Original Article

Liver Function Derangement in Patients with Severe Fever and Thrombocytopenia Syndrome

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Abstract

Background and Aims: Patients with severe fever with thrombocytopenia syndrome (SFTS) commonly show liver function impairment. This study aimed to characterize the liver function indices in SFTS patients and investigate their association with mortality. ***Methods:*** Clinical information and laboratory results of 459 laboratory-confirmed SFTS patients, including 78 deceased and 381 surviving patients, were retrospectively analyzed. To explore the infectivity of SFTS caused by novel Bunyavirus (SFTSV) in hepatocytes, Huh7 human hepatoma cells were infected with various concentrations of SFTSV *in vitro*. ***Results:*** The proportion of SFTS patients developing liver injury during hospitalization was 73.2% (336/459); the hepatocellular injury was the predominant type. The median time to occurrence of liver injury from disease onset was 8 d. Liver injury in the deceased group occurred earlier than that in the surviving group. Alanine aminotransferase (ALT) level between 2–5 times upper limit of normal (ULN) at 4–6 d and between 5–15 ULN at 7–12 d of disease course were independent predictors of mortality. Alkaline phosphatase (ALP) >2 ULN at 7–9 d and elevated ALP at 10–12 days after disease onset were risk factors for death. ALT and aspartate transaminase (AST) levels were correlated with lymphocyte count and platelet-to-lymphocyte ratio (PLR). Total bilirubin (TB), ALT, AST levels showed positive correlation with viral load. In the *in vitro* experiment, SFTSV infected and replicated inside Huh7 cells. ***Conclusions:*** Liver injury is common in SFTS patients. ALT and ALP were independent predictors of SFTS-related mortality. Frequent monitoring and evaluation of liver function indices are needed for SFTS patients.

Introduction

Severe fever with thrombocytopenia syndrome (SFTS) caused by novel Bunyavirus (SFTSV) infection has been reported in many countries and regions in recent years. SFTS is an emerging infectious disease characterized by sudden onset of high fever, thrombocytopenia, and leukopenia accompanied by respiratory or gastrointestinal symptoms. Patients with severe disease often die of multiple organ failure.[1]

The liver, as an important endocrine, digestive, and metabolic organ, is often involved in nonhepatotropic virus infection. For example, 14–53% of patients with Corona Virus Disease 2019 (COVID-19) developed hepatic dysfunction, which was associated with poor outcomes.[2] Dengue virus infection is usually associated with liver injury, which is more pronounced in severe cases.[3] In a previous study, patients with severe SFTS had significantly higher liver enzyme levels than general patients.[4] Postmortem histopathological examination of liver tissue of SFTS patients confirmed liver involvement characterized by focal necrosis, multiple lobular necrosis, and lymphocyte infiltration.[5] In animal models of SFTSV infection, pathological findings showed hepatocyte necrosis with nuclear pyknosis, ballooning degeneration, bridging necrosis of hepatocytes with infiltration of inflammatory cells in the portal area.[6,7] Given the reported findings of altered liver indices and histopathological findings of liver impairment, characterization of liver biochemical changes in SFTS patients and the investigation of their relationship with in-hospital mortality is a key imperative.

However, because of the small number of cases and the under-representation of previous studies, SFTS-related liver injury is not well characterized. Therefore, we retrospectively analyzed the liver function indices in SFTS patients and investigated their relationship with mortality. We also sought to identify the potential correlates of changes in liver enzymes. The effects of SFTSV on hepatocytes were verified by *in vitro* experiments. Our findings may help clarify the impact of SFTSV infection on the liver and guide clinical treatment.
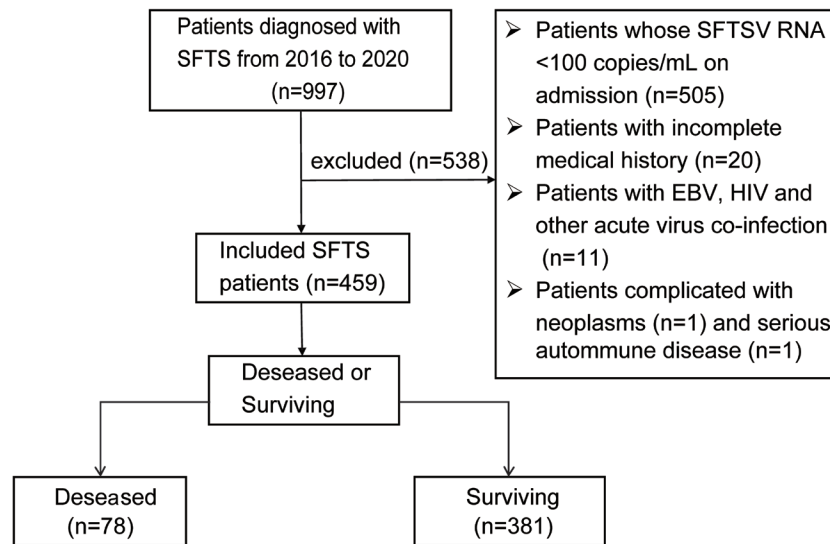
Fig. 1. Flow chart of the study population and patient selection criteria. In total, 459 SFTS patients were enrolled. Seventy-eight patients who died were classified as deceased, while three-hundred and eighty-one surviving patients were classified as surviving. EBV, Epstein-Barr virus; HIV, human immunodeficiency virus; SFTS, severe fever with thrombocytopenia syndrome; SFTSV, severe fever with thrombocytopenia syndrome virus.

Methods

Study design and participants

A total of 997 patients with SFTS were admitted to the Wuhan Union Hospital between January 1, 2016 and December 30, 2020. The demographic, clinical characteristics, medical history, laboratory tests, treatment, and outcome data were retrieved from electronic medical records. The inclusion criteria were: (1) epidemiological history; (2) typical clinical symptoms; (3) case specimen positive for the novel Bunyavirus nucleic acid test following the 2010 Guidelines for the prevention and treatment of severe fever with thrombocytopenia syndrome issued by the Ministry of Health, China.[8] The exclusion criteria were: (1) SFTSV RNA <100 copies/mL at admission; (2) incomplete medical data; (3) concomitant infection with Epstein-Barr virus (EBV), human immunodeficiency virus (HIV), or other acute virus infections; (4) concomitant neoplastic disease or severe autoimmune disease. After screening, 459 patients were included in the study. Patients were divided into deceased and surviving groups by the survival outcome (Fig. 1). The study was approved by the Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology. The requirement for informed consent was waived because of the retrospective nature of study.

SFTSV RNA detection

Viral RNA was extracted from blood samples of all SFTS patients using a virus RNA kit (Daan Gene Company, Guangzhou, China). The viral load of SFTSV RNA was determined using a certified real-time fluorescent quantitative PCR kit (SFDA registration number 340166), following the manufacturer's instructions.

Definition of abnormal liver test and liver injury

The upper limit of normal (ULN) reference range of each liver enzyme in serum are: total bilirubin (TB)=19 µmol/L, alanine aminotransferase (ALT)=40 U/L, aspartate aminotransferase (AST)=40 U/L, alkaline phosphatase (ALP)=150 U/L, and γ-glutamyl transpeptidase (GGT)=50 U/L. The normal reference range of serum albumin (ALB) is 33–55 g/L. An abnormal liver test was defined as an increase in liver enzymes above the ULN and a decrease in ALB <35 g/L. Liver injury was considered if there was an increase of over twice the ULN for ALT or conjugated bilirubin or a combined increase in AST, ALP, and TB provided the level of one of these was more than twice the ULN. The pattern of liver injury was classified by the R ratio, which was calculated as R = (ALT value ÷ ALT ULN) ÷ (ALP value ÷ ALP ULN). An R ratio of >5 defined hepatocellular injury, <2 defined cholestatic injury, and 2–5 indicated a mixed pattern.[9] The liver tests used for the classification of liver damage were the first blood tests available after liver injury. According to clinical guidelines of the American College of Gastroenterology (ACG) for the evaluation of abnormal liver chemistries in blood sample,[10] borderline AST and/or ALT elevation was defined as <2 ULN, mild AST and/or ALT elevation as 2–5 ULN, moderate AST and/or ALT elevation as 5–15 ULN, and severe AST and/or ALT elevation as >15 ULN.

SFTSV infection and replication in hepatocytes

The SFTSV strain WCH/97/HN/China/2011 (CSTR: 16533.06. IVCAS 6.6088) used in this study was originally isolated from an SFTS patient and preserved at the Wuhan Institute of Virology. The $TCID_{50}$ was determined as described by Reed and Mench.[11] The primary polyclonal antibody against SFTSV nucleoprotein was obtained from the National Virus Resource Center. The Huh7 human hepatoma cell line was obtained from the China Center for Type Culture Collection.

Huh7 cells (1×10^6 cells/well) were seeded in six-well plates, incubated in Dulbecco's modified Eagle's medium (DMEM; Sigma, St. Louis, MO, USA) with 2% fetal bovine serum (FBS; Gibco, Australia) at 37°C in 5% CO_2 for 1 day. Subsequently, the cells were infected with SFTSV at multiplicity of infection (MOI) of 0.1, 1, and 10, respectively, and incubated for 3 days at 37°C in 5% CO_2. Then the cells were fixed with 4% paraformaldehyde for 15 m and permeabilized with 0.5% Triton X-100 for 12 m. After three washes, the cells were blocked with 5% bovine serum albumin at 37°C in

Table 1. Baseline characteristics of deceased and surviving SFTS patients

Characteristic[a]	SFTS (*n*=459)	Deceased (*n*=78)	Surviving (*n*=381)	*p*-value[b]
Age, years	60 (52–79)	65 (59–70)	59 (51–67)	<0.001
Sex, female	258 (56.2)	37 (47.4)	221 (58.0)	0.056
Days after disease onset	6.0 (5.0–7.0)	7.0 (5.0–9.0)	6.0 (5.0–7.0)	0.112
Duration of hospitalization, d	9.0 (7.0–12.0)	4.0 (3.0–6.3)	10.0 (8.0–12.5)	<0.001
Disease course, d	15.0 (13.0–19.0)	11.0 (9.0–13.0)	16.0 (14.0–19.0)	<0.001
Underlying disease				
Hypertension	47 (10.2)	10 (12.8)	37 (9.7)	0.414
Diabetes	5 (1.1)	2 (2.6)	3 (0.8)	0.202
Coronary heart disease	13 (2.8)	2 (2.6)	11 (2.9)	1
Chronic hepatitis B	62 (13.5)	9 (11.5)	53 (13.9)	0.717
Laboratory examination				
SFTSV load, $\log_{10}$,	3.61 (2.81–4.48)	4.96 (4.06–5.75)	3.45 (2.66–4.17)	<0.001
WBCs, 10^9/L	2.12 (1.41–3.60)	2.27 (1.39–3.23)	2.11 (1.42–3.78)	0.732
Hemoglobin, g/L	128.00±17.3	127.59±18.84	128.72±17.29	0.609
Neutrophils, 10^9/L	1.37 (0.82–2.58)	1.64 (1.00–2.51)	1.34 (0.80–2.63)	0.785
Lymphocytes, 10^9/L	0.48 (0.32–0.73)	0.41 (0.28–0.67)	0.50 (0.34–0.75)	0.034
Monocytes, 10^9/L	0.10 (0.05–0.23)	0.08 (0.03–0.20)	0.11 (0.06–0.24)	0.004
Platelets, 10^9/L	51.00 (34.00–65.00)	36.50 (23.30–52.30)	52.00 (39.00–68.80)	<0.001
NLR	2.82 (1.51–5.13)	3.78 (2.34–5.25)	2.71 (1.37–5.05)	0.027
PLR	80.64 (36.32–142.66)	66.42 (31.89–136.20)	83.30 (39.41–145.29)	0.346
NMR	12.54 (6.15–30.61)	19.14 (7.39–36.66)	11.79 (6.04–27.79)	0.045
Creatine, μmol/L	73.90 (62.15–93.13)	90.90 (69.78–134.53)	71.75 (61.53–89.70)	<0.001
BUN, mmol/L	5.20 (3.68–7.08)	6.90 (4.94–10.69)	4.88 (3.60–6.60)	<0.001

[a]Data are medians (interquartile range) or *n* (%), as indicated. [b]$p<0.05$ is significant. BUN, blood urea nitrogen; Cr, creatinine; NLR, neutrophil-to-lymphocyte ratio; NMR, neutrophil-to-monocyte ratio; PLR, platelet-to-lymphocyte ratio; WBCs, white blood cells.

5% CO_2 for 2 h. The cells were then incubated overnight with the primary antibody 1:1,000 diluted in phosphate-buffered saline (PBS) at 4°C, followed by incubation with the secondary goat antirabbit serum antibody conjugated with fluorescein isothiocyanate (Abcam, UK) for 1 h. Finally, the nuclei were stained by diamidino-phenylindole for 10 m. The green and blue fluorescence were visualized using a fluorescence microscope (Eclipse TE2000-S; Nikon, Japan).

Huh7 cells were infected with SFTSV at 1 MOI in DMEM with 2% FBS at 37°C in a 5% CO_2 atmosphere for 1 h. The supernatant was discarded and the cells were washed three times with PBS. After that, fresh DMEM with 2% FBS was added and the cells were incubated at 37°C in 5% CO_2 for 3 d. 100 μL supernatant was harvested at 0, 6, 12, 24, 48, and 72 h postinfection, and stored at −80°C.[12] Virus titers were determined as described by Reed and Mench.[11]

Statistical analysis

SPSS 25 (IBM Corp. Armonk, NY, USA) was used for data processing and analysis. Statistical charts and figures were drawn with R 4.0.2 and Graphpad Prism 8.0.1. Normally distributed continuous variables were expressed as mean±standard deviation, and between-group differences were assessed with the *t*-test. Non-normally distributed continuous variables were expressed as medians and interquartile range (IQR) and between-group differences assessed with the Mann-Whitney U test. Categorical variables were expressed as frequencies and percentages (%), and between-group differences were assessed with the chi-squared or Fisher's exact tests. The distribution of liver function indices in the two groups was shown by Kernel density estimation (KDE). Dynamic changes in liver function indices in the deceased group and surviving group were reported by locally weighted scatterplot smoothing (Lowess). Spearman correlation was applied to assess the degree of correlation between parametric variables. The relationship of liver test parameters with all-cause mortality was assessed using a multivariate Cox regression model adjusted for sex, age (≤60 and >60) years, neutrophil-to-lymphocyte ratio (NLR), and concomitant presence of hepatitis B. Two-tailed *p*-values <0.05 were considered statistically significant.

Results

General characteristics of patients with SFTS

A total of 459 patients with SFTS were included in the study. Their median age was 60 (52–79) years) and 258 (56.2%) were women. Table 1 summarizes the demographic and clini-

Table 2. Liver injury in SFTS patients during hospitalization

Variable[a]	Total (*n*=459)	Deceased (*n*=78)	Surviving (*n*=381)	*p*-value[b]
Liver injury	336 (73.2)	61 (78.2)	269 (70.6)	0.174
Type of liver injury				0.054
Hepatocellular	223 (66.3)	41 (61.1)	182 (67.6)	
Cholestatic	19 (5.6)	1 (1.4)	18 (6.6)	
Mixed	93 (27.6)	25 (37.3)	68 (25.2)	
Days after onset	8.0 (6.0–10.0)	7.0 (6.0–10.0)	8.0 (6.6–10.6)	0.019

[a]Data are medians (interquartile range) or *n* (%), as indicated. [b]*p*<0.05 is significant.

cal characteristics of the patients at admission. The patients were classified by their outcome into a surviving group of 381 (83.0%) and a deceased group of 78 (17.0%). There was no significant between-group difference of the time from symptom onset to hospitalization. However, the duration of hospitalization and disease course in the deceased group were shorter than those in surviving group (*p*<0.001). There was no significant between-group difference with respect to underlying diseases. Compared with the surviving group, deceased group had significantly a higher SFTSV load [4.96 (4.06–5.75) vs. 3.45 (2.66–4.17) copies/mL, *p*<0.001] and significantly lower lymphocyte [0.41 (0.28–0.67) vs. 0.50 (0.34–0.75) × 10^9/L], monocyte [0.08 (0.03–0.20) vs. 0.11 (0.06–0.24) × 10^9/L], and platelet [36.50 (23.30–52.30) vs. 52.00 (39.00–68.80) × 10^9/L] counts. Neutrophil to lymphocyte ratio (NLR) and neutrophil to monocyte ratio (NMR), two markers of the inflammatory state, were significantly higher in the deceased than in the surviving group [3.78 (2.34–5.25) vs. 2.71 (1.37–5.05) and 19.14 (7.39–36.66) vs. 11.79 (6.04–27.79), respectively]. Deceased patients also had more critical kidney dysfunction than the surviving group, including creatinine (Cr) [90.90 (69.78–134.53) vs. 71.75 (61.53–89.70)] and blood urea nitrogen (BUN) [6.90 (4.94–10.69) vs 4.88 (3.60–6.60) mmol/L] (*p*<0.05 for all).

Liver injury and liver function derangement in patients with SFTS at admission and during hospitalization

During hospitalization, a total of 336 (73.2%) patients developed liver injury; the proportion of patients developing liver injury in the deceased group (61, 78.2%) was higher than that in the surviving group (269, 70.6%), but the between-group difference was not statistically significant. In patients with liver injury, the most common type was hepatocellular, followed by mixed, and cholestatic injury was the rarest. The median time to the occurrence of liver injury was 8 (6–10) d after disease onset, and it occurred earlier in the deceased than the surviving group [7 (6–10) vs 8 (6.6–10.6), *p*=0.019] (Table 2). Liver function indices were more severely deranged in the deceased than in the surviving group, among which increase in AST level was the most pronounced. At admission, a majority of patients had normal TB (93.4%), ALP (93.8%), and GGT (74.2%), but only a few patients had normal ALB (26.4%). Except for GGT, there were significant between-group differences in the constituent ratios of other liver function indices. Most deceased patients had mild-to-moderate elevation of liver enzyme levels (ALT 2–5 ULN, 41%; AST 5–15 ULN, 43.6%), while majority of surviving patients had borderline-to-mild elevation in liver enzymes (ALT 1–2 ULN, 38.6% and AST 2–5ULN, 39.9%) (Table 3). Similarly, the peak value of liver function indices of the deceased patients was higher than that of the survived group (Supplementary Table 1).

Dynamic profile of liver function parameters in SFTS patients

KDE was used to describe the distribution of peak levels of liver function indices in the deceased and surviving groups. ALT, AST, ALP, and GGT levels were less dispersed in the surviving group than that in the deceased group. AST levels were the most widely distributed indices in the surviving and deceased groups. The ALB level in the deceased group was approximately 4 g/L lower than that in surviving group overall, and was fairly dispersed (Fig. 2). A LOESS model was used to describe the trajectories of TB, ALT, AST, ALP, GGT, and ALB levels in the deceased and surviving patients during hospitalization (Fig. 3). The TB levels of the two groups separated on the tenth day after the disease onset, and the surviving patients maintained approximately normal levels, while the deceased patients had increases to more than twice the ULN. The model showed a significant elevation of AST levels at admission, which was maintained at higher levels in the deceased group than in the surviving group. The ALT levels had similar trends but were not as pronounced as AST. Elevated ALP levels exceeding the ULN were only observed in deceased patients. There was a minor difference in GGT levels between the two groups, and the GGT levels exceeded the ULN on about 5 or 6 days. ALB decreased in all patients, and the decrease was more pronounced in the deceased patients.

Association of abnormal liver function indices with SFTS mortality

After adjusting for potential confounding factors such as age (≤60 and >60) years, sex, NLR, concomitant presence of hepatitis B, a multivariate Cox regression model was used to assess the correlations of liver function indices on days 4–6, 7–9, and 10–12 of disease course with mortality. In case of multiple liver tests in the same period, the first test results were included in the analysis. Patients with mildly elevated ALT at 4–6 d were at almost 4.6-fold greater risk of all-cause mortality compared to those with normal ALT levels [Hazard ratio (HR) 4.598; 95% confidence interval (CI): 1.301–16.247; *p*=0.018]. Those with a moderate ALT elevation at 7–9 d were at 15.41 higher odds of death (HR: 15.411; 95% CI: 2.029–117.027; *p*=0.008), and those with moderate ALT elevation at 10–12 d were at 5.68 higher odds of death (HR: 5.681; 95% CI: 1.264–25.532; *p*=0.023) (Table 4). Compared with patients with normal ALP, all-cause mortality risk significantly increased by eight-fold (HR: 9.031; 95% CI: 697–22.061; *p*<0.001) in patients with ALP >2ULN at 7–9 d after disease onset and increased by 5.2 to 8.7-fold (HR: 6.215; 95% CI 3.496–11.049; *p*<0.001; HR: 9.698; 95% CI: 3.794–24.792; *p*<0.001, respectively) in patients with elevated ALP at 10–12 d after disease onset. GGT >2 ULN and ALB

Table 3. Characteristics of liver abnormalities in patients with SFTS at admission

Variable[a]	Total (*n*=459)	Deceased (*n*=78)	Surviving (*n*=381)	*p*-value[b]
TB, µmol/L	9.10 (6.80–12.40)	10.25 (8.15–13.90)	8.70 (6.70–12.00)	<0.001
Normal	421 (93.4)	69 (88.5)	352 (93.6)	<0.001
1–2 ULN[c]	27 (5.9)	8 (10.3)	19 (5.1)	
>2 ULN	7 (1.5)	6 (7.7)	1 (0.3)	
ALT, U/L	67.00 (42.00–115.00)	92.50 (57.50–175.00)	62.00 (41.50–105.50)	<0.001
Normal	104 (22.9)	10 (12.8)	94 (25.0)	<0.001
1–2 ULN	166 (36.6)	21 (26.9)	145 (38.6)	
2–5 ULN	141 (31.1)	32 (41.0)	109 (29.0)	
5–15 ULN	40 (8.8)	13 (16.7)	27 (7.2)	
>15 ULN	3 (0.7)	2 (2.6)	1 (0.3)	
AST, U/L	173.00 (96.00–339.00)	288.50 (170.50–592.50)	151.00 (89.50–320.00)	<0.001
Normal	19 (4.2)	0 (0.0)	19 (5.1)	<0.001
1–2 ULN	64 (14.1)	4 (5.1)	60 (16.0)	
2–5 ULN	172 (37.9)	22 (28.2)	150 (39.9)	
5–15 ULN	153 (33.7)	34 (43.6)	119 (31.6)	
>15 ULN	46 (10.1)	18 (23.1)	28 (7.4)	
ALP, ULN	63.00 (52.00–80.00)	72.00 (55.00–91.00)	62.00 (51.00–79.00)	0.007
Normal	426 (93.8)	68 (87.2)	358 (95.2)	0.014
1–2 ULN	22 (4.8)	9 (11.5)	13 (3.5)	
>2 ULN	6 (1.3)	1 (1.3)	5 (1.3)	
GGT, ULN	27.00 (18.00–51.00)	37.00 (22.00–87.50)	26.00 (17.00–48.00)	0.003
Normal	337 (74.2)	51 (65.4)	286 (76.1)	0.107
1–2 ULN	52 (11.5)	12 (15.4)	40 (10.6)	
>2 ULN	63 (13.9)	15 (19.2)	48 (12.8)	
ALB	32.60 (29.40–35.30)	30.00 (26.98–33.03)	33.00 (30.00–35.85)	<0.001
>35 g/L	120 (26.4)	9 (11.5)	111 (29.5)	<0.001
>30 g/L, ≤35 g/L	198 (43.6)	29 (37.2)	169 (44.9)	
≤30 g/L	136 (30.0)	40 (51.3)	96 (25.5)	

[a]Data are medians (interquartile range) or *n* (%), as indicated. [b]*p*<0.05 is significant. ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transpeptidase; TB, total bilirubin; ULN, upper limit of normal. [c]ALP ULN=150 U/L, ALT ULN=40 U/L, AST ULN=40 U/L, GGT ULN=50 U/L, TB ULN=19 µmol/L.

<30 g/L on days 10–12 were also associated with increased risk of death (Table 4).

Factors associated with liver impairment

Spearman correlation analysis was used to identify factors associated with abnormal liver function indices. ALT and AST levels showed a significant correlation with lymphocyte count ($r=0.15$, $p=0.001$, respectively) and PLR ($r=-0.26$, $p<0.001$; $r=-0.28$, $p<0.001$, respectively). ALP showed a significant correlation with monocyte count ($r=0.26$, $p<0.001$) and NMR ($r=-0.19$, $p<0.001$; Supplementary Table 2).

In addition, TB, ALT, and AST levels were positively correlated with viral load ($r=0.14$, $r=0.15$, and $r=0.27$, respectively, $p<0.05$ for all, Supplementary Table 2). Thus, higher viral loads were associated with more severe elevation of liver enzymes. In the *in vitro* experiment, Huh7 cells were inoculated with SFTSV and incubated for 3 days. The Immu-nofluorescence image showed accumulation of viral N protein in the cytoplasm of SFTSV-infected Huh7 cells (Fig. 4A), which suggested that SFTSV can directly infect Huh7 cells. The culture supernatant of SFTSV-infected Huh7 cells was collected at different times to determine the virus titer. The virus titer in the supernatant of virus-infected hepatocytes increased, and peaked at 24 h, suggesting extensive replication of SFTSV in the hepatocytes during that period (Fig. 4B).

Discussion

In our cohort, most patients with SFTS had signs of liver injury during hospitalization, among which the hepatocellular type was the most predominant. The most common abnormalities involved ALT, AST, and ALB levels. Both baseline and peak levels of ALT, AST, ALP, GGT in the deceased group were higher than those in the surviving group, while the opposite was observed with ALB level. The elevation of
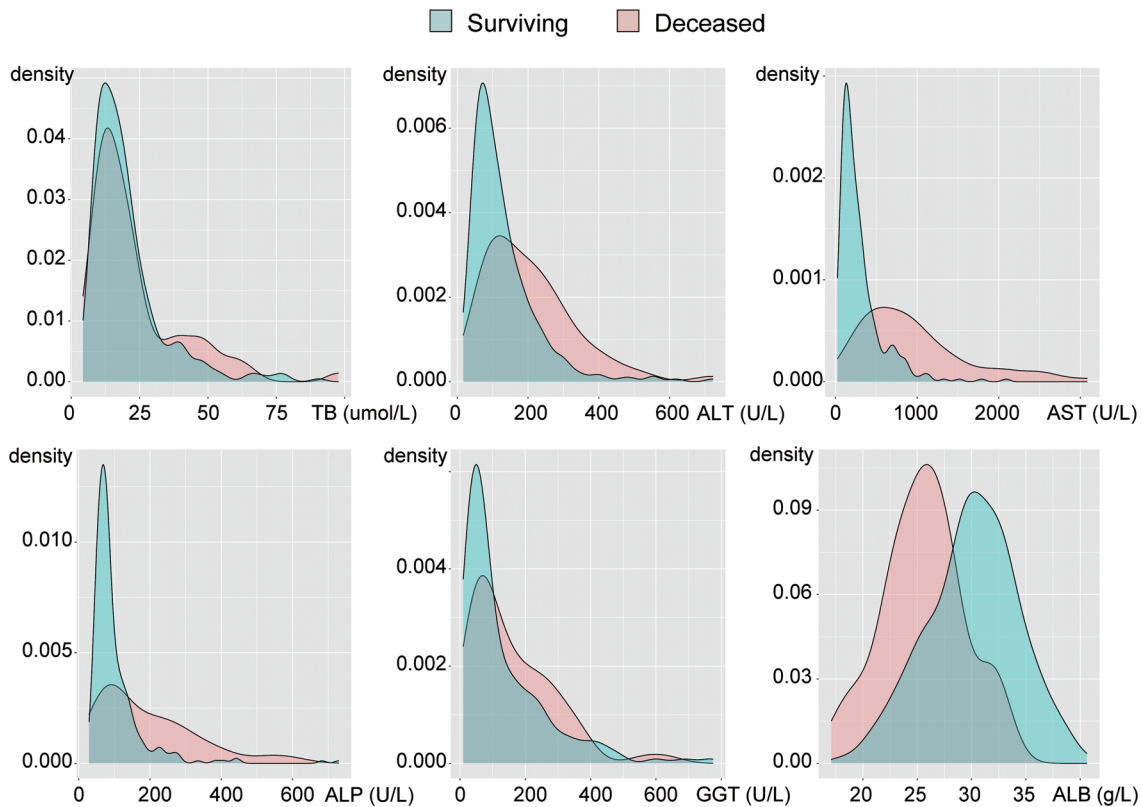
Fig. 2. Distribution of peak levels of liver function indices in patients with SFTS. Kernel density estimation of the overlay of distribution of peak levels of TB, ALT, AST, ALP, GGT, and ALB with patient outcomes in the deceased (pink) and surviving (blue) patients. Liver function indices are on the abscissa, and nuclear density values, indicating the degree of density of data are on the ordinate axis. ALB, albumin ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; GGT, γ-glutamyl transpeptidase; TB, total bilirubin.

ALT and ALP were independent risk factors for mortality in SFTS patients. ALT and AST levels were correlated with lymphocyte count, and PLR; TB, ALT, AST levels were positively correlated with viral load. In addition, SFTSV infected and replicated inside liver cells *in vitro*.

In a previous study, the proportion of SFTS patients with multiple organ failure in the deceased group was much higher than that in the surviving group.[13] Elevated levels of liver enzymes often indicate impairment of liver cells, which is a common phenomenon in SFTS patients.[4,14] Unlike acute disease caused by infection with other RNA viruses, SFTS is more prone to cause liver involvement. In patients with hantavirus infection, 28% had elevated transaminases (ALT or AST) and those with abnormal liver tests had significantly higher mortality than those with normal transaminases.[15] Among patients with SARS-CoV-2 infection, 14–53% of patients had abnormal liver function and liver biochemical indices that were closely related to the disease severity and prognosis.[2,16] However, in our study, 73.2% of the patients had liver injury during hospitalization, with hepatocellular injury being the dominant type of injury. In addition, the degree of elevation and the proportion of patients with elevation were greater with AST compared with ALT, which may be related to more tissue sources of AST, such as liver, cardiac muscle, and skeletal muscle tissue.[17]

ALP and GGT are often referred to as cholangiocyte-related enzymes. However, ALP is a more sensitive marker of cholestatic injury than GGT because of their histological differences.[18,19] In our study, more patients had elevated GGT than elevated ALP. In patients with elevated GGT but normal ALP, drug-induced liver injury, or other organ damage

should be considered, rather than cholestatic type liver injury. In addition, elevated serum ALP level was found to be a predictor of death in SFTS patients. In previous studies, elevated ALP level was associated with adverse outcomes in ST-segment elevated myocardial infarction patients; in addition, elevated ALP level was a risk factor for all-cause mortality in lung cancer patients.[20,21] The decrease in albumin level may not only be related to pre-existing chronic diseases but also to decreased protein synthesis caused by acute liver damage. Increased vascular permeability induced by SFTSV infection may also be a major cause of hypoalbuminema.[22,23] The baseline and peak levels of ALB in the deceased group were lower than those in the surviving group, suggesting that patients with lower ALB are more likely to have a poor prognosis. This conclusion is in line with previous studies in which a low ALB level was found to be a risk factor for poor prognosis.[24] Therefore, nutritional support, including intravenous albumin supplementation, should be strengthened for patients with SFTS.

We observed a positive correlation of abnormal TB, ALT, and AST levels with SFTSV load, which is related to disease severity.[25] In this study, we infected Huh7 cells with SFTSV *in vitro* and cultured them for three days. Experimental results showed that SFTSV could infect and replicate in liver cells, which indicated that direct viral infection of liver cells may be one of the causes of liver damage in SFTS patients. A high SFTSV RNA copy number was detected in the liver tissue[5] of deceased SFTS patients, and virus antigen was found in liver tissue from IFNAR (−/−) alpha/beta interferon receptor knockout mice by immunohistochemistry.[26] On the other hand, correlation analysis also revealed a posi-
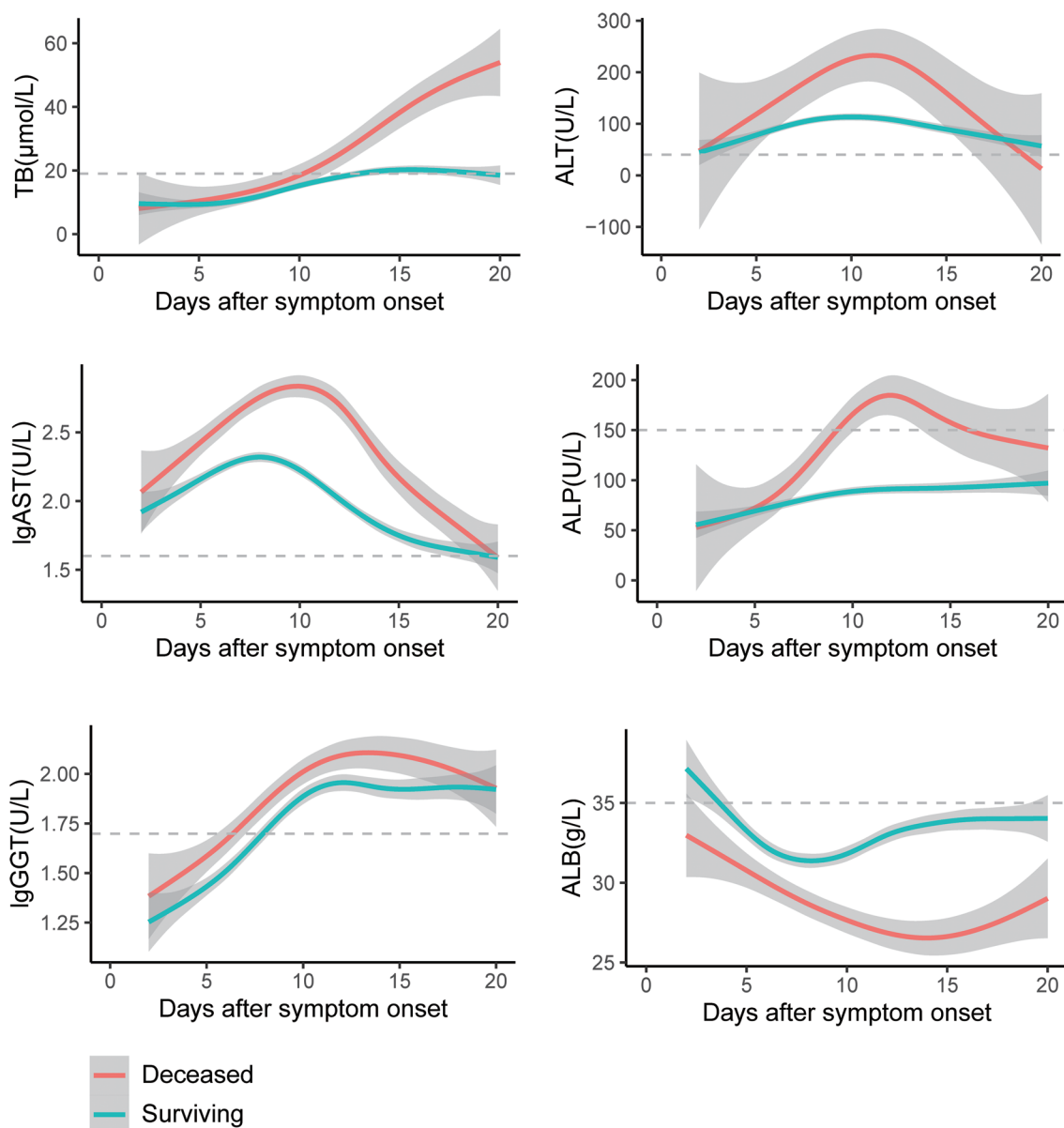
Fig. 3. Dynamic profile of liver test indices in patients with SFTS. Smooth trajectories of mean ALT, AST, ALP, and TB based on a locally weighted scatter plot with 95% confidence intervals according to disease severity for deceased (red) and surviving (blue) patients. Days after disease onset are on the abscissa and liver test indexes are on the ordinate. ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, γ-glutamyl transpeptidase; TB, total bilirubin.

tive correlation of ALT and AST levels with lymphocyte count and PLR, which are often used as markers of inflammatory status. However, ALP was mainly correlated with monocyte count and NMR. Previous studies have documented a marked increase in inflammatory factors such as IL-6, MCP-1,[27] accompanied by hepatic infiltration of lymphocytes and macrophages,[28] suggesting that liver injury in SFTS may be a secondary immunopathological process.[29,30] Systemic inflammation caused by viral infection may also contribute to liver damage. Further studies are required to confirm the mechanism of liver injury in SFTS patients.

In addition, we considered the influence of chronic hepatitis B virus infection (CHB) on SFTS patients. In SFTS patients with CHB, the severity of liver injury, abnormal liver indices, and mortality were not significantly different from those without CHB (Supplementary Tables 3–5, and Supplementary Fig. 1). Our results were consistent with those of previous studies in which concomitant hepatitis B virus infection was not found to aggravate liver impairment or to increase the risk of mortality in COVID-19 patients.[31,32]

Some limitations of this study should be acknowledged. Firstly, we failed to acquire liver biopsy tissue to directly observe SFTSV infection in liver parenchymal cells of patients. That was because SFTS is an acute disease that which is not suitable for liver puncture. Secondly, this was a retrospective cohort study with limited data. Therefore, we could not assess the relationship between liver injury and multiple organ injury. Thirdly, the mechanism of liver damage caused

Table 4. Association between liver function indices and mortality

	4–6 d (n=243)			7–9 d (n=363)			10–12 d (n=325)		
	HR	95% CI	p-value[a]	HR	95% CI	p-value[a]	HR	95% CI	p-value[a]
TB									
<ULN[b]		Reference			Reference			Reference	
1–2 ULN	0.857	0.292–2.514	0.779	2.004	1.088–3.689	0.026	0.789	0.431–1.444	0.442
>2 ULN	NA (0)			0.989	0.303–3.231	0.986	1.443	0.679–3.070	0.340
ALT									
< ULN		Reference			Reference			Reference	
1–2 ULN	3.335	0.965–11.532	0.057	4.621	0.612–34.915	0.138	1.904	0.419–8.641	0.404
2–5 ULN	4.598	1.301–16.247	0.018	6.357	0.8585–47.113	0.070	2.906	0.670–12.607	0.154
5–15 ULN	2.801	0.465–16.870	0.261	15.411	2.029–117.027	0.008	5.681	1.264–25.532	0.023
>15 ULN	NA (0)			509.629[c]	40.994–6,335.568	<0.001	45.289[c]	8.424–243.476	<0.001
AST									
< ULN		Reference			Reference			Reference	
1–2 ULN	976.038	$0.000–2.214\times10^{66}$	0.926	0.715	$0.000–1.087\times10^{63}$	0.996	606.339	$0.000–2.585\times10^{36}$	0.871
2–5 ULN	6,914.001	$0.000–1.549\times10^{67}$	0.905	281.785	$0.000–4.348\times10^{63}$	0.937	2,220.321	$0.000–9.265\times10^{36}$	0.845
5–15 ULN	8,543.362	$0.000–1.913\times10^{67}$	0.903	1,140.472	$0.000–1.758\times10^{64}$	0.922	4,426.846	$0.000–1.846\times10^{37}$	0.832
>15 ULN	7,143.476	$0.000–1.609\times10^{67}$	0.905	2,319.507	$0.000–3.574\times10^{64}$	0.914	35,479.258	$0.000–1.477\times10^{38}$	0.791
ALP									
<ULN		Reference			Reference			Reference	
1–2 ULN	NA (3)			1.673	0.650–4.305	0.286	6.215	3.496–11.049	<0.001
>2 ULN	NA (1)			9.031	3.697–22.061	<0.001	9.698	3.794–24.792	<0.001
GGT									
<ULN		Reference			Reference			Reference	
1–2 ULN	2.274	0.977–5.294	0.057	1.949	0.956–3.976	0.067	1.456	0.635–3.337	0.375
>2 ULN	0.416	0.056–3.077	0.390	1.547	0.866–2.763	0.141	2.323	1.140–4.734	0.020
ALB									
≥35		Reference			Reference			Reference	
30–35	1.218	0.506–2.933	0.660	0.416	0.173–1.003	0.051	5.517	0.7–43.499	0.105
<30	1.988	0.806–4.901	0.136	1.561	0.735–3.317	0.247	28.980	3.963–211.9	0.001

[a]$p<0.05$ is significant. ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; GGT, γ-glutamyl transpeptidase CI, confidence interval; HR., Hazard ratio; NA, not applicable; TB, total bilirubin. [b]ALP ULN=150 U/L; ALT ULN=40 U/L; GGT ULN=50 U/L TB ULN=19.0 μmol/L. [c]Because of the small sample size, statistical analysis was not carried out. There were only two samples in this statistic, so the result was considered meaningless.
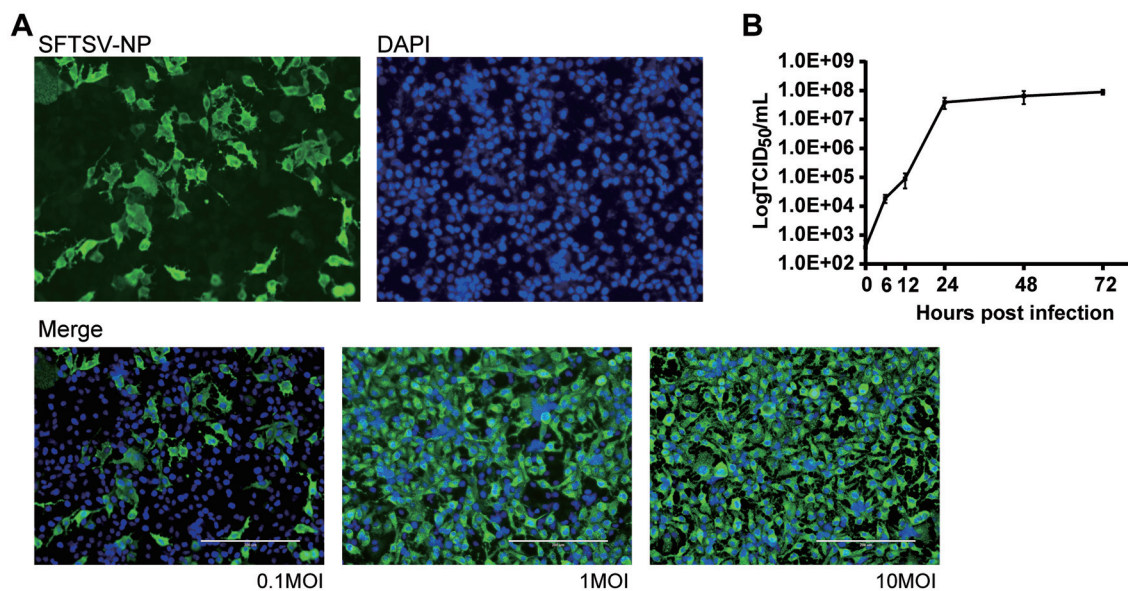
Fig. 4. SFTSV infection and replication in human hepatocellular carcinoma cell line-Huh7 cells. (A) Huh7 cells inoculated with SFTSV at a multiplicity of infection (MOI) of 0.1, 1, or 10. Confocal images of accumulation of viral N protein (green) in the cytoplasm of SFTSV-infected Huh7 cells (blue), magnification 20×; scale bar, 200 µm). (B) Huh7 cells inoculated at an MOI of 1 $TCID_{50}$ units/cell. The supernatant was harvested at 0, 6, 12, 24, 48, and 72 h postinoculation. Viral titers were determined by the method of Reed and Mench. The tests were performed in triplicate. Bars, standard deviations.

by viral infection needs further investigation.

To conclude, our study confirmed that liver injury is a common phenomenon in patients with SFTS. Abnormal ALT and ALP levels during the disease process were independent predictors of SFTS-related mortality. Therefore, frequent monitoring and evaluation of liver function, especially ALT and ALP, are necessary for SFTS patients.

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Conflict of interest

XZ has been an editorial board member of *Journal of Clinical and Translational Hepatology* since 2021. The other authors have no conflict of interests related to this publication.

Author contributions

Study concept and design (XZ, CP), acquisition of data (HW, TW, SuL, JL), analysis and interpretation of data (SiL, LX, BL, LF), drafting of the manuscript (SiL, LX), critical revision of the manuscript for important intellectual content (XZ, CP, BL, TX), administrative, technical, or material support, study supervision (XZ, CP).

Data sharing statement

The raw data used to support the findings of this study are available from the corresponding author upon request.

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