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The world first two cases of severe fever with thrombocytopenia syndrome: An epidemiological study in Nagasaki, Japan

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Abstract

Severe fever with thrombocytopenia syndrome (SFTS) caused by the SFTS virus (SFTSV), a novel phlebovirus belonging to the family Bunyaviridae, was reported in China for the first time in 2009. We observed two cases where the SFTSV was isolated for the first time in Nagasaki, Japan, in 2005. Two males in their 60s, a farmer and a hunter, respectively, living in Nagasaki developed SFTS during the same period. The patients developed similar clinical symptoms and signs, such as fever, loss of consciousness, and multiple organ dysfunction. The farmer died and the hunter survived. A retrospective diagnosis of SFTS was made in 2013, and genetic analysis revealed that the patients were infected with different SFTSV strains. Retrospective analysis of cytokine production in non-fatal case revealed interleukin (IL)-6, IL-8 and interferon-γ level of acute phase was low and could be potential prognostic factors. As there are no epidemiological studies of positive rate of SFTSV antibody in people living in endemic areas in Japan, a field study was performed. Volunteers at high risk for tick bites, such as hunters, farmers, and soldiers, were recruited in 6 regions, including the areas where the SFTS cases occurred. Three hundred and twenty six volunteers in Nagasaki prefecture were examined and none of these tested positive for the SFTSV antibody. Our data indicates that the risk for SFTSV infection is not high in Nagasaki prefecture. Further collection of blood samples from endemic areas is warranted for the prevention of SFTSV infection.

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1. Introduction

An outbreak of 171 cases of severe fever with thrombocytopenia syndrome (SFTS) caused by the SFTS virus (SFTSV), a novel member of the genus phlebovirus in the family Bunyaviridae, was first reported in China in 2009 [1]. A total of 2047 SFTS cases have been reported in China up to the end of 2012 [2]. SFTS cases were also reported in North Korea [3], South Korea [4], and Japan, including our cases [5] in 2009, 2012, and 2013, respectively. A recent phylogenetic study demonstrated that SFTSV isolated from patients in Far East Asia is divided into two clades, which may have evolved separately over time, except for the rare occasion of overseas transmission [6]. This result also suggests that SFTS may have existed without being diagnosed for a long time. We report
evidence comprising 2 cases of SFTS that occurred in Nagasaki, Japan, in 2005 and were diagnosed retrospectively in 2013. The two patients had no history of travel abroad, and the isolated SFTSVs were the types that predominantly occur in Japan according to the genetic analysis [5]. To the best of our knowledge, these represent the first cases of SFTS infection worldwide and precede the cases reported in China. We present these earliest cases of SFTS worldwide, which occurred in same area of the SFTSV infection with analysis of cytokine production during the course.

The crude mortality rate of SFTS syndrome ranges from 10% to 30% and the pathogenesis of this syndrome has been reported. A total of 170 laboratory-confirmed SFTS cases were reported in Western Japan up to 24 February 2016 and, of these, 46 patients are dead (the mortality rate is 27.1%). Haemaphysalis longicornis and Rhipicephalus microplus are the most likely vectors for the transmission SFTSV in China [17]; however, there are no studies regarding the prevalence of SFTSV infection among these ticks in Japan.

Zeng et al. investigated SFTSV sero- and viral prevalence among Chinese blood donors. Only 0.27%–0.54% were positive for the SFTSV antibody, and asymptomatic SFTSV viremia was found in 0.02% of 17 208 samples [8]. The background and past medical history of these patients detecting SFTSV among sero-positive cases are not described in the study. Nine SFTS cases have been reported in Nagasaki, Japan, to date. Currently there are no epidemiological studies on the prevalence of the SFTSV antibody in endemic areas in Japan; therefore, we conducted a field study.

2. Material and methods

2.1. Cytokine measurement in case

Serum samples from two patients with SFTS were stored in a freezer at a temperature of −20 °C from November 2005. Diagnosis was confirmed by SFTSV antibody detection and virus isolation using common methods that have been previously described [5]. Cytokines including interleukin (IL)-6, interferon (IFN)-γ, IL-1 receptor antagonist (RA), macrophage inflammatory protein (MIP)-1α, MIP-1β, and IL-8 in stored serum samples acquired in different timing in Case 2, were measured by standard ELISA method. Due to low volume of serum sample, cytokine production was not measured in Case 1.

2.2. SFTSV antibody titer measurement

For the epidemiological antibody study, volunteers at high risk for tick bites, such as hunters, farmers, and soldiers, were recruited in 6 regions where the documented cases of SFTS had occurred. Written informed consent was obtained from each volunteer along with information including sex, age, medical history, history of the tick-bite, development of symptoms after the tick-bite, and work location. Then, 6 mL of blood samples were collected and antibody levels were measured using an indirect IgG ELISA, with the recombinant SFTSV-N protein, as described previously [9]. Briefly, recombinant SFTSV-N protein and recombinant Rift Valley fever virus nucleocapsid (RVFV-N) protein are used as positive and negative antigens, respectively. Serum samples from one case of confirmed SFTS and from a healthy volunteer were used as positive and negative controls, respectively. A serum sample in this ELISA test was considered positive if the adjusted OD value was greater than or equal to the assay cut-off of 0.2. We adhered to the Japanese ethical guidelines for epidemiological studies, and the ethics committees of Nagasaki University Hospital approved the protocol for this study.

3. Results

3.1. Case reports

The serum samples from two patients with SFTS had been stored at −20 °C since November 2005. Diagnosis was confirmed on the basis of SFTSV detection and antibody detection in Spring, 2013.

3.1.1. Case 1

A 62-year-old farmer who lived in the Northern part of Nagasaki city presented with fever, respiratory, and neurological symptoms. He visited the local hospital (hospital A) on the third day after the onset of fever. There was no past medical history of note. On the fourth day, he developed tremor of the extremities and headaches. He became restless and disoriented on the seventh day. He did not complain of gastrointestinal symptoms during the course. He was admitted to the hospital. On admission, physical examination revealed the following: pulse, 94/minute with sinus rhythm; blood pressure, 130/70 mmHg; body temperature, 38.5 °C; oxygen saturation, 93% (at room atmosphere) and diminished consciousness (Glasgow Coma Scale: E4V3M5). There was no history of a tick bite and no obvious eschar although the patient’s occupation was associated with an increased risk of tick bites. Chest and abdominal radiographs and magnetic resonance imaging (MRI) of the brain revealed no abnormalities. A spinal fluid test showed no increase in the number of cells, an opening pressure of 120 mm H2O, and a closing pressure of 70 mm H2O. Bicytopenia of white blood cells and of platelets, disseminated intravascular coagulation (DIC), liver dysfunction, and renal failure were diagnosed on admission (Table 1). Aseptic encephalomenigitis with multiple organ failure and DIC were suspected, and treatment with electrolyte infusion and nafamostat mesilate were commenced. No antibiotics were administered. The patient developed shock 58 h after admission and died 64 h after admission. (10th day after onset of fever).

3.1.2. Case 2

A 58-year-old hunter with a past history of tick bites, presented with fever 7 days after being bitten by a tick in the mountains of central Nagasaki prefecture during wild boar hunting. He also complained of nausea, vomiting, and diarrhea on the second day after the onset of fever. He was admitted to a local hospital on the third day after the onset of symptoms. He became disoriented and subsequently developed hallucinations and convulsions. He was admitted to our intensive care unit on the sixth day after the infection.
onset of symptoms, and sedation was administered following a convulsion.

Vital signs of the patient on admission were as follows: body temperature 38.8°C; heart rate 62 beats/minute and regular; and blood pressure 128/90 mmHg. On physical examination, there were multiple tick bites on the right thigh, buttocks, and left axillary region. There was no anemia or jaundice. Respiratory, cardiac, and abdominal examination was normal. There was no superficial lymphadenopathy or edema. Neurological examination was not performed owing to a severe convulsion on admission. Bicytopenia of white blood cells and of platelets, DIC, liver dysfunction, and renal failure were diagnosed on admission (Table 1). A spinal fluid test showed no increase in the number of clear cells and cell number 6/3, and an opening pressure of 130 mm H2O and a closing pressure of 90 mm H2O. Chest radiography and computed tomography (CT) of the brain revealed no abnormal findings. Rickettsia infection was suspected and intravenous tetracycline and ciprofloxacin were administered immediately. Although the transaminases declined on the sixth day following the onset of fever, the platelet count decreased and a platelet transfusion was required. Bone marrow aspiration was performed on the fourth day following admission and a hemophagocytic syndrome of unknown etiology was diagnosed. However, steroid treatment was not required because the bicytopenia improved.

On the third day following admission, sedation was reversed. On the following day, painful bilateral lymphadenopathy of the groin was found. On the tenth day after admission, the platelet count returned to normal. The SFTSV viral load and level of cytokine such as IL-6, IFN-γ, IL-1 RA, MIP-1α, MIP-1β, and IL-8 on various days post onset of illness are shown in Table 1. IL-6 levels were elevated until the 10 days after onset of illness but subsequently declined. The level of IFN-γ and IL-8 continued to decrease following admission. The level of IL-1 RA, MIP-1α and MIP-1β were gradually increased until 17 days from onset of illness and remained at almost same level until 41 days.

3.2 Epidemiological study

Three hundred and twenty six volunteers were recruited and blood samples were examined for the SFTS antibody. Subject demographics are shown in Table 3. Subjects were persons who lived in the forest area or entered the ticks’ habitats for specific activities, such as hunting, hiking, forestry, military exercises, or agriculture. Relatives of SFTS cases and some subjects had same activity in the mountain with the patient of Case 2 were also included. Fig. 1 indicates the distribution and number of volunteers in the area. All tests were negative for the SFTS antibody.

4 Discussion

There has been no report of an outbreak of SFTS cases in Japan or familial cases to date. The two cases of SFTS in this report occurred over the same period of time and within a relatively limited geographical area. Interestingly, the patient in Case 2, described above, was referred to the attending physician of the patient in Case 1 and the referral took place on the date of death of the patient in Case 1. The cause of death was recorded as unknown at the time. The attending physician was alerted by the similarity of the clinical presentation and laboratory test results between the two cases and referred the patient to our university hospital for specialized treatment. We suspected an outbreak of a severe transmissible infection of unknown etiology in Nagasaki and requested notification of all similar cases from the local health authorities. However, there was no evidence of multiple cases of SFTS at the time. Retrospective genetic analysis of SFTSV indicated that the two cases were caused by different SFTSV strains [5]. Although SFTS is generally observed in November in Japan, climate conditions may influence the activity of ticks and these two cases occurred at the same period by chance. Interestingly, the serum samples were kept in the freezer for further analysis and attending physicians including us noticed the discovery of novel SFTSV resulting in sending samples to check SFTSV after 8 years after the experience. Impact of clinical manifestations of SFTS was high enough to remind these cases to us as soon as discovery of SFTS and SFTSV were announced worldwide.

Retrospectively, the diagnosis of SFTS for Case 1 still appears problematic. Although the main symptoms were fever, respiratory symptoms, and disturbance of consciousness [1], the common digestive symptoms were not observed. Furthermore, the absence of an obvious tick bite makes the diagnosis of SFTS more difficult. Conversely, the patient in Case 2 presented with the typical symptoms of SFTS such as fever, leukopenia, thrombocytopenia, and digestive symptoms with hematuria and proteinuria. However, jaundice, decrease of urine volume, and edema were not obvious. In 2005, we postulated that these cases were an atypical Rickettsia infection caused by Orientia tsutsugamushi or Rickettsia japonica or other Rickettsia species. In both cases, we identified two of the three signs of typical Rickettsia diseases, such as eschar and fever, but not a rash [10]. The antibody test for Rickettsia was negative and we sent the serum samples to the National Institute of Infectious Diseases in Tokyo, Japan, for further analysis. Differential diagnosis including ehrlichiosis, borrelia infection, tularemia, tick-borne encephalitis, and babesiosis were considered; however, the tests were all negative. While the clinical manifestations and laboratory data of the cases presented here were similar, the outcome was not. According to the definition of multiple organ dysfunction (MOD) and multiple organ failure (MOF) of SFTS by Gai et al. [11], Case 1 and 2 fulfilled 4/7 and 3/7 of MOD factors and both are considered as MOD status. However, only Case 1 fulfilled 2/7 MOF factors during his admission and was considered as having MOF as well. Although the MOF score was negative at the time of admission in Case 1, the patient deteriorated rapidly and died 3 days after admission. Although the incubation period of SFTSV is considered as 7 days, the use of the incubation period as a prognostic factor is controversial. Other factors, including initial SFTSV viral load, may influence the incubation period. A recent study suggested the severity of organ failure [11], age [12], underlying diseases, the SFTS viral load and some cytokine levels on admission [7] as possible prognostic factors. Although pathogenesis of SFTS has not been fully revealed, host cytokine storm is associated with its severity and level of IL-6, IFN-γ, IL-1 RA, MIP-1α, MIP-1β, and IL-8 and others are analyzed in both fatal and non-fatal SFTS cases [13,14]. Additionally, the transition of SFTS viral load and these cytokine levels compared in acute (7-9 days after onset of illness) and convalescent phase (50-150 days after onset of illness) are investigated and some of cytokine levels in acute phase are to be possible indicators of the patient’s outcome [13,14]. Our analysis of stored
serum samples of non-fatal Case 2 revealed that all cytokine levels except MIP-1α on 7 days after onset of illness (acute phase) are below the mean level of those of non-fatal cases previously reported [13]. Cytokine including IL-6, IL-8 and IFN-γ indicated remarkable reduction as the patient recovered suggesting that these cytokine levels could be potential prognostic factors. Although previous analysis indicated that IL-1RA decreased from acute to convalescent phase and both of MIP-1α and MIP-1β increased from acute to convalescent phase, no data of cytokine production between acute and convalescent phase are available. Our data indicated all of IL-1 RA, MIP-1α, and MIP-1β production increased between 10 and 17 days after onset of illness and remained almost same level between 17 and 41 days after onset of illness. Since this data is resulted from single case, it is impossible to draw conclusion. Further accumulation of data during this period is warranted.

The antibody test for SFTSV was negative in our epidemiological study. Zeng et al. reported only 0.27%–0.54% of 17,208 were positive for the SFTSV antibody in China [8]. There was a possibility that one or two sero-positive samples was identified if we adapted this
specific rate of China in our study. The chance to identify the sero-positive samples was speculated even higher in our study, since we attempted to collect samples from individuals with a relatively higher risk of tick bites and actually 51% of volunteers had apparent tick-bite history. Whereas samples were collected from whole blood and apheresis donors during donation process in China and tick-bite history is unknown.

Interestingly, one SFTS case was identified on a small island with a population of approximately 200 (largely representing an elderly population) (Fig. 1). Total of 45 volunteers (12 male and 33 female, average age 69.3 years old) were recruited to measure the SFTSV antibody titer on this island, and no one tested positive. Eight of 45 volunteers (17.8%) possessed apparent tick-bite history and 18 female were housewives. Although other epidemiological studies investigating the SFTSV harboring rate in ticks on this island has not been revealed thus far, the chance of being infected with SFTSV is relatively rare.

A limitation of this epidemiological study is the small number of cases examined; however, these data have never been reported in Japan. Our data indicate that the risk of being infected with SFTSV is not high. The accumulation of patients blood samples from endemic areas is warranted in the future for the prevention of SFTSV infection.

Conflict of interests

No authors declare the conflict of interests in this study.

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