea, vomiting and high fever, as well as prominent hemorrhagic symptoms including petechiae, nasal and internal bleeding typically characterize the severe form. Laboratory findings include elevated blood urea and creatinine levels and leukocytosis with left shift and severe thrompocytopenia [8–10]. The moderate form of the disease has similar but more subtle symptoms. Blood tests also reveal high BUN and creatinine levels and leukocytosis, as well as thrombocytopenia are commonly reported [8,11]. The mild form often goes undiagnosed due to the lesser symptoms, which often include slight headache and low-grade fever. The hemorrhagic symptoms are restricted to small petechiae on the mucosa and skin, while laboratory findings regarding blood-formed elements are typically mild and include thrombocytopenia and proteinuria [8,10].

Each form of HFRS may be characterized by four phases: febrile, oliguric, diuric and convalescent [10]. The febrile phase is distinguished by fine petechiae, flashing over the neck and head, headache, malaise and fever. As the disease advances, subjects enter the oliguric phase, developing oliguria and even anuria.

Future Medicine part of fsg

Severe nausea, vomiting and marked hemorrhages are also often observed. The oliguric phase is considered the most critical due to the high probability of developing life-threatening complications. Clinical recovery begins with the onset of diuresis [10]; however, during this stage, some complications may occur including disseminated encephalomyelitis and hypopituitarism.

Studies of tissue collected postmortem as well as *in vitro* experiments suggest that endothelial cells are the primary targets of hantavirus infection [12]; however, it has also been shown that infections are non-cytopathic [13,14]. Nevertheless, increased levels of serum proinflammatory cytokines have been observed in HFRS cases at the onset and remain elevated throughout the duration of the disease [15]. Additionally, increased numbers of TNF-α-producing cells have been observed in kidney biopsies of HFRS cases [16]. These data suggest that inflammatory cytokines may play a pivotal role in the pathogenesis of *Hantavirus* infection and support the 'cytokine storm' hypothesis [17]. Accordingly, *Hantavirus* infection putatively promotes the upregulation of a host of inflammatory cytokines and chemokines, which lead to tissue inflammation, vascular leakage and kidney injury. In further support of this hypothesis, it has been reported that serum sIL-2R and IL-6 inversely correlate with platelet count and arterial pressure [18]. Additionally, higher serum concentrations of TNF-α, IL-6, IFN-γ, CXCL8 (IL-8) and CCL5 (RANTES) have been observed in the most severe HFRS cases [15,19,20].

In this report, we present serum cytokine data for NE cases in relation to clinical presentation. These data suggest that inflammatory cytokines kinetics define the severity and phase of the disease.

Materials & methods

Study subjects

A total of 137 cases (114 male and 23 female) were admitted to the Infectious Disease Hospital No.2 in the city of Ufa and to the Hemodialysis Unit of the G.G. Kuvatov Republican Clinical Hospital, Republic of Bashkortostan, Russian Federation. A diagnosis of HFRS was established based upon clinical presentation and was serologically confirmed. Sera from 44 (37 male and seven female) healthy individuals were also collected to serve as controls. Informed consent was obtained from each subject according to the clinical and experimental research protocol, approved by the biomedicine ethic expert committee of Bashkir State Medical University (no. 186, 11.07.2006).

Serum

Sera were collected for each case and at each phase of the disease when possible. Detection of anti-*Hantavirus* antibodies was performed using the Dignosticum™ HFRS kit (Institute Poliomyelitis and Encephalitis, Russia), an indirect fluorescence assay that utilizes slides coated with Puumala virus-infected cells. This kit allows for the detection of all species of *Hantavirus* circulating in the Volga region of Russia. Subjects were considered positive when the antibody titers of their second serum sample were increased fourfold over their first.

Cytokine analysis

Serum IL-6, CXCL10, CCL2 and CCL3 were analyzed by Luminex (Luminex. Corp. TX, USA) using the respective SinglePlex cytokine kits (BioRad, CA, USA) according to the manufacturer's instructions. IL-1β, IL-2, IL-4 and TNF-α were evaluated by ELISA (Vector-BEST, Novosibirsk, Russia) according to the manufacturer's instructions.

Statistical analysis

Statistical analysis was conducted using Statistica and XLSTAT software (StatsSoft, Inc., OK, USA, and Addinsoft, Inc. NY, USA, respectively), and differences between the means of compared groups were performed using the Mann–Whitney test for nonparametric data and were considered significant at $p \leq 0.05$.

Results

Clinical presentations

The average hospitalization period was 15.6 ± 4.2 days and the average duration of febrile, oliguric and polyuric phases were 4.4 ± 1.4, 6.5 ± 2.5 and 4.7 ± 2.1 days, respectively. All cases fell into three major categories based on their clinical presentation: moderate, severe and severe with complications (severe w/c). Those with moderate HFRS had less pronounced intoxication syndrome. Laboratory findings included oliguria of 100–400 ml per day for a period of 2 days, mean serum urea levels of 13.4 (minimum–maximum [min–max]: 8.5–17.9) mM/l, mean serum creatinine levels of 172 (min–max: 122–236) μM/l and protein in urine up to 1.65 g/l. Severe cases were characterized by pronounced toxic syndrome with headache, abdominal pain, nausea, vomiting and hypotension, as well as oliguric syndrome with decreased urine output (less than 400 ml per day) for 3–4 days. Additionally, laboratory findings revealed an increased mean serum urea concentration of 26 (min–max: 20.0–31.9) mM/l and mean creatinine of 619.5 (min–max: 368.0–790.0) μM/l. In addition, high protein concentrations in the urine (up to 6.6 g/l) were detected. A separate group was designated for cases with severe w/c. Complications included stage I and II shock (20.4%); acute renal injury requiring hemo-

dialysis (16.8%); DIC (10.2%); acute respiratory distress syndrome (1.5%); erosive gastritis (1.5%); renal hematoma (0.7%); nasal and gastric bleeding (0.7%); and toxic encephalopathy (0.7%).

Serum cytokine/chemokine levels in NE cases

Serum cytokines for IL-1β, IL-2, IL-4, IL-6, TNF-α, CCL2, CCL3 and CXCL10 were measured for all study subjects. When compared with healthy controls, TNF-α levels were significantly elevated for all cases during all phases of the disease (Table 1) and progressively increased with severity (moderate < severe < severe w/c). Additionally, the kinetics of TNF-α expression typically increased from the febrile phase to a maximum value during the oliguric phase and progressively decreased from the polyuric to the convalescent phase, with the exception of those who presented as severe w/c, which peaked at the polyuric phase and remained elevated through the convalescent phase (Table 1). Similar to TNF-α, serum IL-1β levels were also elevated when compared with controls (Table 2). However, in contrast to TNF-α, levels of IL-1β were progressively increased from the febrile phase through the convalescent phase for all clinical presentations (febrile < oliguric < polyuric < convalescent).

We next evaluated each subject for the Th2 cytokine IL-4 with respect to phase and clinical presentation (Table 3). Average IL-4 values were increased during the oliguric and polyuric periods for subjects with the moderate form of the disease and for all phases in subjects with the severe form (Table 3). Interestingly, those who presented with severe w/c showed no increase in serum IL-4 when compared with controls.

Sufficient quantities of serum were not available to include all cases in the final analysis; therefore, we evaluated a subset of subjects for the inflammatory cytokines and chemokines IL-6, CXCL10, CCL2 and CCL3 with respect to each phase of the disease. Average IL-6 levels increased at the onset (febrile phase) but decreased to below control levels by the oliguric phase and remained decreased through the convalescent phase (Table 4). CXCL10 increased tenfold, on average, over the control value at the onset and remained elevated through the oliguric phase, progressively decreasing to a value approximately fivefold over controls by the convalescent phase (Table 4). Serum CCL2 kinetics largely paralleled that of CXCL10 (Table 4). Changes in the levels of CCL3 did not reach statistical significance during the febrile and oliguric phases of the disease; however, they decreased significantly below control levels during the polyuric and the convalescent phases (Table 4).

Discussion

In this report, we have presented the serum cytokine kinetics of subjects with NE. In order to understand the kinetics associated with inflammatory cytokine expression in NE, sera were collected during the four phase of the disease from subjects with three different

Table 1. TNF-α values for nephropathia epidemica subjects by form of disease (pg/ml).

Disease phase	Moderate			Severe			Severe with complications		
	Mean (minimum–maximum)	*n*	*p-value*	*Mean (minimum–maximum)*	*n*	*p-value*	*Mean (minimum–maximum)*	*n*	*p-value*
Febrile	35.5 (26.2–43.8)	11	p = 0.003	41.8 (35.5–59.6)	10	p = 0.004; p_2 = 0.14	58.1 (37.5–71.7)	9	p = 0.003; p_2 = 0.01; p_3 = 0.03
Oliguric	69.9 (61.8–82.8)	15	p = 0.001; p_1 = 0.003	85.8 (77.8–92.4)	16	p = 0.002; p_1 = 0.001; p_2 = 0.01	92.9 (74.4–128.5)	14	p = 0.001; p_1 = 0.009; p_2 = 0.001; p_3 = 0.06
Polyuric	41.4 (38.2–53.9)	16	p = 0.002; p_1 = 0.006	62.7 (54.8–99.9)	16	p = 0.002; p_1 = 0.03; p_2 = 0.001	35.3 (84.8–185.3)	15	p = 0.001; p_1 = 0.07; p_2 = 0.001; p_3 = 0.002
Convalescent	26.7 (19.4–32.2)	13	p = 0.002; p_1 = 0.01	36.2 (32.4–45.1)	14	p = 0.002; p_1 = 0.003; p_2 = 0.01	64.05 (43.4–88.1)	14	p = 0.002; p_1 = 0.01; p_2 = 0.03; p_3 = 0.02

p: Compared with control; p_1: Compared with previous period; p_2: Compared with moderate; p_3: Compared with severe.
Control value: mean: 5.1; minimum–maximum: 3.1–9.5; n = 20.

10.2217/BMM.14.88

clinical presentations. NE is a febrile disease; therefore, based upon their potential role in *Hantavirus* pathogenesis, we selected IL-1β, IL-6 and TNF-α, which are known to be major endogenous pyrogens. We additionally analyzed the minor endogenous pyrogen CCL3. Interstitial infiltration with lymphocytes, plasma cells, monocytes and macrophages is characteristically observed in kidney biopsies of HFRS cases [7]. The migration of mononuclear immune effector cells is governed by a specific set of chemokines including CXCL10 and CCL2 [21,22]; therefore, we also included these cytokines in our study.

Consistent with previous reports [23,24], we observed elevated levels of TNF-α and IL-1β in *Hantavirus*-infected subjects. TNF-α and IL-1β were significantly higher in the sera of subjects with the severe w/c form of NE compared with subjects with the severe and the moderate forms. A higher percentage of cases with acute renal injury, DIC syndrome and various degrees of bleeding characterized the severe w/c forms. Many cells, including lymphocytes, macrophages and endothelial cells, release TNF-α upon activation [25,26]. A local and systemic increase in TNF-α may contribute to the inflammation, shock, increased vascular leakage, DIC syndrome and renal injury associated with hantavirus infection.

Kidney pathology during HFRS is characterized as interstitial nephritis, which often develops as a consequence of renal tissue damage due to immune complexes, hypertension and metabolic disturbances [27]. Previous studies suggest that cytokines play a role in pathogenesis of kidney tissue injury. For example, it has been shown that systemic administration of TNF-α aggravates glomerular injury in a rat model of nephrotoxic nephritis [28,29]. Reduction of proteinuria and leukocyte infiltration, as well as decreased expression of adhesion molecules, has been demonstrated in TNF-α-deficient animals with experimental nephrotoxic nephritis [29,30]. Our results are also consistent with the premise that TNF-α plays a role in the pathogenesis of HFRS-associated kidney injury. We have shown that mean serum concentration of TNF-α was significantly elevated in subjects with the severe w/c form of NE compared with subjects with the moderate form. Additionally, serum TNF-α levels reached their peak in the oliguric phase of the disease, potentially subjecting kidney tissue to the damaging effects of this inflammatory cytokine. TNF-α has been shown to increase vascular permeability *in vivo* and *in vitro* [31]. It is also known to upregulate the adhesion molecules ICAM and VCAM, as well as β1 and β2 integrins on endothelial cells [32], consistent with the promotion of transendothelial migration of leukocytes reported with respect to HFRS [33]. Therefore, prolonged upregulation of serum TNF-α may compromise the integrity of the endothelial monolayer, facilitating leukocyte migration.

In contrast to TNF-α expression, which generally peaked during the oliguric phase and progressively decreased thereafter, IL-1β gradually increased from

Table 2. IL-1β values for nephropathia epidemica subjects by form of disease (pg/ml).

Disease phase	Moderate			Severe			Severe with complications		
	Mean (minimum–maximum)	*n*	*p-value*	*Mean (minimum–maximum)*	*n*	*p-value*	*Mean (minimum–maximum)*	*n*	*p-value*
Febrile	27.8 (19.1–47.3)	9	p = 0.002	42.1 (24.2–62.8)	10	p = 0.001; p_2 = 0.03	48.9 (34.8–67.2)	10	p = 0.001; p_2 = 0.02; p_3 =0.4
Oliguric	35.6 (19.4–52.1)	13	p = 0.001; p_1 = 0.8	77.1 (62.4–92.9)	14	p = 0.001; p_1 = 0.02; p_2 = 0.002	118.1 (84.8–180.3)	16	p = 0.001; p_1 = 0.02; p_2 =0.002; p_3=0.004
Polyuric	48.1 (33.8–62.8)	16	p = 0,001; p_1 = 0.7	88.5 (62.2–113.4)	15	p = 0.001; p_1 = 0.5; p_2 = 0.003	128.7 (84.8–142.8)	13	p = 0.0008; p_1 =0.7; p_2 = 0.001; p_3 = 0.01
Convalescent	59.6 (37.5–80.2)	15	p = 0.001; p_1 = 0.8	99.2 (76.7–120.8)	15	p = 0.001; p_1 = 0.7; p_2 = 0.003	137.05 (114.9–152.8)	14	p = 0.0008; p_1 = 0.8; p_2 =0.001; p_3 = 0.002

p: Compared with control; p_1: Compared with previous period; p_2: Compared with moderate; p_3: Compared with severe.
Control value: mean: 4.3; minimum–maximum: 0.8–6.9; n = 21.

Table 3. IL-4 values for nephropathia epidemica subjects by form of disease (pg/ml).

Disease phase	Moderate			Severe			Severe with complications		
	Mean (minimum–maximum)	*n*	*p-value*	*Mean (minimum–maximum)*	*n*	*p-value*	*Mean (minimum–maximum)*	*n*	*p-value*
Febrile	5.1 (4.1–8.1)	11	p = 0.9	6.2 (3.8–9.9)	10	p = 0.03; p_2 = 0.05	6.7 (3.1–9.2)	9	p = 0.3; p_2 = 0.5; p_3 = 0.4
Oliguric	7.6 (2.3–9.2)	14	p = 0.002; p_1 = 0.2	8.2 (1.4–10.9)	13	p = 0.001; p_1 = 0.7; p_2 = 0.3	4.7 (2.1–12.9)	12	p = 0.7; p_1 = 0.2; p_2= 0.5; p_3 = 0.1
Polyuric	7.9 (4.8–12.3)	13	p = 0.009; p_1 = 0.6	6.2 (4.8–8.5)	15	p = 0.001; p_1 = 0.7; p_2 = 0.7	5.9 (3.3–9.7)	14	p = 0.7; p_1 = 0.6; p_2 = 0.04; p_3 = 0.03
Convalescent	4.9 (2.2–13.6)	16	p = 0.4; p_1 = 0.5	6.5 (1.4–12.9)	16	p = 0.003; p_1 = 0.8; p_2 = 0.2	4.6 (2.3–6.3)	13	p = 0.8; p_1 = 0.5; p_2 = 0.3; p_3 =0.01

p: Compared with control; p_1: Compared with previous period; p_2: Compared with moderate; p_3: Compared with severe.
Control value: mean: 4.4; minimum–maximum: 1.2–5.9; n = 18.

the febrile phase through the convalescent phase and remained elevated for all clinical presentations. IL-1β is secreted by macrophages, monocytes and dendritic cells in a precursor form and must be cleaved by caspase-1 in order to bind to its receptor [34]. The observed kinetics of IL-1β in NE suggest a delayed pro-caspase-1 activation and, in turn, a delayed and protracted IL-1β response to infection. These observations are consistent with previous *in vitro* studies by Tan and Chu, who reported that dengue virus-infected monocytes upregulated functional caspase-1 mRNA and pro-caspase-1 activation as a late response to infection [35]. We also observed that the anti-inflammatory cytokine IL-4 showed no statistical difference between controls and cases who presented with the severe w/c form of NE. In contrast, subjects who presented with the moderate and severe form of the disease displayed a slight but significant upregulation of IL-4 at the oliguric phase, potentially providing a protective effect to the inflammatory consequences of infection.

The most significant change in cytokine expression was observed for the proinflammatory chemokine CXCL10. Mean levels of CXCL10 increased approximately tenfold over controls during the febrile phase, remained at this level during the oliguric phase and decreased to a level approximately fivefold greater than controls by the convalescent phase. We also observed a similar but less pronounced upregulation of the chemokine CCL2. CXCL10 and CCL2 are chemotactic factors produced by monocytes, macrophages, endothelial cells and fibroblasts in response to tissue injury and infection. Previous studies have reported a more pronounced upregulated CXCL10 by lung endothelial cells in response to the highly pathogenic influenza viruses H5N1 and H9N2 when compared with the less virulent H1N1 strain [36]. Notwithstanding, upregulation of CXCL10 and CCL2 in subjects with influenza A/H1N1 infection and subsequent acute respiratory distress syndrome is associated with kidney injury and a higher risk of death [37]. In contrast, Sundstrom *et al.* reported that hantavirus infection promotes the upregulation of CXCL10 without causing increased permeability in lung microvasculature [38].

We also observed a short but significant upregulation of the cytokine IL-6. Mean values of IL-6 during the febrile phase were almost fourfold higher than controls, but declined by the oliguric phase and remained low throughout the convalescent phase. IL-6 has both inflammatory and anti-inflammatory properties; however, the kinetics of IL-6 in NE are that of classical acute-phase responses, suggesting its production in NE is inflammatory in nature and likely the result of the engagement of pattern recognition receptors on infiltrating macrophages. Similar to the kinetics of IL-6, we observed a short, acute response in the upregulation of CCL3. Although slightly elevated, levels of CCL3 did not reach statistical significance during the febrile and oliguric phases of the disease; however, they decreased significantly below control levels during the polyuric and conva-

10.2217/BMM.14.88

Table 4. Cytokines values of the four phases of hemorrhagic fever with renal syndrome phases (ng/ml).

	Control		Febrile			Oliguric			Polyuric			Convalescent		
	Mean (minimum–maximum)	*SD*	*Mean (minimum–maximum)*	*SD*	*p-value*	*Mean (minimum–maximum)*	*SD*	*p-value*	*Mean (minimum–maximum)*	*SD*	*p-value*	*Mean (minimum–maximum)*	*SD*	*p-value*
IL-6	54.7 (21.5–143)	36.4	191.9 (30.5–521)	154.1	p = 0.013	41.8 (5.0–281.0)	61.1	p = 0.048	37.5 (6.0–147.0)	33.5	p = 0.126	41.8 (4.0–389.0)	61.1	p = 0.487
CXCL10	1013.2 (335.0–2407.0)	686.018	11378.2 (3116.5–21386.0)	5799.6	p < 0.0001	11836.6 (677.0–22416.0)	6287.9	p < 0.0001	6823.9 (12.0–15869.0)	4628.6	p = 0.002	5363.0 (5.0–15531.0)	3954.9	p = 0.002
CCL2	484.7 (144.0–1359.0)	358.7	2577.6 (170.5–7488.0)	2297.4	p = 0.013	2563.4 (9.5–17523.0)	4253.5	p = 0.013	1215.3 (11.0–6899.0)	1756.0	p = 0.788	800.6 (11.0–3694.0)	903.3	p = 0.788
CCL3	2150.9 (860.0–4461.0)	1167.7	4347.1 (916.0–10080.0)	3176.1	p = 0.095	1802.8 (27.0–5144.5)	1534.6	p = 0.341	859.4 (22.0–2862.0)	810.6	p = 0.004	627.4 (28.0–1657.0)	414.4	p < 0.0001

p-value of respective cases vs controls by Mann–Whitney test.

lescent phases. CCL3 is an inflammatory chemotactic factor also produced by infiltrating macrophages and plays many roles in orchestrating chronic and acute inflammation. Similar to CXCL10 and CCL2, CCL3 promotes transendothelial migration of monocytes, dendritic cells and natural killer (NK) cells through engagement of the CCR5 receptor [39,40]. Additionally, by activating the CCR5 receptor, CCL3 facilitates Th1 lymphocyte differentiation and recruitment to sites of infection [41]. Our observations support the supposition that their expression is likely the result of an inflammatory cascade initiated at the site of virus infection. Indeed, CXCL10 CCL2 and CCL3 are all known chemoattractants of monocytes, macrophages, T cells, NK cells and dendritic cells [21]. Depending on the host's immune status or genetic background, CXCL10 can, for instance, facilitate infection or prevent virus spread and replication [42,43]. Wang *et al.* reported that subjects with the AG genotype in DC-SIGN (CD209), a C-type lectin, had higher CXCL10 production and a lower rate of dengue virus replication. In another study, Mahanty *et al.* reported that fatal Lassa virus infection is associated with low serum levels of CXCL10 [44]. Upregulation of CXCL10 in Puumala virus-associated NE is consistent with observations of *Hantavirus*-infected individuals by Zhang *et al.* [45], thus suggesting that CXCL10 expression, as well as other inflammatory cytokines, is not unique to the Puumala virus species of *Hantavirus* but reflective of *Hantavirus* pathogenesis in general.

To the best of our knowledge, this study is the first to investigated serum cytokines at different stages of NE. Serum levels of TNF-α and IL-1β were observed to associate with disease severity, while upregulation of IL-6, CXCL10, CCL2 and CCL3 were associated with clinical presentation. These observations may provide clues to the pathogenesis of hantavirus infection and may also identify targets for therapeutic intervention.

Acknowledgements

The authors wish to thank all the subjects for their gracious participation in this study.

Financial & competing interests disclosure

This research was supported in part by the subsidy of the Russian Government to support the Program of Competitive Growth of Kazan Federal University and Russian Foundation for Basic Research grant 13-04-97099. Some of the experiments were conducted with support of Interdisciplinary Center of Collective Use and Pharmaceutical Research and Education Center, Kazan (Volga Region) Federal University, Kazan, Russia. The authors declare that there are no conflicts of interest regarding the publication of this paper. The authors have no other relevant affiliations or financial involvement with any

organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

Background

- Nephropathia epidemica (NE) is a form of hemorrhagic fever with renal syndrome (HFRS) caused by the Puumala virus strain of *Hantavirus*.
- The Republic of Bashkortostan (Russia) is endemic for NE, where more than 1000 cases are reported annually.
- Although the pathogenesis of NE is largely unknown, immune mechanisms are believed to play a major role.

Cytokines analysis

- Serum cytokines were analyzed in 137 HFRS cases that presented with the moderate, severe and severe with complications forms of the disease.
- Serum levels of TNF-α and IL-1β were significantly higher in HFRS cases when compared with healthy controls and their expression generally associated with disease severity.
- Upregulation of IL-6, CXCL10, and CCL2 was observed in NE cases, the kinetics of which associated with the four phases of the disease.

Conclusion & future perspective

- Our data support a role for inflammatory cytokines in the pathophysiology of HFRS and suggest that inflammatory cytokines associate with discrete stages and clinical presentations of the disease.
- *Hantavirus* infections produce no pathological symptoms in the native rodent reservoir; however, they often produce life-threatening pathology in humans.
- Vascular leakage is described for virtually all forms of *Hantavirus* infection, and while the mechanism remains unknown, it is believed to involve inflammatory immune responses.
- Presently, treatment options for NE as well as other *Hantavirus*-associated pathologies are limited [46,47]. For these reasons, future studies into the inflammatory mechanism of hantavirus infection will lead to greater understanding of disease pathogenesis and, in turn, may lead to improved treatment strategies and options for healthcare practitioners.

References

Papers of special note have been highlighted as:
• of interest; •• of considerable interest.

1 Mustonen J, Makela S, Outinen T *et al.* The pathogenesis of nephropathia epidemica: new knowledge and unanswered questions. *Antiviral Res.* 100(3), 589–604 (2013).

2 Korobov LI, Minin GD, Nurgaleeva RG, Stepanenko AG, Mustafin NM. [Morbidity and prophylaxis of hemorrhagic fever with renal syndrome in the Republic of Bashkortostan]. *Zh. Mikrobiol. Epidemiol. Immunobiol.* 4, 58–60 (2001).

3 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.* 11(6), 921–927 (1989).

4 Settergren B, Leschinskaya E, Zagidullin I, Fazlyeva R, Khunafina D, Niklasson B. Hemorrhagic fever with renal syndrome: comparison of clinical course in Sweden and in the Western Soviet Union. *Scand. J. Infect. Dis.* 23(5), 549–552 (1991).

5 Bren AF, Pavlovcic SK, Koselj M, Kovac J, Kandus A, Kveder R. Acute renal failure due to hemorrhagic fever with renal syndrome. *Ren. Fail.* 18(4), 635–638 (1996).

6 Sundberg E, Hultdin J, Nilsson S, Ahlm C. Evidence of disseminated intravascular coagulation in a hemorrhagic fever with renal syndrome-scoring models and severe illness. *PLoS ONE* 6(6), e21134 (2011).

7 Mustonen J, Helin H, Pietila K *et al.* Renal biopsy findings and clinicopathologic correlations in nephropathia epidemica. *Clin. Nephrol.* 41(3), 121–126 (1994).

8 Turcinov D, Puljiz I, Markotic A, Kuzman I, Begovac J. Clinical and laboratory findings in patients with oliguric and non-oliguric hantavirus haemorrhagic fever with renal syndrome: an analysis of 128 patients. *Clin. Microbiol. Infect.* 19(7), 674–679 (2013).

• **Gives comprehensive clinical and laboratory description of hemorrhagic fever with renal syndrome (HFRS) cases.**

9 Tsai TF. Hemorrhagic fever with renal syndrome: clinical aspects. *Lab. Anim. Sci.* 37(4), 419–427 (1987).

10 Bi Z, Formenty PB, Roth CE. Hantavirus infection: a review and global update. *J. Infect. Dev. Ctries* 2(1), 3–23 (2008).

11 Germash EI, Timokhov VS, Zagidullin Sh Z, Zagidullin IM, Ozhgikhin SN. [The pathogenetic therapy of patients with a severe form of hemorrhagic fever and acute kidney failure]. *Ter. Arkh.* 69(11), 26–30 (1997).

10.2217/BMM.14.88

12 Mori M, Rothman AL, Kurane I *et al.* High levels of cytokine-producing cells in the lung tissues of patients with fatal hantavirus pulmonary syndrome. *J. Infect. Dis.* 179(2), 295–302 (1999).

• **Presents data on proinflammatory cytokine-producing cells in *Hantavirus*-infected subjects.**

13 Khaiboullina SF, Rizvanov AA, Otteson E, Miyazato A, Maciejewski J, St Jeor S. Regulation of cellular gene expression in endothelial cells by sin nombre and prospect hill viruses. *Viral Immunol.* 17(2), 234–251 (2004).

14 Pensiero MN, Sharefkin JB, Dieffenbach CW, Hay J. Hantaan virus infection of human endothelial cells. *J. Virol.* 66(10), 5929–5936 (1992).

•• **Seminal publication that describes *Hantaviruses* as noncytopathic**

15 Wang PZ, Li ZD, Yu HT *et al.* Elevated serum concentrations of inflammatory cytokines and chemokines in patients with haemorrhagic fever with renal syndrome. *J. Int. Med. Res.* 40(2), 648–656 (2012).

16 Temonen M, Mustonen J, Helin H, Pasternack A, Vaheri A, Holthofer H. Cytokines, adhesion molecules, and cellular infiltration in nephropathia epidemica kidneys: an immunohistochemical study. *Clin. Immunol. Immunopathol.* 78(1), 47–55 (1996).

17 Hjelle B, Torres-Perez F. Hantaviruses in the americas and their role as emerging pathogens. *Viruses* 2(12), 2559–2586 (2010).

•• **Comprehensive review of *Hantavirus* pathology.**

18 Takala A, Lahdevirta J, Jansson SE *et al.* Systemic inflammation in hemorrhagic fever with renal syndrome correlates with hypotension and thrombocytopenia but not with renal injury. *J. Infect. Dis.* 181(6), 1964–1970 (2000).

19 Outinen TK, Makela SM, Ala-Houhala IO *et al.* The severity of Puumala hantavirus induced nephropathia epidemica can be better evaluated using plasma interleukin-6 than C-reactive protein determinations. *BMC Infect. Dis.* 10, 132 (2010).

•• **Comprehensive review of *Hantavirus* pathology.**

20 Makela S, Mustonen J, Ala-Houhala I *et al.* Urinary excretion of interleukin-6 correlates with proteinuria in acute Puumala hantavirus-induced nephritis. *Am. J. Kidney Dis.* 43(5), 809–816 (2004).

21 Moser B, Loetscher M, Piali L, Loetscher P. Lymphocyte responses to chemokines. *Int. Rev. Immunol.* 16(3–4), 323–344 (1998).

22 Yao Y, Tsirka SE. Monocyte chemoattractant protein-1 and the blood–brain barrier. *Cell Mol. Life Sci.* 71(4), 683–697 (2014).

23 Krakauer T, Leduc JW, Krakauer H. Serum levels of tumor necrosis factor-alpha, interleukin-1, and interleukin-6 in hemorrhagic fever with renal syndrome. *Viral Immunol.* 8(2), 75–79 (1995).

24 Sadeghi M, Eckerle I, Daniel V, Burkhardt U, Opelz G, Schnitzler P. Cytokine expression during early and late phase of acute Puumala hantavirus infection. *BMC Immunol.* 12, 65 (2011).

25 Tumanov AV, Grivennikov SI, Kruglov AA *et al.* Cellular source and molecular form of TNF specify its distinct functions in organization of secondary lymphoid organs. *Blood* 116(18), 3456–3464 (2010).

26 Zhao Y, Burbach JA, Roby KF, Terranova PF, Brannian JD. Macrophages are the major source of tumor necrosis factor alpha in the porcine corpus luteum. *Biol. Reprod.* 59(6), 1385–1391 (1998).

27 Ferluga D, Vizjak A. Hantavirus nephropathy. *J. Am. Soc. Nephrol.* 19(9), 1653–1658 (2008).

28 Khan SB, Cook HT, Bhangal G, Smith J, Tam FW, Pusey CD. Antibody blockade of TNF-alpha reduces inflammation and scarring in experimental crescentic glomerulonephritis. *Kidney Int.* 67(5), 1812–1820 (2005).

29 Vielhauer V, Stavrakis G, Mayadas TN. Renal cell-expressed TNF receptor 2, not receptor 1, is essential for the development of glomerulonephritis. *J. Clin. Invest.* 115(5), 1199–1209 (2005).

30 Ryffel B, Eugster H, Haas C, Le Hir M. Failure to induce anti-glomerular basement membrane glomerulonephritis in TNF alpha/beta deficient mice. *Int. J. Exp. Pathol.* 79(6), 453–460 (1998).

31 Anda T, Yamashita H, Khalid H *et al.* Effect of tumor necrosis factor-alpha on the permeability of bovine brain microvessel endothelial cell monolayers. *Neurol. Res.* 19(4), 369–376 (1997).

32 Sheridan AM, Force T, Yoon HJ *et al.* PLIP, a novel splice variant of Tip60, interacts with group IV cytosolic phospholipase A(2), induces apoptosis, and potentiates prostaglandin production. *Mol. Cell Biol.* 21(14), 4470–4481 (2001).

33 Furie MB, Mchugh DD. Migration of neutrophils across endothelial monolayers is stimulated by treatment of the monolayers with interleukin-1 or tumor necrosis factor-alpha. *J. Immunol.* 143(10), 3309–3317 (1989).

34 Contassot E, Beer HD, French LE. Interleukin-1, inflammasomes, autoinflammation and the skin. *Swiss Med. Wkly* 142, w13590 (2012).

35 Tan TY, Chu JJ. Dengue virus-infected human monocytes trigger late activation of caspase-1, which mediates pro-inflammatory IL-1beta secretion and pyroptosis. *J. Gen Virol.* 94(Pt 10), 2215–2220 (2013).

36 Law AH, Lee DC, Yuen KY, Peiris M, Lau AS. Cellular response to influenza virus infection: a potential role for autophagy in CXCL10 and interferon-alpha induction. *Cell Mol. Immunol.* 7(4), 263–270 (2010).

37 Bautista E, Arcos M, Jimenez-Alvarez L *et al.* Angiogenic and inflammatory markers in acute respiratory distress syndrome and renal injury associated to A/H1N1 virus infection. *Exp. Mol. Pathol.* 94(3), 486–492 (2013).

38 Sundstrom JB, Mcmullan LK, Spiropoulou CF *et al.* Hantavirus infection induces the expression of RANTES and IP-10 without causing increased permeability in human lung microvascular endothelial cells. *J. Virol.* 75(13), 6070–6085 (2001).

39 Xu LL, Warren MK, Rose WL, Gong W, Wang JM. Human recombinant monocyte chemotactic protein and other

C-C chemokines bind and induce directional migration of dendritic cells in vitro. *J. Leukoc. Biol.* 60(3), 365–371 (1996).

40 Jiang L, Newman M, Saporta S *et al.* MIP-1alpha and MCP-1 Induce Migration of Human Umbilical Cord Blood Cells in Models of Stroke. *Curr. Neurovasc. Res.* 5(2), 118–124 (2008).

41 Murai M, Yoneyama H, Harada A *et al.* Active participation of $CCR5^{+}CD8^{+}$ T lymphocytes in the pathogenesis of liver injury in graft-versus-host disease. *J. Clin. Invest.* 104(1), 49–57 (1999).

42 Deng G, Zhou G, Zhang R *et al.* Regulatory polymorphisms in the promoter of CXCL10 gene and disease progression in male hepatitis B virus carriers. *Gastroenterology* 134(3), 716–726 (2008).

43 Wang L, Chen RF, Liu JW *et al.* DC-SIGN (CD209) Promoter -336 A/G polymorphism is associated with dengue hemorrhagic fever and correlated to DC-SIGN expression and immune augmentation. *PLoS Negl. Trop. Dis.* 5(1), e934 (2011).

44 Mahanty S, Bausch DG, Thomas RL *et al.* Low levels of interleukin-8 and interferon-inducible protein-10 in serum are associated with fatal infections in acute Lassa fever. *J. Infect. Dis.* 183(12), 1713–1721 (2001).

45 Zhang Y, Liu B, Ma Y *et al.* Hantaan virus infection induces CXCL10 expression through TLR3, RIG-I, and MDA-5 pathways correlated with the disease severity. *Mediators Inflamm.* 2014, 697837 (2014).

46 Vaheri A, Strandin T, Jaaskelainen AJ *et al.* Pathophysiology of a severe case of Puumala hantavirus infection successfully treated with bradykinin receptor antagonist icatibant. *Antiviral Res.* 111C, 23–25 (2014).

47 Antonen J, Leppanen I, Tenhunen J *et al.* A severe case of Puumala hantavirus infection successfully treated with bradykinin receptor antagonist icatibant. *Scand. J. Infect. Dis.* 45(6), 494–496 (2013).

10.2217/BMM.14.88