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Dynamic changes of laboratory parameters and peripheral blood lymphocyte subsets in severe fever with thrombocytopenia syndrome patients

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ABSTRACT

Objectives: The aim of this study was to dynamically investigate laboratory parameters and peripheral blood lymphocyte subsets in severe fever with thrombocytopenia syndrome (SFTS) patients at different stages, to evaluate the significance of these changes in the infection process and its influence on prognosis.

Methods: Case-control study was used in the research. Sixty-nine confirmed thrombocytopenia syndrome virus(SFTSV) infected patients were enrolled. They were divided into two groups, recovery group and poor prognosis group, according to the clinical prognosis of the diseases. The laboratory parameters were measured by matched fully-automatic detector. The dynamic lymphocyte subsets of each group were tested by flow cytometry. Independent-group Student's t-test, Bonferroni test and Nemenyi test were used to compare the mean value of every group.

Results: The clinical manifestations typically became worse on about the 7th day. Most of them had multi organ dysfunction, and part of them had hemophagocytic lymphohistiocytosis histiocytosis (HLH). The characteristic laboratory findings in the early stage were the drop of platelets (PLT), while the increase of alanine aminotransferase (ALT), aspartate amino transferase (AST), creatine kinase (CK), and lactate dehydrogenase (LDH). SFTSV viral loads reached the highest on Days 7–10 after onset of fever in SFTS patients. CD3+, CD3 + CD4+ T cell counts were significantly reduced in poor prognosis group, more so on Days 7–10 after onset of fever. CD3–CD19+ (B cell) counts in SFTS patients were significantly higher than that of healthy controls. 11 days after illness onset, symptoms were improved, accompanied by resolution of laboratory abnormalities.

Conclusions: These results indicated that SFTS had an acute onset and self-limited course. It was a systemic infection. The host immune response caused tissues and organs injury. The improvement of symptoms and laboratory tests was consistent with the elimination of the virus and recover of immune response. Further investigation should be done in order to reveal the mechanisms of SFTSV pathogenesis and guide the clinical treatment.

Introduction

Severe fever with thrombocytopenia syndrome (SFTS) is a newly emerging infectious disease reported for the first time in China in 2010. The etiological agent of SFTS has been revealed to be a novel phlebovirus in the family Bunyaviridae, termed severe fever with thrombocytopenia syndrome virus (SFTSV).[1] The typical clinical presentation of SFTS is acute fever and thrombocytopenia (platelet count less than 100,000/ml), in addition to other non-specific features including muscle pain, severe malaise, nausea, vomiting, and diarrhea,[1–5] while severe patients might develop multiple organs dysfunction and even death. A high mortality rate (ranging from 12%–30%) has been reported for SFTSV-infected patients.[1,3,6] However, the pathogenic mechanism in patients with SFTSV infection is still unclear.

For an emerging infectious disease with high case fatality rate, the clinical and laboratory parameters that might predict adverse disease outcome have been investigated with high interest. Laboratory tests on serum samples of SFTS patients commonly

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showed elevated alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and creatine kinase (CK). On the other hand, viral interaction with the innate immune system played a core role in determining the outcomes of the infection.[7] Some authors found that SFTS patients were at least partly immune-mediated, which might be important in determining the severity and clinical outcome[3] Lymphocytes, which played an important role in the induction of cellular immunity in organism, could stimulate the organism to produce immune response against viral antigens when organism was infected with SFTSV.[8]

In the current report, changes in SFTSV viral load, platelets and white blood cell counts, levels of key serum enzymes, and changes in peripheral blood lymphocyte subset populations were measured in SFTSV patients at different stages. Information learned from the current study provides a better understanding on the relationship between clinical disease progression and key clinical lab and immunological parameters. Such information is also useful to guide a more in-depth investigation on the mechanisms of SFTSV pathogenesis.

Materials and Methods

Participants

Between May 2014 and September 2015, sixty-nine patients(33 males and 36 females), with confirmed SFTSV infection based on diagnostic guidelines from the Chinese Ministry of Health,[3] were admitted to the Jinan Infectious Diseases Hospital, Jinan, China. These patients were aged between 40 and 78 years (mean: 59 years). They were divided into two groups, recovery group and poor prognosis group, according to the clinical prognosis of the diseases. 56 patients of the recovery group were admitted to the hospital at the earliest on the3th day from onset of illness. 13 patients of the poor prognosis group, including patients who died in hospital and died after leaving hospital, were admitted to the hospital at the earliest on the 7th day from onset of illness. Some patients had ever been exposed to ticks. Serum anti-SFTSV IgM and SFTSV RNA were positive in all infected patients. In addition to the SFTSV-infected patients, twenty-five healthy volunteers (16 males and 9 females) were also enrolled in this study to serve as normal controls. They were from the healthy physical examination center of the Jinan Infectious Diseases Hospital. These volunteers were aged between 40 and 78 years (mean: 59 years).

Sample collection and processing

Blood samples coated with EDTA −K2 were collected from patients on admission during the acute phase of SFTSV infection, while serum samples were collected at the same time and stored at −80 °C until analyzed. In addition, blood samples from healthy donors were collected at a single time point at the time of enrollment.

Laboratory tests

Blood cell and platelet counts were measured by fully-automatic blood cell analyzer. A fully-automatic biochemical detector was used to determine levels of alanine transaminase (ALT), aspartate transaminase (AST), lactic dehydrogenase (LDH), and creatine kinase (CK).

SFTSV RNA was detected by real-time reverse transcription polymerase chain reaction (RT-PCR) using a certificated SFTSV RT-PCR kit. The SFTSV-specific immunoglobulin M (IgM) insera were detected by enzyme-linked immunosorbent assay (ELISA) using a SFTSV IgM kit.

Flow cytometry

The peripheral blood samples were processed for an analysis of lymphocyte subsets by flow cytometry within 2 h. Lymphocyte subsets were identified by the following monoclonal antibodies: anti-CD45-PerCP, anti-CD3-FITC,anti-CD8-PE, anti-CD4-APC, anti-CD16/56-PE and anti-CD19-APC. Cell suspension was incubated in a dark place at room temperature for 30 min. The red blood cells were removed by using 500 μl lysis buffer in a dark place at room temperature for 10 min. Finally, cells were analyzed with FACS Canto Flow Cytometer and CellQuest software (BD BioScience). Up to 10000 total events were collected per sample. The lymphocyte population was selected by gating lymphocytes. The number of positive cells including the expression of CD3+, CD4+ and CD8+ T lymphocytes, B cells and NK cells was analyzed as a percentage of the cells in the lymphocyte gate.

Statistical analysis

The results were analyzed using statistical software SPSS17.0 for Windows (SPPS, an IBM Company). Shapiro–Wilkes test and Levene's test were used to assess normality and variance homogeneity. Independent-group Student's t-test and Bonferroni test were used to compare the mean value of every group. Mann–Whitney test, Kruskal–Wallis test and Nemenyi test were used for the non-normality or unequal variance. $P < 0.01$ was considered statistically significant.

Table 1
Study cohorts.

Groups	No. of Subjects	Age range (average)	Gender (M/F)	Day of admission (range, median)*
Recovery group	56	27–83 (58)	24/32	3–17 (9)
Poor prognosis group	13	40–70 (59)	9/4	7–12 (9)
Healthy control group	25	40–78 (59)	16/9	NA

* Days after symptom onset.

Table 2
Comparison of clinical parameters in different conditions of SFTS patients.

Parameter	Recovery group Mean ± SE	Poorprognosis group Mean ± SE	*p value*
WBC, $\times 10^9$/L	9.98 ± 4.83	6.27±	*>0.05*
1.24			*<0.01*
PLT, $\times 10^9$/L	92.98 ± 9.78	37.00±	*<0.01*
4.59			*<0.01*
ALT, U/L	95.38 ± 10.52	251.46±	*<0.01*
66.80			*<0.01*
AST, U/L	188.86 ± 28.04	633.69±	*<0.01*
136.32			*>0.05*
CK, U/L	598.21 ± 130.23	2933.31	
±1233.50			
LDH, U/L	664.29 ± 64.02	1508.00	
±164.02			
Viral load, $\times 10^3$IU/ml	14.54 ± 5.18	310.24±	
143.52			
IgM	0.76 ± 0.10	0.63±	
0.18			

Data were analyzed by Mann–Whitney test.