Clinical and Etiological Characteristics of Severe Hemorrhagic Fever Caused by Coinfection of Thrombocytopenia Syndrome Bunyavirus and Hemorrhagic Fever Virus

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Abstract

Objective: Severe fever with thrombocytopenia syndrome (SFTS) and hemorrhagic fever with renal syndrome (HFRS) usually have different infection routes, and coinfection is relatively rare. The clinical and etiological characteristics of coinfection by the two pathogens will provide important references for clinical diagnosis and treatment. **Methods**: Blood samples and epidemiological data on HFRS patients were collected and classified into severe and non-severe groups according to clinical severity. The differences in clinical characteristics and levels of pathogens were evaluated and compared. **Results**: A total of 22 HFRS patient cases were collected from December 2021 to October 2022. Of these patients, 16 were non-severe and 6 severe. Patients with rodent exposure history, muscle and joint pain, weight loss, pharyngeal and conjunctival hyperemia, and positive urine protein and antibody IGM had a high severe rate ($P< 0.05$). Molecular tests on blood samples showed that 3 of the 6 severe patients were positive for hantavirus, 2 of the 3 hantavirus positives were positive for bunyavirus. **Conclusion**: Severe HFRS patients have different epidemiological, clinical, and laboratory characteristics. The coinfection of hantavirus and bunyavirus leads to severe HFRS. These findings have implications and references for diagnosis and treatment of coinfected severe cases.

Clinical and Etiological Characteristics of Severe Hemorrhagic Fever Caused by Coinfection of Thrombocytopenia Syndrome Bunyavirus and Hemorrhagic Fever Virus

——Coinfection of SFTSV with HTNV may lead to severe HFRS

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40-word summary of the article's main point:

In areas where both ticks and mice are endemic, physicians should consider the possibility of SFTSV and HTNV coinfection when diagnosing severe forms of HFRS in order to achieve precise treatment.

Abstract

Objective : Severe fever with thrombocytopenia syndrome (SFTS) and hemorrhagic fever with renal syndrome (HFRS) usually have different infection routes, and coinfection is relatively rare. The clinical and etiological characteristics of coinfection by the two pathogens will provide important references for clinical diagnosis and treatment.

Methods : Blood samples and epidemiological data on HFRS patients were collected and classified into severe and non-severe groups according to clinical severity. The differences in clinical characteristics and levels of pathogens were evaluated and compared.

Results : A total of 22 HFRS patient cases were collected from December 2021 to October 2022. Of these patients, 16 were non-severe and 6 severe. Patients with rodent exposure history, muscle and joint pain, weight loss, pharyngeal and conjunctival hyperemia, and positive urine protein and antibody IGM had a high severe rate ($P< 0.05$). Molecular tests on blood samples showed that 3 of the 6 severe patients were positive for hantavirus, 2 of the 3 hantavirus positives were positive for bunyavirus.

Conclusion : Severe HFRS patients have different epidemiological, clinical, and laboratory characteristics. The coinfection of hantavirus and bunyavirus leads to severe HFRS. These findings have implications and references for diagnosis and treatment of coinfected severe cases.

Key Words: Hemorrhagic fever with renal syndrome (HFRS); Severe fever with thrombocytopenia syndrome (SFTS); Hantaan orthohantavirus (HTNV); Severe fever with thrombocytopenia syndrome bunyavirus (SFTSV); Rodent; Tick; Co-infection

Introduction

Hemorrhagic fever with renal syndrome (HFRS) is predominantly attributed to Hantaan ortho-hantavirus (HTNV), a naturally occurring focal infectious disease primarily transmitted through the inherent host of rodents. HTNV was initially discovered in South Korea in 1978 and is prevalent in regions in China, South Korea, Russia, and Vietnam[1, 2]. The endemic strains in Northeast China encompass HTNV and Seoul virus (SEOV). Human infection occurs via exposure to wild mice's blood, saliva, urine, and feces or through the transmission of vector organisms such as fleas settling on the exterior of wild mice. It has been reported that there were over 100,000 annual infection cases globally, with China being among the most severely

affected countries, accounting for more than 90% of these infections[3]. The recognizable HFRS course is divided into five stages: fever, hypotensive shock, oliguria, polyuria, and recovery[1]. These disease stages are well-defined in severe HFRS, but the boundaries are not distinct or may overlap in mild and moderate cases[4]. During the initial phase of the disease, there are typically pronounced "three pains"(headache, low back pain, orbital pain) and "three reds"(face red, neck red, and chest red), but it is significantly relieved after the polyuria stage. Based on the severity of fever, toxic symptoms, bleeding, shock, and kidney damage, the clinical cases were divided into five categories: mild, moderate, severe, critical, and atypical, with a mortality rate ranging from 0.5% to 40%. The severity of the disease is also dependent on variants of virulent agents[5]. HFRS can be instigated by Hantaan ortho-hantavirus (HTNV), Seoul virus (SEOV), Dobrava-Belgrade virus (DOBV), and Puumala virus (PUUV). HTNV and DOBV frequently infect and cause severe HFRS, while SEOV mainly leads to moderate HFRS[1, 4]. Apart from clinical manifestations and epidemiological history, diagnosis relies on laboratory testing methods and techniques such as serology, polymerase chain reaction, immunochemistry, and virus culture.

Severe fever with thrombocytopenia syndrome bunyavirus (SFTSV) is an emergent viral hemorrhagic fever with a significant fatality rate. It was initially identified and documented in China in 2009 and was subsequently isolated and named as severe fever with thrombocytopenia syndrome (SFTS) in 2010, referred to as novel Bunyavirus[6]. It belongs to the family Bunyaviridae and the genus Phlebovirus. SFTS occurrences were primarily concentrated in East Asian countries such as China, Japan, South Korea, and Vietnam. It has been reported that the cumulative number of confirmed cases was 5360 in China by 2016, 866 in South Korea by 2018, and 467 in Japan by 2019, respectively[7]. The pathogen is predominantly transmitted through blood infection, and tick-to-human transmission is the main route of human infection, with Haemaphysalis longicornis being the most common transmission vector. The infection manifests as severe fever, platelet and leukopenia, obvious neurological symptoms, and gastrointestinal symptoms with high mortality.

The various hemorrhagic fevers (HFRS and SFTS) demonstrate similar epidemic characteristics and clinical symptoms. Regarding epidemiological characteristics, spatial and temporal distribution mirror the attributes of susceptible populations. Meanwhile, the clinical characteristics are similar in the early stage of the disease, such as general discomfort, abrupt onset of fever, and irregular coagulation function[8]. Despite overlapping similarities in clinical features, instances of co-infection with both viruses are extremely rare.

Liaoning Province is recognized as a high-prevalence area for HFRS and SFTS, exhibiting a recent upward trend. Additionally, the incidence rate of SFTS persistently escalates, engendering significant public health problems. The epidemic zones are mainly distributed in Dandong, Dalian, Benxi, and other parts of Liaodong, particularly Fengcheng City and Kuandian County, which are under the jurisdiction of Dandong. At the same time, there exists considerable overlap between the endemic areas and the vector dispersion. Liaoning Province harbors high tick density, increasing the risk for humans and animals. Consequently, Liaoning Province provides an ideal research environment for investigating diseases.

It is an important scientific question of effectively diagnosing viral hemorrhagic fevers with similar characteristics. Presently, most studies independently study analyze the clinical characteristics and diagnostic methods of the two viral hemorrhagic fevers in isolation or establish diagnostic models rooted in clinical prediction and identification, failing to comprehensively combine clinical data and specimens for a thorough analysis of the correlation between the two viral hemorrhagic fevers. Besides, some research on HFRS mainly consist of descriptive studies on case reports, nursing experience, and clinical characteristics, with minimal studies on severe HFRS[9, 10]. Therefore, utilizing clinical data and combining it with the detection of patient samples, this study conducted the statistical analysis of severe cases of HFRS and explored the relationship between co-infection and disease severity combined with laboratory detection.

The present study collected the clinical case information and blood specimens of 22 patients clinically diagnosed with HFRS admitted to Dandong Infectious Diseases Hospital of Liaoning Province from December 2021 to October 2022 in Northeast China, where two types of infectious diseases are highly endemic. These cases were categorized into severe and non-severe groups. Statistical description and inference were applied to compare and analyze the clinical characteristic differences between the two groups. Meanwhile, molecular

biological detection of common vector infectious disease pathogens was carried out on the blood samples of patients, molecular biological detection of their cell cultures was carried out, and the pathogens were isolated. The severity of HFRS was identified under different factors. Moreover, the co-infection of HFRS and SFTS was also discussed to provide scientific reference for the early and timely differential diagnosis, effective prevention, and treatment of severe cases.

Methods

Clinical specimens

The blood specimens of HFRS patients were collected by the Dandong Infectious Diseases Hospital of Liaoning Province, which diagnosed and confirmed HFRS according to the combination of epidemiological history, clinical symptoms, blood biochemical markers, and conventional colloidal gold test strips (Xiamen Bosheng Biotechnology Co., LTD.). The details information to the patient sample are shown in Table 1.

Table 1 The sample information of the HFRS cases

No.	Case	Gender	First Admission. Date of Collection	Rat's Exposure	Tick's Exposure	Antibody Detection	Antibody Detection	Diagnosis	Severity Status	Fever	Pharyngeal Hyperaemia	Conjunctival Congestion	WBC (10^9
						IgM	IgG						
1	A	Male	2021.12.07	Yes	No	+	-	HFRS	Yes	Yes	Yes	Yes	13.93
2	B	Male	2021.12.10	Yes	No	+	+	HFRS	Yes	Yes	Yes	Yes	24.17
3	C	Female	2021.12.11	Yes	No	+	-	HFRS	Yes	Yes	Yes	Yes	19.14
4	D	Male	2021.12.13	Yes	No	+	+	HFRS	Yes	Yes	Yes	Yes	14.60
5	E	Male	2021.12.15	Yes	No	+	+	HFRS	Yes	Yes	Yes	Yes	7.41
6	F	Male	2021.12.16	Yes	No	+	+	HFRS	Yes	Yes	Yes	Yes	17.33
7	G	Male	2022.9.16	Yes	No	+	+	HFRS	No	Yes	No	No	19.62
8	H	Male	2022.9.18	No	No	+	+	HFRS	No	Yes	No	No	1.16
9	I	Female	2022.10.18	Yes	No	+	+	HFRS	No	Yes	No	No	8.57
10	J	Female	2022.9.11	No	Yes	+	+	HFRS	No	Yes	No	No	1.85
11	K	Male	2022.9.18	No	Yes	+	+	HFRS	No	Yes	No	No	1.32
12	L	Male	2022.8.28	No	Yes	+	+	HFRS	No	Yes	No	No	1.71
13	M	Male	2022.10.14	No	No	+	+	HFRS	No	Yes	No	No	2.53
14	N	Female	2022.8.28	No	No	+	+	HFRS	No	Yes	No	No	2.26
15	O	Male	2022.8.26	No	Yes	+	+	HFRS	No	Yes	No	No	2.89
16	P	Male	2022.8.26	No	No	+	+	HFRS	No	No	No	No	2.73
17	Q	Male	2022.9.28	No	No	+	+	HFRS	No	Yes	No	No	1.12
18	R	Male	2022.9.27	Yes	No	+	+	HFRS	No	Yes	No	No	1.91
19	S	Male	2021.12.20	Yes	No	+	+	HFRS	No	Yes	Yes	Yes	47.38
20	T	Female	2021.12.20	Yes	No	+	+	HFRS	No	No	No	Yes	11.31
21	U	Male	2021.12.23	Yes	No	+	+	HFRS	No	Yes	No	No	5.19
22	V	Male	2021.12.07	Yes	No	+	+	HFRS	No	Yes	No	No	14.69

Nucleic acid extraction and reverse transcription

Patient total nucleic acids were extracted from serum and whole blood specimens utilizing the commercial

nucleic acid extraction Kit (EasyPure® Viral DNA/ RNA Kit ER201-01). The reverse transcription of nucleic acids was performed employing the Kit (HiScript III 1st Strand cDNA Synthesis Kit). All operations and procedures are performed in accordance with the commercial kit's instruction manual.

Pathogen detection

Samples collected from patients and wild mice were detected for common rodent and tick-borne zoonotic pathogens. PCR was used to assay bacterial pathogens with total nucleic acid such as Rickettsia, Coxiella, anaplasmosa and Borrelia burgdorferi. Hantavirus, Seoul virus, novel Bunyavirus, Semliki Forest virus, Dabieshan orthohantavirus, new Alongshan virus (tick-borne flavivirus found for the first time in Liaoning Province) and other viral pathogens were detected by PCR with retrotranscriptional products. PCR reaction system: 20ul, reaction procedure: 94 5min, 94 30s, 54 20s, 72 30s, 72 10min. The sequence of primers mentioned above is detailed in Appendix 1. The amplified PCR products were subjected to agarose gel electrophoresis, and the positive products were sent to Sangong Bioengineering (Shanghai) Co., Ltd. for sequencing.

Cell culture and pathogen isolation

Serum samples were collected from 6 critically ill patients and inoculated into Vero and Bhk cells for routine culture and blind passage 3. Cell cultures were frozen-thawed once, nucleic acids were extracted and reverse transcribed for PCR detection. The amplified PCR products were subjected to agarose gel electrophoresis, and the positive products were sent to Sangon Bioengineering (Shanghai) Co., LTD for sequencing.

Statistical analysis

The patients with hemorrhagic fever with renal syndrome were divided into severe and non-severe type groups. The general demographic characteristics and epidemiological history were statistically described. Fisher's exact probability and Chi-square tests was employed to compare and analyze the differences in various variables (demographic characteristics, clinical symptoms, laboratory tests, etc.) between the two groups for statistical inference. $P < 0.05$ was considered statistically significant.

Results

Demographic characteristics of HFRS cases

A total of 22 HFRS clinical cases were enrolled in this study. The patients' mean age was 58.91 +- 11.69 years, predominantly males, accounting for 77.3% (17/22). In addition, 3 patients were co-infected with other infectious diseases, accounting for 9.1% (2/22). The severe and non-sever cases accounted for 27.2% (6/22) and 86.4% (16/22), respectively, as shown in Table 2 below.

Table 2 Demographic characteristics of HFRS clinical cases

Variable	n (%)/Mean±SD
Age(Years)	**58.91±11.69**
Gender	
Male	**17 (77.3)**
Female	**5(22.7)**
Tick's Exposure	
Yes	**4 (18.2)**
No	**18 (81.8)**
Rat' Exposure	
Yes	**13 (59.1)**
No	**9 (40.9)**
Co-infection	
Yes	**2 (9.1)**
No	**20 (90.9)**

Variable	n (%)/Mean±SD
Severe or Non-severe	
Yes	**6 (27.3)**
No	**16 (72.7)**
No	**22 (100.0)**

Severe HFRS showed different clinical and laboratory characteristics

Statistical analysis showed that HFRS patients with a history of rat contact history (χ^2=5.712,$P = 0.024 < 0.05$), muscle and joint pain (χ^2= 14.438,$P = 0.000 < 0.01$), weight loss (χ^2=14.438,$P = 0.000< 0.01$), pharyngeal (χ^2= 17.679,$P = 0.000 < 0.01$) conjunctival(χ^2= 14.438,$P = 0.000< 0.01$) hyperemia, abnormal white blood cell count (χ^2= 9.900,$P = 0.003 < 0.01$), urine protein (χ^2= 9.900,P =0.003 < 0.01) and antibody IGM (χ^2= 11.917,$P = 0.001< 0.01$) positive cases had a higher severe rate. The differences between the two groups were statistically significant ($P < 0.05$).

Table 3 The univariate analysis of HFRS clinical cases among severe and non-severe group

Various	Severe or Non-severe group	Severe or Non-severe group	Severe or Non-severe
	Yes (%)		**No(%)**
Co-infection			
Yes	2 (33.3)		3 (66.7)
No	0 (0.0)		16 (100.0)
Rat' Exposure			
Yes	6 (100.0)		0 (0.0)
No	7 (43.8)		9 (56.3)
Muscle and Joint Pain			
Yes	6 (100.0)		0 (0.0)
No	2 (12.5)		14 (87.5)
Weight Loss			
Yes	6 (100.0)		0 (0.0)
No	2 (12.5)		14 (87.5)
Pharyngeal Hyperaemia			
Yes	6 (100.0)		0 (0.0)
No	1 (6.3)		15 (93.8)
Conjunctival Congestion			
Yes	6 (100.0)		0 (0.0)
No	2 (12.5)		14 (87.5)
WBC			
Abnormal	6 (100.0)		0 (0.0)
Normal	4 (25.0)		12 (75.0)
Urine Protein			
Positive	6 (100.0)		0 (0.0)
Negative	4 (25.0)		12 (75.0)
IGM			
Positive	6 (100.0)		0 (0.0)
Negative	3 (18.8)		13 (81.3)

Etiological survey of severe HFRS patients

The blood samples were detected for HTNV and other viruses. The antigen detection showed that 3 severe

patients (No. 2, 5 and 6) were positive for HTNV. All other detection assay pertaining to SEOV and other common vector-borne infectious diseases were negative. DNA sequencing results confirmed the presence of HTNV (Appendix 2). To further confirm the pathogen, blood samples were subjected to virus isolation. Samples from two patients (No. 5 and 6) were psotive for SFTSV, and no other common vector-borne viruses were identified. DNA sequencing of the amplification product confirmed the presence of SFTSV (Appendix 3).

Phylogenetic characteristics of the pathogen of severe HFRS

The sequences were BLAST and aligned with related sequences to infer the phylogenetic location of the virus. The SFTSV positive product exhibited sequence homology of 98.11% with SFTSV isolated from ticks in SFTS patients and endemic regions in our lab in 2019 (NCBI accession: MT 232961.1), consisting of genotype A with minimal mortality[11]. The HTNV sequences derived from specimens of severe patients (No 2, 5, and 6) indicated that patient No. 5 and No. 6 share identical DNA, while Patient No. 2 possessed a unique sequence. Appendix 2 illustrates the DNA sequence of Patient No. 2, with specific bases highlighted in sequences 5 and 6. Homeland and international reference strains sourced from the NCBI website served as reference sequences for construction of the phylogenetic tree using MEGA7.0. The results showed an affinity with strains KC576787 and KC5767862 (both isolated in Jilin Province) at 94.78%. The phylogenic tree of HTNV is shown in Figure 1.

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`image1.emf` available at https://authorea.com/users/773964/articles/867649-clinical-and-etiological-characteristics-of-severe-hemorrhagic-fever-caused-by-coinfection-of-thrombocytopenia-syndrome-bunyavirus-and-hemorrhagic-fever-virus

Note: The system evolution parameters are set as follows:

1.Constructed using Neighbor-Joining method; 2.Test of Phylogeny: Bootstrap method;

3.No. of Bootstrap Replications: 1000;

4.Gaps/Missing Data Treatment: Complete deletion.

Figure 1 Phylogenetic analysis based on the partial nucleotide sequences

of the M segment of HTNV

Discussion

Hemorrhagic fever with renal syndrome (HFRS), a global public health concern with a high fatality rate, has been reported in various countries. Approximately 90% of all the worldwide cases have been reported in China distributed in different regions, except Qinghai Province [5, 8, 12]. The highest incidences of HFRS in Shenyang, Anshan, Dandong, Jinzhou, Yingkou, and Huludao of Liaoning Province in China[13]. Meanwhile, another viral hemorrhagic fever infectious disease, SFTS, also affected Liaoning province. Therefore, exploring the relationship between co-infection of both epidemic viral haemorrhagic fever in severe clinical cases will contribute to differential diagnosis and treatment of the disease.

This study systematically analyzed the clinical and etiological characteristics of severe SFTS in Dandong City of Liaoning province. The results showed that the severe rate among HFRS patients co-infected with viral hemorrhagic fevers was higher than independent infection cases with no statistically significant difference. However, blood diagnostic detections revealed that 2 of the 3 severe HFRS patients who tested positive for HYNV were simultaneously positive for SFTSV. The results suggested that co-infection with both viral hemorrhagic fevers is associated with the occurrence of severe cases. Historically, there has been a consistent prevalence of vector-borne infectious diseases in Dandong, located in the Changbai Mountain region, which exhibits high forest coverage and encompasses abundant vectors such as rats and ticks. Previous studies indicated that the dominant tick variety in this region is Haemaphysalis longicornis, the carrier of the zoonotic pathogen SFTSV[11, 14]. This agent resides on the surfaces of rats' bodies and transfers infectious diseases

to humans through host activity. Moreover, ticks can spread disease rapidly and over long distances with migratory bird hosts, potentially leading to widespread disease outbreaks[15]. Various livestock, poultry, wild mammals, and rodents can naturally acquire SFTSV and present seropositivity under subclinical infection, revealing brief viremia and complete viral clearance after recovery[16,17]. This conflicts with our negative nucleic acid analysis of 167 captured wild mouse samples, indicating that wild mice are not directly involved in SFTSV transmission but may contract it from ticks feeding them.

Some previous studies have shown that the spatio-temporal distribution characteristics and clinical symptoms of the above two viral hemorrhagic fevers are comparable, leading to missed and erroneous clinical practice diagnoses. Inaccuracy of diagnosis affects effective disease treatment, hinders disease prognosis, and increases mortality risks. A retrospective analysis study conducted by Rui Qi et al. [18] revealed that SFRS patients were misdiagnosed as HRFS based on 73 (57.0%) having HTNV-IgM antibodies, and 4 (7.3%) were positive for both HTNV-IgM and SFTSV-IgM antibodies after evaluating 128 clinical HFRS patients.

In clinical practice, it is difficult to differentiate between HFRS and SFTS patients because of similar presentations. Patients with HFRS experience typical or atypical symptoms. Recently, atypical presentations predominate, showing mild symptoms similar to influenza, such as fever, fatigue, and headache, inviting potential misinterpretation [19, 20]. Moreover, HFRS and SFTS are both viral hemorrhagic fevers with similar mechanisms. The core of HFRS pathogenesis is endothelial cell infection by hantavirus, triggering a severe and rapid immune response resulting in vascular injury and enhanced microvascular permeability[21]. Systemic inflammatory response syndrome may also account for SFTS pathogenesis[22]. Similar pathogenic mechanisms between these viral hemorrhagic fevers may contribute to the severity of the disease. It is well known that HFRS and SFTS share similar clinical characteristics, such as thrombocytopenia, renal insufficiency, abnormal biochemical indicators, etc[23]. The simultaneous attack further increases the severity of the disease. In 2014, Korean scholar Sun Whan Park et al. [24] reported an HTNV/SFTSV co-infection case verified by serological tests, but molecular biology detection and virus isolation were not performed. In 2019, Liuwei et al. [25] conducted a retrospective analysis of 1546 febrile patients (603 HFRS and 943 SFTS patients), revealing that the co-infection rate of HTNV-SFTSV (0.6%, 9 of 1546 cases) was lower than predicted based on single HTNV and SFTSV infection rates. The results showed that the trend of co-infection between the two pathogens was low. The proportion of clinical features was not significantly higher in the HTNV-SFTSV co-infection group than in the HTNV or SFTSV infection groups alone, indicating that co-infection with both pathogens did not lead to more severe outcomes. This study confirmed for the first time that co-infection of HTNV and SFTSV caused severe HFRS, and the epidemic trend of HFRS had begun in Dandong. Therefore, co-infection of HFRS with other viral hemorrhagic fevers may lead to the emergence of critical cases.

Our findings identified a significant risk for severe HFRS in patients exposed to rodents, harboring the primary source of infection and host of HFRS. Mice contribute to disease spread via direct human exposure, exchange of virus-containing excreta (urine, feces, and saliva), or inhaled aerosols. The HTNV and SEOV positivity percentages were notably high in study areas. Rural environments often present poor housing and sanitary conditions, along with elevated rodent density during harvesting seasons, amplifying opportunities for contact with rodents, thus heightening the risk of direct or indirect transmission. Patients who have directly contact rodent history have high vigorous virus loads. Related research indicates severe/critical HFRS patients typically exhibit higher plasma virus levels in the early stages of the disease (5.90 vs. 5.03 $\log^{10}$ copies/mL, P =0.001), indicating a correlation between viral loads and disease severity[26].

The results also demonstrated that clinical features such as pharyngeal hyperemia, conjunctival congestion, abnormal white blood cells, urine protein, and IGM antibody positive significantly affected the severity of HFRS cases. Pharyngeal hyperemia and conjunctival congestion correlated with disrupted coagulation function in HFRS patients. Recent evidence suggested platelet counts may predict coagulation function and disease severity, thereby expanding prognostic capabilities and mitigating risk[27]. Moreover, renal dysfunction is a significant complication in HFRS as proteinuria appears. This urinary indicator reflects the severity of the disease[28]. Hantavirus infection engenders an inflammatory response. Cytokines associated with

inflammation regulation positively correlate with white blood cell count and disease severity[29]. Several studies report HFRS shows acute kidney injury with transient proteinuria, with proteinuria reflecting the severity of the disease. Increased local heparanase activity in kidneys induced by hantavirus infection may disrupt endothelial glycocalyx, facilitating protein extravasation through the glomerular filtration barrier and leading to severe proteinuria[27]. In addition, this study revealed a higher severity rate for IGM positivity. Comparative investigations of cytokine levels in IGM-positive, -negative, and healthy groups identified elevated cytokines (IL-1ra, IL-12p70, IL-10, IP-10, IL-17, IL-2, and IL-6) in the IgM-positive group, suggesting disease progression[30]. Thus, specific clinical features contribute to the escalation of HFRS severity and impact initial clinical management.

This research corroborated that HTNV and SFTSV dual infection was determinant for severe HFRS cases combined molecular biology with virus isolation, emphasizing clinicians need to pay attention to the presence of multiple pathogens in HFRS severe case management. The severe patients primarily manifested HFRS symptoms but lacked SFTS respiratory and neurological manifestation. Normal or high counts of white cells indicate that SFTSV has not yet attacked the patient's tissues and organs (Appendix). Hence, it was considered that HTNV is the primary pathogen of severe cases, and SFTSV is the synergistic pathogen, but there was the probability that the disease severity would exacerbate with viral load increase. HTNV and SFTSV destroy platelets in large quantities, making distinguishing what pathogen caused the platelet decline difficult. HTNV co-infections with different pathogens have been reported, such as an instance of HTNV co-infection with Dengue virus in Shenzhen in 2021. The patient had rodent contact, absent dengue fever epidemiology, and the source of infection was likely rodents[31]. Moreover, HTNV infection in elderly patients frequently accompanies other pre-existing conditions, predisposing to complications, critical-type incidence, and high death rate.

In this study, patient serum samples were evaluated using Vero and BHK cell lines, yielding two SFTSV isolates, but no HTNV. While the Vero-E6 cell line is typically employed for HTNV studies, the successful isolation rate remains subpar despite elevated viral burden during an early infection phase (febrile stage). There is an immediate need to identify a novel cell line exhibiting superior susceptibility to HTNV, facilitating its isolation. Moreover, numerous SFTSV isolates were obtained from ticks and SFTS patients in our institution over recent years, indicating ease of adaptation and proliferation on Vero cells. Simultaneously, it was observed that SFTSV proliferates rapidly upon inoculation with ticks harboring SFTSV and other viruses, potentially leading to complications.

According to the International Committee on Taxonomy of Viruses, there were 7 genera and 53 species of Hantaviridae with persistent novel species. HTNV is the primary pathogen of HFRS [1], a single-stranded negative RNA virus comprising three segments (L, M, S) that encode an RNA polymerase, glycoprotein, and nucleocapsid protein. In this study, the target gene for HFRS diagnosis is the glycoprotein sequence of segment M of the HTNV, which tends to undergo genetic mutations and modify virulence. Although the evolutionary analysis of the nucleic acid sequence of the fragment revealed that it aligned at approximately 95% identity with strains isolated in Jilin Province, Liaoning Province, it is insufficient to classify the virus as a novel subtype of Hantaan or evaluate the impact on pathogenicity. Limited sample sizes prevented complete gene amplification, requiring more sample collection and virus isolation for exploration. In addition, we isolated multiple SFTSV strains bearing genotype A in Liaoning Province, highly aligned with Anhui Province and Zhejiang Province, which is less virulent and has lower lethality in humans compared to other genotype strains prevalent in South Korea, Japan[14].

Conclusion

This study identified severe cases of haemorrhagic fever with renal syndrome (HFRS) with different epidemiological, clinical and laboratory characteristics. Meanwhile, hantavirus and Bunyavirus co-infection was confirmed by experimental verification in severe HRFS cases, suggesting that co-infection with viral haemorrhagic fever may affect the severity of the disease. The results of this study provide scientific reference and insights for the prevention, diagnosis and treatment of co-infection with viral haemorrhagic fever.

Notes

Contributors : Feng Jiang, Xiaohu Han, Yongxiang Zhao and Zeliang Chen conceptualized the initial hypothesis and conceived and designed the study. Feng Jiang, Xiaohu Han, Ya Wen, Yao Chen, Hua Deng, Qing Xin, Yudan Bi and Yicheng Zhou carried out the sample collection and the molecular testing. Yongxiang Zhao, Yao Chen, Hua Deng and Ya Wen collected and sorted out the data. Ruihao Peng edited the table. Qijun Chen, Xiaohu Han, Ruihao Peng conducted literature search and participated in the writing part. Xiaohu Han, Ruihao Peng and Zeliang Chen did the statistical analysis and wrote the first draft of the manuscript. Qijun Chen and Zeliang Chen revised the manuscript. All authors contributed substantially to data acquisition and interpretation, and revision and editing of the manuscript. The Sixth People's Hospital of Dandong City provided patient samples and related data for this project.

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Availability of data and materials : All data and codes supporting the findings of this study are derived from open databases.

Ethical approval : No.01(2022)of The Sixth People's Hospital of Dandong City.

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