

Severe Fever with Thrombocytopenia Syndrome Complicated by Co-infection with Spotted Fever Group Rickettsiae, China

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During 2013–2015 in central China, co-infection with spotted fever group rickettsiae was identified in 77 of 823 patients infected with severe fever with thrombocytopenia syndrome virus. Co-infection resulted in delayed recovery and increased risk for death, prompting clinical practices in the region to consider co-infection in patients with severe fever with thrombocytopenia syndrome.

In recent years, new tickborne pathogens have increasingly emerged, creating public health challenges. Co-infection may occur in humans either through the bite of 1 tick co-infected with multiple pathogens or bites of multiple ticks, each carrying a different pathogen (1).

In 2009, severe fever with thrombocytopenia syndrome virus (SFTSV) was identified in humans in China, and since then, the virus has been detected in 19 provinces (2). The most highly affected region is in central China, where over one third of cases have been reported. Another tickborne pathogen, *Candidatus Rickettsia tarasevichiae*, classified among the spotted fever group rickettsiae (SFGR), was first identified in 2012 in the northeastern area of China, but is now infecting humans in the more densely populated central region (3). SFGRs have been detected in *Haemaphysalis longicornis* ticks (3,4), which also serve as a competent vector for SFTSV (5). In 2014, *Candidatus R. tarasevichiae* infection was detected in SFTSV-infected persons in eastern central China, indicating that co-infection with SFGR might be common among SFTSV-infected persons in the region (3). To determine the effects of co-infection with SFGR in SFTSV-infected persons, we compared clinical characteristics and laboratory findings for patients with SFTSV infection only with those for patients co-infected with SFTSV and *Candidatus R. tarasevichiae*.

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DOI: <http://dx.doi.org/10.3201/eid2211.161021>

The Study

During 2013–2015, we conducted a retrospective investigation at the 154 Hospital of the People's Liberation Army in Xinyang City, Henan Province, China. All patients meeting the criteria for having suspected severe fever with thrombocytopenia syndrome (SFTS) were enrolled (6). Serial serum and anti-coagulated blood samples were collected from patients throughout hospitalization and during convalescence.

RNA detection by reverse transcription PCR and serologic testing by ELISA were performed for diagnosis of SFTSV infection (6). SFTSV infection was determined by the detection of viral RNA in serum, seroconversion, or a 4-fold increase in SFTSV-specific IgG titers in paired serum samples collected ≥ 2 weeks apart. We used an indirect immunofluorescence assay (Focus Diagnostic, Cypress, CA, USA) to detect *Rickettsia rickettsii* IgG. Acute SFGR infection was defined as seroconversion or a 4-fold increase in *R. rickettsii* IgG titers in paired serum samples. We measured serum levels of cytokines and chemokines by using a Bio-Plex Pro Human Cytokine 27-plex Assay (Bio-Rad, Hercules, CA, USA).

For the study, we recruited 823 SFTS patients who had paired serum samples available for testing (online Technical Appendix 1, <http://wwwnc.cdc.gov/EID/article/22/11/16-1021-Techapp1.pdf>). Of those patients, 77 (8.5%) also had serologic evidence of SFGR infection: 45 showed seroconversion, and 32 had a 4-fold increase in IgG titers. Those 77 patients represented the SFTSV–SFGR co-infection group (online Technical Appendix Table 2); the other 746 patients represented the SFTSV single-infection group.

Influenza-like symptoms were the most common clinical manifestations in both groups, and, except for fever, which was more prolonged in the co-infection group ($p = 0.039$), symptoms were comparable in the groups (online Technical Appendix Table 3). Ascites and hemorrhagic signs were more common in the co-infection than the single-infection group ($p = 0.002$ and $p = 0.003$, respectively). The frequencies of other complications, including gastrointestinal, respiratory, and neurologic syndromes, were similar in the 2 groups.

At hospital admission, the co-infection group had longer prothrombin times (Table). For both groups, thrombocytopenia occurred starting at 4 days after symptom onset and persisted for as long as 2 weeks (Figure 1). Using

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Table. Laboratory test results for patients with severe fever with thrombocytopenia syndrome with and without co-infection with spotted fever group rickettsiae

Characteristics	Single infection, n = 77	Co-infection, n = 746	p value
Laboratory parameters on admission, no. (%) patients			
Leukocyte count <4 × 10 ⁹ /L	60 (77.9)	613 (82.2)	0.358
Platelet count <100 × 10 ⁹ /L	64 (83.1)	624 (83.7)	0.905
Neutrophils >70%	36 (46.8)	348 (46.7)	0.986
Lymphocytes <20%	27 (35.1)	276 (37.0)	0.738
Hemoglobin <110 g/L	9 (11.7)	113 (15.2)	0.416
Aspartate aminotransferase >40 U/L	62 (80.5)	621 (83.4)	0.527
Alanine aminotransferase >40 U/L	40 (52.0)	421 (56.6)	0.435
Albumin <35 g/L	10 (13.0)	90 (12.1)	0.817
Alkaline phosphatase >150 U/L	3 (3.9)	48 (6.4)	0.378
Gamma-glutamyl transpeptidase >50 U/L	22 (28.6)	161 (21.6)	0.164
Lactate dehydrogenase >245 U/L	60 (85.7)	599 (83.9)	0.691
Creatine kinase >200 U/L	49 (63.6)	465 (62.3)	0.822
Blood urea nitrogen >7.14 mmol/L	22 (28.6)	244 (32.9)	0.442
Total bilirubin >17.1 μmol/L	10 (13.0)	77 (10.3)	0.469
Ceatinine >97 μmol/L	14 (20.0)	130 (18.2)	0.716
Serum amylase >115 U/L	30 (56.6)	242 (52.4)	0.560
Calcium <2.1 mmol/L	34 (61.8)	389 (61.8)	0.997
Coagulation parameters, median (interquartile range)			
Prothrombin time, s	11.5 (10.4–12.3)	10.9 (10.2–11.6)	0.017
Thrombin time, s	20.6 (17.4–22.7)	19.7 (17.6–22.4)	0.318
Activated partial thromboplastin time, s	45.5 (40.5–58.5)	46.2 (38.8–56.6)	0.797
Fibrinogen, g/L	2.8 (2.2–3.2)	2.7 (2.3–3.2)	0.775
International normalized ratio	1.0 (0.9–1.1)	1.0 (0.9–1.0)	0.136
Prothrombin time activity, %	84.0 (76.2–92.3)	87.7 (79.7–98.1)	0.124
D-dimer, ng/mL	1,014 (356–1,432)	647 (381–1,373)	0.603

log₁₀-transformed data with the generalized estimating equation model, we showed that platelet count and leukopenia recovery were delayed in the co-infection group compared with the single-infection group ($p = 0.045$ and $p = 0.027$, respectively). The generalized estimating equation model also showed that the co-infection group had higher levels of serum creatine kinase ($p = 0.047$) and lactate dehydrogenase ($p = 0.022$) during those recovery processes.

Based on the dynamic patterns at 2-day intervals, virus loads in the single-infection group peaked at day 5 after symptom onset and gradually decreased thereafter. Virus loads in the co-infection group peaked at day 7 and then decreased at a lower rate than that for the single-infection group after we adjusted for sex, age, and time from symptom onset to hospital admission ($p = 0.028$) (Figure 2, panel A).

At weeks 1 and 2 after symptom onset, SFTSV-specific IgG titers and positivity rates were not significantly different between the 2 groups (Figure 2, panels C, D). At week 3, the co-infection group had a significantly lower rate of SFTSV positivity ($p = 0.007$). Antibody titers at week 4 were not significantly different between the groups (Figure 2, panel C).

We conducted laboratory testing for 34 patients with SFTSV-SFGR co-infection, 30 sex- and age-matched patients with SFTS only, and 25 controls who were negative for both pathogens by molecular and antibody testing. Levels of interleukin (IL)–1 receptor agonist, IL–8–10, IL–17, interferon- γ , monocyte chemoattractant protein 1,

monocyte chemoattractant protein α 1, granulocyte colony-stimulating factor, fibroblast growth factors, and tumor necrosis factor- α were similar in the single-infection and co-infection groups and significantly elevated compared with levels in the control group (online Technical Appendix Figure). IL-6 and IL-15 levels were elevated in both infection groups, but they were significantly higher in the SFTSV single-infection group. Platelet-derived growth factor-BB and RANTES (regulated on activation, normal T cell expressed and secreted) were decreased in both groups, but we observed intergroup differences only for RANTES.

Altogether, 87 (10.6%) patients died. The case-fatality rate in the co-infection group (16.9% [13/77]) was insignificantly higher than that in the single-infection group (9.9% [74/746]) ($p = 0.058$). The association between co-infection and higher case-fatality rate was significant after adjustment for sex, age, and interval from disease onset to hospital admission (odds ratio 1.992, 95% CI 1.025–3.873; $p = 0.042$) (online Technical Appendix Table 4).

Conclusions

Our retrospective investigation in an SFTSV-endemic region of China identified SFTSV-SFGR co-infection in \approx 8.5% of SFTSV-infected patients and a higher frequency of fatal outcome and delayed recuperation in the co-infected patients. These findings highlight the importance of considering SFGR infection in the differential diagnosis for patients in SFTSV-endemic regions.

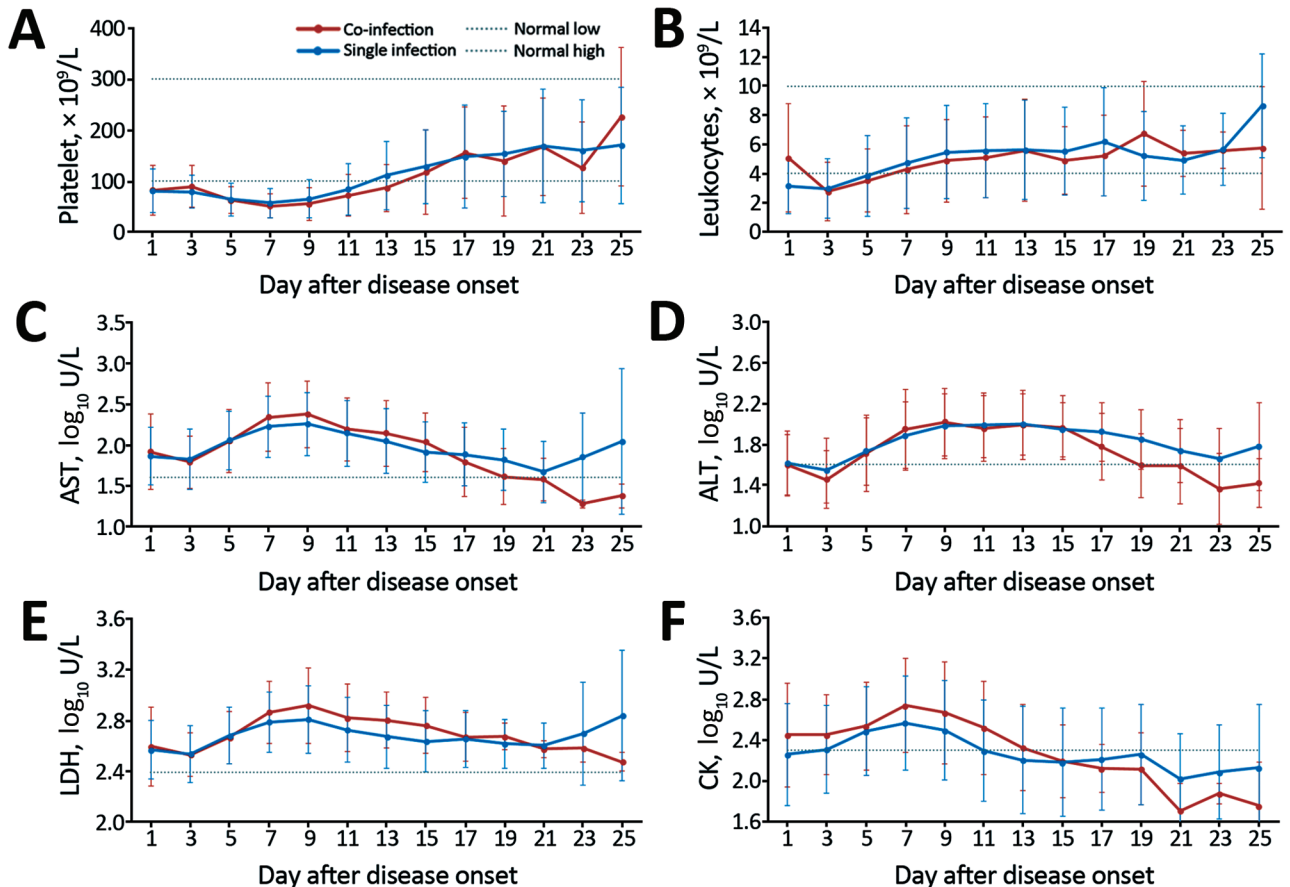


Figure 1. Dynamic profiles for 6 selected laboratory parameters for hospitalized patients with severe fever with thrombocytopenia syndrome virus (SFTSV) infection only or with SFTSV and spotted fever group rickettsiae co-infection, China, 2013–2015. A–B) Mean counts and 95% CIs (error bars) for platelets (A) and leukocytes (B). C–F) \log_{10} -transformed median level of and interquartile ranges (error bars) for aspartate aminotransferase (AST) (C); alanine aminotransferase (ALT) (D); lactate dehydrogenase (LDH) (E); and creatine kinase (CK) (F). Dashed lines indicate the reference level for each parameter. Parameters were compared by using the generalized estimating equation model.

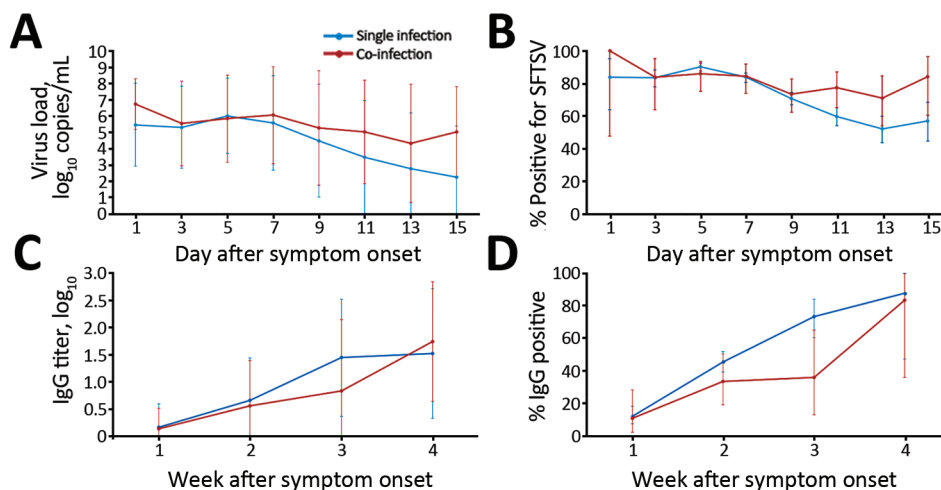
SFTSV infection can cause a wide variety of signs and symptoms, ranging from influenza-like illness to more severe complications and even life-threatening disease (7). Complications usually involve neurologic and hemorrhagic manifestations, which can progress to multiple organ dysfunction in critically ill patients. Rickettsial infections are clinically difficult to distinguish from many virus infections (8), and our results showed that symptoms common to SFTSV- and SFGR-infected patients (e.g., influenza-like illness, gastrointestinal symptoms) are not intensified in co-infected patients. In contrast, less common hemorrhagic signs, especially gastrointestinal hemorrhages, are exacerbated in co-infected patients. Previous studies have shown that in patients with SFTS, blood coagulation parameters are prolonged, as characterized by activated partial thromboplastin time and thrombin time (9,10). Thrombocytopenia, a common laboratory finding in patients with SFTS, can contribute to hemorrhage, and hemorrhagic signs have

also been observed in patients infected with SFGR species (e.g., *R. rickettsii* and *R. conorii*) (12–14); however, SFGR mainly invade the vascular endothelial cells, causing vascular inflammation and increased vascular permeability (11). Also, based on prolonged thrombocytopenia and longer prothrombin times that have been observed in co-infected persons, we hypothesize that the additive effect from 2 pathogens might lead to aggravated hemorrhage.

Doxycycline is the recommended therapeutic regimen for rickettsia infection (15) and could be administered in cases of SFTSV–SFGR co-infection. From a public health perspective, intensified efforts should be made to detect SFTSV–SFGR co-infection in regions where *H. longicornis* ticks predominate and carry both SFTSV and SFGR.

This study was supported by the Natural Science Foundation of China (81222037, 81473023), the China Mega-Project for Infectious Diseases grants (2013ZX10004-202), and the Youth Talent

Figure 2. Dynamic profiles for severe fever with thrombocytopenia syndrome virus (SFTSV) RNA and SFTSV-specific IgG in hospitalized patients with SFTSV infection only or with SFTSV and spotted fever group rickettsiae co-infection, China, 2013–2015. A) \log_{10} -transformed SFTSV virus loads. B) Percentage of patients positive for SFTSV. C) \log_{10} -transformed SFTSV IgG titers. D) Percentage of patients positive for SFTSV IgG. Comparisons were performed using the generalized estimating equation model. The error bars, which show the standard deviation for \log_{10} -transformed SFTSV virus loads and \log_{10} -transformed SFTSV IgG titers, represent the 95% CI for the percentage of patients positive for SFTSV and SFTSV IgG.



Support Program by School of Public Health, Peking University. The funding agencies had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; or preparation, review, or approval of the manuscript.

The authors had the following roles in the study and preparation of the manuscript: Q.-B.L., W.L., and W.-C.C. conceived and designed the experiments; Q.-B.L., H.L., P.-H.Z., N.C., Y.-D.F., X.-M.C., J.-G.H., C.-T.G., and X.-A.Z. performed the experiments; Q.-B.L., H.L., P.-H.Z., N.C., and W.L. analyzed the data; N.C. and Z.-D.Y. contributed materials; and Q.-B.L., H.L., P.-H.Z., N.C., and W.L. prepared the manuscript.

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Severe Fever with Thrombocytopenia Syndrome Complicated by Co-infection with Spotted Fever Group Rickettsiae, China

Technical Appendix

Methods

SFTSV Detection and Quantitation by Real-time PCR

Serial anti-coagulated blood and serum samples were collected from clinically suspected patients, at entry into the hospital and during their hospitalization. Viral RNA was isolated from serum samples using QIAamp Viral RNA Mini Kit (Qiagen, Germantown, MD, USA), according to the manufacturer's instructions. One-step Primer Script RT-PCR Kit (TaKaRa Bio dalian Inc., Japan) was used according to the manufacturer's instructions for SFTSV detection in a volume of 20 μ L containing 10 μ L of One Step RT-PCR Buffer (2 \times), 0.4 μ L of TaKaRa Ex Taq HS (5 U/ μ L) and 0.4 μ L of PrimeScript RT Enzyme MixII (TaKaRa Bio dalian Inc., Japan), 1 μ L of PCR primer mix (20 μ M of sense and antisense each) and 0.5 μ L of Probe (10 μ M), total RNA 2 μ L and RNase free dH₂O (5.7 μ L). PCR was carried with one cycle of 42°C for 5 min and 95°C for 10 s, followed by 40 cycles of 95°C for 5 s and 55°C for 20 s in a LightCycler Real Time PCR apparatus (Roche Diagnostics, Mannheim, Germany). The real-time PCR primers and probe were targeted at the S-segment of the SFTSV. The sequences were as follows:

forward: TTCACAGCAGCATGGAGAGG; reverse: GATGCCTTCACCAAGACTATCAATG;

Probe: AACTTCTGTCTTGCTGGCTCCGC. Nested RT-PCR and sequencing of the M-segment were performed on randomly selected positive samples to verify the real-time RT-PCR

results. We have adopted several strategies to prevent contamination. All negative extraction controls were included to detect cross-contamination events within each extraction. To rule out contamination of the PCR reagents or cross-contamination of the PCR plate, water was included as a control, and RNA from the original specimens was capped before the addition of any positive control materials.

Quantitation of virus was performed using quantitative RT-PCR targeting the same gene segments. Real-time PCR was performed in a volume of 20 μ L by use of the One-step Primer Script RT-PCR Kit (TaKaRa Bio dalian Inc., Japan) in the CFX96 instrument (Bio-Rad, California, USA). Standard curves or absolute RNA quantification were included in every assay and were generated by using RNA transcripts produced by in vitro transcription of cDNA that included the real time qPCR assay amplicons. Standard curves included five dilutions and three replicate wells for each dilution. All samples were quantified in at least duplicate wells. Levels of SFTSV RNA concentrations were expressed as copies/mL.

SFTSV-specific IgG antibody detection by ELISA

SFTSV-specific IgG antibodies were detected by enzyme-linked immunosorbent assay (ELISA) using the same protocol described previously (*1*). Briefly, 96-well polystyrene microtiter plates were coated with purified SFTSV antigen. Serially diluted serum samples starting with 1:10 were added, and after incubation and washing, antihuman IgG conjugates were added. Substrate solution was added for color development. The optical density at 450 nm (A₄₅₀) of each well was measured. The cutoff limit was 0.1 plus the optical density of the negative control, according to the manufacturer's instructions.

Determination of serum cytokine/chemokine concentration

Serum levels of 27 cytokines and chemokines were measured with the use of Bio-plex Pro Human 27-plex cytokine panel (Bio-plex Pro Human 27-plex cytokine panel, Bio-Rad, CA, USA). The tested cytokines included: interleukin (IL)-1 β , 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 chemoattractant protein-1 (MCP-1), macrophage inflammatory protein 1a (MIP-1 α), macrophage inflammatory protein 1 β (MIP-1 β), 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 (VEGF). Following manufacturer's instruction, a multiplex-biometric immunoassay containing fluorescent microspheres conjugating with monoclonal antibodies specific for target cytokines was performed to test serum cytokine levels. Cytokine concentrations were calculated from standard curves of known concentrations of recombinant human cytokines. Levels were analyzed using Bio-Plex Manager 6.0 software.

Data Collection

We performed a chart review of all recruited patients, collected information on demographic characteristics, symptoms and signs, clinical laboratory test results and treatment regimens, etc. These data were drawn from the medical database by a group of trained physicians using a standardized format and entered into an EpiData database. The data were further reviewed for accuracy and consistency by a group of epidemiologists.

Main Outcome Measurements

The main outcome measurements, including hemorrhagic manifestations, presence of plasma leakage, neurologic manifestations, respiratory manifestations and death, were consecutively reviewed during the whole hospitalization. The hemorrhage manifestations were defined by presence of petechiae, ecchymosis, hemoptysis, epistaxis, hematuria, hematemesis, melena, vaginal, and gingival bleeding. Plasma leakage was determined by the presence of pleural and/or ascitic fluid or haemoconcentration. Deaths were classified based on clinical diagnoses of the proximate cause of death by the physician.

Statistical Analyses

Categorical variables were assessed using the χ^2 test or the Fisher exact test. Continuous variables were compared by Student's t-test and assessed with the nonparametric Mann-Whitney U test in case of skewed variables. The comparisons of laboratory parameters every 2 days from disease onset to discharge were performed by generalized estimating equation (GEE) with adjustment for the variables of age, sex and the intervals from disease onset to hospital admission. All analyses were performed by SAS software, version 9.1.3 (SAS Institute, Cary, North Carolina, USA). All p values were 2-tailed and $p < 0.05$ was considered statistically significant.

Results

In total 1080 laboratory-confirmed SFTS patients were hospitalized during the study period, among them 823 patients who had convalescence serum samples collected were recruited into the study. Of the 823 patients, 25 (3.0%) patients were negative for SFTSV RT-PCR and had seroconversion ($n = 18$) or a 4-fold increase ($n = 7$) in titers of IgG antibody (Technical Appendix Table 1). Other 798 patients were positive for SFTSV RT-PCR assay. Seventy-seven (8.5%) of the 823 patients provided serologic evidence of SFGR infection according to seroconversion ($n = 45$) or 4-fold increase of antibody titer ($n = 32$), composing as the SFTSV-SFGR coinfection group (Technical Appendix Table 2).

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<http://dx.doi.org/10.1056/NEJMoa1010095>

Technical Appendix Table 1. The titers of IgG antibody in the SFTS patients with RT-PCR negative result tested by ELISA

Patient No.	Titer of IgG antibody at acute stage	Titer of IgG antibody at recovery stage
1	1:10	1:1280
2	<1:10	1:640
3	<1:10	1:640
4	<1:10	1:10
5	1:20	1:160
6	<1:10	1:640
7	<1:10	1:80
8	<1:10	1:20
9	<1:10	1:20
10	<1:10	1:10
11	<1:10	1:10
12	1:10	1:40
13	<1:10	1:10
14	<1:10	1:80
15	<1:10	1:20
16	1:10	1:80
17	<1:10	1:10
18	1:80	1:320
19	1:10	1:320
20	1:10	1:320
21	<1:10	1:40
22	<1:10	1:10
23	<1:10	1:160
24	<1:10	1:10
25	<1:10	1:20

Technical Appendix Table 2. The titers of IgG antibody for spotted fever group rickettsiae tested by IFA

Patient No.	Titer of IgG antibody at acute stage	Titer of IgG antibody at recovery stage
3	<1:64	1:256
4	<1:64	1:128
9	1:64	1:512
16	<1:64	1:512
18	<1:64	1:256
26	<1:64	1:64
27	<1:64	1:64
28	<1:64	1:64
29	<1:64	1:64
30	<1:64	1:64
31	1:64	1:256
32	1:64	1:256
33	<1:64	1:64
34	<1:64	1:128
35	<1:64	1:128
36	<1:64	1:128
37	<1:64	1:128
38	<1:64	1:128
39	<1:64	1:128
40	<1:64	1:128
41	<1:64	1:128
42	<1:64	1:128
43	<1:64	1:128
44	<1:64	1:256
45	<1:64	1:256
46	<1:64	1:256
47	<1:64	1:256
48	<1:64	1:128
49	<1:64	1:128
50	<1:64	1:512
51	<1:64	1:512
52	<1:64	1:128
53	1:64	1:512
54	1:64	1:256
55	1:64	1:256
56	1:64	1:256

Patient No.	Titer of IgG antibody at acute stage	Titer of IgG antibody at recovery stage
57	1:64	1:512
58	1:64	1:512
59	1:64	1:256
60	1:64	1:512
61	1:64	1:256
62	1:64	1:1024
63	1:64	1:256
64	1:64	1:512
65	1:64	1:256
66	1:64	1:512
67	1:64	1:1024
68	1:64	1:512
69	1:64	1:512
70	<1:64	1:512
71	<1:64	1:256
72	<1:64	1:256
73	<1:64	1:256
74	<1:64	1:256
75	<1:64	1:256
76	<1:64	1:256
77	1:64	1:256
78	1:64	1:256
79	1:64	1:256
80	<1:64	1:128
81	<1:64	1:128
82	<1:64	1:128
83	<1:64	1:128
84	<1:64	1:128
85	<1:64	1:128
86	<1:64	1:128
87	<1:64	1:128
88	<1:64	1:128
89	<1:64	1:64
90	<1:64	1:64
91	<1:64	1:64
92	<1:64	1:64
93	<1:64	1:64
94	<1:64	1:64

Patient No.	Titer of IgG antibody at acute stage	Titer of IgG antibody at recovery stage
95	<1:64	1:64
96	<1:64	1:64
97	<1:64	1:64

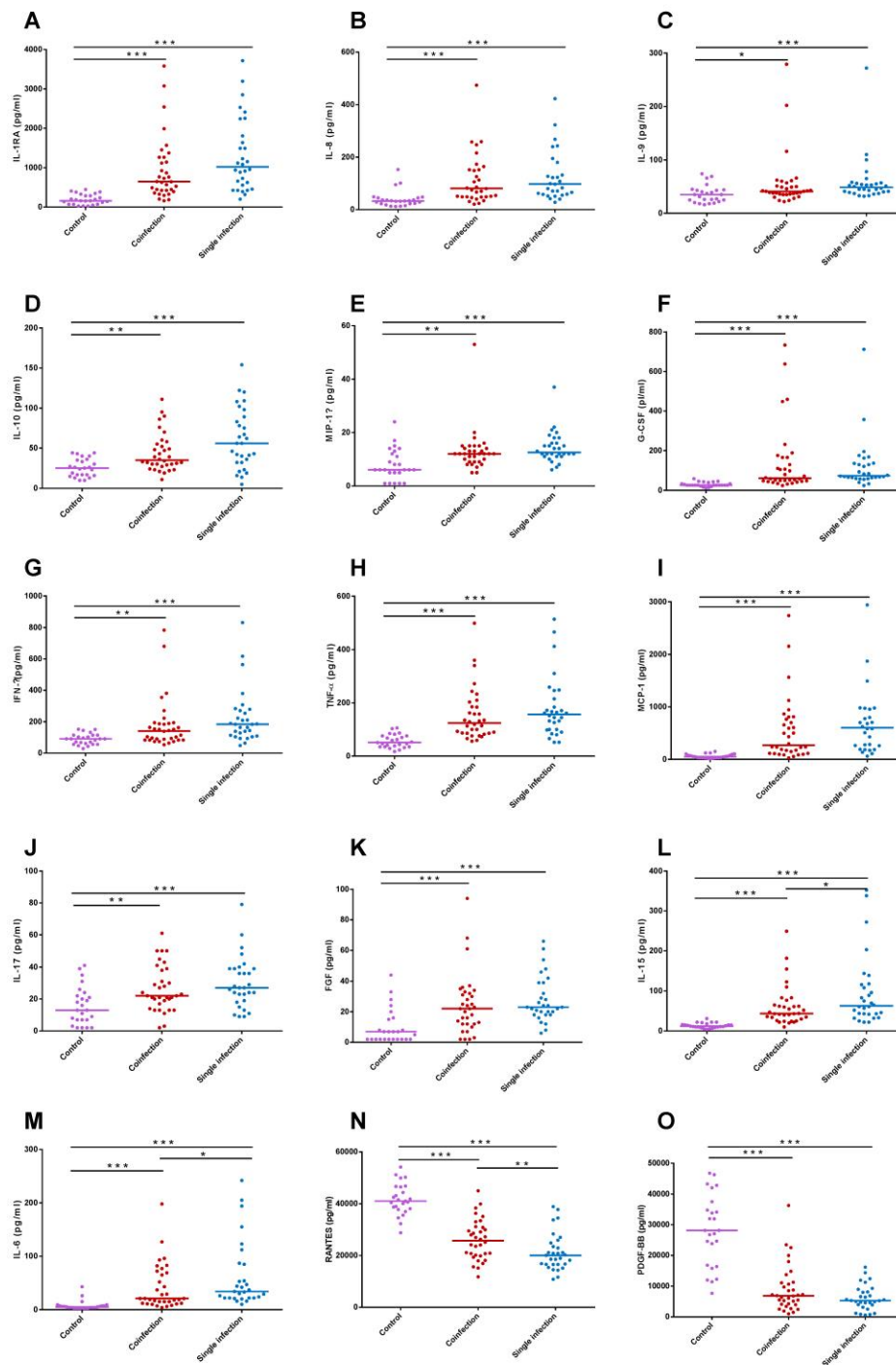
Technical Appendix Table 3. Demographic and clinical characteristics of the patients with SFTSV-SFG rickettsiae co-infection vs. SFTSV single infection

Characteristics	Co-infection (n = 77)	Single infection (n = 746)	P value
Demographic characteristics			
Age, mean \pm SD, years	61.4 \pm 12.6	61.8 \pm 12.2	0.809
Sex, female, n (%)	42 (54.6)	442 (59.3)	0.425
Hospital duration, median (IQR)	8(5–10)	7 (5–9)	0.137
Delay days from onset to admission, median (IQR)	5 (4–7)	5 (4–7)	0.917
Days>5 from disease onset, n (%)	38 (49.4)	355 (47.6)	0.768
Tick bite history, n (%)	10 (13.0)	87 (11.7)	0.731
Underlying diseases, n (%)	32 (41.6)	244 (32.7)	0.016
Cardiovascular and cerebrovascular diseases	11 (14.3)	75 (10.1)	0.248
Diabetes	18 (23.4)	144 (19.3)	0.392
Active hepatitis	7 (9.1)	46 (6.2)	0.320
Chronic respiratory disease	2 (2.6)	14 (1.9)	0.663
Clinical manifestations			
Influenza like illness	77 (100)	746 (100)	1.000
Febrile, n (%)	76 (98.7)	744 (99.7)	0.256
Fever duration, days, median (IQR)	7 (5–9)	6 (4–8)	0.039
Lymphadenectasis, n (%)	33 (42.9)	334 (44.8)	0.748
Myalgia, n (%)	57 (74.0)	608 (81.5)	0.113
Weakness, n (%)	73 (94.8)	722 (96.8)	0.362
Dizziness, n (%)	16 (20.8)	121 (16.2)	0.307
Headache, n (%)	10 (13.0)	54 (7.2)	0.073
Chills, n (%)	7 (9.1)	60 (8.0)	0.749
Arthralgia, n (%)	2 (2.6)	10 (1.3)	0.381
Gastrointestinal illness, n (%)	56(96.1)	509 (68.2)	0.418
Abdominal pain	3 (3.9)	25 (3.4)	0.802
Nausea	53 (68.8)	469 (62.9)	0.398
Diarrhea	20 (26.0)	146 (19.6)	0.183
Vomit	24 (35.2)	196 (26.3)	0.848
Diarrhea and Vomit	36 (46.8)	288 (38.6)	0.164
Plasma leakage, n (%)	9 (11.7)	70 (9.4)	0.513
Hydrothorax	8 (10.4)	64 (8.6)	0.592

Characteristics	Co-infection (n = 77)	Single infection (n = 746)	P value
Hydropericardium	1 (1.3)	12 (1.6)	0.836
Ascites	3 (3.9)	4 (0.5)	0.002
Respiratory syndrome, n (%)	34 (44.2)	377 (50.5)	0.286
Dyspnea	5 (6.5)	4 (2.7)	0.172
Sputum	22 (28.6)	264 (35.4)	0.232
Cough	33 (42.9)	355 (47.6)	0.429
Hemorrhage manifestations, n (%)	38 (49.4)	244 (32.7)	0.003
Melena	8 (10.4)	34 (4.6)	0.027
Gingival bleeding	9 (11.7)	60 (8.0)	0.272
Hemoptysis	2 (2.6)	15 (2.0)	0.730
Haematemesis	2 (2.6)	29 (3.9)	0.571
Hematuria	13 (16.9)	63 (8.5)	0.074
Petechial/ecchymosis	20 (26.0)	119 (16.0)	0.025
Epistaxis	0 (0)	7 (0.9)	0.393
Neurologic symptom, n (%)	29 (37.7)	243 (32.6)	0.366
Coma	1 (1.3)	50 (6.7)	0.061
Dysphoria	11 (14.3)	71 (9.5)	0.184
Lethargy	1 (1.3)	40 (5.4)	0.690
Blurred mind	13 (16.9)	133 (17.8)	0.836
Convulsion	11 (14.3)	99 (13.3)	0.803
Apathy	15 (19.5)	143 (19.2)	0.947

Technical Appendix Table 4. The association between death and the related variables

Variables	Univariate		Multivariate	
	OR (95% CI)	P	OR (95% CI)	P
Model 1 in all the patients				
Group (coinfection/single)	1.845 (0.970–3.508)	0.062	1.992 (1.025–3.873)	0.042
Age	1.061 (1.036–1.087)	<0.001	1.061 (1.036–1.867)	<0.001
Sex (male/female)	1.882 (1.203–2.945)	0.006	1.716 (1.082–2.720)	0.022
Interval from onset to admission	1.142 (1.055–1.235)	0.001	1.107 (1.020–1.202)	0.015
Model 2 in the coinfection group				
Age	1.082 (1.014–1.155)	0.017	1.109 (1.023–1.202)	0.012
Sex (male/female)	9.167 (1.870–44.922)	0.006	12.491 (1.977–78.910)	0.007
Interval from onset to admission	0.757 (0.160–4.963)	0.895	0.860 (0.585–1.265)	0.443
Model 3 in SFTSV single group				
Age	1.063 (1.037–1.090)	<0.001	1.057 (1.030–1.084)	<0.001
Sex (male/female)	1.517 (0.938–2.454)	0.090	1.314 (0.799–2.160)	0.282
Interval from onset to admission	1.182 (1.086–1.286)	<0.001	1.142 (1.047–1.247)	0.003



Technical Appendix Figure. Comparison of cytokine production in two groups of patients. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Altogether 34 coinfecting patients, 30 age and gender comparable SFTS patients and 25 healthy controls who showed no positive SFTSV or *R. rickettsii* detection by either molecular or antibody tests were measured. The 27 cytokines were identified with different patterns of production.

Cytokines IL1-RA, IL-8, IL-9, IL-10, IFN- γ , MCP-1 α , G-CSF, FGF, TNF- α , MCP-1 and IL-17 were significantly elevated in two groups than controls, yet showing no inter-group difference (Figure, panels A–K). Cytokine IL-6 and IL-15 were elevated in both groups, with SFTSV single infection attaining significantly higher level than the coinfection group (Figure, panels L, M). PDGF-BB and RANTES was decreased in both groups (Figure, panels N, O), and showing inter-group difference for RANTES only (Figure, panel N). The remaining cytokines were within the normal level and displayed no significant difference between two groups. Abbreviations: G-CSF, granulocyte colony-stimulating factor; IL, interleukin; RANTES, regulated on activation and normally T-cell expressed. PDGF: platelet-derived growth factor.