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Research note

# Severe fever with thrombocytopenia syndrome-associated encephalopathy/encephalitis

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ABSTRACT

*Objectives:* Severe fever with thrombocytopenia syndrome (SFTS) virus has a variety of central nervous system (CNS) manifestations. However, there are limited data regarding SFTS-associated encephalopathy/encephalitis (SFTSAE) and its mechanism.
*Methods:* All patients with confirmed SFTS who underwent cerebrospinal fluid (CSF) examination due to suspected acute encephalopathy were enrolled in three referral hospitals between January 2013 and October 2016. Real-time RT-PCR for SFTS virus and chemokine/cytokines levels from blood and CSF were analysed.
*Results:* Of 41 patients with confirmed SFTS by RT-PCR for SFTS virus using blood samples, 14 (34%) underwent CSF examination due to suspected SFTSAE. All 14 patients with SFTSE revealed normal protein and glucose levels in CSF, and CSF pleocytosis was uncommon (29%, 4/14). Of the eight patients whose CSF was available for further analysis, six (75%) yielded positive results for SFTS virus. Monocyte chemoattractant protein-1 (MCP-1) and interleukin-8 (IL-8) level in CSF were significantly higher than those in serum (geometric mean 1889 pg/mL in CSF versus 264 pg/mL in serum for MCP-1, $p = 0.01$, and geometric mean 340 pg/mL in CSF versus 71 pg/mL in serum for IL-8, $p = 0.004$).
*Conclusions:* The CNS manifestation of SFTS as acute encephalopathy/encephalitis is a common complication of SFTS. Although meningeal inflammation was infrequent in patients with SFTSAE, SFTS virus was frequently detected in CSF with elevated MCP-1 and IL-8. These findings indicate that possible direct invasion of the CNS by SFTS virus with the associated elevated cytokine levels in CSF may play an important role in the pathogenesis of SFTSAE. **S.Y. Park, Clin Microbiol Infect 2018;24:432.e1–432.e4**


## Introduction

Severe fever with thrombocytopenia syndrome (SFTS) has various central nervous system (CNS) manifestations such as headache, confusion and seizure [1–4]. It is important to determine the detailed features and mechanisms underlying the CNS manifestations of SFTS. However, to date, there are few reports on CNS complications with detailed CNS examination profiles or a possible pathogenesis [1,5,6]. We, therefore, investigated the clinical, virological and immunological markers (chemokines/cytokines in plasma and cerebrospinal fluid (CSF)) in patients with SFTS in whom acute encephalopathy was suspected.

## Materials and methods

We retrospectively reviewed the medical records of patients with confirmed SFTS who underwent CSF examination at the discretion of attending physicians in three referral hospitals between 2013 (the

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year in which the first confirmed case of SFTS was reported in Korea [7]) and 2014. In addition, we prospectively enrolled patients with confirmed SFTS who underwent CSF examination for suspected acute encephalopathy (any diffuse cerebral dysfunction without associated inflammation) or encephalitis (fever, headache, peripheral leucocytosis associated with meningeal inflammation) in the same hospitals from 2015 to 2016 (Supplemental material, Fig. S1). We used the criteria of acute encephalopathy/encephalitis to trigger CSF examination since 2015, i.e. the presence of decreased consciousness, seizures, altered mental status, focal neurological signs, or intractable headache. This study was approved by the Institutional Review Board of our hospital. All prospective participants were informed of the nature of the study and provided their written informed consent before enrolment. However, informed consent was waived for participants who were enrolled retrospectively. We used a BD™ Cytometric Bead Array containing fluorescent microspheres coated with antibodies specific for each soluble protein to measure the levels of 18 cytokines/chemokines in CSF and plasma samples according to the manufacturer's instructions (BD Biosciences, San Jose, CA, USA). To measure SFTS virus (SFTSV) load in CSF and plasma, total viral RNA was extracted from the CSF and plasma using a QIAamp® Viral RNA Mini Kit (Qiagen Inc., Chatsworth, CA, USA). SFTSV load was measured by one-step multiplex real-time RT-PCR (see Supplementary material, Appendix S1 and Fig. S2).

## Results

During the study period, we identified 41 individuals (14 retrospectively, between 2013 and 2014, and 27 prospectively, between 2015 and 2016) with confirmed SFTS in three referral hospitals. A total of 31 (76%) patients had symptoms or signs of CNS manifestations. Of these 31 individuals, 17 had transient or tolerable headache ($n = 10$), or rapidly fatal disease ($n = 7$) and so did not undergo CSF examination and were excluded, and the remaining 14 (34%) who underwent CSF examination were analysed (see Supplementary material, Fig. S1). The clinical manifestations that led to CSF examination were altered mental status ($n = 11$), seizure ($n = 2$) and intractable persistent headache ($n = 1$). The clinical characteristics of the 14 patients with SFTS-associated encephalopathy/encephalitis (SFTSAE) are shown in the Supplementary material (Table S1). The median duration of illness was 5 days (range: 3–7 days). CSF examination was performed within a median of 3 days after hospital admission (range 1–7 days). One patient had a neurological complication of gait disturbance (case 7). The in-hospital mortality rate (overall mortality during the hospital stay) was 21% (3/14). The median duration of follow up of the 11 survivors was 2 months (interquartile range 1–4 months).

Detailed CSF findings in the 14 patients who underwent CSF examination are shown in Table 1. Most patients (10/14, 71%) had no pleocytosis (<5 cells/mm$^3$). The median CSF protein level was 57.3 mg/dL (range 24–151.4 mg/dL, reference range 9–58 mg/dL). The median CSF-to-blood glucose ratio was 0.6 (range 0.4–0.8, reference range 0.5–0.8). Brain imaging was performed in nine patients. No newly developed focal lesions were found in the brain parenchyma.

Measurements regarding SFTSV in CSF samples were performed in eight patients. Of these eight patients, six (75%) showed positive PCR results for SFTSV. The median SFTSV loads were $5.1 \times 10^3$ copies/mL (range $2.0 \times 10^3$ to $6.0 \times 10^6$ copies/mL) in CSF and $3.0 \times 10^6$ copies/mL (range $1.6 \times 10^6$ to $3.2 \times 10^{11}$ copies/mL) in serum.

We performed chemokine/cytokine analyses on the plasma and CSF of six patients (patients 8, 9, 10, 11, 12 and 14) whose paired plasma and CSF samples were available for further analyses. Plasma samples from ten healthy volunteers were used as controls. The CSF

**Table 1**
Initial clinical, cerebrospinal fluid, image findings and outcomes in patients with severe fever with thrombocytopenia syndrome-associated encephalopathy/encephalitis

| Case no. | Age/ Sex | Neurological findings | Duration of illness (days) | Serum SFTSV[a] load (log copies/mL) | CSF SFTSV load (log copies/mL) | CSF findings | | | Outcome |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Opening pressure, cm $H_2O$ | WBC (/mm$^3$) | RBC (/mm$^3$) | |
| 1 | 80/ M | Headache, altered mental status | 3 | NA | ND | 17 | 0 | 8 | Death |
| 2 | 59/ M | Headache, altered mental status, disorientation | 3 | NA | ND | NA | 0 | 0 | Alive |
| 3 | 59/F | Headache, vomiting | 7 | NA | ND | 8 | 0 | 180 | Alive |
| 4 | 66/ M | Altered mental status, tremor, disorientation, dysarthria | 5 | NA | ND | 20 | 2 | 50 | Alive |
| 5 | 56/ M | Altered mental status, seizure | 5 | NA | ND | NA | 207 | 148 | Alive |
| 6 | 68/F | Headache, altered mental status | 5 | NA | ND | 40 | 8 | 366 | Death |
| 7-1 | 61/F | Headache, altered mental status, disorientation | 5 | 8.48 | ND | 15 | 1 | 13 | Alive[b] |
| 7-2 | | | | | 6.78 | NA | 0 | 480 | |
| 8 | 63/ M | Headache, altered mental status, disorientation, dysarthria | 7 | 6.18 | None | 7 | 2 | 1 | Alive |
| 9 | 64/ M | Headache, altered mental status, disorientation, dysarthria | 5 | 7.44 | 3.65 | 16 | 5 | 1 | Alive |
| 10 | 68/ M | Altered mental status, tremor, disorientation, dysarthria | 7 | 7.26 | 3.85 | 11.5 | 30 | 30 | Alive |
| 11 | 66/ M | Headache, altered mental status | 5 | 11.50 | 3.76 | 20 | 1 | 0 | Death |
| 12 | 69/ M | Headache, seizure | 6 | 7.07 | 4.86 | 15.5 | 0 | 0 | Alive |
| 13 | 59/ M | Headache, altered mental status, disorientation | 3 | 6.97 | None | 14 | 1 | 1 | Alive |
| 14-1 | 66/ M | Altered mental status, disorientation, dysarthria | 6 | 7.43 | 3.30 | 18 | 4 | 4000 | Alive |
| 14-2 | | | | | | NA | 0 | 160 | |

Abbreviations: CSF, cerebrospinal fluid; NA, not available; ND, not done; RBC, red blood cells; SFTSV, severe fever with thrombocytopenia syndrome virus; WBC, white blood cells.

[a] Peak SFTSV loads during the course of admission.

[b] The patient had sequelae of gait disturbance.

and plasma samples were collected simultaneously in four patients and within 1 day in two patients. The median time interval between the onset of symptoms and CSF sample collection was 8 days (range: 6–10 days). The detailed data on chemokine/cytokine levels are shown in the Supplementary material (Fig. S3 and Table S2). The median ratios of CSF-to-serum chemokine/cytokine concentrations, in descending order, were as follows: monocyte chemoattractant protein-1 (MCP-1) (7.1), interleukin-8 (IL-8) (4.6), and interferon-γ (IFN-γ)-induced protein 10 (IP-10) (1.4) (see Supplementary material, Fig. S4). MCP-1 and IL-8 levels were significantly higher in CSF than in serum (geometric mean 1889 pg/mL in CSF versus 264 pg/mL in serum for MCP-1, $p = 0.01$, and geometric mean 340 pg/mL in CSF versus 71 pg/mL in serum for IL-8, $p = 0.004$).

An electroencephalogram was performed in nine patients, data from which are shown in the Supplementary material (Table S3). Most patients (8/9, 89%) had a slow (delta-to-theta) background rhythm, which is a common feature of encephalopathy. One patient (case 7) showed epileptiform discharge in the left frontal area for 4 days.

## Discussion

The diagnosis of SFTS-associated encephalopathy or encephalitis was usually made on clinical grounds. However, there are few data on the CSF profiles of patients with CNS manifestations of SFTS. Cui *et al.* reported that 103 (19%) of 538 patients with SFTS developed encephalitis, and found evidence of SFTSV by viral isolation in the CSF of one of two patients with SFTS; however, the authors did not make any mention of CSF pleocytosis [5]. Deng *et al.* reported that 15 (13%) of 115 patients with SFTS met the case definition for suspected encephalitis [1]. They also showed that 7 of 11 patients revealed no or little pleocytosis in their CSF, and all 11 patients exhibited normal protein and glucose levels in their CSF [1]. However, they did not present any straightforward evidence of CNS invasion by SFTSV such as viral isolation or detection of the viral genome by RT-PCR in CSF or brain tissue [1]. Our analysis of the CSF profiles of 14 patients confirmed to have SFTS showed that most of the patients who had altered mental status did not have pleocytosis, and had normal CSF levels of protein and glucose, which favours encephalopathy, whereas the minority of such patients had CSF pleocytosis, which favours encephalitis. Our study demonstrated the presence of SFTSV nucleic acid in CSF from 75% (six out of eight) of SFTS patients with CNS complication. However, a positive CSF PCR result does not necessarily indicate true CNS infection related with direct viral invasion because of a break-down of the blood–brain barrier, contamination of the CSF with blood, or the presence of bystander infected leucocytes [8]. Therefore, further studies on the demonstration of direct viral invasion by showing viral particles by electron microscopy, or culture isolation of virus are needed. Several studies have suggested that serum cytokines as well as high viral loads of SFTSV are associated with disease severity in patients with SFTS [3,5,9]. However, to date, no study has evaluated both CSF and serum cytokine levels in patients with SFTS; we found that the CSF cytokines MCP-1 and IL-8 were elevated compared with those in serum in all six patients with SFTSAE. Our findings are consistent with the results of a previous study on influenza-associated acute encephalopathy, which showed that the levels of IL-8 and MCP-1 were three times higher in CSF than in plasma [10]. Collectively, the pathogenesis of CNS manifestations in SFTS is unknown, but our data suggest that the possible direct invasion of SFTS virus into CNS associated with hypercytokinaemia may play important roles in the pathophysiology of SFTSAE.

Among cytokines and chemokines, IFN-α, IL-6, IL-8, IL-10, MCP-1, macrophage inflammatory protein-1 and IP-10 levels in plasma specimens were high. Virus-infected cells release type I IFN such as IFN-α causing nearby cells to heighten their antiviral defence [11]. Hence, our finding of high levels of IFN-α in patients with SFTS indicates that IFN-α might be involved in the control of SFTSV. Proinflammatory cytokines such as IL-6, and chemokines such as IP-10, IL-8 and MCP-1 were elevated, showing that cytokine storm plays an important role in the pathophysiology of SFTS. Interleukin-10 inhibits the release of proinflammatory cytokines in various infections [12,13]. However, IL-10 may play an important role in the amplification of humoral responses by inducing activated B cells to secret large quantities of IgG, IgA and IgM [14]. Therefore, this finding of high IL-10 level emphasizes the importance of the balance between IL-10-mediated T-cell inhibition and IL-10-mediated B-cell activation. Interleukin-8, or CXCL8, is a chemokine produced by macrophages and other cell types such as epithelial cells, airway smooth muscles and endothelial cells. Regarding SFTSV, splenic macrophages have been shown to harbour infected viruses in a mouse model [15]. This study implies that monocytes/macrophages may support persistent SFTSV infection. Therefore, it is possible that SFTSV tropism to monocytes/macrophages might be associated with elevated IL-8 in CSF. In addition, MCP-1 is one of the key chemokines that regulate the migration and infiltration of monocytes/macrophages [16]. Therefore, the tropism of SFTSV to monocytes/macrophages might partially contribute to the elevated MCP-1 in CSF. Further studies are needed in this area.

Among the *Bunyaviridae* family, the *Orthobunyavirus* (La Crosse virus and Rift Valley fever virus) and *Phlebovirus* (Toscana virus) have been known as neurotropic viruses [17]. However, the mechanisms underlying CNS manifestations in bunyavirus infection are not known. In a mouse model, another widely distributed tick-borne virus within the family *Bunyaviridae*, Crimean–Congo haemorrhagic fever virus, which is a member of the genus *Nairovirus*, was found to infect the brain [18]. However, there are limited data on the CNS manifestations of Crimean–Congo haemorrhagic fever. Further studies are needed on the CNS manifestations of other bunyaviruses and the underlying immunopathological mechanisms such as viral replication and cytokine concentrations in CSF.

There are some limitations to our study. First, most of the patients in this study were male and more than 50 years of age. Previous studies, however, revealed that the median age of SFTS patients was around 60 years [5,19,20]. In addition, some studies reported a male predominance among SFTS patients [5,19], whereas another showed no gender predominance [21]. Therefore, cautious interpretation is needed because our study cohort did not represent the typical patient cohort seen in other studies. However, it is not known whether CNS complications in patients with SFTS may have gender or age preference. Further studies are needed in this regard. Second, we did not investigate the differences in cytokine/chemokine expression of early versus late samples due to the limited range of sampling time. We only performed cytokine/chemokine analysis on plasma and CSF samples taken during the early admission period. Further studies are needed on the late admission period, as well as a comparative analysis between early and late hospital periods. Third, analyses on SFTSV or chemokine/cytokines in CSF were performed only in the patients with SFTSAE, which might introduce a certain kind of selection bias. Hence, in the study design CSF samples for further analysis were only available during the prospective study period of between 2015 and 2016. Therefore, it is unlikely that our findings were significantly affected by selection bias.

In conclusion, we presented 14 patients with SFTSAE with a detailed profile of their CSF analyses, imaging and electroencephalogram findings. Although meningeal inflammation was infrequent among patients with SFTSAE, our data show that the possible direct invasion of SFTSV and cytokines such as MCP-1 and IL-8 may

play a role in the pathogenesis of CNS complications in SFTS. These findings provide a further understanding of SFTSAE and might help in developing therapeutic targets for the management of SFTSAE.

## Transparency declaration

The authors declare no conflict of interest.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.cmi.2017.09.002.

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