

Severe fever with thrombocytopenia syndrome: a newly discovered emerging infectious disease

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Abstract

Severe fever with thrombocytopenia syndrome (SFTS) is a newly discovered emerging infectious disease that has recently become epidemic in Asia. The causative agent of SFTS is a novel phlebovirus in the family *Bunyaviridae*, designated SFTS virus (SFTSV). SFTS clinically presents with high fever, thrombocytopenia, leukocytopenia, gastrointestinal disorders, and multi-organ dysfunction, with a high viral load and a high case-fatality rate. In human infection, SFTSV targets macrophages, replicates in the spleen of infected mice, and causes thrombocytopenia and a cytokine storm. The tick disseminates virus to humans and animals, forming a special transmission model in nature. Person-to-person transmission through direct contact with patient blood has been frequently reported. Measurements of viral RNA and antibodies have been established for diagnosis, but vaccines and specific therapeutics are not available so far.

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Introduction

An emerging infectious disease with an unknown agent was noted in 2009 in China; its major characteristics are severe fever, thrombocytopenia, leukocytopenia, gastrointestinal symptoms, and a high case-fatality rate [1]. The disease has been named severe fever with thrombocytopenia syndrome (SFTS) [1–3]. A novel phlebovirus was isolated from acute patients, ticks, and domestic animals, and was considered to be the causal agent of the disease. The newly discovered virus was named SFTS virus (SFTSV), and belongs to the genus *Phlebovirus*, family *Bunyaviridae* [1–3]. Recently, confirmed SFTS cases have been reported from Japan and South Korea [4,5]. Another novel phlebovirus, Heartland virus, was isolated in the USA from two febrile patients with thrombocytopenia and leukocytopenia [6].

Virology

The causative agent of SFTS is SFTSV, which is a tick-borne virus in the family *Bunyaviridae*, genus *Phlebovirus*. The first isolation of SFTSV was from an acute patient's blood in 2010 [1,2]; more strains of SFTSV were isolated later in China, Japan, and Korea [4,5,7,8]. Vero, Vero E6, DH82, L929, THP-1 and U937 cells are susceptible to infection with SFTSV [1,9–11]. As SFTSV is a newly identified member of the family *Bunyaviridae*, its morphology is compatible with that of a bunyavirus. SFTS is a spherical particle with a lipid bilayer envelope and a diameter of ~100 nm; it contains a tripartite single-stranded negative-sense RNA genome [1,12,13]. The genome of SFTSV consists of three segments, designated S (small, 1744 nucleotides), M (medium, 3378 nucleotides), and L (large, 6368 nucleotides), based on the relative nucleotide lengths; it encodes the nucleocapsid protein (N, 246 amino acids) and non-structural protein (NSs, 294 amino acids) by an ambisense strategy, glycoprotein precursor (which eventually matures into the envelop glycoproteins Gn and Gc, of 582 amino acids and 512 amino acids, respectively) through one open reading frame, and viral RNA-dependent RNA polymerase (2084 amino acids) through one open

reading frame. Each segment possesses non-coding regions located at the 3' and 5' termini. The most terminal nucleotide sequences of each segment are highly conserved. Base-pairing of the terminal nucleotides formed panhandle structures that circular RNAs to stabilize viral RNA and originate viral replication [1,12,13]. Intragenic recombination and genomic segment reassortment have been found in SFTSV [14–16]. SFTSV forms plaques in Vero cell culture, and the plaque reduction neutralization test has been used to detect neutralizing antibody. The pseudovirus expressing SFTSV glycoproteins expressed infects human lung, kidney, liver, colon, retinal epithelium, and glioblastoma cell lines, as well as human monocyte-derived dendritic cells, with high efficiency [11]. The glycoproteins of SFTSV use the lectin DC-SIGN as a receptor for host cell entry, and the entry is pH dependent [11,14]. SFTSV-infected human macrophage cell lines THP-1 and U937 showed that the viral nucleocapsid protein co-localized with the Golgi apparatus, and was closely surrounded by endoplasmic reticulum in the perinuclear region, suggesting that the Golgi complex and endoplasmic reticulum are probably the sites for formation and maturation of SFTSV viral particles [10].

SFTSV proteins show 33%, 30–36%, 30–41% and 11–13% similarity to either Rift Valley fever virus (RVFV) or Uukuniemi virus (UUKV), RNA polymerase, glycoproteins, and N and NSs

proteins, respectively. No putative NSm protein has been found in the glycoprotein precursor. SFTSV sequences form a new clade within the genus *Phlebovirus* [17].

Epidemiology

From 2011 to 2014, >3500 SFTS cases were reported in China (Fig. 1), with an average case-fatality rate of 7.8%; the case-fatality rate varied between different regions and years (<http://www.cdpc.chinacdc.cn>). The cases were distributed in 20 provinces of China. Japan and South Korea reported confirmed SFTS cases in 2013 [4,5]. A febrile patient from North Korea working in Dubai presented with SFTS clinical features, but without a laboratory diagnosis [18]. The earliest infection was traced back to 2005 in Japan [4]. In China, the gender ratio of SFTS was 0.94 (male/female); >86% patients were farmers [1–3,13] (<http://www.cdpc.chinacdc.cn>), and most of them lived in wooded and hilly regions for planting of grains or tea. The age composition of SFTS patients varied widely, from 8 months to 93 years; 90.84% of patients were aged 40–79 years (<http://www.cdpc.chinacdc.cn>). A broad seasonal distribution of SFTS was obvious; cases arose from March, and ended around November, with the peak of incidence being in May to July in

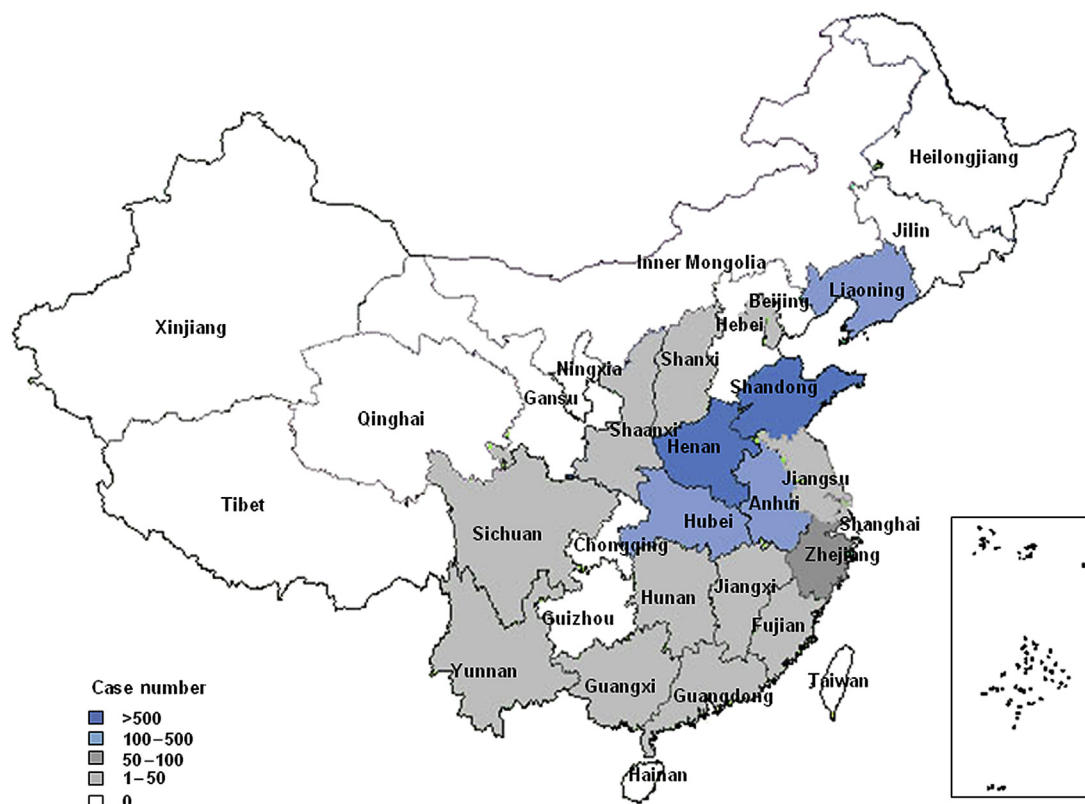


FIG. 1. Geographical distribution of SFTS in China.

China [1,19–21]. The epidemiological characteristics of SFTS matched tick, and not mosquito, activities in endemic areas [1]. Some SFTS patients had a history of being recently tick-bitten. SFTSVs were detected and isolated from *Haemaphysalis longicornis* ticks, including those collected from grass, domestic animals, and SFTS patients [1,8,21,22]. The nucleotide sequences of SFTSVs isolated from ticks showed high homology with those of SFTSVs from patients' blood [8,23,24]. Two SFTSV strains isolated from the ticks removed from the body and blood of an SFTS patient showed 99.7% homology of nucleotide sequences of M segments [24]. *H. longicornis* is distributed in at least 21 provinces of China [25], and is the dominant species in the endemic areas [26]. The above findings suggest that the tick vector plays a major role in the SFTSV life cycle and SFTSV transmission. The situation regarding SFTSV infection of sheep, cattle, dogs, pigs and chicken has been investigated [8,19,22]. It was confirmed that SFTSV is circulating among several species of domesticated animals, with high infection rates in endemic areas of China. In Shandong province, SFTSV infection rates (viral RNA or antibody positive) were as follows: 73.31% (346/472) of sheep, 64.61% (544/842) of cattle, 43.18% (155/359) of dogs, 5.7% (48/839) of pigs, 49.15% (259/527) of chickens [8]. No animal illness or death has been observed [8,22]. Like some arboviruses, such as Venezuelan equine virus and Japanese encephalitis virus, the domesticated animals may act as amplifying hosts for SFTSV, and sheep may be the main hosts in this process. SFTSV most likely circulated between ticks and sheep and other vertebrates, and was transmitted to humans via tick bites [1,13,27–29]. Theoretically, humans can be infected through contact with viraemic animal blood. However, SFTSV infection of domesticated animals results in only transient viraemia, with a low virus load ($1.7\text{--}2.0 \times 10^4$ viral gene copies/mL) [8]. So far, no stockbreeding and slaughtering associated professional infections of SFTS have been reported. Person-to-person transmission of SFTSV has been demonstrated in China [21,30–36]. Of all person-to-person transmission events, index case was fatal with a high virus load in blood. A patient with virus load of 3.55×10^{10} (viral gene copies/mL) in blood was reported [32]. The dying and dead SFTS patients produced blood or bloody secretions from the mouth, nose or vagina, and person-to-person infection happened by contact with the contaminants. The secondary cases were usually family members, relatives, neighbours, medical care workers, and mortuary beauticians [30–36]. All of the secondary cases came into contact with SFTS patients' blood or bloody secretions without effective personal protection [19,29–31]. The risk assessment revealed that direct contact with patients' blood was the major risk factor for person-to-person transmission of SFTSV; there is no evidence suggesting that airborne and faecal–oral transmission play a role in person-to-person transmission [32].

Person-to-person transmission of SFTSV was similar to that of viral haemorrhagic fevers caused by Ebola virus, Marburg virus, and Crimean–Congo haemorrhagic fever virus, and the viruses causing South America haemorrhagic fevers.

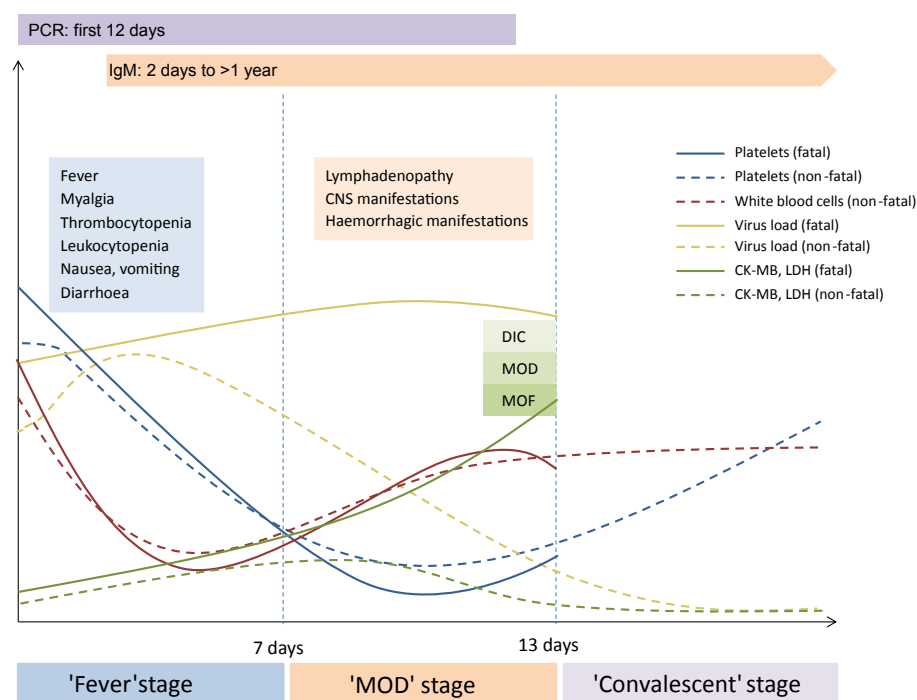
The infection rates of healthy populations ranged from 0.44% to 6.37%, as determined with recombinant NP-based ELISA to detect antibodies [8,21,37,38]. This reflects the fact that latent infection with SFTS varies widely between different endemic regions.

Clinical manifestations, pathology, and pathogenesis

The incubation period of SFTS remains unclear, because most of the patients denied a history of tick bites or did not recall the exact date of tick bites, patients have been tick bitten in 2 weeks. The limited information on person-to-person transmission suggests that the incubation period of SFTS is 5–15 days, and on average 7–13 days [30–32]. Because the infection route and dose of virus were different, the incubation period for person-to-person transmission may not fully reflect that of tick-bitten cases.

SFTS shows a spectrum of severity, from mild, through multi-organ failure, disseminated intravascular coagulation (DIC), and death. The basic clinical manifestations of SFTS are high fever, thrombocytopenia, leukocytopenia, and gastrointestinal disorders; multi-organ failure and DIC occur in severe cases [1,16,39–41]. The clinical course of SFTS can be divided into three overlapping stages: the fever stage, the multi-organ dysfunction stage, and the convalescent stage (Fig. 2). During the fever stage, patients experience 1 week of fever and acute non-specific symptoms. The disease initially presents with an abruptly raised temperature. At the same time, the patients present non-specific manifestations, such as fatigue, headache, myalgia, anorexia, nausea, vomiting, diarrhoea, abdominal pain, lymphadenopathy, thrombocytopenia, and leukocytopenia. The findings of platelet, white blood cell, coagulation, biochemical and other laboratory tests are often abnormal, beginning later in this phase. Serum levels of alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, creatinine kinase and creatinine kinase-MB begin to increase. This is followed by the multi-organ dysfunction stage; during this stage, liver and muscle enzymes are significantly elevated, notable proteinuria and haemorrhage appear, and multi-organ failure and DIC develop in patients with severe SFTS. The survivors enter the convalescent stage, and all of the clinical signs, symptoms and laboratory parameters gradually return to normal [39]. The temperature of most SFTS cases usually exceeds 38°C, and this elevation lasts for 7–10 days; in some patients, the fever

FIG. 2. Clinical course of SFTS. CK, creatinine kinase; CNS, central nervous system; DIC, disseminated intravascular coagulation; LDH, lactate dehydrogenase; MOD, multi-organ dysfunction; MOF, multi-organ failure.



continues for 2 weeks [39,40]. Over 70% of the patients have high fever of $>39^{\circ}\text{C}$ [39,40]. Platelet counts and leukocyte counts of some SFTS patients are reduced to $<50\,000/\text{cm}^3$ and $<4000/\text{cm}^3$, respectively [1,39–41]. The most common lymphadenopathy comprises painful, swollen unilateral sub-inguinal lymph nodes [1]. The central nervous system symptoms include apathy, lethargy, muscular tremor, and convulsions [1,39,40]. Coma frequently occurs in the terminal stage of the disease. Haemorrhagic manifestations consist of petechiae, ecchymosis, injection site haematoma, gingival bleeding, haematemeses, haematuria, and melena [1,39–41]. Haemorrhage in SFTS patients is not usually lethal, with the exception of DIC-associated massive haemorrhage and vital organ haemorrhage, such as pneumorrhagia. Serum levels of alanine aminotransferase, aspartate aminotransferase and lactate dehydrogenase are elevated [1,39–42]. No cytological changes are seen in the bone marrow of SFTS patients, suggesting that the thrombocytopenia and leukopenia of SFTS do not result from damage to haematopoietic cells [43].

The levels of a broad spectrum of serum cytokines, including interleukin (IL)-1RA, IL-6, IL-10, granulocyte colony-stimulating factor, interferon (IFN)- γ -induced protein 10, and monocyte chemoattractant protein-1, are elevated in the acute phase in SFTS patients, and these cytokines are present in higher levels in fatal cases than in non-fatal cases [16,44]. The numbers of CD69^+ T-cells, HLA-DR^+ cells and CTLA4^+ T-cells are elevated. High

SFTSV viral loads are associated with variations in platelet, serum enzymes, and proinflammatory and anti-inflammatory cytokines [44,45].

SFTSV NP was detected in the cytoplasm of blastic cells and necrotic regions in the cortical area of lymph nodes. Severe necrotizing lymphadenitis with massive necrosis, which comprised nuclear debris and eosinophilic ghosts but not granulocytes, was distributed throughout the cortical area; the depletion of small lymphocytes, and severe infiltration of histiocytes and immunoblasts, were observed in the lymph nodes [4]. Two autopsy cases were reported in Japan. The pathognomonic histological feature was necrotizing lymphadenitis of systemic lymphoid tissue with SFTSV and viral RNA [46].

An infectious model of SFTS in C57/BL6 mice was established, and the animals developed symptoms of thrombocytopenia and leukocytopenia. Viral RNA and histopathological changes were identified in the spleen, liver, and kidney. Moreover, the numbers of macrophages and platelets were significantly increased in the spleen, and SFTSV co-localized with platelets in the cytoplasm of macrophages in the red pulp. *In vitro* assays further revealed that SFTSV adhered to platelets and facilitated the phagocytosis of platelets by mouse macrophages. Both of these findings suggest that the host splenic macrophage clearance of virus-bound platelets reduced the number of circulating platelets, causing thrombocytopenia [47]. Lethal infection was induced in α/β -IFN receptor knockout

mice, and all mice died within 3–4 days after subcutaneous inoculation of SFTSV. The virus antigen was found in the liver, intestine, kidney, spleen, lymphoid tissue, and brain, but not in the lungs [48]. In rhesus macaques, SFTSV causes fever, thrombocytopenia, leukocytopenia and increased levels of transaminases and myocardial enzymes in blood, without severe symptoms or death. IgG, IgM and neutralizing antibodies against SFTSV were induced in infected macaques. Levels of the cytokines IFN- γ , eotaxin, tumour necrosis factor- α and macrophage inflammatory protein-1 β were significantly elevated. Minor pathological lesions were observed in the liver and kidney during the late stages of infection [49].

The NSs protein of some phleboviruses suppresses IFN and INF- β promoter activities [50]. The SFTSV NSs protein also plays a major role in immune evasion. NSs was found to be associated with TANK-binding kinase 1, and may inhibit the activation of downstream interferon regulatory factor and nuclear factor- κ B signalling through this interaction [9]. The viral NSs forms inclusion bodies in SFTSV-infected and SFTSV-transfected cells. NSs was able to sequester IKK, interferon regulatory factor 3 and RIG-I signalling proteins into inclusion bodies or virus-induced cytoplasmic structures, resulting in reduced IFN- β induction, suggesting a novel mechanism for SFTSV evasion of innate immunity [51,52].

Diagnosis and treatment

The diagnosis of SFTS based on clinical manifestations has been shown to be unreliable, and should be confirmed by laboratory tests, because the clinical syndrome of SFTS can be confused with Hemorrhagic Fever with Renal Syndrome and some haematonoses.

Isolation of SFTSV is not difficult, but usually takes 1–3 weeks, so virus isolation is not routinely performed for SFTS diagnosis [1,4,5].

Taqman qPCR assays have been established to detect L, M and S segments of SFTSV in the acute-phase serum of patients; the assay sensitivities for sera collected 2–10 days and 11–20 days after the onset of symptoms were 100% and 93.8%, respectively [53]. The S segment-based Taqman qPCR has been licensed in China [53]. The sensitivity and specificity of the assay are 98.6% and 99%, respectively, and the detection limit of the assay is 100 copies/mL or 1 TCID₅₀/mL [53]. A loop-mediated isothermal amplification assay targeting the S segment of SFTSV has been developed; with this assay, the entire procedure was completed within 30 min, and the sensitivity of the assay was 94.4% [54,55]. An RT-cross-priming amplification with a vertical flow visualization strip assay based on the M segment of SFTSV, with a sensitivity and specificity of 94.1% and 100.0%,

respectively, has been developed [56]. A multiplex real-time RT-PCR assay has been developed for simultaneous detection of SFTSV, Hantaan virus, Seoul virus and Dengue virus for patients 2–12 days after the onset of symptoms; the sensitivity was 100% [57]. A qPCR detecting both the Chinese and Japanese clades of SFTSV was established in Japan [58]. A purified recombinant NP-based IgM capture ELISA, an indirect ELISA, a double-sandwich ELISA, an immunochromatography assay [1,8,58,59] and an immunofluorescence assay (IFA) were developed and optimized to determine virus-specific IgM, IgG and total antibodies in sera of human and animals, and used for SFTS diagnosis and surveillance. Indirect IFAs were also established to determine virus-specific antibodies. The results of an ELISA-based method (microtitre) to titre SFTSV were consistent with those obtained by IFA ($R = 0.99$) and plaque-forming ($R = 0.95$) methods [60]. Neutralizing antibody was determined with a plaque reduction neutralization test and a microtitre-based microneutralization assay.

Multiplex detection of viral haemorrhagic fevers, including SFTS, were developed by means of the Luminex technique to detect viral RNAs or antigens [61,62]. The SFTS patient's sera were tested by real-time PCR; 64.3% of the patients were viral RNA positive during the first week after onset, and 37.5% were RNA positive during the second week [63]. Other studies indicated that the viral RNA-positive rates of the patients were 60% (81/135) and 60.61% (20/33) [64,65].

So far, there is no specific treatment for SFTS; supportive and symptomatic treatments are applied routinely. Ribavirin has been used to treat haemorrhagic fevers caused by arenaviruses and bunyaviruses [66,67]. *In vitro*, ribavirin inhibits SFTS replication in cell culture [2,68]. The effectiveness of ribavirin in treating SFTS cases was investigated in a cross-sectional study, but there were no significant differences in case fatality rate, platelet counts and viral load between patients who received ribavirin and those who did not [42,69]. During the acute phase of infection, the serum levels of multiple proinflammatory cytokines were abnormal in patients with SFTS; plasma exchange may decrease the cytokine imbalance. Two severe cases of SFTS were successfully treated with plasma exchange and ribavirin in South Korea.

It is difficult to prevent or control SFTS infection in animals and ticks, as the tick–animal–tick cycle usually goes unnoticed, and the infection in domestic animals is usually not apparent. The development of cell culture-based inactivated SFTS vaccines is in progress in China.

Transparency declaration

The author declares that there is no conflicts of interest.

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