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Case Report

Viral load and inflammatory cytokine dynamics associated with the prognosis of severe fever with thrombocytopenia syndrome virus infection: An autopsy case☆

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ABSTRACT

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging tick-borne disease caused by a novel bunyavirus. The mechanism underlying disease progression remains unknown, and effective treatment strategy for SFTS is yet to be completely established, making its increasing incidence and subsequent mortality a great concern. Here, we present the autopsy case of a patient with rapidly progressed, fatal SFTS infection. Her viral titer and serum cytokines levels were measured daily and compared with the values of a survivor of the infection. Our findings elucidate the clinical features and pathophysiology of SFTS.

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1. Introduction

Severe fever with thrombocytopenia syndrome virus (SFTSV) was identified for the first time in Central and Northeast China in 2009 [1]. In Japan, SFTS was first reported in 2013, mainly from the western regions [2]. In total, 375 patients with SFTS have been diagnosed, and the mortality rate has decreased from 35.0% in 2013 to 9.8% in 2017 since mild infections could be detected; however, total mortality rate of 16.8% has been reported until August 2018 [3]. Clinical studies have indicated that cytokine storms (production of certain cytokines at high concentrations) are associated with numerous severe viral infections, including SFTS, but evidence combining viral loads and histopathological findings from autopsies remains limited.

Here, we present an autopsy case of an SFTSV-infected patient, which was managed by plasma exchange and steroid pulse therapy

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransaminase; CNS, Central nervous system; CRP, C-reactive protein; CSF, Cerebrospinal fluid; LDH, Lactate dehydrogenase; RT-PCR, Reverse transcriptase-polymerase chain reaction; SFTS, Severe fever with thrombocytopenia syndrome; SFTSV, Severe fever with thrombocytopenia syndrome virus; TNF-α, Tumor necrosis factor alpha.

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albeit without success. Moreover, we present dynamics of viral load and inflammatory cytokine levels during the course of hospitalization. Our report will help clarify the association of disease progression with viral load and inflammation level. These findings can be a basis for further detailed investigation into mechanisms of SFTSV pathogenesis, which can guide establishment of a treatment strategy.

2. Case report

A 77-year-old woman presented to a community hospital with a 6-day history of fever and myalgia. She was transferred to our hospital 10 days after the development of continuous high fever, leukopenia, thrombocytopenia, and consciousness disturbance. On admission, her body temperature was 38.9 °C and she presented frequent diarrhea. Her mental status was mildly diminished (Glasgow coma scale score at 13: E4 V3 M6). Tick bite wounds were noted on the inner surface of right femoral area, and the right inguinal lymph node (1.5 cm) could be palpated in the absence of other skin lesions. Her laboratory findings revealed leukopenia (500/μL); thrombocytopenia (2.2×10^4/μL); and elevated serum levels of alanine aminotransferase (ALT; 117 IU/L), aspartate aminotransferase (AST; 345 IU/L), lactate dehydrogenase (LDH; 994 IU/L), creatine phosphokinase (CPK; 467 IU/mL), creatinine (1.1 mg/dL) and C-reactive protein (CRP; 0.13mg/dL). Her serum levels of total protein (7.2 g/dL), albumin (4.0 g/dL), and total bilirubin (0.5 mg/dL) were normal. Coagulation study revealed prolonged activated partial thromboplastin time (67.9 s) and high D-dimer levels (12.8 μl/mL). The cytokines and chemokines concentration of serially collected serum samples were tested by ELISA. Plain computed tomography of the abdomen revealed no hepatosplenomegaly, and chest X-ray was clear on admission. During initial medical examination, diagnosis of rickettsial infection could not be ruled out, and simultaneous administration of tetracycline and ciprofloxacin with gamma globulin was initiated until reaching a definitive diagnosis. Subsequently, SFTSV infection was confirmed by reverse transcriptase-polymerase chain reaction (RT- PCR) of a serum sample using SFTSV-specific primers. In addition, serum ferritin level was extremely high (19,248 ng/mL), and hemophagocytosis was diagnosed by bone marrow aspiration; treatment with methylprednisolone (1000 mg/day for 3 days) was initiated. On 4th clinical day, plasma exchange was performed to prevent the progression of cytokine storm, but the clinical status became worse. On 5th clinical day, despite repeated plasma exchanges, hemodynamical and oxygenation statuses became unstable, with a bilateral pleural effusion evident on chest X-ray. Her consciousness disturbance became worse, and convulsions suspected of being opsoclonus myoclonus syndrome were noted. However, cerebrospinal fluid (CSF) test was not performed due to progressing thrombocytopenia. Although her white blood cell counts started to improve, platelet counts and AST, ALT, LDH, and CPK levels did not improve (Table 1). Refractory metabolic acidosis was detected, and unfortunately the patient died on 7th day of admission (Fig. 1). A postmortem examination, including autopsy, was performed with the informed consent of the patient's family. In the autopsy, a subcutaneous hemorrhage was observed in the right cervical regions, both sides of the cubital regions, and the left chest region. Hemorrhagic spots were observed in gastrointestinal mucosa and the liver; however, there were no sources of bleeding. The loss of basic lymph nodal architecture, which was replaced by massive necrosis leaving cell debris in the inguinal lymph nodes and the spleen, due to necrotizing lymphadenitis (Fig. 2A and C) was noted. Paraffin-embedded blocks of various tissues (liver, spleen, kidneys, lymph nodes, brain, cardiac muscle, small intestine, colon, and thyroid) were prepared for immunohistochemistry. Numerous SFTSV-positive cells were observed in the right inguinal lymph node (Fig. 2B and D) and the spleen, whereas few SFTSV-positive cells were found in histiocytes but not in the parenchymal cells of each organ. In addition we also detected SFTSV RNA in brain (2.3×10^6 copies/g), cardiac muscle (1.2×10^8 copies/g), kidney (3.6×10^8 copies/g) and CSF (2.5×10^5 copies/g) by RT-PCR. Histopathological findings of myocarditis and encephalitis or encephalopathy were not noted.

Table 1
Changes in laboratory findings during the course of hospitalization.

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
WBCs (/μl)	500	600	4300	10,900	15,300	13,900
Ab-Ly (%)	3	20	8	6	3	0
Platelets ($\times 10^4$/μl)	2.2	1.6	5.4	2.6	3.3	4.2
AST (IU/L)	345	543	671	707	656	1454
ALT (IU/L)	117	155	167	178	139	375
LDH (IU/L)	994	1604	2560	3661	3506	4598
CK (IU/mL)	467	825	789	649	475	1189
CRP (mg/dL)	0.13	0.17	0.10	0.09	0.28	0.19

WBC, white blood cells; Ab-Ly, abnormal lymphocytes; AST, aspartate aminotransaminase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; CK, creatine kinase; CRP, C-reactive protein.

3. Discussion

Here, we described the case of a woman with SFTS and consciousness disturbance managed by plasma exchange and steroid pulse therapy. Some viruses belonging to *phlebovirus* of the family *Bunyaviridae*, e.g., Rift Valley fever virus, are known to cause febrile infections in patients with encephalitis [4], but few reports have that confirmed that SFTS induced encephalitis histopathologically. In a previous report, Kaneko et al. showed results of microscopic examination of the brain tissue in which SFTS-positive cell infiltrations were observed in the vascular lumina but not in parenchymal cells [5]. In our case SFTSV was detected in the CSF however the other reports with autopsies have failed to detect SFTSV in the CSF or brain [6,7], considering that consciousness disturbance may not be induced by direct infection of the CSF or brain by SFTSV but rather be indirectly triggered by SFTSV infection-induced cytokine storm.

During the acute phase of infection, serum levels of multiple proinflammatory cytokines were found to be abnormal in patients with SFTS who occasionally develop hemophagocytic syndrome and multiple organ failure and correlated with disease severity and mortality [8,9]. In our study, only a single severe patient was analyzed, but we were able to determine the important findings of this case. As shown in Fig. 3, interleukin-10 (IL-10) and interferon-gamma (IFN-γ) levels were highest on admission, but they decreased rapidly contrary to the level of granzyme B (gzm B), which was elevated rapidly at day 5 whereas IL-6, tumor necrosis factor-alfa (TNF-α) and interferon inducible protein 10 (IP-10) sustained high. IL-10 inhibits the proinflammatory cytokine release in various infections and plays an important role in the amplification of humoral responses [10,11]. Therefore, this consistent finding of decreased IL-10 levels in this case may impair IL-10-mediated T cell inhibition and neutralize antibody production. IFN-gamma is a cytokine known to inhibit microbial replication, particularly viral replication, indicating that reduced levels of IFN-gamma in our case may damp the antiviral immunity and induce continuous viral replication. Gzm B is one of the key effector molecules in host defense systems against viruses and intracellular bacteria. A previous study demonstrated that gzms-deficient mice resist lipopolysaccharide-induced septic shock [12]. Additionally, cytotoxic T lymphocyte are activated in severe sepsis and express

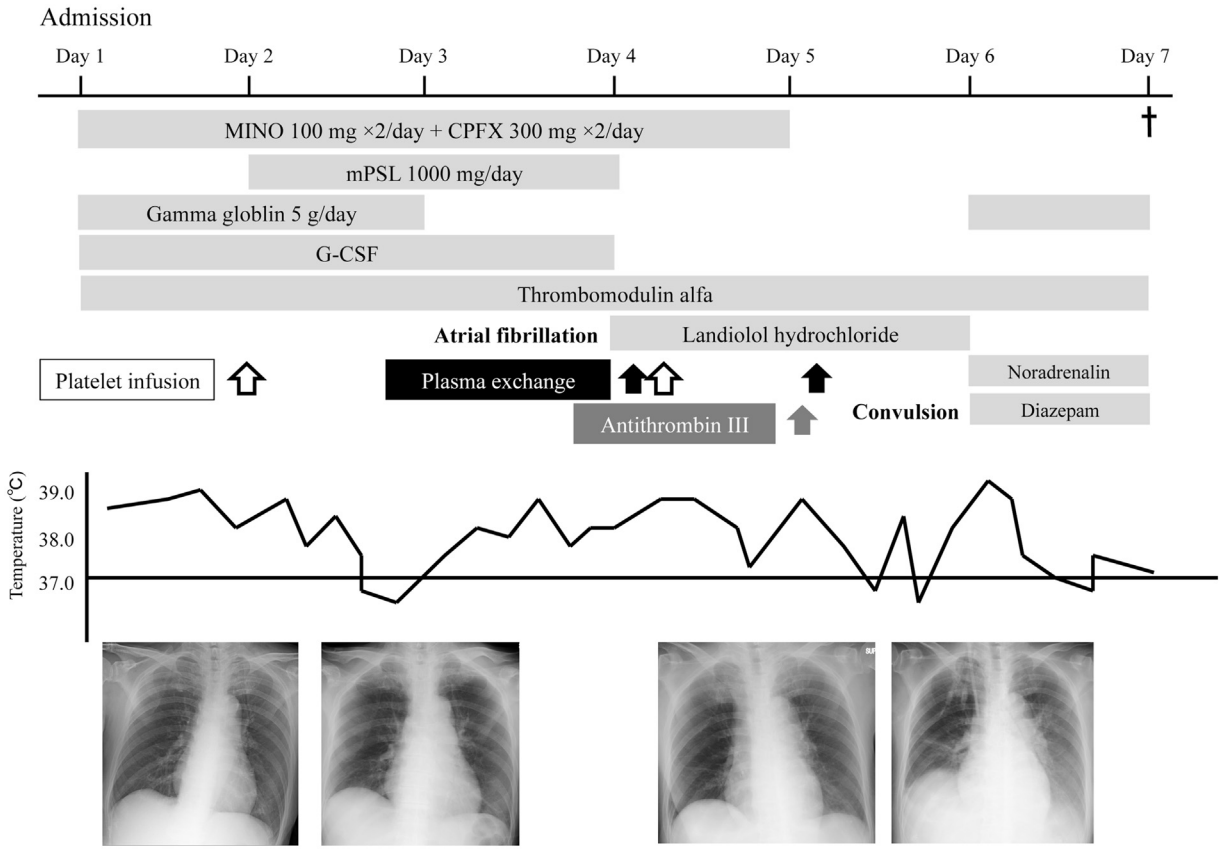

Fig. 1. Clinical course. A 77-year-old woman was admitted on the 6th day of symptom onset. She developed consciousness disturbance, convulsions, and circulatory failure on the 5th clinical day. Methylprednisolone (mPSL, 1000 mg/day for 3 days) and repeated plasma exchange were administered. In addition to supportive care, she received antibiotics, gamma globulin, and recombinant thrombomodulin, but her clinical status became worse rapidly and she died on the 7th day of admission despite the comprehensive treatment.

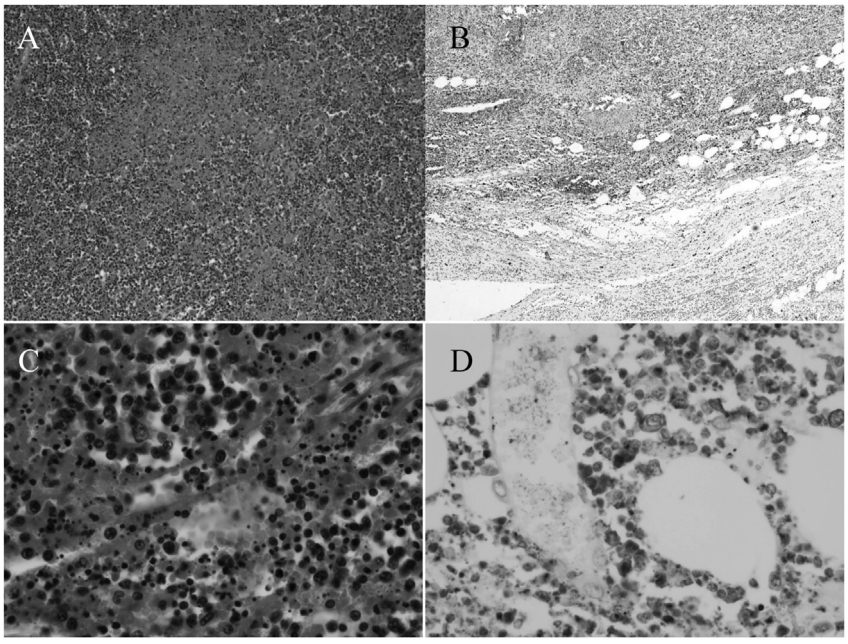

Fig. 2. Histopathological findings of the right inguinal lymph nodes. (A,C) Hematoxylin–Eosin staining showed necrotizing lymphadenitis with loss of the basic lymph nodal architecture and presence of massive necrosis leaving cell debris. A: × 10, C: × 40 (B, D) Immunohistochemistry revealed numerous SFTS-positive cells in the inguinal lymph node. B: × 10, D: × 40.

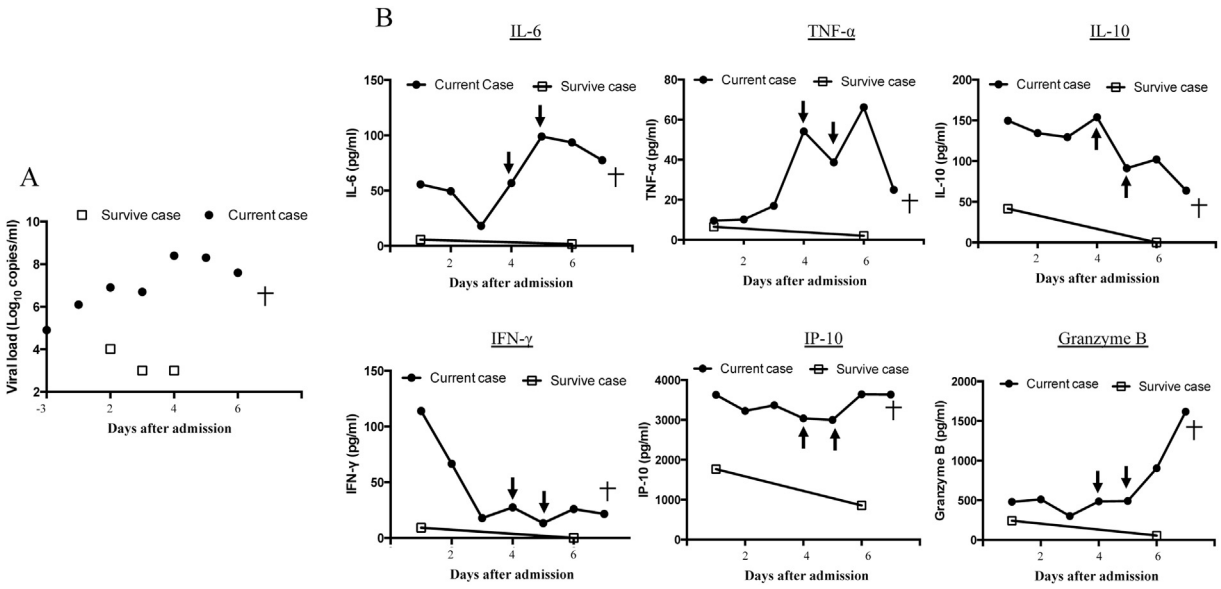

Fig. 3. Dynamics of viral load and inflammatory cytokines during the course of hospitalization. (A) Viral load in the case remained high throughout treatment and until death, but it decreased rapidly in the survivor. (B) Levels of inflammatory cytokines were higher in the case than in the survivor. Levels of TNF-α, IL-6, IP-10, and granzyme B in the case remained elevated despite repeated plasma exchange (arrow).

significantly higher intracellular levels of gzmB and correlated with disease severity [13], suggesting delayed elevation of gzm B may be associated with uncontrolled sepsis and poor prognosis.

In clinical settings, steroid pulse therapy is widely used to suppress excessive cytokine production. A recent study by Nakamura et al. described three successful cases of steroid pulse therapy for SFTSV-induced clinical encephalopathy due to cytokine storm [14]. Although it is difficult to determine whether steroid pulse therapy has any positive effect because of the limited data available, steroid pulse therapy is considered one of the treatment options for hemophagocytic syndrome induced by cytokine storm; thus, it is reasonable that steroid pulse therapy may be beneficial for patients with SFTSV infection.

Recently, the efficacy of plasma exchange has been reported [15,16]. In our case, compared with another survivor of the infection, the viral load was high and did not decline with treatment despite combination treatment with repeated plasma exchange and steroid pulse therapy. This discrepancy in outcomes may be due to individual patients differences (such as presence of mortality risks) and delay in plasma exchange administration as 10 days had elapsed between symptom onset and hospital admission of our case. A study of 24 cases treated with plasma exchange has showed an inverse association between early plasma exchange administration and 30-day mortality [17]. Whether plasma exchange actually improves outcomes is debatable, yet it may be a therapeutic approach against rapidly progressing SFTS.

Finally, appropriate infection control procedures, including both standard and transmission-based precautions should be performed to avoid direct contact of skin with infected tissue or blood. Oral ribavirin prophylaxis can be offered to anyone at high risk of infection, such as those who are directly exposed to the blood of SFTS patients through contact or needlestick injury.

In conclusion, we presented our tracking of daily viral loads of SFTSV and cytokine levels during the course of hospitalization. In addition to supportive care, development of new treatment strategies, such as the use of antiviral agents and/or anti-inflammatory therapy, is imperative, especially for patients at a high risk of mortality.

Conflicts of interest disclosures

None.

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