

Original Article



The Evaluation of Surrogate Laboratory Parameters for Predicting the Trend of Viral Loads in Patients with Severe Fever with Thrombocytopenia Syndrome: Cross-Correlation Analysis of Time Series

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ABSTRACT


Background: There is a correlation between the severe fever with thrombocytopenia syndrome (SFTS) viral load and disease severity; however, measurement of viral load is difficult in general laboratory and it takes time to obtain a viral load value. Here, the laboratory parameters for predicting the dynamic changes in SFTS viral load were identified. In addition, we tried to evaluate a specific time point for the early determination of clinical deterioration using dynamic change of laboratory parameters.

Materials and Methods: This observational study included SFTS patients in Korea (2013 - 2020). Cross-correlation analysis at lagged values was used to determine the temporal correlation between the SFTS viral loads and time-series variables. Fifty-eight SFTS patients were included in the non-severe group (NSG) and 11 in the severe group (SG).

Results: In the cross-sectional analyses, 10 parameters -white blood cell, absolute neutrophil cell, lymphocyte, platelet, activated partial thromboplastin time (aPTT), C-reactive protein, aspartate aminotransferase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH), and creatine phosphokinase (CPK)- were assessed within 30 days from the onset of symptoms; they exhibited three different correlation patterns: (1) positive, (2) positive with a time lag, and (3) negative. A prediction score system was developed for predicting SFTS fatality based on age and six laboratory variables -platelet, aPTT, AST, ALT, LDH, and CPK- in 5 days after the onset of symptoms; this scoring system had 87.5% sensitivity and 86.0% specificity (95% confidence interval: 0.831 - 1.00, $P < 0.001$).

Conclusion: Three types of correlation patterns between the dynamic changes in SFTS viral load and laboratory parameters were identified. The dynamic changes in the viral load could be predicted using the dynamic changes in these variables, which can be particularly helpful in clinical settings where viral load tests cannot be performed. Also, the proposed scoring system could provide timely treatment to critical patients by rapidly assessing their clinical course.


Keywords: Severe fever with thrombocytopenia syndrome; *Banyangvirus*; Tick-borne disease; Fatality prediction; Cross-correlation analysis

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Conflict of Interest

No conflict of interest.

Author Contributions

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INTRODUCTION

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging tick-borne disease caused by the SFTS virus (SFTSV) belonging to the genus *Bunyavirus*, family *Phenuiviridae*, and order *Bunyavirales* [1]. The clinical course after SFTSV infection is variable; from asymptomatic with only positive SFTS antibody to death [2]. The mortality rate of SFTS estimates range between 5 – 30% in East Asia [3, 4]. High mortality rate and lack of specific treatments mandate early diagnosis and the identification of prognostic factors associated with severity. In previous studies, high SFTS viral load in blood and cytokine storm were found to be high-risk factors for mortality [5-7]. However, it is difficult to determine the SFTS viral load and cytokine levels in general laboratory. Activated partial thromboplastin time (aPTT) and the levels of C-reactive protein (CRP), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatinine kinase, and creatinine are associated with severity and fatality [8-10]. Additionally, loss of T, B, and natural killer cells progress during early SFTSV infection; reduced humoral immunity and CD4+ T cell deficiency are associated with disease severity [11, 12]. However, these variables cannot optimally predict fatal SFTS before clinical course worsens because the baseline values of these variables may be different and fluctuate depending on other factors such as the underlying disease except for the severity of the patient. Therefore, if physicians could identify a laboratory variable that assessed the dynamic changes in SFTS viral load during the early stage of the disease, they may be able to prescribe appropriate treatments for critical patients with SFTS. This study aimed to identify the laboratory parameters that can predict the dynamic changes in the SFTS viral load and a specific time point suitable for predicting the clinical course of SFTS before it worsens.

MATERIALS AND METHODS

1. Study design

A longitudinal panel data analysis was conducted on the data from the electronic medical records of confirmed SFTS patients at Jeju National University hospital, a single tertiary hospital in Jeju Island, Korea, between May 2013 and December 2020.

2. Ethics statement

This study was approved by the Jeju National University Hospital Institutional Review Board (IRB) [2021-03-012]. This study was a retrospective study, therefore the patient informed consent was waived from the IRB.

3. Patients

SFTS was confirmed based on the detection of the S and M segment gene of the SFTSV RNA using reverse transcription polymerase chain reaction (RT-PCR). Patients were divided into the severe group (SG) and non-severe group (NSG). The SG included patients with three or more organ dysfunction defined by Sequential Organ Failure Assessment (SOFA) score; lung – PaO₂/FiO₂ <400 or patients requiring mechanical ventilation, liver – serum bilirubin ≥1.2 mg/dL, kidney – serum creatinine ≥1.2 mg/dL, urine output <500 ml/day or patients requiring hemodialysis, cardiovascular system – mean arterial pressure <70 mmHg or patients requiring vasopressor, central nervous system – Glasgow Coma Scale <15, or deceased patients. Otherwise, the NSG included patients not included in the SG. A patient was excluded if the age or final clinical outcome of the patient could not be determined or if the patient was deceased on arrival.

4. Data collection and measurements

1) Demographic and clinical characteristics

The demographic and clinical characteristics data were obtained from the electronic medical records and included the patient demographics, activity at the time of exposure, history of tick bites, presence of initial symptoms (fever, chills, fatigue, headache, myalgia, dizziness, poor oral intake, nausea, vomiting, abdominal pain, diarrhea, cough, sputum, hemoptysis, and dyspnea), vital signs, past medical history, Charlson Comorbidity Index score (CCI), and multiple organ dysfunction score (MODS) during hospitalization and after 72 hours of treatment (inotropics, intubation, renal replacement therapy, and plasma exchange), disposition after hospital admission (discharge, inter-hospital transfer, death, and others), and 30 day mortality.

2) SFTS viral loads and laboratory parameters

Blood samples were collected at the first visit to the hospital and then at regular intervals (every two days in the acute phase, twice a week in the recovery phase during the hospitalization, and every 2 to 3 months after discharge).

The presence of SFTSV and its RNA copies were evaluated using RT-PCR. Viral RNA was extracted from the first acute-phase serum using a QIAamp Viral RNA Mini kit (Qiagen Inc., Mainz, Germany). The extracted RNA was preserved in elution buffer at -70°C . RT-PCR of the partial S and M segments of SFTSV was performed [5]. The RT-PCR mixture contained 8 μl of one-step RT-PCR premix, 7 μl of detection solution, and 5 μl of the RNA template (total volume of 20 μl). The following cycling conditions were used: 30 min at 45°C , 10 min at 90°C , and 45 cycles of 15 s at 95°C and 30 s at 48°C . The products were sequenced using a BigDye Terminator Cycle Sequencing kit (Perkin Elmer Applied Biosystems, Warrington, UK).

The 10 laboratory parameters evaluated were: white blood cell (WBC), absolute neutrophil cell (ANC), lymphocyte, and platelet (PLT) counts; aPTT; and the levels of CRP, AST, alanine transaminase (ALT), LDH, and creatine phosphokinase (CPK). Lymphocyte subpopulations and T cell profiles in the peripheral blood mononuclear cells were analyzed using flow cytometry with florescent-conjugated T cell surface markers.

5. Statistical analysis

Descriptive statistics were presented as frequencies and percentages for categorical variables and as means, standard deviations, medians, and interquartile ranges for continuous variables. We compared baseline demographics, clinical characteristics, and initial laboratory results between the study population with or without fatality using either a Student's *t*-test, a Wilcoxon's rank-sum test, Chi-squared test, or a Fisher's exact test. The level of comorbidities was assessed using the CCI based on the previous diagnosis of each patient.

The variables recorded intermittently from each SFTS patient were standardized. The SFTS viral loads, body temperature (BT), and 10 laboratory parameters, which were measured multiple times for each SFTS patient, were converted into a daily time-series data structure, with median values at each date from the date of symptom onset (day 0). The descriptive summaries of the initial measurement and the number of serial measurements for SFTS viral loads, BT, and 10 laboratory parameters were calculated. SFTS viral load was considered as a dependent variable, and the bivariate time-series plots were constructed. Cross-correlation analysis at lagged values (± 3 days) was used to determine the temporal correlation between the SFTS viral loads and other time-series variables (BT and the 10 laboratory parameters).

Receiver operating characteristic (ROC) curve was generated, and area under the curve (AUC) analyses were performed to assess the predictive power of the constructed model. AUC was used as the measure of accuracy of the predictive score. An AUC of 0.5 suggested no discrimination, 0.7 to 0.8 was considered acceptable, 0.8 to 0.9 was considered excellent, and > 0.9 was considered outstanding [13]. All statistical analyses were performed using Stata 14.0 (Stata Corp, College Station, TX, USA) and SPSS version 20.0 (IBM Corp., Armonk, NY, USA).

RESULTS

1. Baseline characteristics

Seventy-three SFTS patients were included in the analysis; 11 in the SG and 58 in the NSG; four patients were excluded because they were asymptomatic. The baseline clinical characteristics are summarized in **Table 1**. The mean initial SFTS viral load was significantly higher in the SG compared to that in the NSG. The mean initial MODS and MODS at 72-hour after diagnosis were significantly higher in the SG compared to that in the NSG (5.6 ± 4.0 vs. 1.9 ± 1.3 , $P < 0.001$; 12.1 ± 4.2 vs. 2.9 ± 1.9 , $P < 0.001$, respectively). Mortality rate was significantly higher in the SG compared to that in the NSG (63.6% vs. 1.7%, $P < 0.001$). Most of the initial laboratory findings were not significantly different between the groups except for aPTT, which was significantly higher in the SG.

2. Viral kinetics and the serial changes in the laboratory parameters in the NSG and SG

The baseline values of clinical and laboratory parameters during 30 days are summarized in **Supplementary Table 1**. The overall peak time of the median SFTS viral load was 2 days from the onset of symptoms; it was 3 and 2 days in the SG and NSG, respectively ($P = 0.873$). The high or low peak time of the median clinical or laboratory parameters in the SG and NSG are summarized in **Supplementary Figure 1**.

3. Kinetics of CD4+ T cells in the NSG and SG

The serial changes in SFTS viral load and CD4+ T cell populations did not show any correlation between the groups. However, in the SG, the median CD4+ T cell proportions exhibited a decreasing trend in the early phase (1 - 10 days from the onset of symptoms); it was lower in the SG compared to that in the NSG (28.3% vs. 41.4%, $P = 0.104$). It decreased below the normal range between 6 - 10 days of symptom onset (**Supplementary Fig. 2**).

4. Association between the dynamic changes in SFTS viral load and the laboratory parameters

The 10 parameters were retrospectively analyzed in comparison with SFTS viral load using a cross-sectional test on the data obtained within 30 days from the onset of each symptom. Three different correlation patterns were observed: (1) positive correlation, (2) positive correlation with time lag, and (3) negative correlation.

1) Positive correlation

BT and aPTT were positively correlated with the viral load at different time points (**Fig. 1A, 1B**). The correlation was the highest at the time lag "0" day. These laboratory parameters exhibited an increasing pattern with increasing SFTS viral load; they decreased with the decrease in the viral load during the recovery phase.

Surrogate laboratory parameters for SFTS viral load

Table 1. Baseline clinical characteristics of patients with severe fever with thrombocytopenia syndrome between severe group (SG) and non-severe group (NSG)

Variables	All (n = 69)	NSG (n = 58)	SG (n = 11)	P-value
Age, years (mean ± SD)	63.3 (± 13.8)	62.0 (± 13.8)	70.5 (± 12.0)	0.061
Male gender, n (%)	39 (56.5)	32 (55.2)	7 (63.6)	0.745
CCI ± SD	0.4 (± 0.7)	0.4 (± 0.6)	0.6 (± 1.0)	0.410
Initial SFTS viral load (mean ± SD) ^a	1,672,346 (± 6,434,249)	228,603 (± 532,862)	10,013,974 (± 17,912,878)	<0.001
Initial SFTS viral load (median, IQR) ^a	48,767 (3,088 - 257,944)	31,480 (2,276 - 236,823)	186,606 (11,793 - 19,272,690)	0.067 ^b
Exposure type, n (%)				0.019
Agriculture	31 (44.9)	28 (48.3)	3 (27.3)	
Livestock	7 (10.1)	4 (6.9)	3 (27.3)	
Leisure activity	8 (11.6)	8 (13.8)	0 (0.0)	
Grave	6 (8.7)	6 (10.3)	0 (0.0)	
Person-to-person transmission	1 (1.4)	1 (1.7)	0 (0.0)	
Other	7 (10.1)	3 (5.2)	4 (36.4)	
Tick bite, n (%)	34 (49.3)	30 (51.7)	4 (36.4)	0.513
Seasonal occurrence, n (%)				0.745
Spring	12 (17.4)	10 (17.2)	2 (18.2)	
Summer	38 (55.1)	33 (56.9)	5 (45.5)	
Autumn	19 (27.5)	15 (25.9)	4 (36.4)	
Winter	0 (0.0)	0 (0.0)	0 (0.0)	
From onset of illness to admission (mean ± SD)	4.0 (± 2.2)	3.9 (± 2.3)	4.2 (± 1.2)	0.736
Tsutsugamushi co-infection, n (%)	7 (10.1)	4 (6.9)	3 (27.3)	0.075
MODS (mean ± SD)				
Initial	2.5 (± 2.4)	1.9 (± 1.3)	5.6 (± 4.0)	<0.001
72 hours after diagnosis	4.4 (± 4.1)	2.9 (± 1.9)	12.1 (± 4.2)	<0.001
Plasma exchange, n (%)	27 (39.1)	20 (34.5)	7 (63.6)	0.095
Duration of onset time to death (mean ± SD)	11.6 (± 8.4)	8 (± 0.0)	12.4 (± 8.9)	0.660
30-days Mortality, n (%)	8 (11.6)	1 (1.7)	7 (63.6)	<0.001
Clinical findings, n (%)				
Fever	63 (91.3)	56 (96.6)	7 (70.0)	0.020
Chill	42 (44.9)	28 (48.3)	3 (30.0)	0.326
Fatigue	20 (29.0)	15 (25.9)	5 (50.0)	0.145
Headache	18 (26.1)	16 (27.6)	2 (20.0)	0.720
Myalgia	28 (40.6)	26 (44.8)	2 (20.0)	0.179
Dizziness	11 (15.9)	11 (19.0)	0 (0.0)	0.197
Poor oral intake	29 (42.0)	26 (44.9)	3 (30.0)	0.498
Nausea	20 (29.0)	17 (29.3)	3 (30.0)	0.965
Vomiting	9 (13.0)	8 (13.8)	1 (10.0)	0.744
Abdominal pain	4 (5.8)	4 (6.6)	0 (0.0)	0.392
Diarrhea	28 (40.6)	24 (41.4)	4 (40.0)	0.935
Cough	3 (4.3)	3 (5.2)	0 (0.0)	0.462
Sputum	3 (4.3)	3 (5.2)	0 (0.0)	0.462
Hemoptysis	2 (2.9)	1 (1.7)	1 (10.0)	0.274
Dyspnea	2 (2.9)	0 (0.0)	2 (20.0)	0.020
Laboratory findings (mean ± SD)				
WBC	2,271 (± 1,586)	2,130 (± 1,471)	3,014 (± 2,010)	0.090
ANC	1,343 (± 1,195)	1,284 (± 1,147)	1,673 (± 1,460)	0.347
Lymphocyte fraction	36.7 (± 37.9)	37.3 (± 16.1)	33.4 (± 13.7)	0.450
Platelet	775,443 (± 1,250,935)	748,957 (± 1,312,058)	287,750 (± 558,182)	0.240
CRP	0.7 (± 0.9)	0.7 (± 1.3)	2.1 (± 2.2)	0.053
aPTT	40.2 (± 12.5)	37.8 (± 9.1)	52.8 (± 19.3)	0.028
AST	172 (± 284)	125 (± 166)	419 (± 556)	0.112
ALT	75 (± 85)	64 (± 62)	130 (± 153)	0.190
LDH	997 (± 1,174)	796 (± 597)	2,021 (± 2,399)	0.123
CPK	1,117 (± 2,097)	828 (± 1,501)	2,884 (± 3,917)	0.157

^an = 61/69.

P-values are derived from simple t-test or Mann-Whitney test (^b) for continuous data and Chi-square test for categorical date.

Continuous data is presented as means and standard deviations, only initial SFTS viral load is also presented as median and Q1 - Q3; categorical data is presented as counts and percentages.

CCI, comorbidity index score (calculated by Charlson comorbidity index); MODS, multiple organ dysfunction score; WBC, white blood cell; ANC, absolute neutrophil count; CRP, C-reactive protein; INR, international normalized ratio; aPTT, activated partial thromboplastin time; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; CPK, creatinine phosphokinase.

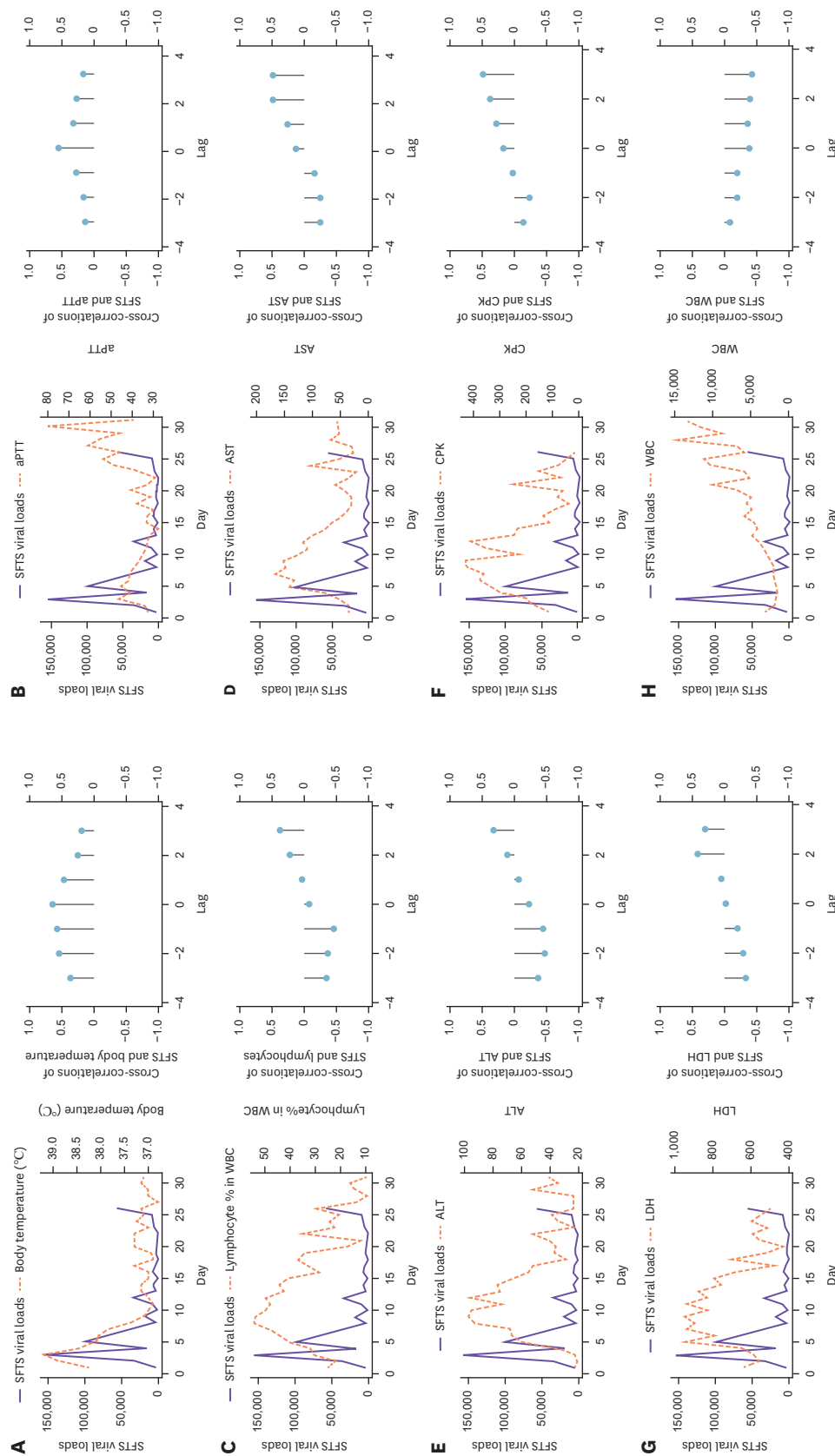
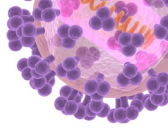


Figure 1. The association of dynamic changes between laboratory parameters and SFTS viral load in SFTS patients. (A-B) positive correlation - body temperature, aPTT; (C-G) positive correlation with time lag - lymphocytes fraction in WBC, AST, ALT, CPK, and LDH; (H-K) negative correlation - WBC, ANC, Platelet, and CRP.

(A) Body temperature; (B) aPTT; (C) lymphocytes fraction in WBC; (D) AST; (E) ALT; (F) CPK; (G) LDH; (H) WBC; (I) ANC; (J) Platelet; (K) CRP. SFTS, severe fever with thrombocytopenia syndrome; aPTT, active partial thromboplastin; WBC, white blood cell; AST, aspartate aminotransferase; ALT, alanine transaminase; CPK, creatinine phosphokinase; LDH, lactate dehydrogenase; ANC, absolute neutrophil counts; CRP, C-reactive protein.

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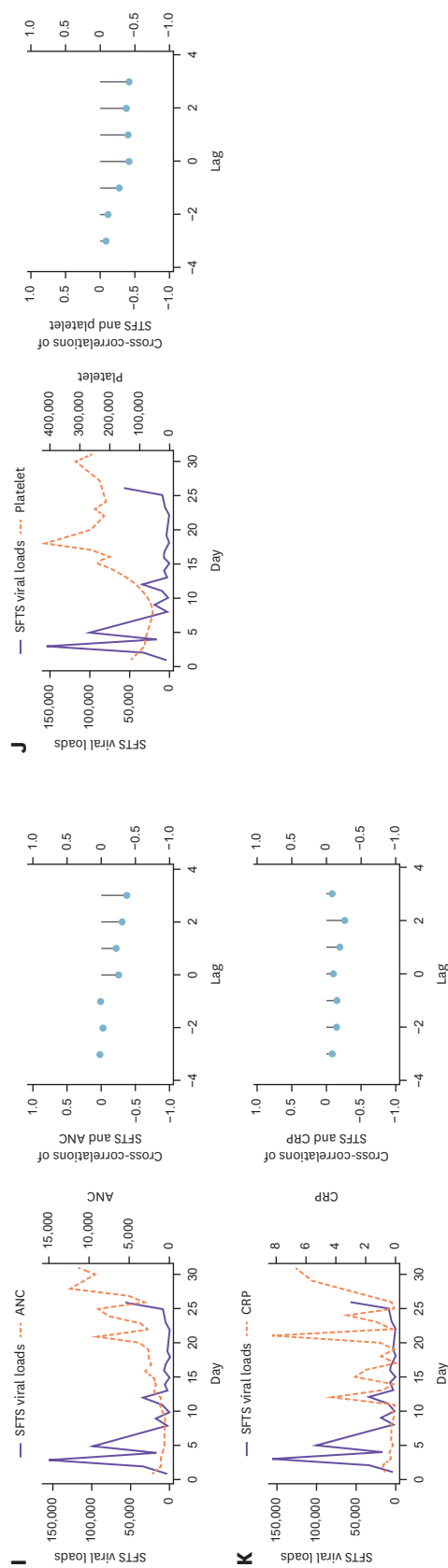
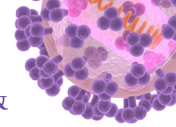


Figure 1. (Continued) The association of dynamic changes between laboratory parameters and SFTS viral load in SFTS patients. (A-B) positive correlation - body temperature, aPTT; (C-G) positive correlation with time lag - lymphocytes fraction in WBC, AST, ALT, CPK, and LDH; (H-K) negative correlation - WBC, ANC, Platelet, and CRP. (A) Body temperature; (B) aPTT; (C) lymphocytes fraction in WBC; (D) AST; (E) ALT; (F) CPK; (G) LDH; (H) WBC; (I) ANC; (J) Platelet; (K) CRP. SFTS, severe fever with thrombocytopenia syndrome; aPTT, active partial thromboplastin; WBC, white blood cell; AST, aspartate aminotransferase; ALT, alanine transaminase; CPK, creatinine phosphokinase; LDH, lactate dehydrogenase; ANC, absolute neutrophil counts; CRP, C-reactive protein.

2) Positive correlation with time lag

The lymphocyte fraction and the levels of AST, ALT, LDH, and CPK were positively correlated with the changes in the SFTS viral load (Fig. 1C-1G). The correlation was the highest at time lag "2 - 3" days for lymphocyte fraction (3 days), AST (3 days), ALT (3 days), CPK (3 days), and LDH (2 days) levels.

3) Negative correlation

WBC, ANC, PLT, and CRP levels were negatively correlated with the changes in the SFTS viral load (Fig. 1H-1K). These laboratory parameters decreased as the SFTS viral load increased during the early phase and they increased during the recovery phase. The correlation was the highest at time lag "2 - 3" days for WBC (3 days), ANC (3 days), PLT (3 days), and CRP (2 days) levels.

5. Time point for predicting clinical course of SFTS

The ROC curve was used to determine whether a combination of laboratory parameters and the recovery trend of laboratory values at a specific time could better predict the clinical course of SFTS patients. A prediction score for the fatality in SFTS was assigned based on a combination of variables: age ≥ 60 , non-recovery of platelet counts, aPTT, AST, ALT, LDH, and CPK. It was assigned 0 point if the laboratory parameters exhibited a recovery trend 5 days after the onset of symptoms and 1 point if laboratory parameters did not recover or exhibited a worsening trend 5 days after the onset of symptoms; the score ranged from 0 to 7. The ROC curve indicated that the optimal cut-off score for the model was ≥ 5 points. A score ≥ 5 had 87.5% sensitivity and 86.0% specificity for the SG, with ROC AUC being 0.921 (95% confidence interval: 0.84 - 1.00, $P < 0.001$) (Fig. 2). When the group in which the laboratory parameters entered a recovery trend on the 6 days of symptom onset was defined as the recovery group and the group in which the laboratory parameters did not recover was defined as the non-recovery group, the MODS and laboratory parameters at 6 days after the onset of symptoms were not significantly different between the two groups (Supplementary Table 2).

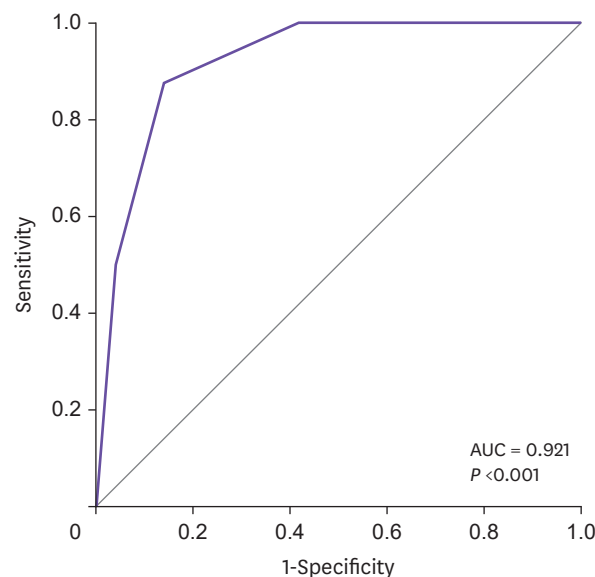


Figure 2. Receiver operating characteristics curve for the prediction score for the fatality of SFTS. Prediction score system = non-recovery trend after day 5 from the onset of symptoms of (1 \times platelet count) + (1 \times aPTT level) + (1 \times AST level) + (1 \times ALT level) + (1 \times LDH level) + (1 \times CPK level). SFTS, severe fever with thrombocytopenia syndrome; AUC, area under the curve; aPTT, active partial thromboplastin; AST, aspartate aminotransferase; ALT, alanine transaminase; LDH, lactate dehydrogenase; CPK, creatinine phosphokinase.

Although there was no clinical difference at the early phase, all critical patients with SFTS were included in the non-recovery group. Among patients in the non-recovery and recovery groups, there were patients who died only in non-recovery group (24.1% vs. 0.0%, $P < 0.006$).

DISCUSSION

The laboratory parameters of SFTS patients showed three types of correlation patterns (positive, positive with a time lag, and negative) for predicting the dynamic changes in SFTS viral load. BT and aPTT increased with increasing viral load and decreased during the recovery phase. Lymphocyte fraction and the levels of AST, ALT, LDH, and CPK increased or decreased directly with the increase or decrease in the viral load, with a lag of 2 - 3 days. The WBC, ANC, PLT, and CRP levels decreased with increasing viral load and recovered with the decrease in viral load, with a lag of 2 - 3 days. A predictive score for the fatality in SFTS, based on the age and serial changes in six common laboratory variables that could be predicted at the beginning of the clinical course, is proposed. If the age ≥ 60 years and six laboratory parameters (PLT, aPTT, AST, ALT, LDH, and CPK) did not show a recovery trend 5 days after the symptom onset, the SFTS patients would progress rapidly on the clinical course.

To the best of our knowledge, this is the first study to statistically evaluate the correlation between the clinically measurable parameters and the daily dynamic changes in the SFTS viral load. Impairment of liver function is associated with SFTS [14]. Serum levels of AST, ALT, LDH, and CPK, which reflect liver dysfunction, peak at approximately 10 days. They gradually decrease and reach the normal range in non-fatal cases, whereas they gradually increase in the fatal case. PLT counts are low at diagnosis and steadily improve between days 7 - 10 and return to the normal range 11 days after disease onset [15]. In the present study, the AST, LDH, and CPK levels peaked at 7 - 8 days after symptom onset in all patients. PLT count showed a low peak at 7 days and then it steadily recovered. The dynamic changes in the laboratory parameters are similar; however, earlier reports did not directly correlate these parameters with the dynamic change in the SFTS viral load. This study suggests that the dynamic changes in the SFTS viral load can be predicted based on the changes in the laboratory parameters.

The subsets of peripheral blood lymphocyte were altered in SFTS patients. The total T cell count and CD4+ population were significantly reduced in SFTS patients [16]. In addition, the CD4+ population was significantly lower in the acute phase (first week of disease onset) and in severe SFTSV infection compared to that in convalescent (2 weeks after the disease onset) and mild SFTSV infection. In the present study, CD4+ T cell proportions showed no significant differences over the course of the disease. However, it showed a decreasing trend during the early course of the disease in the SG; it was lower in the SG compared to that in the NSG.

The factors associated with the fatal outcome in SFTS are elucidated; this includes older age, neurologic symptoms, and respiratory symptoms including acute respiratory distress syndrome during the illness [17, 18]. In addition, higher serum levels of liver transaminase, CPK, and LDH and prolonged coagulation panel are associated with the progression of multi-organ failure and fatal outcomes; these laboratory parameters correlate with the viral RNA levels [5, 6, 8]. There was no significant difference in the initial values of the laboratory parameters between the SG and NSG, and the SFTS viral load was not known at the time of initial hospitalization. The mortality rate was very high in the SG; therefore, early initiation

of supportive management such as plasma exchange or immunoglobulin administration should be considered more seriously in patients with a worsening prognosis, predicted using the scoring system proposed in this study [19, 20]. In previous multicenter studies on SFTS in Korea, an application of early plasma exchange within 7 days had a beneficial effect on the clinical outcome of SFTS patients [21]. We suggest that it will be possible to identify patients with severe SFTS and improve their prognosis by prescribing effective treatments at an early stage by applying this score system.

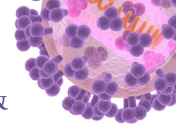
There is an overlap among the risk factors reported in various studies; however, they are not consistent. Therefore, it is difficult to predict disease fatality based on one or two specific laboratory values. The fatality and prognosis could be predicted more sensitively by evaluating several significant laboratory parameters. Several scoring models based on a combination of variables for predicting fatality in SFTS patients are proposed. One of them includes SFTS viral load, neurologic symptoms, respiratory symptoms, and lower level of monocytes; however, the clinical symptoms could be subjectively interpreted [22]. The SFTS viral load cannot be tested in all medical facilities, and confirming the test results are time consuming; therefore, a clinical scoring model with three factors — older age and aPTT and BUN levels at hospitalization — which are easily measurable, was proposed [23]. However, there was no statistically significant difference in the initial laboratory parameters at diagnosis, and mean values by each day during the first 10 days after symptom onset between the SG and NSG and the baseline of the laboratory parameters of each patient could differ depending on the underlying disease or clinical situation of the patient. Therefore, predicting fatality by applying the scoring systems only with the cut-off values at the initial phase or at specific time points could be a limitation. Laboratory parameters change dynamically depending on the clinical course; therefore, a scoring system incorporating the recovery trend rather than the cut-off values of the laboratory variables at the early stage of disease could be useful for rapidly predicting the disease course.

This study has limitations. First, it is a single center study with a small sample size, which could affect the predictive efficacy of the indices and models. Second, SFTS viral load was not measured at regular intervals compared to that of the other laboratory parameters. This could influence the mean and other statistical values. Third, the sample sizes evaluated for T and B cell proportions were smaller compared to that for the other laboratory parameters.

In conclusion, this study presented three different correlation patterns between the daily dynamic changes in the SFTS viral load and clinical parameters. The dynamic change in viral load can be predicted based on the changes in easily measurable laboratory parameters. This study has proposed a scoring system based on these clinical parameters to predict the clinical outcome in SFTS at a specific early time point. This scoring system could be helpful in treating critical patients by rapidly judging the clinical course for the patient based on the recovery trend of six laboratory parameters at the early stage of the disease.

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SUPPLEMENTARY MATERIALS

Supplementary Table 1

Mean and median value of baseline clinical and laboratory parameters during 30 days in all SFTS patient's variables

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Supplementary Table 2

Comparison of clinical characteristics between recovery group and non-recovery group in patients with SFTS at 6 days from symptom onset

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Supplementary Figure 1

Dynamic change of SFTS viral load and laboratory parameters during 30 days between non-severe group and severe group in patients with SFTS.

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Supplementary Figure 2

Dynamic changes in CD4+ T cell count in the SFTS patients during illness from symptom onset.

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