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ORIGINAL RESEARCH

Changes in Cytokine Levels in Patients with Severe Fever with Thrombocytopenia Syndrome Virus

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Purpose: To characterize the cytokine profile of patients with severe fever with thrombocytopenia syndrome (SFTS) in relation to disease severity.

Patients and Methods: 60 laboratory-confirmed SFTS patients and 12 healthy individuals from multi-centers in Shandong Province of China were included, and all patients were divided into fatal patients (9) and recovered patients (51) due to their final outcomes. Multiplex-microbead immunoassays were conducted to estimate levels of 27 cytokines in the sera of patients and controls.

Results: The results showed that levels of IL-2, IL-4, IL-6, IL-7, IL-8, IL-15, IL-1RA, G-CSF, GM-CSF, IFN-γ, TNF-α, basic FGF, PDGF-BB, RANTES, IP-10, MIP-1α, MIP-1β, MCP-1, and Eotaxin differed significantly among the SFTS fatal patients, recovered patients, and the healthy controls (all $p<0.05$). Compared to the healthy controls, the fatal patients and recovered patients had reduced levels of IL-2, IL-4, IL-7, PDGF-BB, RANTES, and Eotaxin, while the levels of PDGF-BB and RANTES were significantly lower in fatal patients compared to recovered patients. The increasing levels of IL-6, IL-8, IL-15, IL-1RA, G-CSF, GM-CSF, IFN-γ, TNF-α, basic FGF, IP-10, MIP-1α, MIP-1β, and MCP-1 were observed in fatal patients (all $p<0.05$), and the levels of IL-6, IP-10, MIP-1α, and MCP-1 were significantly higher than other two groups. The Spearman correlation analysis indicated a positive correlation between platelet count and PDGF-BB levels ($p<0.05$), while the white blood cell count had a negative correlation with MIP-1 level ($p<0.05$).

Conclusion: The research exhibited that the SFTS virus (SFTSV) caused an atypical manifestation of cytokines. The levels of IL-6, IP-10, MIP-1α, and MCP-1 had been observed a positive association with the severity of the illness.

Keywords: SFTS, SFTSV, cytokines, fatal patients, correlation analysis

Introduction

Severe fever with thrombocytopenia syndrome (SFTS) was initially identified in Central and North-Eastern China in 2010.[1] Since then, the disease has been increasingly reported in China, and known to affect at least 23 provinces of China.[2] SFTS has also been reported in several other countries, including Korea, Japan, Vietnam, and Myanmar,[3–6] and has once been included in the World Health Organization's (WHO) List of Blueprint priority diseases.[7] The causative pathogen of the disease is Dabie bandavirus, formerly referred to as SFTS virus (SFTSV), which is usually tick-borne, and occasionally transmitted between individuals through oral or ocular mucous membrane contact.[8] SFTS commonly have clinical manifestations of high temperature, a decrease in platelet count, reduced white blood cell count,[1] and sometimes can lead to the rapid development of multiple organ dysfunction syndrome (MODS) ultimately resulting in death within two weeks.

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The inflammatory response, which is mediated by cytokines, is critical in the progression of SFTS.[9] The viral infection may lead to complications or even fatalities due to an excessive release of pro-inflammatory cytokines, commonly known as a "cytokine storm".[10] Cytokine storms often arise within the framework of specific ailments, conditions, and treatments, including anaphylaxis, graft-versus-host disease, acute respiratory distress syndrome (ARDS), and systemic inflammatory response syndrome (SIRS),[11] which often lead to poor disease outcomes.

The initial two weeks after the onset of SFTSV infection are commonly referred to as the "acute phase" in multiple studies. This phase marks the progression of the infection.[9] In patients with SFTS, an unusually high level of 17 inflammatory mediators was detected compared to healthy individuals.[12] This observation remained consistent regardless of the severity of the disease or the patient's survival status, indicating the potential involvement of these inflammatory mediators in the development of the disease. Additionally, it was noted that lower levels of the chemokine RANTES and the growth factor PDGF were associated with an increased risk of mortality.[12] In contrast to the control group comprising healthy individuals, the group of patients exhibited elevated levels of cytokines (IL-6, IL-10, IP-10, MCP-1, and IFN-γ), while displaying decreased levels of IL-8, TGF-β1, and RANTES. Notably, the severe patients demonstrated higher levels of IL-6, IL-10, IP-10, and MCP-1 compared to the mild patients.[13] Other studies have also reported comparable findings.[14,15] However, there is no clear and unified conclusion on the differential changes in cytokine levels in SFTS patients. The objective of this study was to quantify changes in serum cytokine levels of SFTS patients, to evaluate the association between cytokine levels and laboratory test parameters, and to better understand the importance of cytokines in SFTS prognosis.

Materials and Methods

Study Design

This was a multi-center observational study, which recruited 60 SFTS patients from eight hospitals in Shandong Province of China from April to August 2014. All recruited patients met the definition of laboratory-confirmed SFTS and were diagnosed with only SFTSV infection during hospitalization. Patients who were hospitalized for multiple diseases or unwilling to cooperate with the study were excluded. Meanwhile, 12 individuals in good health were randomly chosen from Shandong Province as controls. The Shandong Center for Disease Control and Prevention (Shandong CDC) has granted ethical approval according to the guidelines set by the Ethics Committee (no.2011–12). All patients involved in this study have voluntarily provided their informed and written consent by signing the necessary documents.

Laboratory-confirmed SFTS patient was defined according to the guideline issued by the Chinese Ministry of Health (now renamed as the National Health Commission of China):[16] showing an acute onset of fever >38 °C, with an exposure history and thrombocytopenia (<100 × 10^9 platelets/L) or leucopenia (<4 × 10^9 leukocytes/L), and having at least one of the laboratory test criteria: a positive SFTS virus culture; a positive result for viral RNA; seroconversion or a four-fold increase in specific antibody between acute and convalescent sera. All 60 patients were SFTS patients group, and divided into fatal patients group and recovered patients group by their final outcomes. The healthy control group were individuals with no clinical evidence of SFTS and negative results for both viral RNA and specific antibodies against SFTSV. Individuals with other infections or chronic diseases were excluded.

Collection of Serum Samples and Data

We aimed to collect serum samples when SFTS patients were admitted to hospitals in the acute phase. Data on basic information, clinical manifestations, and laboratory test results were collected at the same time. All the patients were followed-up and confirmed if they were survival or fatal. Serum samples were collected from healthy controls in the same way, and basic information was collected in the meantime. The serum samples isolated in the local laboratory were immediately frozen and transported to Shandong CDC as soon as possible. The samples were stored at −80°C until use.

Tests of Serum Cytokines

We utilized the Bio-plex Pro Human cytokine 27-plex Assay kit (Bio-Rad, USA) to quantify the levels of cytokines in serum samples collected during the hospitalization of SFTS patients. This kit incorporates fluorescent microspheres that are conjugated with monoclonal antibodies that specifically target the cytokines of interest. To ensure accurate results, we strictly followed the instructions provided by the manufacturer, and all cytokines were tested using the same method. The following cytokines underwent testing: interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), Eotaxin, macrophage inflammatory protein 1β (MIP-1β), MIP-1α, monocyte chemotactic protein-1 (MCP-1), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (basic FGF), granulocyte-macrophage colony-stimulating factor (GM-CSF), inducible protein-10 (IP-10), granulocyte colony-stimulating factor (G-CSF), interleukin (IL)-1β, IL-17, IL-15, IL-13, IL-12, IL-10, IL-9, IL-8, IL-7, IL-6, IL-5, IL-4, IL-2, IL-1 receptor antagonist (IL-1RA), regulated on activation and normally T-cell expressed (RANTES), and platelet-derived growth factor (PDGF-BB). Processed samples were subjected to testing using the Bio-Plex 3D system, which is a flow cytometry-based method.

Statistical Analysis

The data analyses were conducted using GraphPad Prism 9.5.0 software. To describe continuous variables that adhered to a normal distribution, we employed means and standard deviations (SDs), while variables with abnormal distribution were described using medians and interquartile (IQ) ranges. To estimate the differences between groups, the student's *t*-test for a continuous variable, and χ^2-test or a Fisher's exact test for a categorical variable were used. Comparisons among three groups were conducted using a nonparametric one-way analysis of variance. When there was an abnormal distribution of data, a nonparametric test was employed to evaluate the disparities. The correlation analysis of laboratory parameters and serum cytokines involved the calculation of Spearman test values for the correlation of two variables. A two-sided p value less than 0.05 was considered to be significant.

Results

Characterization of SFTS Patients and Healthy Controls

A total of 60 SFTS patients were included in the study, of whom 9 died of the disease, and 51 recovered. In comparison to the 12 individuals in the control group, SFTS patients had no statistical differences in age and gender (Table 1). We then compared the demographic and clinical characteristics of recovered and fatal patients. There was no significant difference in age and gender between recovered and fatal patients. The recovered patients experienced significantly more body soreness than fatal cases (p=0.03) (Table 1). Notably, all the patients had a fever, and the fatal patients had significantly higher maximum temperatures than the recovered cases (p=0.002) (Table 1). The platelet count exhibited a significant decrease in the fatal group in comparison to the recovered group (p=0.01), while the total white blood cell count as well as lymphocyte count, and neutrophil count did not demonstrate any differences between the two groups (Table 1).

Comparison of Cytokine Levels Between SFTS Patients and Healthy Controls

Among the 27 cytokines tested in this study, 19 showed significantly different levels between SFTS patients and controls. Sixteen cytokines, including IL-2, IL-4, IL-5, IL-7, IL-9, IL-17, IL-1RA, G-CSF, GM-CSF, IFN-γ, TNF-α, basic FGF, PDGF-BB, RANTES, MIP-1β, and Eotaxin in the sera were significantly lower in SFTS patients compared to the healthy controls (all p<0.05) (Figure 1A). On the contrary, three cytokines (IL-6, IP-10, and MCP-1) showed a significant increase in SFTS patients (all p<0.05) (Figure 1B).

Comparison of Cytokine Levels Between Fatal and Recovered Patients in Relation to Controls

We conducted a comparative analysis of cytokine production between fatal and recovered patients in relation to healthy controls. Significant differences were observed in 19 cytokines among the three groups. The analyses revealed three distinct patterns of cytokine changes. The first pattern displayed that the levels of IL-2, IL-4, IL-7, PDGF-BB, RANTES, and Eotaxin, were decreased in both fatal and recovered patients compared to healthy individuals, with statistical significance for PDGF-BB and

Table 1 Comparison of Demographic and Clinical Characteristics in General

	SFTS patients		Healthy Controls	*p* value[a]	*p* value[b]
	Recovered Patients (n=51)	Fatal Patients (n=9)			
Age, year n(%)				0.06	0.28
Median, year, IQR	60(54.00–70.04)	64(58.06–73.74)	64(57.50–69.75)		
Gender n(%)				0.84	0.21
Male	17(33.33)	5(55.56)	4(33.33)		
Female	34(66.67)	4(44.44)	8(66.67)		
Occupation n(%)				0.02	0.75
Farmer	47(92.16)	8(88.89)	9(75.00)		
Others	4(7.84)	1(11.11)	3(25.00)		
Clinical manifestations n(%)					
Fever	51(100.00)	9(100.00)			N/A
Shiver	25(49.02)	3(33.33)			0.39
Headache	23(45.10)	2(22.22)			0.21
Fatigue	45(88.24)	8(88.89)			0.96
Body soreness	31(60.78)	2(22.22)			0.03*
Conjunctival hemorrhage	8(15.69)	2(22.22)			0.63
Skin ecchymosis	3(5.88)	1(11.11)			0.57
Poor appetite	23(45.10)	4(44.44)			0.97
Nausea	25(49.02)	3(33.33)			0.39
Vomiting	18(35.29)	1(11.11)			0.16
Abdominal Pain	6(11.76)	2(22.22)			0.40
Abdominal distension	4(7.84)	1(11.11)			0.75
Diarrhea	13(25.49)	1(11.11)			0.36
Renal pain	2(3.92)	0(0.00)			0.55
Lymphadenopathy	11(21.57)	0(0.00)			0.12
Maximum temperature, °C **Median (25–75%)**	39(38.50–39.00)	40(38.90–39.85)			0.002**
Laboratory tests Median (25–75%)					
WBC count, 10^9/L	2.29(1.76–3.02)	3.56(1.43–4.88)			0.58
PLT count, 10^9/L	68.00(53.00–84.00)	48.50(37.75–59.50)			0.01*
NEU count, 10^9/L	1.39(0.95–2.00)	1.74(1.00–2.84)			0.12
LYM count, 10^9/L	0.70(0.48–1.10)	1.14(0.39–1.50)			0.10

Notes: [a]The SFTS patients compared with the healthy controls. [b]The recovered patients compared with the fatal patients. Significant difference is designated by an asterisk (*).*p<0.05, **p<0.005. Medians and interquartile (IQ) ranges were used for abnormal distribution. The categorical variables were compared with χ 2 test. The continuous variables were compared with the student's t-test.

Abbreviations: IQR, interquartile range; WBC, white blood cell; PLT, platelet; NEU, neutrophil; LYM, lymphocyte; N/A, not applicable.

RANTES between fatal patients and controls (Figure 2A). The second pattern involved IL-8, IL-15, IL-1RA, G-CSF, GM-CSF, IFN-γ, TNF-α, basic FGF, and MIP-β, which were significantly increased in fatal patients than in recovered patients. However, no significant difference was observed between fatal patients and healthy controls. IL-1RA, G-CSF, GM-CSF, IFN-γ, and basic FGF exhibited a significant difference between the recovered patients and the healthy controls. While IL-8, IL-15, TNF-α, and MIP-β did not show any significant difference in the two groups (Figure 2B). In the third pattern, fatal patients exhibited substantially higher levels of cytokines, including IL-6, IP-10, MIP-1α, and MCP-1, in comparison to both recovered patients and healthy individuals (Figure 2C). Only the level of IP-10 showed significant differences among the three study groups.

Cytokine Levels at Different Phases

Considering the sera were collected at various times after disease onset, we compared cytokine levels of SFTS patients at different phases. A total of 9 patients' serum samples were collected within three days of onset, 28 from four to seven days, 20 from eight to fourteen days, and three over fourteen days. Thirteen of 27 cytokines, including IL-6, IP-10, IL-

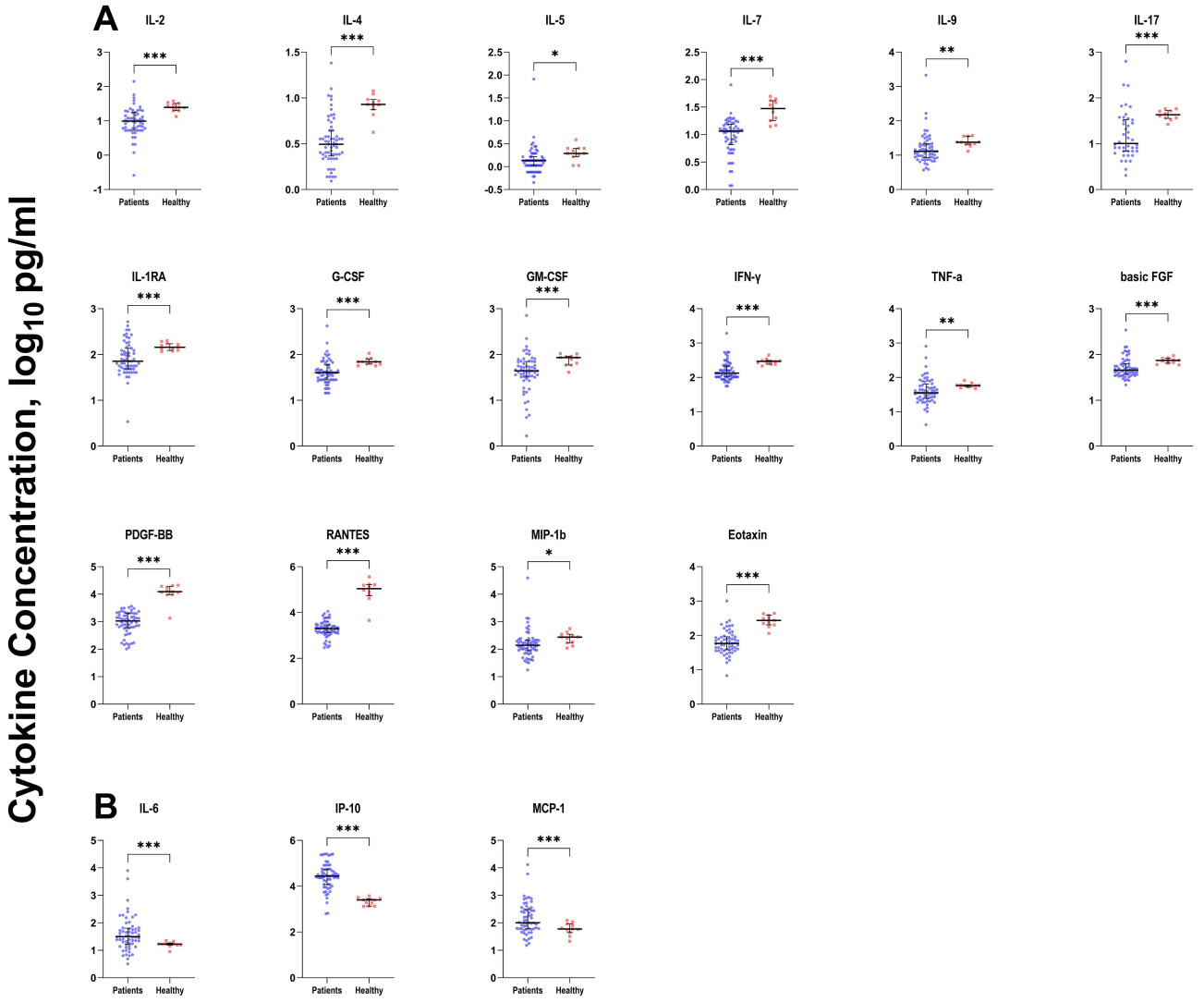

Figure 1 Comparison of cytokine production in SFTS patients and healthy individuals.

Notes: (**A**) Shows that SFTS patients had lower levels of cytokines compared to the healthy controls; (**B**) shows that SFTS patients had higher levels of cytokines compared to the healthy controls. Cytokines were detected in the acute phase serum samples from SFTS patients and healthy donors by multiplex-microbead immunoassays. The cytokine levels were compared among the groups of 60 patients and 11 healthy controls. One sample from the healthy donors was excluded due to abnormal results (out of range above) on cytokine levels testing. The parametric test was performed to evaluate the differences after the logarithmic conversion of cytokine levels. Each dot shows the cytokine concentration in an individual. Horizon bars indicate the respective group median and IQR. The significant difference between the two groups is designated by an asterisk (*). *p<0.05, **p<0.005, ***p< 0.001.

Abbreviations: G-CSF, granulocyte colony-stimulating factor; IL, interleukin; RANTES, regulated on activation and normally T-cell expressed; PDGF-BB, platelet-derived growth factor; IFN-γ, interferon-γ; VEGF, vascular endothelial growth factor; IP, inducible protein.

1RA, IL-4, Eotaxin, basic FGF, G-CSF, GM-CSF, IFN-γ, TNF-α, IL-15, MCP-1, and RANTES, were continuously decreased as the disease progresses (Figure 3A). In contrast, MIP-1β, IL-8, and IL-9 generally kept increasing with time after disease onset (Figure 3B). Four cytokines, including IL-10, IL-12, IL-17, and VEGF, were increased within seven days of disease onset and then decreased (Figure 3C). The other seven cytokines, including IL-2, IL-5, IL-7, IL-Iβ, IL-13, MIP-1α, and PDGF-BB, had variable levels from time to time after disease onset (Figure 3D).

Correlation Between Cytokines and Laboratory Parameters

In addition to our previous findings, we conducted further analysis to find the correlation between cytokines and laboratory parameters in all 60 SFTS patients during viral infection. Our correlation analyses revealed that the levels of MCP-1 in the serum positively correlated with white blood cell count (p=0.045). Specifically, the correlation

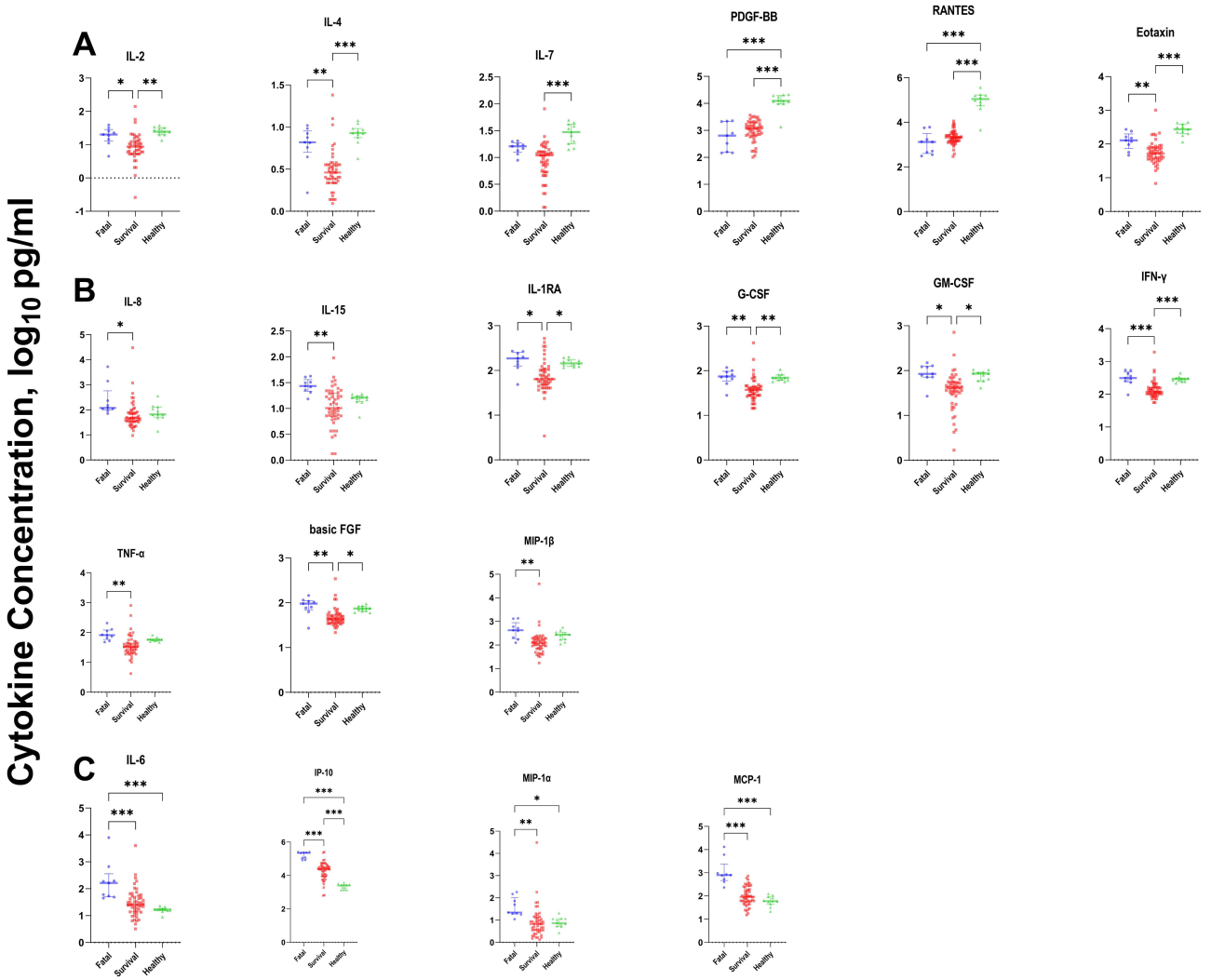

Figure 2 Comparison of cytokine levels in fatal, nonfatal patients and healthy people.

Notes: In Figure 2A, it was observed that the levels of certain cytokines were reduced in both fatal and recovered patients compared to healthy individuals. Figure 2B showed that the levels of some cytokines were higher in fatal patients than in recovered patients. Figure 2C indicated that fatal patients had higher levels of certain cytokines than both recovered patients and healthy individuals. Cytokines were detected in the acute phase serum samples from SFTS patients and healthy donors by multiplex-microbead immunoassays. The cytokine levels were compared among the groups of 9 fatal patients, 51 survival patients, and 11 healthy controls. One sample from the healthy donors was excluded due to abnormal results (out of range above) on cytokine levels testing. The parametric test was performed to evaluate the differences after the logarithmic conversion of cytokine levels. Each dot shows the cytokine concentration in an individual. Horizon bars indicate the respective group median and IQR. The significant difference between the two groups is designated by an asterisk (*).*p<0.05, **p<0.005, ***p< 0.001.

coefficient for MCP-1 was −0.276 (Table 2). The results of our research indicate a substantial link between the white blood cell count and the concentrations of MCP-1 in individuals affected by SFTS. Platelet count positively correlated with PDGF-BB serum levels, with the respective correlation coefficient of 0.303 (p=0.028) (Table 2). The positive correlation indicates that as platelet count decrease, the cytokine level also decreases.

Discussion

The immune response greatly influences the outcome of infectious diseases, especially those caused by viruses.[12] Many patients with severe SFTS progress rapidly to bad outcomes within 1–2 weeks after illness onset. The primary cause of death during the acute phase of viral infections is widely believed owing to cytokine storm.[17] Patients with SARS have experienced cytokine storms, which have been linked to unfavorable outcomes.[18] It has been suggested that the occurrence of a cytokine storm could potentially play a role in the development of severe COVID-19 cases.[19]

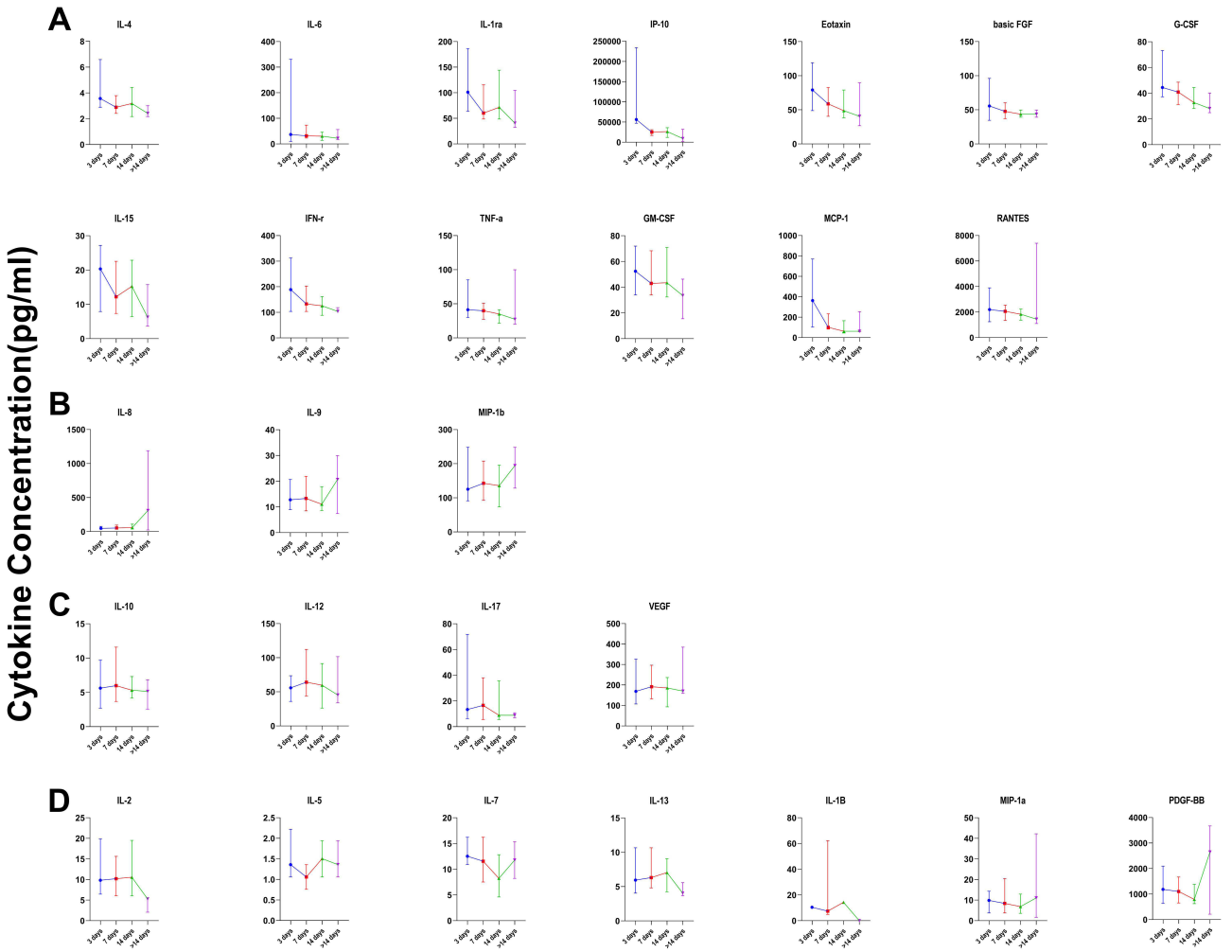

Figure 3 Cytokine levels at different phases.

Notes: Figure 3A indicates that cytokine levels decreased consistently as the disease progressed. However, Figure 3B showed a contrasting trend. In Figure 3C, some cytokine levels initially increased and then decreased. In Figure 3D, the levels of certain cytokines constantly changed. Cytokines were detected in the acute phase serum samples from SFTS patients and healthy donors by multiplex-microbead immunoassays. The one-way ANOVA was used to evaluate the differences in cytokine levels at different phases. Each dot indicates the group median. Horizon bars indicate the 95% CI of each group.

Cytokines are minute soluble proteins that are excreted by immune and tissue cells. These proteins play a crucial role in cell growth, differentiation, and regulation of various physiological processes by binding to corresponding receptors. Cytokines also regulate immune responses and can be involved in the occurrence of various diseases, such as inflammation, under certain conditions.[20] Cytokines have also exhibited a correlation with other infectious diseases. It has been uncovered that different types of cytokines, including TNF-α and IP-10, exhibit a correlation with the gravity of hantavirus-induced hemorrhagic fever with renal syndrome.[21] Significant discrepancies in cytokine levels were observed when examining fatal cases in contrast to both infected survivors and uninfected control groups. The venous virus family, to which the Rift Valley fever virus belongs, demonstrated remarkable variations in cytokine levels. Specifically, there were significant increases in several cytokines (such as MCP-1, and IL-10). Conversely, the RANTES level decreased in response to the virus.[22] Interestingly, this finding suggests that the presence of the Rift Valley fever virus triggers a substantial immune response characterized by the up regulation of certain cytokines.

The levels of some cytokines have been previously reported among SFTS patients.[13–15] However, there was no consensus on the effects of SFTS on cytokine levels. Therefore, analyzing the changes in cytokine levels in SFTS

Table 2 Correlation Analysis of Cytokine Levels and Laboratory Parameters in SFTS Patients

	WBC	PLT	NEU	LYM
IL-1β	0.174 (0.750)	0.143 (0.803)	0.257 (0.658)	0.522 (0.300)
IL-1RA	−0.026 (0.851)	−0.107 (0.450)	0.08 (0.569)	−0.045 (0.753)
IL-2	0.045 (0.759)	0.019 (0.899)	0.079 (0.592)	−0.005 (0.973)
IL-4	−0.15 (0.279)	−0.125 (0.371)	−0.049 (0.730)	−0.114 (0.423)
IL-5	−0.148 (0.286)	−0.14 (0.317)	0.026 (0.851)	−0.12 (0.395)
IL-6	0.015 (0.914)	−0.11 (0.435)	0.182 (0.192)	−0.165 (0.242)
IL-7	−0.226 (0.107)	0.148 (0.301)	−0.061 (0.669)	−0.118 (0.414)
IL-8	0.177 (0.200)	−0.133 (0.342)	0.139 (0.321)	0.046 (0.749)
IL-9	−0.149 (0.288)	−0.036 (0.797)	−0.075 (0.599)	−0.218 (0.124)
IL-10	0.01 (0.945)	0.015 (0.913)	0.054 (0.705)	0.108 (0.453)
IL-12	0.023 (0.871)	0.151 (0.292)	0.048 (0.739)	0.097 (0.503)
IL-13	−0.147 (0.293)	0.03 (0.835)	−0.048 (0.737)	0.095 (0.508)
IL-15	−0.01 (0.946)	−0.189 (0.185)	0.142 (0.321)	−0.023 (0.873)
IL-17	−0.049 (0.770)	−0.166 (0.328)	−0.098 (0.564)	−0.068 (0.691)
Eotaxin	−0.006 (0.968)	−0.003 (0.983)	0.088 (0.534)	−0.027 (0.853)
Basic FGF	−0.139 (0.320)	0.01 (0.946)	−0.114 (0.421)	−0.078 (0.584)
G-CSF	−0.092 (0.510)	−0.047 (0.738)	−0.018 (0.901)	0.009 (0.951)
GM-CSF	−0.018 (0.900)	−0.171 (0.222)	0.046 (0.745)	0.002 (0.989)
IFN-γ	−0.109 (0.431)	−0.064 (0.651)	−0.001 (0.996)	−0.084 (0.555)
IP-10	−0.104 (0.455)	−0.212 (0.128)	−0.02 (0.889)	−0.141 (0.320)
MCP-1	−0.276 (0.045)*	0.035 (0.806)	−0.166 (0.241)	−0.254 (0.073)
MIP-1α	0.107 (0.447)	−0.196 (0.165)	0.013 (0.929)	0.006 (0.967)
MIP-1β	−0.045 (0.746)	−0.032 (0.823)	−0.06 (0.670)	−0.131 (0.354)
PDGF-BB	−0.077 (0.580)	0.303 (0.028)*	−0.041 (0.772)	0.023 (0.871)
RANTES	−0.04 (0.773)	0.159 (0.257)	−0.036 (0.799)	0.09 (0.527)
TNF-α	−0.032 (0.819)	−0.063 (0.654)	0.061 (0.666)	−0.045 (0.752)
VEGF	−0.104 (0.459)	0.204 (0.148)	−0.168 (0.233)	−0.031 (0.828)

Notes: For each correlation analysis, the respective correlation coefficient (*r* value) and *p* value of significance (shown in brackets) are presented, and $p < 0.05$ is considered as significant. Significant correlation between the two indicated parameters is designated by an asterisk (*). *$p<0.05$.
Abbreviations: WBC, white blood cell; PLT, platelet; NEU, neutrophil; LYM, lymphocyte.

patients is crucial to understand the pathological mechanisms of the disease, and suggest appropriate intervention strategies. In this study, we detected 27 cytokines in SFTS patients and healthy controls.

In nonfatal patients, the initial cytokine pattern revealed marked reductions in IL-2, IL-4, IL-7, PDGF-BB, RANTES, and Eotaxin, compared to healthy individuals. Additionally, compared to healthy individuals, PDGF-BB and RANTES were significantly lower in fatal patients. RANTES, a chemotactic factor, is released by activated platelets,[23] while PDGF-BB, a growth factor, is abundant in platelet-rich plasma.[24] The platelet levels are closely correlated with the levels of PDGF-BB and RANTES. The previous reports have indicated that cytokines PDGF-BB and RANTES are decreased in SFTS patients compared to healthy individuals,[12,15] which was consistent with our findings. The decline in platelet levels in SFTS patients might be the cause behind the reduction in serum levels of PDGF-BB and RANTES. Platelets, acting as significant containers of PDGF-BB and RANTES in the peripheral circulation, are likely responsible for this.[14] T-cell-associated cytokines like IL-2, IL-4, and IL-7 hold importance in immune responses to viral infections. The stimulating effects of IL-2 on T-cell growth and proliferation, along with its ability to enhance the activity of natural killer (NK) cells, have been widely demonstrated. On the other hand, IL-7 plays a crucial role in promoting the differentiation and viability of immature T cells, while also actively participating in the production and maintenance of Memory T cells. Lastly, IL-4 is indispensable for the development and functionality of T helper cells (Th2 cells). IL-2 and IL-7 have a collaborative interaction, which is crucial for promoting the optimal proliferation and survival of

activated CD4+ T cells. This interaction between these two interleukins is considered significant for the functioning of immune cells.[25] Another study indicated a close correlation between CD4 T-cell deficiency and the intensity of SFTS, with the disease becoming increasingly severe as CD4 T-cell levels decrease.[26] This elucidates the reason why SFTS patients exhibited notably diminished levels of IL-2, IL-4, and IL-7 in comparison to healthy individuals. In a prior investigation, it was discovered that there was a noticeable increase in the blood Eotaxin level right before the clinical decline in individuals who had been confirmed to have encephalitis.[27] However, our results differed from this study. Eotaxin is a chemokine that primarily attracts Eosinophils (such as Eosinophils and TH2 cells) to the site of inflammation. In SFTS patients, there may be an inhibitory effect on the inflammatory response, leading to reduced expression and release of Eotaxin. We assumed that the cytokine storm disrupted the immune system and caused overexpression of other chemokines, potentially reducing the chemotaxis of Eotaxin on white blood cells.

In fatal patients, the levels of nine cytokines, namely IL-8, IL-15, IL-1RA, G-CSF, GM-CSF, IFN-γ, TNF-α, Basic FGF, and MIP-1β exhibited a significant increase compared to nonfatal patients. However, the elevation was only marginally higher than that observed in healthy individuals. This higher level trend of IL-1RA, G-CSF, GM-CSF, IFN-γ, and Basic FGF was observed. IFN-γ is a cytokine known for its antiviral and immune regulatory functions. IFN-γ elevating levels in fatal patients may indicate an active immune response to resist infection and control inflammatory reactions.[28] TNF-α, on the contrary, is a cytokine responsible for promoting inflammation and regulating the immune system. Previous research has indicated that during the acute phase of fatal patients, increased levels of IFN-γ and TNF-α have been observed.[14,29] Through our scientific investigation, we have gathered noteworthy evidence indicating a potential correlation between elevated IFN-γ levels and the magnitude of SFTSV infection. Additionally, previous studies have hinted at a connection between elevated TNF-α levels and the severity of the ailment, further substantiating our findings.[30,31] G-CSF and GM-CSF are both colony-stimulating factors. G-CSF promotes the generation and release of granulocytes in the bone marrow, whereas GM-CSF enhances the generation and function of granulocytes and macrophages in the bone marrow. The white blood cell count decreases gradually when the SFTS virus infects the body.[32,33] The production of colony-stimulating factors may help boost white blood cell count to fight against SFTSV infection. The increase in basic FGF and GM-CSF may indicate the need for tissue damage and repair in deceased patients.[34] Basic FGF and GM-CSF were released to promote angiogenesis and cell proliferation for tissue repair. IL-1RA and IL-10, essential cytokines with anti-inflammatory properties, play a crucial role in the modulation of the body's inflammatory response. Often, when there is an overwhelming production of inflammatory mediators, the body tends to counterbalance by producing higher levels of anti-inflammatory mediators.[12] IL-1RA can inhibit the inflammatory effect of interleukin-1. Its increase may be a protective response of the body to regulate the inflammatory response. IL-8 was first discovered in 1987 as a unique type of neutrophil-activating cytokine. Monocytes and macrophages produce IL-8 when stimulated by IL-1α, IL-1β, TNF-α, IL-3, and GM-CSF.[35] Consequently, the level of IL-8 rises in correlation with the levels of these cytokines. IL-15, a crucial immune modulator, is implicated in the regulation of diverse immune reactions. In fatal SFTS cases, increased levels of IL-15 might be linked to an abnormal immune system, potentially leading to heightened inflammatory response. A previous study indicated that elevated serum levels of IL-15 could promote the development, activation, and function of NK cells, potentially enhancing their effector functions in SFTS patients.[36] As the body's inflammatory response became stronger, the levels of IL-15 increased.

According to previous studies, the serum levels of IL-6, IP-10, MIP-1α, and MCP-1 in fatal patients were considerably elevated when compared to both healthy individuals and nonfatal patients.[15,37] This finding aligns with the third pattern observed in our research. The systemic inflammatory response syndrome (SIRS) has been observed in severe/fatal diseases, including viral hemorrhagic fevers.[38] The excess production of pro-inflammatory SIRS in individuals suffering from SFTS is widely acknowledged.[39] Furthermore, the combination of systemic inflammatory response syndrome (SIRS) and mixed or compensatory anti-inflammatory response syndrome (MARS/CARS) can cause tissue damage, abnormalities in coagulation, and leakage in the blood vessels. These factors can lead to hemorrhage, eventual failure of multiple organs, and ultimately, death.[7] Several studies have confirmed that the presence of TNF-α, IFN-γ, IL-6, and IP-10 has a substantial effect on the severity and potential fatality of SFTSV infection.[14] Various studies have previously documented a substantial increase in MIP-1α and MIP-1β levels among fatal patients and nonfatal outcome patients.[12,37] MIP-1α, MIP-1β, MCP-1, and IP-10 belong to the chemokine family and have the ability to attract and activate various immune cells (such as Monocytes,

Eosinophils, and T cells) to the site of pathogen entry.[40] The SIRS could lead to the excessive activation of T-cells and macrophages in affected patients.[39] Chemokines are essential for the proper functioning of the immune system as they serve as regulators, controlling the behavior of immune cells. One of their main functions is to guide lymphocytes toward lymph nodes, where they can effectively search for any invading pathogens. This guidance is achieved through the interaction between chemokines and antigen-presenting cells that reside in these lymphoid tissues. The presence of chemokines ensures that lymphocytes efficiently reach the sites where they are needed to carry out their immune response activities.[41] Severe viral infections can cause tissue damage and cell death. When cells become infected, they release chemokines that signal other immune cells to come to the affected area and increase the inflammatory response. This localized inflammation and tissue damage can result in higher levels of chemokines. By combining the results from different sampling times, we suggested the medical personnel could utilize four specific cytokines, MIP-1α, MCP-1, IL-6, and IP-10 as biomarkers to assess the severity of SFTS in patients. This information could be valuable in determining the appropriate course of treatment and improving patient outcomes. IL-10 is known as an important anti-inflammatory cytokine.[42] Some studies revealed that in patients with nonfatal severe disease, the IL-10 levels were found to be lower compared to patients with fatal severe disease. Additionally, the levels of these cytokines exhibited a decrease in nonfatal severe disease patients.[43,44] And suggested that the destiny of individuals (who suffer from severe illness with a final result of either recuperation or demise) is predominantly influenced by IL-6 and IL-10, rather than by the viral load and the concentration of neutralizing antibodies. However, according to our research data, there was no significant statistical difference in cytokine IL-10 levels between the fatal patients and the survival patients.

Our research also unveiled a negative correlation between the white blood cell and the MCP-1 serum level and a positive correlation between the platelet count and the PDGF-BB serum level. As we described above, MCP-1 belongs to the chemokine family, and it controls the recruitment of leukocytes in inflammation and tissue injury.[45] MCPs, also known as Monocyte Chemoattractant Proteins, exhibit a wide range of effects on different types of cells in the body. These effects are mediated through their interaction with receptors found on various leukocyte types. The expression of MCP receptors on leukocytes allows for their cellular communication and response to MCP signals.[46] The white blood cell count decreases gradually when the SFTS virus infects the body.[32,33] In order to recruit more white blood cells to the SFTSV-affected area, more chemokines will be released, so there is a negative correlation between white blood cell count and MCP-1 level. As for the positive correlation between platelet count and PDGF-BB, we have already described enough about their relationship earlier.

Our research presents certain constraints. Firstly, our investigation comprised a comparatively limited cohort of individuals. However, our sampling plan aimed to include as many cases as possible in Shandong Province during the initial discovery of the disease. Secondly, we did not design our study to continuously sample the same patient and observe the continuous dynamic changes in cytokine levels. This aspect will be explored in future research.

Conclusion

During our investigation into the host's immune response, it was discovered that SFTSV infection resulted in the activation of 19 various cytokines. These cytokines exhibited three distinct patterns that were directly correlated with the pathogenesis of the disease. The disease's intensity correlates strongly with elevated concentrations of IL-6, IP-10, MIP-1α, and MCP-1, while diminished PDGF-BB and RANTES synthesis. Our findings showed a significant negative correlation between white blood cell count and MCP-1, and a positive correlation between platelet count and PDGF-BB. These findings enhance our understanding of SFTS immune response kinetics and shed light on the potential significance of these cytokines in disease progression. Examining alterations in the particular cytokine profile holds significance in suggesting targeted approaches to enhance SFTS patients' prognosis.

Ethics Approval and Informed Consent

This study was approved by the Ethics Committee of the Shandong Center for Disease Control and Prevention (no.2011-12). Following the principles of the Helsinki Declaration, the patients/participants provided their written informed consent to participate in this study, and we obtained consent from the close relatives of the patients who passed away.

Consent for Publication

We have read and agreed to the final draft before submission.

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Disclosure

The authors report no conflicts of interest in this work.

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