Host Cytokine Storm Is Associated With Disease Severity of Severe Fever With Thrombocytopenia Syndrome

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Background. Severe fever with thrombocytopenia syndrome (SFTS) is an emerging viral disease in China, caused by SFTS virus (SFTSV). Severe SFTS patients can quickly proceed to multiorgan dysfunction and death; however, underlying pathogenic mechanisms remain unclear.

Methods. Serum samples from 15 fatal and 44 nonfatal SFTS cases were subjected to multiplex-microbead immunoassays to detect a broad spectrum of cytokines. The viral load and virus-specific IgG titers were also tested by real-time PCR and ELISA, respectively.

Results. Cytokines IL-1RA, IL-6, IL-10, G-CSF, IP-10, and MCP-1 were elevated in SFTS patients and produced at robust levels in fatal cases. In contrast, cytokines PDGF-BB and RANTES decreased in SFTS patients. These cytokines reverted to normal ranges during the convalescent phase of SFTSV infection. Cytokines IL-1β, IL-8, MIP-1α, and MIP-1β showed a unique pattern of elevation in fatal cases but not in nonfatal cases. However, these cytokines increased in the convalescent phase of nonfatal SFTS cases. Our regression analysis revealed that the serum viral load correlated with these cytokines. Moreover, levels of these cytokines correlated with various clinical parameters and virus-specific IgG titers.

Conclusion. The study demonstrates that SFTSV infection induces a cytokine storm with abnormally expressed cytokine profiles, which are associated with the disease severity.

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging viral disease recently identified in multiple provinces in China [1]. 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]. SFTS is an acute illness with clinical presentations of abrupt fever, thrombocytopenia, leukocytopenia, gastrointestinal symptoms, neural disorders, proteinuria, hematuria, and bleeding tendency [1, 2]. Laboratory tests on serum samples of SFTS patients commonly show elevated alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), creatinine (Cr), lactate dehydrogenase (LDH), creatine kinase (CK), creatine kinase MB fraction (CK-MB), as well as prolonged activated partial-thromboplastin time (APTT). These abnormally changed laboratory parameters are indicative of pathological lesions involved in liver, kidney, heart, and coagulation systems of SFTS patients. For critically ill SFTS patients, their clinical symptoms usually become exacerbated during the acute phase of the disease and quickly proceed to multiple organ dysfunction syndromes (MODS), disseminated intravascular coagulation (DIC), and ending in death around 1–2 weeks after the onset of the disease [2, 3]. When this new viral disease was initially identified, the case fatality

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rate was reported to reach 30% [1]. However, to date, the pathogenesis mechanisms underlying the fast proceeding of SFTS symptoms and the fatal outcomes of severe patients remain unclear.

Clinical studies have indicated that cytokine storm, characterized by production of certain cytokines at high concentrations, is associated with the severe forms of several viral infections, including viral hemorrhagic fevers [4–7]. Because peripheral inflammatory leukocytes did not markedly increase in critically ill SFTS patients, we hypothesized that a substantial elevation of small bioactive mediators in serum, such as proinflammatory cytokines, might lead to the fast and systematic exacerbation of symptoms in SFTS patients. Therefore, various cytokines were tested in serum samples from the acute phase of 15 fatal cases and in paired serum samples from the acute and convalescent phases of 44 nonfatal cases. Additionally, the viral loads in the acute phase serum samples of fatal and nonfatal cases were detected to reveal the possible role of virus amount on triggering immunoactive cytokines. The virus-specific immunoglobulin G (IgG) titers in the convalescent phase serum samples of nonfatal cases were also tested to identify cytokines relating to recovery of the disease. Moreover, all these SFTS patients had complete medical records of laboratory tests, which enabled us to analyze correlations between clinical parameters and serum levels of cytokines in SFTS patients, and to reveal potential factors associated with severe pathological lesions in SFTS patients. Our data clearly showed that SFTSV infection triggered cytokine storm during the acute phase of disease in SFTS patients and that the serum viral load correlated with substantially elevated cytokine levels. Furthermore, by logistic regression analysis, we revealed that the abnormally regulated proinflammatory cytokines correlated with the exacerbation of thrombocytopenia, leukocytopenia, and blood biochemical parameters in the acute phase, as well as with the production of virus-specific IgG in the convalescent phase of SFTS patients.

MATERIALS AND METHODS

Patients and Clinical Samples
The 59 SFTS patients were admitted to hospitals located in 5 provinces of China in 2010. All patients were confirmed positive for SFTSV infection by detection of viral RNA using a certificated real-time polymerase chain reaction (RT-PCR) kit for clinical diagnosis [8] (SFDA Registration 340166, China). To further confirm their infectious status, these SFTS patients were also subjected to immunofluorescent test where a 4-fold or greater increase of virus-specific IgG titers in paired sera from the acute and convalescent phases of infection was verified or virus isolation. All 59 confirmed SFTS cases had complete medical records of laboratory tests. For all fatal cases, the acute phase serum samples were collected around 6–9 days

post the onset of illness. For all nonfatal cases, paired serum samples were collected in the acute phase of around 6–9 days after onset of illness and in the convalescent phase ranging from 50 to 150 days after onset of illness. In addition, serum samples from 20 healthy volunteers were also analyzed as healthy controls.

Quantitative Real–Time PCR
The viral load of SFTSV RNA copies in serum samples of acute phase SFTS patients and healthy donors were detected using a certified real-time PCR kit as described above, which was demonstrated to have 98.6% sensitivity and 99.1% specificity [8].

Immunofluorescence Assay
The immunofluorescence assay (IFA) was performed to detect SFTSV-specific IgG antibodies as previously described [1]. Briefly, Vero cells were infected with the SFTSV strain HB29, and viral antigen slides (8 wells) were prepared by dropping cells on slides and fixed in acetone. Serum samples from SFTS patients were serially diluted by 2-folds and incubated on the slides for 30 minutes at 37°C. Then, the slides were washed by phosphate-buffered saline (PBS) and probed with FITC-conjugated anti-IgG secondary antibodies (Sigma). Positive reactions were identified as cells exhibiting bright green cytoplasmic fluorescence against black background. The antibody titer corresponds to the highest dilution factor that yields a positive reading.

Multiplex-Microbead Immunoassay
A multiplex-biometric immunoassay containing fluorescent microspheres conjugating with monoclonal antibodies specific for target cytokines was performed to test serum cytokine levels following manufacturer's instruction (Bio-plex Pro Human 27-plex cytokine panel, Bio-rad). The cytokines tested are as follows: interleukin (IL)-1β, IL-1 receptor antagonist (IL-1RA), IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, eotaxin, granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF), basic FGF, interferon (IFN)-γ, IFN-γ-inducible protein (IP-10), monocyte chemotactic protein-1 (MCP-1), macrophage inflammatory protein 1a (MIP-1α), macrophage inflammatory protein 1β (MIP-1β), platelet-derived growth factor (PDGF-BB), regulated on activation and normally T-cell expressed (RANTES), tumor necrosis factor (TNF)-α, and vascular endothelial growth factor

Hierachical Clustering Analysis
The hierachical clustering analysis [9, 10] in this study was performed using the software Cluster 3.0 (open source software [11]). Median centering of cytokine data was performed once to align data before clustering. Clusters are calculated using average linkage based on Euclidian distance. Cluster

results are graphically represented and high through low expression levels are presented as a 2-color spectrum that allows identification of groups of interesting genes through visual pattern recognition. Columns (cytokines) are rearranged to place columns with similar response patterns near each other. Rows are serum samples divided into 4 study groups.

Statistical Analyses

Graphpad prism 5 software was used to compare values in groups and calculate the correlation coefficients and significance values of 2 variables. Comparison of clinical parameters between fatal and nonfatal cases was calculated by unpaired t test (for continuous variables) or Pearson χ^2 test on cross table (for categorical variables). Comparisons among 3 groups were calculated by nonparametric one-way analysis of variance. Comparisons of paired serum samples were calculated by paired t test. Serum virus load and antibody titers were subjected to correlation analysis after logarithmic transformation. The correlation analysis of 2 variables among virus load, clinical parameters, and serum cytokines were calculated using Pearson test. The correlation analysis of antibody titers with individual serum cytokines were calculated using Spearman test.

Ethics Statement

According to the medical research regulation of the Ministry of Health of China, all studies involving human samples were reviewed and approved by the ethics committee of China CDC, which uses international guidelines to ensure confidentiality, anonymity, and informed consent. The written informed consent was agreed by patients.

RESULTS

Characterization of Fatal and Nonfatal SFTS Cases

The demographic data showed that the average age of fatal cases was significantly higher than nonfatal cases. The case fatality rate was slightly higher in females than that in males, but the difference was not statistically significant (Table 1). The slight difference might be due to the comparatively small study groups of SFTS patients in this study and needs further clarification. In the acute phase, around 6–9 days after onset of illness, the average viral load in fatal cases was substantially higher than in nonfatal cases. In comparison with nonfatal cases, the platelet counts were markedly lower in fatal cases, whereas the white blood cell counts, as well as the highest fever temperature and duration of fever, were comparable (Table 1). Laboratory tests found that serum levels of AST, LDH, CK, and CK-MB were significantly elevated in fatal cases, but serum levels of ALT, BUN, Cr, and APTT did not show difference between the 2 study groups (Table 1). These observations clearly indicated that during the acute phase, SFTS patients with fatal outcomes had markedly higher viral load and presented more severe disease status than SFTS patients recovered from the disease.

Table 1. Comparison of Demographic and Clinic Parameters in Fatal and Nonfatal Cases of Severe Fever With Thrombocytopenia Syndrome

Index[a]	Normal range[b]	Fatal (N = 15)	Nonfatal (N = 44)	P[c]
Age, years	N/A	74 (59.5–78.5)	55 (50–68)	.019*
Male, %	N/A	33	48	.332
Highest temperature, °C	36.4–37.2	38.9 (38.1–39.6)	39 (38.5–39.2)	.787
Duration of fever, days	N/A	5 (3–6)	6 (4–7)	.427
Viral load, copies/mL	N/A	$10^{7.1}$ ($10^{6.6}$–$10^{8.0}$)	$10^{5.2}$ ($10^{4.3}$–$10^{6.1}$)	<.001*
Platelet count, 10^9/L	100–300	28 (21–47)	45 (34–73)	.013*
White blood cell count, 10^9/L	4–10	1.6 (1.1–2.4)	1.7 (1.2–2.3)	.588
Alanine aminotransferase, U/L	0–40	274.2 (85.5–509.5)	99.5 (73.3–151)	.095
Aspartate aminotransferase, U/L	0–40	871.2 (340–1430)	130 (65.5–130)	.010*
Blood urea nitrogen, mmol/L	3.2–7.1	9.9 (5.7–13)	5.3 (4.0–8.0)	.062
Creatinine, μmol/L	31.7–93.3	90 (64–98.3)	87 (69–100.4)	.406
Lactate dehydrogenase, U/L	120–240	1625 (1242–1999)	419 (225–635)	.004*
Creatine kinase, U/L	38–174	1032 (589–2459)	171.1 (78–687)	.041*
Creatine kinase MB fraction, U/L	0–25	97 (41–150)	21 (15–52)	.026*
Activated partial-thromboplastin time, U/L	23.5–35.7	52.6 (47.2–74)	44.4 (34.1–47)	.195

Abbreviation: N/A, not applicable.

[a] Except for the proportion of males, all other data are presented as median (25% to 75%).

[b] Normal physiological levels for clinical diagnosis.

[c] Statistic significance between fatal and nonfatal cases, $P < .05$ was considered as significant and labeled with an asterisk (*) at the top corner of the P value.

* The parameter shows significant difference between the fatal and non-fatal groups.

Distinct Patterns of Cytokine Production in Fatal and Nonfatal SFTS Cases

To identify potential cytokine responses in SFTSV infection, the acute phase serum samples from 15 fatal cases and the paired serum samples from the acute and convalescent phases of 44 nonfatal cases, as well as serum samples from 20 healthy donors, were subjected to 27-plex cytokine immunoassay. In order to acquire overall patterns of cytokine production, a hierarchical clustering analysis was performed to classify 27 cytokines according to different disease outcomes and stages. The hierarchical clustering analysis clearly identified 3 clusters of cytokines that had distinct patterns (Figure 1). The first cluster of cytokines included IL1-RA, IL-6, IL-10, G-CSF, IP-10, and MCP-1, which were induced in the acute phase of both fatal and nonfatal cases, while produced at minimal levels in the convalescent phase of nonfatal cases and healthy donors. The second cluster of cytokines including PDGF-BB and RANTES had an opposite pattern. These cytokines were normally high in the convalescent phase of nonfatal cases and healthy donors, but markedly reduced in the acute phase of both fatal and nonfatal cases. The third cluster of cytokines, including IL-1, IL-8, MIP-α, and MIP-1β, were produced at high levels in the acute phase of fatal cases and in the convalescent phase of nonfatal cases, but produced at comparatively low levels in the acute phase of nonfatal cases and healthy donors. It was also noted that the cytokine expression patterns were visually associated with serum SFTSV load detected in the acute phase of SFTS patients (Figure 1).

Furthermore, we statistically analyzed the production of these cytokines in the acute phase of fatal and nonfatal cases as well as in healthy donors. For the first cluster of cytokines including IL1-RA, IL-6, IL-10, MCP-1, G-CSF, and IP-10, both fatal and nonfatal cases showed higher serum levels than healthy donors, and fatal cases had a more robust production than nonfatal cases (Figure 2A). For the second cluster of cytokines, PDGF-BB and RANTES, the production in the acute phase of both fatal and nonfatal cases was similarly reduced than in healthy donors (Figure 2B). The third cluster of cytokines, IL-1β, IL-8, MIP-1α, and MIP-1β, had significantly higher production in fatal cases than in nonfatal cases and

Figure 1. Hierarchical clustering analysis on cytokine production in patients with severe fever with thrombocytopenia syndrome (SFTS). Each cell in the 2-dimentional graph indicates the measure of a single mediator in one sample, with standardized levels scaled by color in the top corner. Cytokine clustering is depicted on the top of the graph, and the name of each cytokine is labeled at the bottom of the graph. The serum samples in each study group are described on the right side of the graph and displayed according to the serum viral load (Log$_{10}$ viral RNA copies/mL, left side of the graph) from high to low. NA and NG indicate either the data not available or negative data, respectively. Abbreviations: G-CSF, granulocyte colony-stimulating factor; IFN, interferon; IL, interleukin; MCP, monocyte chemotactic protein; TNF, tumor necrosis factor.
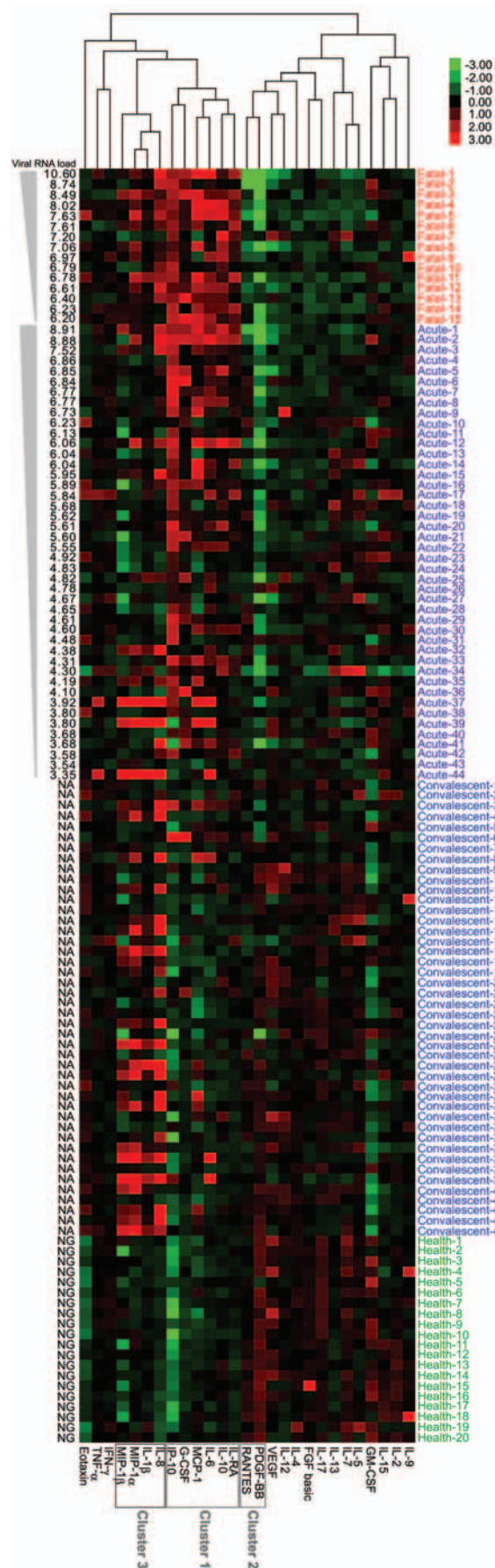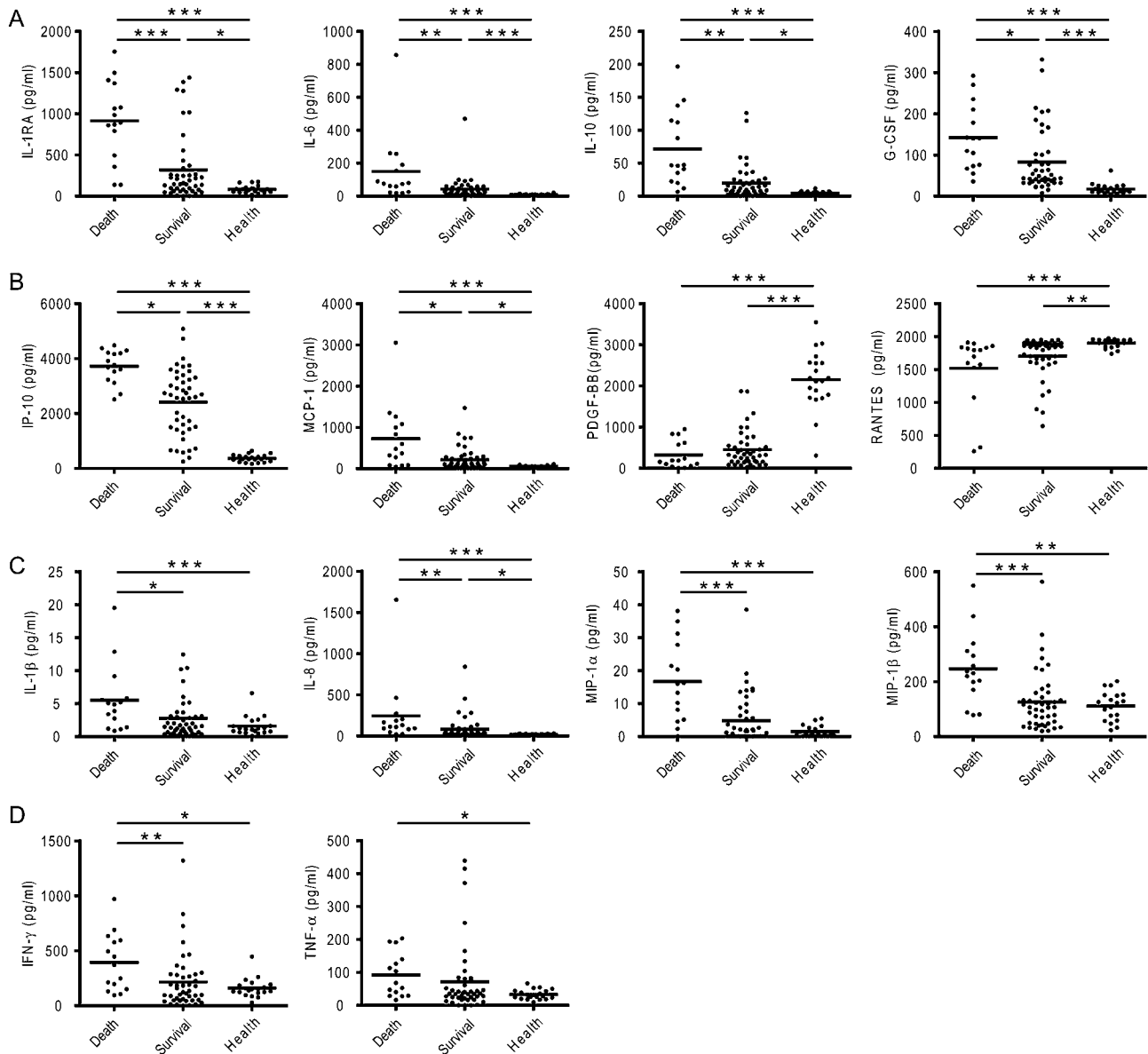
Figure 2. Comparison of cytokine production in the acute phase of fatal and nonfatal severe fever with thrombocytopenia syndrome (SFTS) cases. Cytokines in cluster 1 (*A*), cluster 2 (*B*), cluster 3 (*C*), as well as cytokines of interferon (IFN)-γ and tumor necrosis factor (TNF)-α (*D*), were detected 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 15 fatal cases, 44 nonfatal cases, and 20 healthy donors. Each dot shows the cytokine concentration in an individual. Horizon bars indicate the respective group mean. *$P < .05$, **$P < .005$, ***$P < .001$. Abbreviations: G-CSF, granulocyte colony-stimulating factor; IL, interleukin; RANTES, regulated on activation and normally T-cell expressed.

healthy donors, except for IL-8, which had a higher production in recovering patients than in healthy donors, the production of IL-1β, MIP-1α, and MIP-1β in recovering patients was comparable to healthy donors (Figure 2*C*). Because the cytokines IFN-γ and TNF-α are involved in many viral infections [5, 12, 13], serum levels of IFN-γ and TNF-α in SFTS patients were also examined. The results showed that the production of IFN-γ and TNF-α during the acute phase was comparable

between nonfatal cases and healthy donors, and only slightly elevated in the fatal cases (Figure 2*D*).

We also statistically compared cytokine levels between the 2 phases of the disease progress. The analysis showed that the first cluster of cytokines, IL1-RA, IL-6, IL-10, MCP-1, G-CSF, and IP-10, decreased in the convalescent phase to near normal levels (Figure 3*A*). In contrast, the second cluster of cytokines, PDGF-BB and RANTES, increased in the
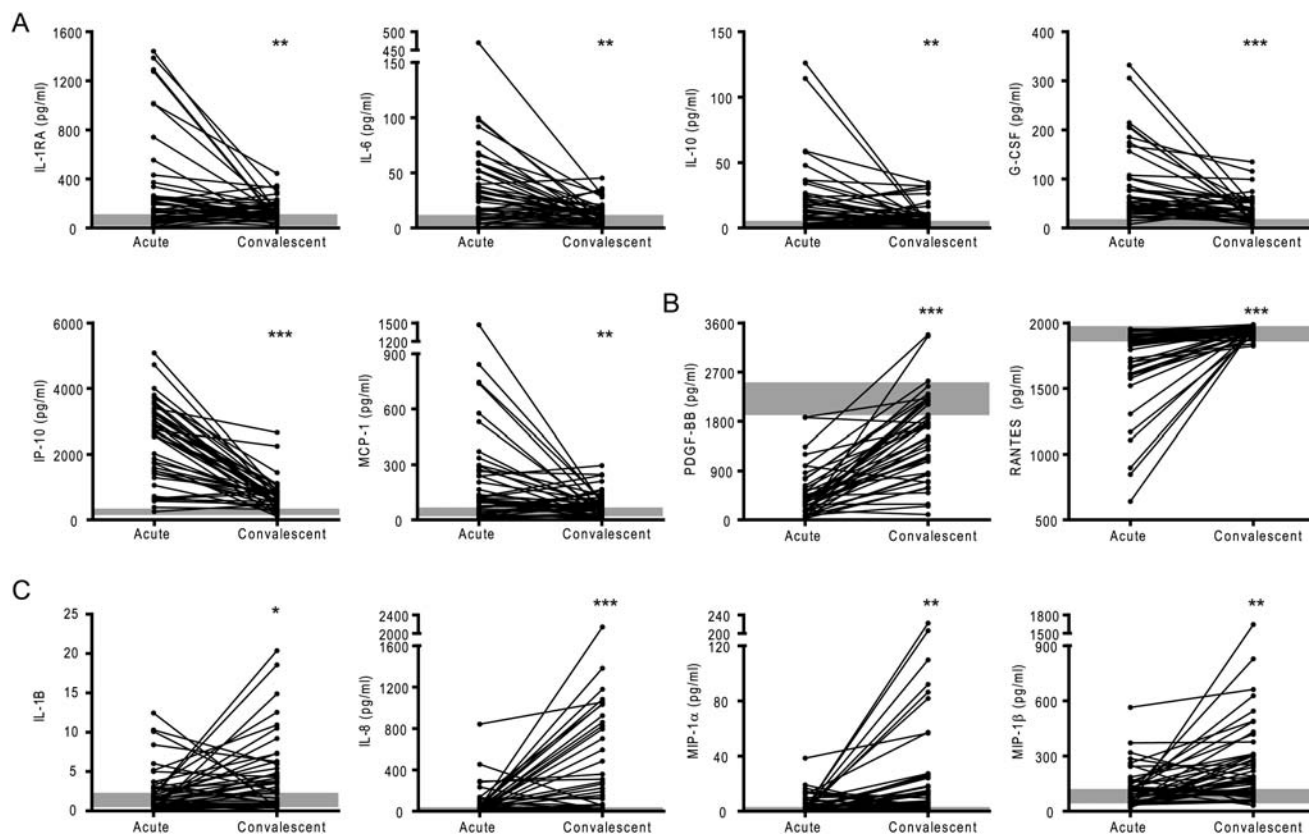
Figure 3. Comparison of cytokines produced in the acute phase and convalescent phase of nonfatal severe fever with thrombocytopenia syndrome (SFTS) cases. Serum levels of cytokines of cluster 1 (*A*), cluster 2 (*B*), and cluster 3 (*C*) were compared in the paired serum samples from the acute phase and convalescent phase of nonfatal cases. Each dot shows the cytokine concentration in an individual. Each line connects a pair of serum samples collected from the acute phase and convalescent phase from the same case. Gray shadows indicate physiological levels of cytokines in healthy donors. *$P < .05$, **$P < .005$, ***$P < .001$. Abbreviations: IP, inducible protein; MCP, monocyte chemotactic protein; PDGF, platelet-derived growth factor; RANTES, regulated on activation and normally T-cell expressed.

convalescent phase to near normal levels (Figure 3*B*). This dynamic analysis indicated that the reversion of these proinflammatory cytokines back to physiological ranges was associated with the recovery of SFTS patients. Interestingly, the cytokines in the third cluster, IL-1β, IL-8, MIP-1α, and MIP-1β, did not revert to their physiological ranges during the convalescent phase but increased instead (Figure 3*C*).

On the whole, the hierarchical clustering analysis and statistical comparisons between different patient groups and different disease stages identified 3 unique clusters of proinflammatory cytokines in SFTS patients.

Viral Load Correlated With Cytokine Levels in the Acute Phase of SFTSV Infection

Because the cytokine expression patterns were visually associated with SFTSV RNA viral load detected in acute phase serum samples from SFTS patients, correlation analyses were performed to investigate possible relationship between viral load and the cytokines abnormally regulated in SFTS patients.

The correlation analyses showed that, during the acute phase of SFTSV infection, positive correlations existed between viral load and the first cluster of cytokines IL-1RA, IL-6, IL-10, MCP-1, G-CSF, and IP-10 (Figure 4*A*), as well as the third cluster of cytokines IL-8, MIP-1α, and MIP-1β (Figure 4*C*). In contrast, negative correlations existed between viral load and the second cluster of cytokines PDGF-BB and RANTES (Figure 4*B*). The statistical correlations between viral load and cytokines suggested that higher amount of circulating virus in the acute phase was capable of inducing higher levels of IL1-RA, IL-6, IL-10, MCP-1, G-CSF, IP-10, IL-8, MIP-1α, and MIP-1β, while repressing the production of PDGF-BB and RANTES to a larger extent.

Correlations of Cytokines With Clinical Symptoms and Laboratory Parameters in SFTS Patients

The 3 clusters of cytokines identified in SFTS patients have been reported to function in causing pathological lesions [14–16]. Therefore, we analyzed correlations between these cytokines
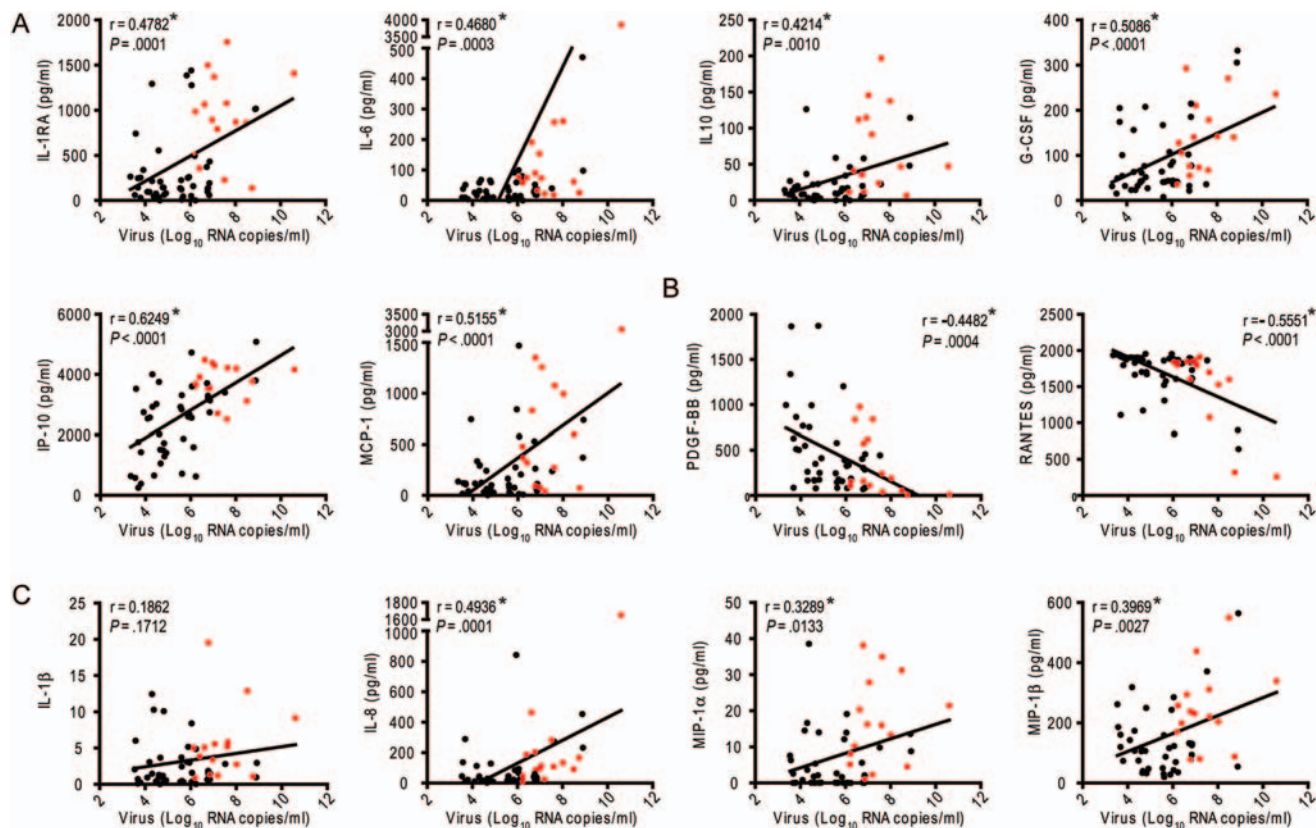
Figure 4. Correlations between the serum viral load and cytokines in the acute phase. Logistic regression analysis was used to show correlations between the serum virus load (Log$_{10}$ viral RNA copies/mL) and cytokines of cluster 1 (*A*), cluster 2 (*B*), and cluster 3 (*C*) detected in the acute phase of severe fever with thrombocytopenia syndrome (SFTS) patients. Red dots indicate fatal cases, and black dots indicate nonfatal cases. The best-fit lines are shown on each graph. On the graphs, *r* and *P* indicate the correlation coefficient and the *P* value of significance, respectively. A *P* value of <.05 is considered as significant and labeled with an asterisk (*) on the *r* value. Abbreviations: IL, interleukin; MCP, monocyte chemotactic protein; PDGF, platelet-derived growth factor; RANTES, regulated on activation and normally T-cell expressed.

and key clinical parameters of SFTS patients during the acute phase of viral infection. The correlation analyses showed that blood platelet counts positively correlated with G-CSF serum levels but were negatively correlated with PDGF-BB and RANTES (Table 2) serum levels. White blood cell counts positively correlated with IL-1β levels. Liver transaminase AST positively correlated with IL-1RA, G-CSF, and IL-8 levels, while ALT values only correlated with G-CSF levels. BUN, a parameter that can indicate impairment of kidney function, positively correlated with elevated levels of multiple cytokines including IL-1RA, IL-6, IL-10, MCP-1, G-CSF, IP-10, IL-8, MIP-1α, and MIP-1β, but negatively correlated with PDGF-BB and RANTES levels. Cardiac enzymes LDH, CK, and CK-MB, which are released during heart tissue damage, were variously affected by IL1-RA, IL-6, IL-10, G-CSF, IP-10, MCP-1, IL-8, MIP-1α, MIP-1β, PDGF-BB, and RANTES (Table 2). APTT, the parameter indicating coagulation function, was correlated negatively with PDGF-BB and RANTES levels. Therefore, correlations between these cytokines and key laboratory

parameters in SFTS patients indicated that these cytokines were associated with the exacerbation of multiorgan functions.

The relation between cytokines and virus-specific IgG antibody titers was also examined to identify cytokines associated with recovery of SFTS. The analyses showed that IgG titers in the convalescent phase had no correlation with cytokine levels produced in the acute phase. However, IgG titers positively correlated with RANTES and negatively correlated with G-CSF and IP-10 produced in serum samples of the convalescent phase (Table 2). Because higher IgG titers generally indicate a better recovery, this finding suggested that a high serum level of RANTES in the late phase was associated with better recovery from the disease, whereas high serum levels of G-CSF and IP-10 in the late phase might be associated with a negative effect on the recovery.

Because we found that SFTSV viral load correlated with production of cytokines, and cytokine levels correlated with clinical parameters, we further analyzed the correlation between viral load and clinical parameters. The data showed that the

Table 2. Correlation Matrix of Clinical Parameters With Cytokines or Viral Load in Patients With Severe Fever With Thrombocytopenia Syndrome

	PLT	WBC	ALT	AST	BUN	LDH	CK	CK-MB	APTT	IgG[a]
IL-1RA	−0.223[b] (0.220)	0.226 (0.213)	0.193 (0.297)	0.379 (0.039)*	0.558 (0.001)*	0.543 (0.001)*	0.478 (0.006)*	0.299 (0.096)	0.105 (0.687)	−0.020 (0.901)
IL-6	−0.327 (0.068)	0.222 (0.223)	0.131 (0.484)	0.348 (0.059)	0.358 (0.044)*	0.462 (0.008)*	0.441 (0.012)*	0.318 (0.077)	0.066 (0.801)	0.010 (0.951)
IL-10	−0.069 (0.706)	0.120 (0.515)	0.136 (0.466)	0.243 (0.195)	0.426 (0.015)*	0.406 (0.021)*	0.320 (0.074)	0.213 (0.243)	0.096 (0.715)	0.077 (0.629)
G-CSF	−0.44 (0.013)*	−0.219 (0.236)	0.381 (0.038)*	0.571 (0.001)*	0.576 (0.001)*	0.631 (<0.001)*	0.506 (0.004)*	0.4 (0.026)*	−0.218 (0.400)	−0.309 (0.041)*
IP-10	−0.257 (0.156)	0.152 (0.406)	0.030 (0.871)	0.279 (0.136)	0.587 (<0.001)*	0.467 (0.007)*	0.336 (0.060)	0.401 (0.023)*	−0.022 (0.933)	−0.516 (<0.001)*
MCP-1	−0.070 (0.703)	0.308 (0.086)	0.061 (0.744)	0.255 (0.173)	0.430 (0.014)*	0.438 (0.012)*	0.426 (0.015)*	0.331 (0.064)	0.010 (0.970)	0.205 (0.192)
PDGF-BB	0.462 (0.008)*	0.328 (0.067)	−0.035 (0.851)	−0.305 (0.101)	−0.367 (0.039)*	−0.511 (0.003)*	−0.461 (0.008)*	−0.208 (0.253)	−0.576 (0.016)*	0.173 (0.273)
RANTES	0.440 (0.012)*	0.245 (0.176)	−0.147 (0.431)	−0.360 (0.051)	−0.426 (0.015)*	−0.501 (0.003)*	−0.379 (0.032)*	−0.240 (0.187)	−0.650 (0.005)*	0.544 (<0.001)*
IL-8	−0.193 (0.297)	0.262 (0.155)	0.192 (0.310)	0.421 (0.036)*	0.421 (0.018)*	0.440 (0.013)*	0.461 (0.009)*	0.445 (0.012)*	0.476 (0.062)	0.039 (0.502)
MIP-1α	−0.034 (0.853)	0.190 (0.297)	0.164 (0.379)	0.221 (0.241)	0.252 (0.164)	0.318 (0.076)	0.426 (0.015)*	0.294 (0.102)	0.250 (0.333)	0.093 (0.559)
MIP-1β	0.121 (0.518)	0.197 (0.287)	−0.009 (0.963)	0.087 (0.652)	0.189 (0.308)	0.272 (0.024)*	0.282 (0.124)	0.288 (0.045)*	−0.056 (0.837)	0.070 (0.661)
IL-1β	0.044 (0.810)	0.353 (0.048)*	0.026 (0.891)	0.147 (0.437)	0.106 (0.563)	0.247 (0.174)	0.312 (0.082)	0.040 (0.828)	0.395 (0.117)	0.039 (0.808)
Viral load[c]	−0.5290 (0.001)*	−0.120 (0.511)	0.250 (0.176)	0.4230 (0.020)*	0.507 (0.003)*	0.597 (<0.001)*	0.4810 (0.005)*	0.577 (0.001)*	0.319 (0.213)	−0.063 (0.690)

Abbreviations: ALT, alanine aminotransferase; APTT, activated partial-thromboplastin time; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CK, creatine kinase; CK-MB, creatine kinase MB fraction; IgG, immunoglobulin G; IL, interleukin; IP, inducible protein; LDH, lactate dehydrogenase; MCP, monocyte chemotactic protein; MIP, macrophage inflammatory protein; PDGF, platelet-derived growth factor; RANTES, regulated on activation and normally T-cell expressed.

[a] Except for the IgG titers, other clinical parameters were regressed with serum cytokine levels and viral load in the acute phase of both fatal and nonfatal cases. The data in the most right column show correlations between IgG titer (in the format of Log_2 IgG titer) and cytokine levels tested in convalescent phase of nonfatal cases, or correlations between IgG titer and viral load tested in the acute phase of nonfatal cases.

[b] For each correlation analysis, 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 (*).

[c] The viral load (in the format of Log_{10} viral RNA copies/mL) was subjected to the correlation analysis with clinical parameters and IgG titers.

circulating viral load positively correlated with serum levels of AST, BUN, CK, CK-MB, and LDH, and negatively correlated with blood platelet counts, but not correlated with serum levels of ALT, APTT, IgG titers, and blood white blood cell counts.

DISCUSSION

As a newly discovered viral infection, the underlying pathogenesis mechanisms of SFTS remain unknown. Many severe SFTS patients quickly proceed to MOD, DIC, and even death around 7–14 days after the onset of illness. In an effort to identify potential immune mediators contributing to disease severity, we found that the physiological balance of multiple proinflammatory cytokines was substantially changed in SFTS patients during the acute phase of viral infection. However, most of these cytokines returned to physiological range during the recovery phase. Cytokine storm occurring in the acute phase of the disease has been widely hypothesized to be the main cause of morbidity and mortality for several viral infections [17]. Previously, it was thought that inflammatory cytokines and chemokines could promote activation and chemoattraction of lymphocytes and exert antiviral effects. However, recent findings indicated that a cytokine storm, characterized by an overwhelming and imbalanced profile of cytokines, could become excessive and harmful [5, 14]. A study on cytokine storms triggered in dengue fever demonstrated that the cytokine storm that follows severe primary dengue infection is mostly associated with the exacerbation of disease rather than protection against severe infection [18]. Additionally, clinical research on asymptomatic Ebola patients showed that the initial robust responses of cytokines rapidly faded in these patients as they recovered [6].

In several clinical studies of hemorrhagic fever diseases, the cytokine storm was shown to be associated with disease severity. In acute dengue infection, a panel of cytokines including IL-6, IL-8, IL-10, MCP-1, MIP-1α, MIP-1β, IP-10, RANTES, IFN-γ, and TNF-α, were reported to correlate with severe dengue disease [4, 13], and serum levels of IL-1RA were reported to be higher in fatal dengue cases than in the survivors [19]. Chikungunya severity was shown to correlate with cytokines of IL-1β, IL-6, and RANTES produced in the acute phase [20]. Recent clinical studies on Crimean-Congo hemorrhagic fever (CCHF) patients also showed higher levels of IL-6 and TNF-α in patients with fatal outcome than in survivors [21].

In our study, 12 cytokines with 3 distinct patterns of production were identified by the hierarchical clustering analysis. In the acute phase, cytokines IL1-RA, IL-6, IL-10, MCP-1, G-CSF, and IP-10 were elevated in nonfatal cases and produced at more robust levels in fatal cases, similar to previously reported studies [22]; cytokines IL-1β, IL-8, MIP-1α, and MIP-1β were only elevated in fatal cases but not in nonfatal cases;

whereas cytokines PDGF-BB and RANTES were reduced in both fatal and nonfatal cases. Therefore, we postulated that the elevation of IL1-RA, IL-6, IL-10, MCP-1, G-CSF, and IP-10 combined with the reduction of PDGF-BB and RANTES could serve as biomarkers of acute phase SFTS patients. Additionally, the elevation of serum IL-1β, IL-8, MIP-1α, and MIP-1β in the acute phase could serve as biomarkers to predict a fatal outcome for SFTS patients. We noticed that the level of IFN-γ in the acute phase of SFTSV infection, unlike previously reported studies [22], was not significantly different between nonfatal cases and healthy people and only had a slight elevation in fatal cases. Our finding that IFN-γ was not efficiently elevated in SFTSV infection was identical with previous reports that some bunyaviruses are weak IFN-γ inducers [5, 23, 24]. In the convalescent phase, cytokines IL1-RA, IL-6, IL-10, MCP-1, G-CSF, IP-10, PDGF-BB, and RANTES reverted to physiological levels, but cytokines IL-8, MIP-1α, and MIP-1β increased during the convalescent phase of recovering patients. The findings that serum IL-8, MIP-1α, and MIP-1β, which elevated in the convalescent phase of recovering patients suggested that, if combined with virus-specific IgG titers, these cytokines may serve as chronic biomarkers to confirm a previous SFTSV infection.

Serum virus load was shown to correlate with the production of multiple peripheral cytokines in SFTS patients, and these cytokines were further found to correlate with clinical parameters, indicating impairments in organ functions. These observations suggested that the abnormal production of cytokines during the acute phase of SFTSV infection was induced by active viral replication. When initial immune responses could not restrain viral replication, the virus may induce target cells to release overwhelming amounts of cytokines and thus lead to pathological lesions. In fact, multiorgan dysfunctions caused by acute infections are believed to result from an inappropriate cytokine response in the host to a variety of acute insults [14]. For instance, MCP-1 and IL-8 are important for progressive kidney injury [16], IL-1RA and IL-6 are associated with the severity of nephropathia epidemica [25], MCP-1 and IP-10 can cause liver inflammation and fibrosis [15], and IL-8 has activity to enhance vascular permeability [26]. Low serum levels of RANTES and PDGF-BB in SFTS patients might be due to the decrease in circulating platelets, which are major reservoirs of these 2 cytokines in the peripheral circulation [27]. And low levels of RANTES were reported to be associated with disease severity in viral infections [20]. So far, it has not been clear which type of cells is responsible for producing and regulating a cytokine storm. However, a recent study on influenza virus-induced cytokine storm suggested that endothelial cells may play a central role in orchestrating the cytokine amplification, and the sphingosin-1-phosphate agonism could suppress cytokine storms and provide protection against pathogenic influenza virus [28]. To date,

knowledge on the cytokine storm induced by bunyaviruses has been limited, and different strategies might be used for SFTSV to trigger cytokine storms. Moreover, although the correlation between cytokine storms and the disease severity of SFTSV infection was established in our study, evidence is still lacking to demonstrate a cause-effect relation.

In summary, our study on the host cytokine response revealed that multiple cytokines triggered during the acute phase of SFTSV infection are associated with the disease severity. In this respect, the treatment using antagonists against proinflammatory cytokines to block the cytokine storm might relieve the disease severity to a certain extent. Additionally, the unique cytokine patterns identified in this study could serve as biomarkers to aid physicians evaluating the disease severity of SFTS patients.

Notes

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