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Kinetics of viral load and cytokines in severe fever with thrombocytopenia syndrome

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ABSTRACT

Background: Severe fever with thrombocytopenia syndrome (SFTS) is an emerging infectious disease in China, Japan, and Korea, which is characterized by high fever, thrombocytopenia, and high mortality. It is hypothesized that a cytokine storm plays an important role in the pathophysiology of SFTS. However, limited data have been published on the detailed kinetics of the viral load and cytokine profiles throughout the course of this disease.
Objectives: We investigated the patterns of changes in cytokines and viral load in SFTS patients.
Study design: During the admission period of patients, RNA was extracted from plasma and quantified by reverse transcription polymerase chain reaction. In addition, cytokine bead arrays were performed for the 18 cytokines and chemokines selected for testing.
Results: The median time from admission to the negative conversion of SFTS viremia was 17.0 days. When censored patients were found to be negative for viral load at discharge, the median duration of viral shedding was 13.0 days (95% CI, 5.4–20.6). Interferon (IFN)-α, interleukin (IL)-10, and IFN-γ-induced protein (IP)-10 concentrations significantly increased in the early course of disease and then decreased during the hospital stay. However, the concentrations of tumor necrosis factor-α, IL-1β, IL-12p40, IL-13, IL-17A, Regulated on Activation and Normally T-cell Expressed and Secreted (RANTES), and vascular endothelial growth factor (VEGF) increased during the late course of disease. Initial IP-10 levels during hospital days 1–4 were the most significantly correlated with initial viral load ($r = 0.88$, $P < .01$).
Conclusion: SFTS viremia persisted until weeks 2–3 and was highly correlated with initial plasma IP-10 levels. In addition, IFN-α, IL-10, and IP-10 were associated with the initial cytokine storm in SFTS.

1. Background

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging infectious viral disease in China, Japan, and Korea. The SFTS virus (SFTSV) belongs to the genus *Phlebovirus* of the family Bunyavirus and was first identified by a group of Chinese medical researchers [1]. The major clinical signs and symptoms of SFTS are fever, abdominal pain, vomiting, nausea, thrombocytopenia, and leukocytopenia. Multiple organ failure can sometimes occur, with a mortality rate of 6–30% [1–3]. A previous study that used a pathogenic mouse model of SFTS reported that the viral RNA was detected and enriched primarily in the spleen during the acute phase of the disease. The study also suggested that because the virus was attached to the surface of the platelet and was detected in the cytosol of macrophages, macrophages may phagocytose SFTSV-attached platelets [4]. In an IFN-α/β knock-out mouse model, dendritic cells, megakaryocytes, monocytes, macrophages, neutrophils, and endothelial cells were not targeted by the virus; rather, reticular cells in the lymphoid tissues of the intestine and spleen were the major target cells [5]. In humans, however, the pathogenesis of the disease is poorly understood due to insufficient data.

2. Objectives

It has been hypothesized that the cytokine storm plays an important role in the pathophysiology of SFTS [6–9]. However, limited data have been published on the detailed kinetics of viral load and cytokine

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profiles concerning the course of disease [3,10,11]. For this reason, we investigated the patterns of changes in cytokines and viral loads in SFTS patients during the course of disease.

3. Study design

3.1. Patients and clinical samples

From July 2015 to October 2016, we prospectively enrolled 11 patients with SFTS in Asan Medical Center. SFTSV infection was confirmed by using positive real-time reverse transcription polymerase chain reaction (RT-PCR) for the detection of viral RNA. Plasma samples were obtained from patients during their hospitalization period and placed in an EDTA-treated collection tube. Samples were immediately frozen at −80 °C until further analysis. Plasma samples from 10 healthy volunteers were analyzed as healthy controls for cytokine analysis. The study protocol was approved by the Institutional Review Board of Asan Medical Center.

3.2. Quantification of viral RNA

The viral load of SFTSV was measured using one-step multiplex real-time RT-PCR. For detection, RNA was extracted from plasma samples using Qiagen RNeasy Mini Kit (Qiagen, Hilden, Germany). To detect SFTSV, segment S and M genes were quantified, and human beta actin gene was used as an internal control. The sequences of primers and probes used in this study were as follows: SF 5′-CGAGAGAGCTGGCCT ATGAA-3′, SR 5′-TTCCCTGATGCCTTGACGAT-3′, SP 5′-FAM-TGTCTTT GCCCTGACTCGAGGCA-BHQ1-3′, MF 5′-ATGCTTGTCGTGAAGAA GGC-3′, MR 5′-CTAGACTTCCCACTGCCACA-3′, MP 5′-Cy5-ACTTTTGA TGGATACGTAGGCTGGGGC-BHQ2-3′, BAF 5′-ACTAACACTGGCTCGT GTGA-3′, BAR 5′-CTTGGGATGGGGAGTCTGTT-3′, and BAP 5′-HEX-AGGCTGGTGTAAAGCGGCCTTGG-BHQ1-3′. The reaction mixture was prepared with the LightCycler Multiplex RNA Virus Master (Roche Diagnostics, Indianapolis, IN), and real-time RT-PCR was conducted using the LightCycler 96 System (Roche Diagnostics, Indianapolis, IN) according to the manufacturer's instructions with slight modification. SFTSV RNA copy number was determined based on a standard curve made from Ct values of *in vitro* transcript RNA. The limit of quantitative PCR reaction was 4.3 copies/μL of plasma samples.

3.3. Measurement of plasma cytokines

We measured the 18 cytokine and chemokine levels in plasma samples of 11 patients and 10 healthy volunteers simultaneously by cytometric bead array based on microspheres for detecting cytokine/chemokine in accordance with the manufacturer's instructions (BD Biosciences, San Jose, CA). We measured granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF), interferon (IFN)-α, IFN-γ, tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, IL-8, IL-10, IL-12p40, IL-13, IL-17A, monocyte chemotactic protein (MCP)-1, macrophage inflammatory protein (MIP)-1α, MIP-1β, regulated on activation and normally T-cell expressed and secreted (RANTES), IFN-γ-induced protein (IP)-10, and vascular endothelial growth factor (VEGF). Data were acquired using the FACS CANTO II flow cytometer, FACSDiva software (BD Biosciences, San Jose, CA), and FlowJo software (FlowJo LLC, Ashland, OR).

3.4. Statistical analysis

Statistical analyses were performed using GraphPad Prism 5.01 (GraphPad Software, Inc., La Jolla, CA) and SPSS for Windows software package, version 23 (SPSS Inc., Chicago, IL). We classified disease progression as follows: hospital days 1–4, 5–10, 11–16, and 17–24. Statistical differences among periods were analyzed using the Kruskal–Wallis test. The viral load of the survivors and non-survivor were compared using the Mann–Whitney *U* test. The Spearman test was used to calculate the correlation coefficient between cytokine/chemokine levels and viral RNA load. The median time from admission to negative conversion of viremia was analyzed using Kaplan–Meier survival analysis. All tests of significance were two-tailed, and *p* values less than .05 were considered statistically significant.

Table 1
Baseline clinical characteristics of the 11 patients with SFTS.

	SFTS (n = 11)
Age mean years ± SD	60 ± 7
Male sex	8 (73)
Season (months)	
Spring–summer (3–8)	4 (36)
Fall (9–11)	7 (64)
Eschar	3 (27)
Clinical characteristics	
Fever	11 (100)
Skin rash	0 (0)
Headache	5 (45)
Altered mental status	7 (64)
Underlying disease	
Previous healthy	3 (27)
Diabetes	2 (18)
Solid tumor	0
Chronic liver disease	0
Chronic kidney disease	0
Immunosuppressive condition	0
Leukocytosis (WBC > 10,000/mm^3)	0
Leukopenia (WBC < 4000/mm^3)	10 (91)
Thrombocytopenia (platelet, < 150 × 10^3/mm^3)	11 (100)
Normal CRP (≤1.0 mg/dL)	10 (91)
Renal dysfunction	4 (36)
Clinical course	
ICU admission	6 (55)
In-hospital mortality	1 (9)
Treatment	
Doxycycline	9 (82)
Ribavirin	8 (73)

Abbreviations: *WBC*, white blood cell; *CRP*, C-reactive protein; *ICU*, intensive care unit; *SFTS*, severe fever with thrombocytopenia syndrome; *SD*, standard deviation. ***Note:*** Data are no. (%) of patients, unless otherwise indicated.

4. Results

4.1. Clinical characteristics of the patients

A total of 11 patients with SFTS confirmed by SFTSV-specific RT-PCR testing of plasma specimens were enrolled in this study. Among these patients, eight were men, and the mean age (± standard deviation) of the group was 60 (± 7) years. No underlying disease was present in three of the 11 SFTS patients. Four cases occurred in spring to summer, and seven cases occurred in fall. All patients had fever and thrombocytopenia, and seven patients showed an altered mental status. Ten patients survived and one died during the course of hospitalization. The detailed clinical characteristics of these patients are shown in Table 1. The mean age (± standard deviation) of the 10 control subjects was 22.5 (± 0.9) years, and 8 of 10 were male.

4.2. Viral load kinetics

The detailed viral load kinetics of each patient are shown in Fig. 1A. The initial viral load was measured at the day of admission in plasma specimens from 10 patients, including nine survivors and one non-survivor. In the nine survivors, the mean initial viral load was 3.70 log copies/μL (± 1.03), and the viral RNA level gradually decreased over the course of disease. However, the initial viral load was 6.51 log copies/μL, and it increased up to the time of death in the non-survivor