Case Report

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Sporadic case infected by severe fever with thrombocytopenia syndrome bunyavirus in a non-epidemic region of China

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Summary **We report here a clinical and molecular study on a case suffer from severe fever with thrombocytopenia syndrome (SFTS) due to a new type of bunyavirus, named SFTS bunyavirus (SFTSV), in Zhejiang Province China. The key clinical features of this patient include fever, lymphocytopenia and thrombocytopenia. We carried out a serological and molecular investigation in the indicated case and on relatives with close contact. The SFTSV infection was confirmed through amplification of viral genetic material using the polymerase chain reaction (PCR) from the patient's serum, but not relatives with close contact. Subsequently direct sequence of PCR product demonstrated a homology of 94-96% in the nucleotide sequence compared to a reference sequence previously reported, in which the majority of patients originated from an epidemic area of Central and Northeast China. Our results suggest that SFTSV can occur in a non-epidemic area due to a similar strain of SFTSV that apparently affect the blood system, implying the importance of dissecting the pathogenesis of SFTS as well as mode of infection.**

Keywords: Severe fever with thrombocytopenia syndrome bunyavirus (SFTSV), molecular diagnosis, sporadic infection

1. Introduction

Severe fever with thrombocytopenia syndrome bunyavirus (SFTSV) infection, a type of RNA virus and a new member of Bunyaviridae belonging to *phlebovirus*, was first identified by the Chinese Center for Disease Control and Prevention in 2010 (*1*). severe fever with thrombocytopenia syndrome (SFTS) mainly occurs in spring and summer, with middle-aged residents in hilly regions most susceptible. The major manifestations of SFTSV infection include fever, thrombocytopenia, gastrointestinal symptoms, and leukocytopenia (*2*). However, the pathogenesis of SFTSV remains unclear and the epidemiological data are still limited. Up to September 2010, SFTS had only been reported in Central and Northeast China (*2*). We report here a patient with suspected SFTSV infection who was admitted to Zhoushan People's Hospital, Zhejiang Province of China in the middle of June 2011, and was finely diagnosed using the polymerase chain reaction (PCR) of the target genes of the RNA-dependent RNA polymerase, the glycoprotein and N protein in the conserved core region of novel bunyavirus, and nucleotide sequence analysis.

2. Case report

The patient was a 72-year-old female who had a

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sudden onset of fever (the highest temperature was 39.1°C), accompanied by chills and pharyngeal pain. After accepting intravenous ribavirin and clindamycin for one day without remission, she was referred to the local hospital with a preliminary diagnosis of fever and thrombocytopenia of undetermined origin. Thereafter she was treated with intravenous piperacillin/tazobactam, levofloxacin tablets, solu-medrol and frozen fresh plasma for a week, but no clinical improvement was observed. On the eighth day, since she developed melena and generalized myalgia, the patient was subsequently transferred to the emergency room of the general hospital located in Zhoushan city for further treatment. Upon arrival, she was conscious but listless and pale, with temperature of 37.5°C, and pulse rate of 98 beats/min Her respiratory rate was 19 times/min, and her blood pressure was 104/68 mmHg. There were no overt sign of jaundice, and no petechia or ecchymoses were noted. Enlarged lymph nodes were palpable on both sides of the neck, in the axillary and inguinal regions. Her posterior pharyngeal wall was congested, and her vesicular breath sounds were abnormal. Her abdomen was soft, without rebound tenderness, shifting dullness or percussion tenderness over the hepatic and renal regions and no hepatosplenomegaly. Her lower limbs were not edematous, and neurological signs were negative.

Hematology showed leukopenia (total count 2.6 × 10^9/L, lymphocytes 0.36 × 10^9/L and neutrophils 1.98 × 10^9/L) and thrombocytopenia (48 × 10^9/L). Her lymphocyte subsets count showed that $CD4^+$ and $CD8^+$ T cells were significantly decreased, especially the $CD4^+$ T cells (52.4 × 10^6/L). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were elevated to 710 U/L and 129 U/L, respectively. Creatine kinase (CK) was 671 U/L, albumin 19.8 g/L, and lactate dehydrogenase (LDH) 3,669 IU/L. Urinalysis showed moderate proteinuria and hematuria. Fecal occult blood test was strongly positive, consistent with the melena. A non-contrast CT scan of the chest showed mild inflammation in the superior lobe of the right lung, as well as emphysema in both lungs with pleural thickening and adhesions indicative of chronic bronchitis. The non-contrast CT scan of the abdomen was normal (Figure 1).

Considering the high probability of active gastrointestinal bleeding, intravenous cefmetazole and omeprazole sodium for injection were prescribed, along with reduced glutathione tablets. No improvement was seen after three days' treatment. The patient's platelet count decreased further to 12 × 10^9/L on the 11th day. She also experienced mild renal insufficiency, hypokalemia and hyponatremia. Her thrombin time was mildly abnormal and fibrinogen degradation products were slightly elevated. All of the signs and symptoms suggested that the patient was suffering from SFTS. Meanwhile, the patient was prescribed bed rest and was given a semi-liquid diet, along with symptomatic and supportive therapy as well as close monitoring of vital signs and urine output. Intravenous ribavirin and broad-spectrum antibiotics were prescribed for the empiric treatment of viral and bacterial infection. Since serum albumin levels and platelet count were severely depressed, and symptoms of gastrointestinal bleeding persisted, nutritious supportive therapy was also used. In addition, human immunoglobulin was injected intravenously to enhance the immunity of the patient. The treatments were effective and the patient was discharged after 20 days.

The patient had worked as a farmer for more than fifty years in hilly regions of Zhoushan Island, Zhejiang province, and had not travelled to other places for many years. She was not rearing poultry. She had worked in the cornfield two weeks before the onset of fever, but had no history of tick bites or contacts with individuals who had similar manifestations. There were rodents living in the areas she inhabited, and poor sanitary condition in her domicile. No other individuals in her village or neighboring villages presented with similar symptoms.

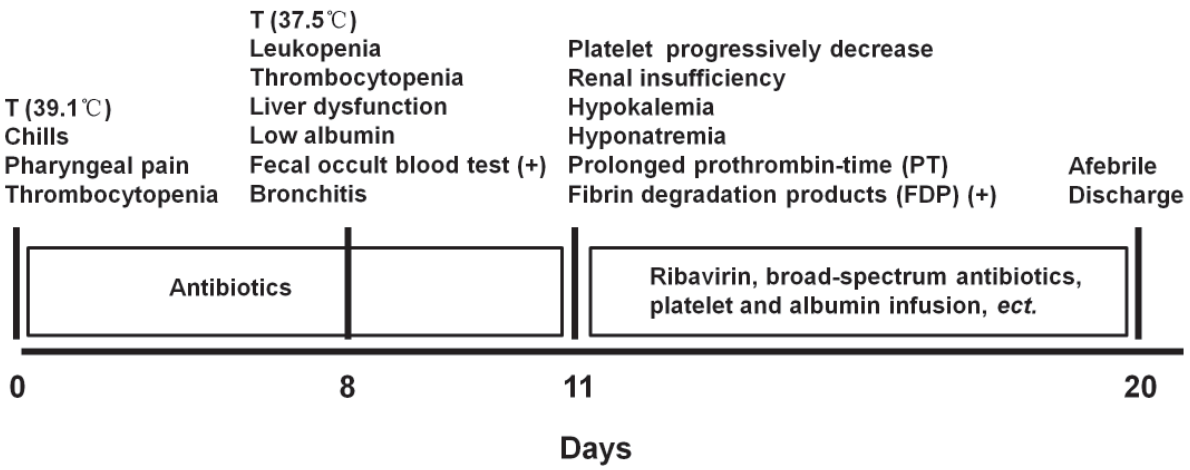

Figure 1. Summary of clinical findings.

Table 1. Sequences of primers

Direction	Sequence (5'-3')
bun rdrp-S	ATGGACAACCCTGCATTCG
bun rdrp-AS	TCAGCTTCTAGGCTAAAACCAG
bun gly 601-S	AAGAGTTTTAGCCAAAGTGAATTCCC
bun gly 1753-AS	ACATTCCTTCATATTTCCGCTCCC
bun NS 1043-S	CTTCAGCCACTTCACCCGAAC
bun NS 1646-AS	GCAGCAGCTCAATTTGACT

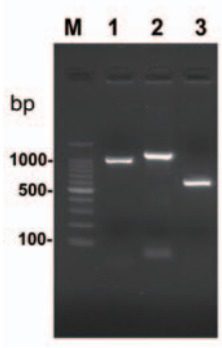

Figure 2. PCR amplification of RNA-dependent RNA polymerase, glycoprotein and N protein genes from blood DNA of a patient with severe fever and thrombocytopenia syndrome (SFTS). The amplified DNA fragment of RNA-dependent RNA polymerase with primer bun rdrp-S and bun rdrp-AS (**lane 1**). The amplified DNA fragment of glycoprotein with primer bun gly601-S and bun1753-AS (**lane 2**), and the amplified DNA fragment of N protein with primer bun NP 69-S and bun NP 606-AS (**lane 3**). **M**, DNA size marker (100 bp ladder).

The diagnosis of SFTSV infection was confirmed by DNA assay using detection of the target genes of the RNA-dependent RNA polymerase, the glycoprotein and N protein in the conserved core region of a novel bunyavirus by PCR. Viral RNA was extracted from the blood sample of the patient with a QIAampMinElute virus spin kit according to the manufacturer's instructions, and used to synthesize the single-strand cDNA. The single-strand cDNA was amplified by RT-PCR with random primers by using a ProtoScriptFirst Strand cDNA synthesis kit (New England Biolabs, Beijing, China), and used as the template for PCR. Three primer pairs were designed to target the RNA-dependent RNA polymerase genes, the glycoprotein genes and N protein genes in the conserved core region of a novel bunyavirus in China (Table 1). The PCR products were analyzed using 2% agarose gel electrophoresis (Figure 2). Then, the products were sequenced with an ABI Prism 3100 genetic analyzer (Applied Biosystems, Foster City, CA, USA). The mRNA sequence of these genes deposited in GeneBank (genes of the RNA-dependent RNA polymerase accession No. AB678796, genes of glycoprotein accession No. AB678797 and genes of Nproteinaccession No. AB678798). The sequences of these genes PCR products were compared with known sequences of novel bunyavirus in the GenBank database.

The results of PCR product electrophoresis patterns indicated that the products have identical patterns with SFTSV ones, and the sequence analysis demonstrated that the nucleotide sequences of the RNA-dependent RNA polymerase, the glycoprotein and Nprotein DNA fragments from this case had a 96%, 94% and 95% homology to nucleotide sequences of novel Bunyavirus found in blood samples of patients with SFTS in Central and Northeast China, respectively.

3. Discussion

It is known that SFTS needs to be differentiated clinically from human anaplasmosis, hemorrhagic fever with renal syndrome, and leptospirosis. Patients with human anaplasmosis have fever, but leucocytopenia, thrombocytopenia, and gastrointestinal symptoms are infrequent. Leptospirosis may be confused with SFTS because of the initial fever, chills, headache, myalgia, and abdominal pain, but common symptoms of leptospirosis, such as rash and jaundice (*3*), are rare in patients with SFTS. Most phleboviruses are associated with sand flies, and, in such cases, there is evidence of transdermal transmission. SFTSV RNA was detected in some ticks, indicating that ticks may serve as one of the candidate vectors of SFTSV (*2*).

In this case, the patient was an aged farmer who had been living in the hilly areas of Zhoushan Island, a relatively isolated location in Zhejiang province. The patient presented with high fever and thrombocytopenia accompanied by chills and pharyngeal pain. There was no history of tick bites or contacts with individuals who had similar manifestations. The sanitary condition of her house was poor and it was probably infested by rodents. No similar cases were reported in the village where she lived. According to retrospective investigations of the local medical clinic that the patient first visited, there were about 15 similar cases annually in the past decade, and patients were mostly elderly. Despite the lack of laboratory results, these patients may be identified as probable SFTS cases according to their similar clinical features. Tick bite may be one way of transmission (*1*), and reports by Wenyuan Tao (*4*) of six cases of SFTS occurring in a cluster around a single initial SFTSV patient suggest contact transmission may occur. Further research is required to determine whether there are other routes of transmission.

The laboratory tests of this patient showed that white blood cell and platelet counts decreased progressively during the early stage. The platelet count reached a nadir of 12×10^9/L on the 11th day after the onset of the disease. Liver dysfunction and cardiac dysfunction became more evident. It is known that SFTSV can invade heart, liver, kidney, gastrointestinal tract and other vital organs as well as the bloodstream, as indicated by the elevated levels of serum amylase, lipase, blood glucose and the abnormalities in routine urinalysis. This might be the reason for multiple system

organ failure and deaths caused by severe SFTS (*5*). A review of the current case suggests (*6*), rapid loss of both $CD4^+$ and $CD8^+$ T lymphocytes during the acute phase of the severe respiratory syndrome, which might be associated with the damage of cellular immune function. On the 9th day after the onset of illness, this patient's $CD4^+$ and $CD8^+$ T cells decreased. Thus, it was concluded that the decline of $CD4^+$ and $CD8^+$ T cells may be related to the degree of damage to cell-mediated immune function by the SFTSV. More studies are needed to determine the specific mechanism of this phenomenon.

SFTS was previously identified in six provinces of China, which were Henan, Hubei, Shandong, Anhui, Jiangsu and Liaoning, and surveillance for SFTS was carried out outside of these regions. Our finding indicates that SFTSV is the probable cause of a SFTS case outside of the reported endemic regions. Although this first case of SFTSV caused SFTS found in Zhejiang had no travel history to the above endemic regions of SFTSV in China, the patient lived in a wooded and grassy area with lots of chances for tick exposure and was infected with the existing SFTSV. It is most likely that SFTS had not only been prevalent in the above 6 provinces, but also including Zhejiang Province, which is adjacent to Jiangsu and Anhui. Our findings suggest more research is needed to determine the extent to which this disease occurs, especially in the area near the endemic regions.

SFTS as an emerging infectious disease in China is progressive in nature and potentially fatal. The epidemiology, pathogenesis and route of transmission of this disease are still unclear, the clinical manifestations can be systemic and the treatment is challenging. More emphasis should be given to this disease and further training of medical personnel should be carried out to prevent misdiagnosis, especially in the epidemic areas.

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