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Longitudinal analysis of cytokine dynamics in severe fever with thrombocytopenia syndrome patients — high-incidence regions of China (2010–2023)

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Abstract: Severe fever with thrombocytopenia syndrome (SFTS) is a life-threatening tick-borne disease characterized by cytokine dysregulation and immune-mediated hyperinflammation. This multicenter retrospective study analyzed the dynamics of 17 cytokines across acute and recovery phases using 287 serum samples collected between 2010 and 2023 from high-incidence regions of China, evaluating their associations with disease severity, sex, age, and antibody responses. The results demonstrated that elevations of IL-6, IFN-α, IL-8, and IP-10 during the acute phase were associated with hyperinflammation, while IL-10 balanced inflammatory control and may have contributed to viral persistence. During recovery, most cytokines declined; however, IL-8 and IP-10 remained elevated longer in some patients, reflecting heterogeneity in recovery trajectories. Severe cases exhibited significantly higher levels of IL-10, IFN-γ, IL-6, IFN-α, TNF-α, IL-8, and IP-10, underscoring their potential as biomarkers for disease severity prediction. Sex-based differences revealed higher IFN-γ and IL-8 levels in females, potentially due to hormonal and genetic factors, while older patients exhibited elevated IL-10, IFN-γ, and IFN-α, reflecting immune dysregulation and age-related shifts in adaptive immunity. Correlation analysis revealed distinct immune response patterns, with IL-10 strongly correlating with IFN-γ and minimal antibody-cytokine associations observed during the acute phase. In contrast, in the recovery phase, IgG negatively correlated with IL-10, IFN-γ, and IP-10, and IgM positively correlated with IL-10, IFN-γ, IL-6, IFN-α, TNF-α, IL-8, and IP-10, reflecting dynamic immune regulation and the interplay between humoral and cellular immunity. These findings provide critical insights into the immunopathogenesis of SFTS, supporting the development of cytokine-targeted therapies and advanced diagnostic tools to improve clinical outcomes.

Keywords:

Severe fever with thrombocytopenia syndrome (SFTS)

Cytokine dynamics

Antibody-cytokine correlations

Disease severity biomarkers

1. Introduction

Severe Fever with Thrombocytopenia Syndrome (SFTS), caused by the Severe Fever with Thrombocytopenia Syndrome virus (SFTSV), is a highly lethal tick-borne disease that was first identified in China in 2010 [1, 2]. Since its discovery, SFTS has become endemic in East Asia, with high-incidence regions including Shandong, Anhui, Hubei, and Jiangsu provinces [3–6]. Its rising annual incidence poses significant public health challenges in these areas [7,8]. It is transmitted primarily by tick bites, but human-to-human transmission through contact with infected blood or bodily fluids has also been documented, complicating control measures, especially in healthcare settings. Clinically, the disease is characterized by high fever, thrombocytopenia, leukopenia, and multi-organ dysfunction. Severe cases are frequently complicated by disseminated intravascular coagulation (DIC) and multiple organ dysfunction syndrome (MODS), conditions associated with high mortality rates [9, 10].

Cytokines are critical mediators of immune regulation, playing diverse roles in coordinating cellular responses and signaling pathways. They are not only produced in viral infections but are also essential for the normal development and response of the immune system. Secreted by activated cells, cytokines serve as crucial signaling molecules, directing the immune system's response to pathogens and maintaining immune homeostasis. However, an imbalance in cytokine production can occur in various disease states, including tumors, leading to immune dysfunction and contributing to infectious disease progression and chronic inflammatory conditions [11–13]. In SFTS, immune dysregulation, particularly the uncontrolled release of pro-inflammatory cytokines, or "cytokine storm," has been strongly implicated in disease severity and progression [14, 15]. This hyperinflammatory state exacerbates tissue damage and systemic inflammation, ultimately contributing to life-threatening complications such as DIC and MODS. Despite their recognized importance, large-scale, longitudinal studies investigating cytokine dynamics, interactions, and their associations with disease severity are limited. Most prior studies, constrained by small sample sizes or cross-sectional designs, have failed to capture the temporal complexity of cytokine-mediated immune responses during the disease course [16–22]. The lack of an effective vaccine or targeted immunomodulatory therapies further underscores the urgent need for comprehensive research in endemic regions [23, 24].

This multicenter retrospective study analyzed the dynamics of 17 cytokines, including IL-10,

IL-12P70, IFN-γ, IL-6, IL-5, IFN-α, IL-2, IL-4, IL-17A, IL-1β, TNF-α, IL-8, G-CSF, MCP-1, MIP-1α, MIP-1β, and IP-10, using serum samples collected from 2010 to 2023 in high-incidence regions of China. Longitudinal analysis of cytokine levels was performed, with systematic comparisons across severity groups, age cohorts, and sexes using healthy controls as a baseline, and correlation analyses between antibody levels, cytokine concentrations, and inter-cytokine relationships were conducted separately for the acute and recovery phases. By addressing critical gaps in existing knowledge, this study aims to enhance the understanding of cytokine dynamics and their contributions to disease progression, ultimately informing the development of evidence-based strategies for diagnosis, immune monitoring, and targeted therapeutic interventions.

2. Materials and methods

2.1. Study design and patient enrollment

This study included 199 laboratory-confirmed SFTS patients and 30 healthy donors from high-incidence provinces in China (Shandong, Anhui, Hubei, and Jiangsu) between 2010 and 2023. 287 serum samples were collected, including 257 from the SFTS patients (143 acute-phase and 114 recovery-phase samples) and 30 from healthy donors. Among the 199 SFTS patients, 58 provided paired acute- and recovery-phase samples (totaling 116), while the remaining 141 patients contributed a single sample. Diagnoses were confirmed by positive SFTSV nucleic acid detection or a ≥fourfold increase in IgG antibody levels from the acute phase (1–14 days post-onset) to the recovery phase (≥15 days post-onset). Exclusion criteria included patients with concurrent infections caused by Rickettsia, epidemic hemorrhagic fever (EHF), or human immunodeficiency virus (HIV). Cytokine positivity rates and concentrations were analyzed by stratifying patient samples into five intervals based on symptom onset: 1–7, 8–14, 15–21, 22–180, and >180 days. Among the 199 SFTS patients, 91 (41 severe, 50 mild) had disease severity assessed by attending physicians based on complications such as MODS, DIC, or other life-threatening conditions, providing a single acute-phase serum sample for cytokine comparisons among the mild, severe, and healthy groups. Acute-phase patient samples were further stratified by sex and age groups (<50 years, 50–70 years, >70 years) for additional analyses. Correlations among antibody levels, cytokine concentrations, and inter-cytokine relationships were assessed separately for the acute and recovery phases. Paired samples were used to compare immune responses across phases. The study was approved by the National Institute for Viral Disease Control and Prevention ethics committee, Chinese CDC (IVDC2022-011), and all patients provided informed consent.

2.2. Sample collection and processing

Serum samples were collected with local Centers for Disease Control and Prevention (CDC) and designated partner hospitals. Following the acquisition of informed consent and confirmation of patient identity, 10 mL of venous blood was drawn into EDTA-coated tubes under sterile conditions. The samples were gently inverted immediately after collection to prevent clot formation

and subsequently processed within two hours. The serum was separated via centrifugation at 3,000 revolutions per minute (RPM) for 10 minutes at 4 ℃ and aliquoted into cryogenic vials. These vials were stored at – 80 ℃ until further analysis to ensure sample integrity. All procedures adhered strictly to biohazard safety protocols, with comprehensive documentation maintained throughout to guarantee full traceability and compliance with ethical standards.

2.3. NP-specific IgM detection by IgM-capture ELISA

NP-specific Immunoglobulin M (IgM) antibodies were detected using a validated IgM capture enzyme-linked immunosorbent assay (ELISA) developed by our laboratory, with healthy control sera as negative controls. Briefly, 96-well plates were coated with 100 μL of anti-human IgM (μ-chain specific) antibodies per well and incubated overnight at 4 ℃. Following five washes with phosphate-buffered saline containing Tween-20 (PBST), plates were blocked with bovine serum albumin (BSA) (Beijing Biodragon Immunotechnologies Co., Ltd., China) at 37 ℃ for 1 h. Next, 100 μL of 1:100 diluted patient serum and control samples were added and incubated at 37 ℃ for 30 minutes. After five additional washes, 100 μL of 1:500 diluted horseradish peroxidase (HRP)-conjugated SFTSV NP antigen was added and incubated for 30 minutes. Following five more washes, 3,3′,5,5′-tetramethylbenzidine (TMB) substrate was added for color development in the dark. After 5–10 minutes, the process was stopped, and the optical density (OD) was measured at 450 nm using the Thermo Scientific Varioskan Flash Reader (Thermo Fisher Scientific, USA). Samples were considered positive if their OD was ≥2.1 times the average OD of the negative controls.

2.4. NP-specific IgG detection by indirect ELISA

NP-Specific Immunoglobulin G (IgG) antibodies were identified using a well-established indirect ELISA approach using healthy samples as negative controls. SFTSV nucleocapsid protein (NP) antigen was coated at 500 ng per well on 96-well plates and incubated overnight at 4 ℃. After five washes with PBST, the plates were blocked using BSA blocking solution (Beijing Biodragon Immunotechnologies Co., Ltd., China) for 1 hour at 37 ℃. Place 100 μL of each sample, including patient sera and controls diluted 1:100, into wells and incubate for 30 minutes at 37 ℃ after blocking. After washing the plates, 100 μL of 1:5,000 HRP-conjugated antibody was added to each well, followed by a 30-minute incubation. After incubation and washing, TMB substrate was added to wells for color development. After 5–10 minutes, the process was stopped, and the optical density (OD) was measured at 450 nm using the Thermo Scientific Varioskan Flash Reader (Thermo Fisher Scientific, USA). Samples were considered positive if their OD was ≥2.1 times the average OD of the negative controls.

2.5. Quantitative measurement of 17 cytokines in SFTS patients

Cytokine levels were measured using the NovaPlex-1200 system (Hubei Xinzongke Virus Disease Engineering Technology Co., Ltd., China), a flow cytometry-based detection platform that received Class II medical device registration (Chongqing Medical Device Registration No. 20242220247). Following the manufacturer's instructions, a 20-fold concentrated washing solution was diluted, and 50 μL of either a gradient standard or a 2-fold diluted serum sample was added to each well along with 5 μL of microspheres. The plate was gently mixed and incubated at 37 ℃ in the dark for 1 h. After incubation, the microspheres were separated using a magnetic plate, and the supernatant was carefully removed. Wells were washed and dried before adding 50 μL of detection antibody solution, followed by a 30-minute incubation at 37 ℃ in the dark. After washing, 50 μL of fluorescein solution was added, and the plate was incubated for 15 minutes at room temperature. Finally, 70 μL of dispersion solution was added, and the plate was shaken gently for 2 minutes to resuspend the beads evenly. The prepared samples were analyzed by the NovaPlex-1200 system, which requires only 10 μL of serum per sample and can process up to 120 samples per hour. Cytokine concentrations were determined based on manufacturer-provided cutoff values, which were established and validated for each cytokine to ensure accuracy and consistency.

2.6. Statistical analysis

All statistical analyses were performed using GraphPad Prism (version 10.1.2, La Jolla, CA) and R (version 4.2.3). Data distribution was assessed for normality using the Shapiro-Wilk test. For non-normally distributed data, the Mann-Whitney U test was used to compare cytokine levels between two groups (e.g., male vs. female SFTS patients). Kruskal-Wallis test followed by Bonferroni correction for multiple comparisons was applied to compare the three groups (e.g., mild vs. severe SFTS patients vs. healthy controls, or patients aged <50, 50–70, and >70 years), the Paired sample data (e.g., longitudinal measurements from the same individuals) were evaluated using the Wilcoxon signed-rank test. Heatmaps illustrating the temporal dynamics of cytokine positivity rates were generated using the ggplot2 package in R. Correlation analyses were conducted in R using the corrplot package, employing Spearman's method to assess monotonic relationships between cytokines. Spearman's correlation coefficients (r) ranged from -1 to 1 and were classified as strong ($|r| \geq 0.6$), moderate ($0.4 \leq |r| < 0.6$), or weak ($|r| < 0.4$). Statistical significance thresholds for probability values (P values) were labeled as $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$, and "ns" denoted non-significant results. All reported P values were two-tailed, and a P value <0.05 was considered statistically significant.

3. Results

3.1. Dynamic changes in cytokine positivity rates in SFTS patients

The dynamic positivity patterns of 17 cytokines throughout SFTS were analyzed in 257 serum samples from 199 patients, excluding 30 healthy control samples from the total of 287, with the samples categorized into five-time intervals based on symptom onset (1–7 days, 8–14 days, 15–21 days, 22–180 days, and >180 days). (Fig. 1). IL-10 and IP-10 exhibited consistently high positivity

rates during the acute phase. Specifically, IL-10 showed a slight decline from 90.24 % (74/82) on Day 1–7 to 85.25 % (52/61) on Day 8–14, while IP-10 displayed a marginal decrease over the same period, from 90.24 % (74/82) to 88.52 % (54/61). In contrast, IL-6 demonstrated an increasing trend, with its positivity rising from 82.93 % (68/82) on Day 1–7 to 91.80 % (56/61) on Day 8–14. Similarly, IL-8 maintained consistently high positivity rates, reaching 97.56 % (80/82) on Day 1–7 and peaking at 98.36 % (60/61) on Day 8–14. On the other hand, IFN-γ and IFN-α exhibited moderate positivity rates during the acute phase, with both showing a slight decline. Specifically, IFN-γ dropped from 48.78 % (40/82) on Day 1–7 to 45.90 % (28/61) on Day 8–14, while IFN-α decreased from 51.22 % (42/82) to 45.90 % (28/61). Conversely, TNF-α and MCP-1 demonstrated increasing positivity rates over the same period. MCP-1 positivity rose from 25.61 % (21/82) on Day 1–7 to 42.62 % (26/61) on Day 8–14, while TNF-α increased from 31.71 % (26/82) to 44.26 % (27/61). A notable decline in cytokine positivity rates was observed during the recovery phase. Specifically, IL-10 and IL-6 positivity rates decreased to 66.67 % (14/21) and 61.90 % (13/21) on Day 15–21, dropping further to below 40 % beyond 22 days. In contrast, IL-8 and IP-10 demonstrated prolonged positivity, with rates remaining high at 95.24 % (20/21) and 80.95 % (17/21), respectively, on Day 15–21 and 96.30 % (52/54) and 77.78 % (42/54) on Day 22–180. However, both cytokines eventually fell below 40 % beyond 180 days. On the other hand, the positivity rates of IFN-γ, IFN-α, MCP-1, and TNF-α declined more rapidly during the recovery phase, with all cytokines dropping below 40 % as early as 15 days onward. Furthermore, other cytokines—including IL-2, IL-1β, MIP-1α, MIP-1β, G-CSF, IL-12P70, IL-5, IL-4, and IL-17A—exhibited consistently low positivity rates, remaining below 40 % across all disease phases without notable fluctuations.

3.2. Dynamic changes in cytokine levels throughout SFTS in patients

Dynamic changes in cytokine levels throughout SFTS were analyzed in 257 serum samples from 199 patients (excluding 30 healthy control samples from a total of 287), with samples categorized into five-time intervals based on symptom onset: 1–7 days, 8–14 days, 15–21 days, 22–180 days, and >180 days (Fig. 2). Notably, IL-10 and IL-6 exhibited distinct temporal profiles, rapidly rising to detectable positivity levels within the first week after symptom onset and peaking in concentration during the acute phase. Subsequently, their concentrations declined markedly by Day 15–21, remaining above positivity thresholds during this period, and ultimately returned to normal ranges by Day 22–180. IFN-α followed a similar trajectory, reaching positivity within the first week and peaking between Day 8 and 14. However, it declined more rapidly than IL-10 and IL-6, returning to normal ranges by Day 15–21. In contrast, IL-8 and IP-10 exhibited sustained elevations, displaying distinct patterns of prolonged activity. Both cytokines reached positivity levels within the first week and peaked during Day 8–14. Unlike IL-10, IL-6, and IFN-α, which sharply declined after their peaks, IL-8 and IP-10 decreased gradually through Day 15–21 and 22–180, consistently remaining above positivity thresholds and normalizing to baseline levels only after 180 days. Interestingly, IFN-γ and TNF-α levels approached positivity thresholds during the acute phase without exceeding them, reflecting a relatively lower contribution to cytokine elevation at this stage. However, other cytokines, including IL-12P70, IL-5, IL-2, IL-4, IL-17A, IL-1β, MCP-1, MIP-1α, MIP-1β, and G-CSF, consistently remained below positivity thresholds throughout all time

intervals, showing minor fluctuations that neither approached positivity thresholds nor suggested active involvement in disease progression.

3.3. Comparison of cytokine levels across disease severity, sex, and age groups in acute-phase SFTS patients

Cytokine levels across different disease severities, sexes, and age groups were compared during the acute phase. Of the 287 total samples, 257 were from SFTS patients (143 acute-phase and 114 recovery-phase), and 30 were from healthy controls. For severity comparisons, we focused on the 91 acute-phase samples from 91 SFTS patients with known severity (41 severe and 50 mild) and compared them with 30 healthy controls. For sex- and age-based comparisons, the 143 acute-phase samples were further stratified by sex (male, female) and age groups (<50 years, 50-70 years, and >70 years). Fig. 3 summarizes significant differences in IL-10, IFN-γ, IL-6, IFN-α, TNF-α, IL-8, and IP-10 levels between mild and severe SFTS cases. Among the cytokines analyzed IL-10, IL-6, IL-8, and IP-10 levels were markedly elevated in both mild and severe SFTS cases compared to healthy controls ($P < 0.0001$) and exceeded positivity thresholds, with significantly higher levels observed in severe cases compared to mild cases ($P < 0.05$). IFN-γ levels were significantly elevated in the severe group compared to both mild cases ($P < 0.001$) and healthy controls ($P < 0.0001$), with positivity observed exclusively in severe cases, while mild cases remained negative. Similarly, IFN-α and TNF-α levels were significantly higher in severe cases ($P < 0.0001$) compared to mild cases *(*$P < 0.01$) and healthy controls ($P < 0.0001$), with positivity observed only in severe cases, whereas mild cases remained negative. Sex-based comparisons revealed significantly higher IFN-γ levels in females than males ($P < 0.05$), with positivity observed exclusively in females, while IL-8 levels were slightly elevated in females ($P < 0.05$) but exceeded positivity thresholds in both sexes. Age-based comparisons revealed that IL-10 levels were significantly higher in patients >70 years compared to those <50 years ($P < 0.01$) and 50–70 years ($P < 0.05$), with all age groups exceeding positivity thresholds, while IFN-γ levels were significantly elevated in the 50–70 years group compared to the <50 years group ($P < 0.05$), where negativity was observed, but positivity was detected in both the 50–70 years and >70 years group; similarly, IFN-α levels were significantly higher in the >70 years group compared to the <50 years group ($P < 0.01$), with the <50 years group negative and positivity observed in the 50–70 years and >70 years group (Supplementary Fig.1).

3.4. Correlation analysis of antibody levels and cytokine concentrations in acute and recovery SFTS patients

The correlations among antibody levels, between antibodies and cytokine concentrations, and among cytokines provide valuable insights into the immunopathogenesis of SFTS. Fig. 4 shows the correlation analysis between antibody levels and cytokine concentrations, as well as inter-cytokine relationships, during both the acute (Fig. 4A) and recovery (Fig. 4B) phases of SFTS. In the acute phase, IL-10 displayed a robust positive correlation with IFN-γ ($r = 0.623$, $P < 0.0001$). Moderate correlations ($r = 0.452–0.551$, $P < 0.0001$) were observed between IL-10 and IP-10, TNF-α, IFN-α, and IL-6 individually; IFN-γ and IP-10, TNF-α, IFN-α, and IL-6 individually; as well as IL-6 and

TNF-α and IL-8 individually. Weak correlations (r = 0.180–0.360, $P < 0.05$ to $P < 0.0001$) were observed between IL-10 and IL-8; IFN-γ and IL-8; IL-6 with IP-10 and IFN-α; and IFN-α with TNF-α as well as IL-8. Antibody levels showed limited correlations with cytokines, although IgM and IgG exhibited a moderate positive correlation (r = 0.536, $P < 0.0001$). In the recovery phase, IgG displayed significant negative correlations with IL-10 (r = – 0.310, $P < 0.001$), IFN-γ (r = – 0.201, $P < 0.05$), and IP-10 (r = – 0.219, $P < 0.05$). In contrast, IgM exhibited significant positive correlations with multiple cytokines, including IL-10, IFN-γ, IL-6, IFN-α, TNF-α, IL-8, and IP-10 (r = 0.363–0.608, $P < 0.0001$). Notably, strong inter-cytokine correlations (r = 0.621–0.727, $P < 0.0001$) included IL-10 with IL-6, TNF-α, IP-10, and IFN-γ; IFN-γ with TNF-α; IFN-α with both TNF-α and IL-8; and IL-6 with TNF-α. Moderate correlations (r = 0.478–0.596, $P < 0.0001$) were also observed between IL-10 and IL-8, and between IFN-γ and each of IL-6, IL-8, and IP-10.

3.5. Comparison of cytokine levels in paired serum samples from SFTS patients

The comparison of cytokine levels in paired serum samples from SFTS patients is crucial for understanding individual immune dynamics. Fig. 5 shows the analysis of 17 cytokines in paired serum samples from 58 SFTS patients across the acute and convalescent phases. Cytokine dynamics were categorized into three distinct patterns: (1) increased concentrations (IC), characterized by a transition from negative to positive or sustained positivity with elevated levels; (2) decreased concentrations (DC), defined as a transition from positive to negative or sustained positivity with reduced levels; and (3) remaining negative concentrations (RNC), where cytokines were negative in both phases. Except for MIP-1β, all cytokines exhibited significantly lower levels during the convalescent phase than the acute phase ($P < 0.05$ to $P < 0.0001$). Notably, IL-10, IL-6, IL-8, and IP-10 showed the highest proportions of patients with reductions, observed in 74.14 % (43/58), 77.59 % (45/58), 77.59 % (45/58), and 75.86 % (44/58) of patients, respectively, who either transitioned from positive to negative or exhibited sustained positivity with reduced levels during the convalescent phase. In contrast, IFN-γ, IFN-α, and TNF-α showed reductions in 48.28 % (28/58), 39.66 % (23/58), and 36.21 % (21/58) of patients, respectively, while 44.83 % (26/58), 55.17 % (32/58), and 55.17 % (32/58) of patients, respectively, maintained negative levels across both phases. For IL-2, MCP-1, MIP-1α, MIP-1β, and G-CSF, reductions were observed in 32.76 % (19/58), 29.31 % (17/58), 27.59 % (16/58), 31.04 % (18/58), and 20.69 % (12/58) of patients, respectively, while negative levels across both phases were noted in 62.07 % (36/58), 63.79 % (37/58), 63.79 % (37/58), 58.62 % (34/58), and 75.86 % (44/58), respectively. Cytokines such as IL-12P70, IL-5, IL-4, IL-17A, and IL-1β predominantly remained undetectable, with 98.28 % (57/58), 100 % (58/58), 100 % (58/58), 98.28 % (57/58), and 87.93 % (51/58) of patients, respectively, maintaining negative levels during both phases.

4. Discussion

This study provides a comprehensive and methodologically rigorous analysis of cytokine dynamics in Severe Fever with Thrombocytopenia Syndrome (SFTS), addressing pivotal gaps in the current understanding of its immunopathogenesis. In contrast to prior research limited by static

or cross-sectional analyses, the present work adopts a longitudinal framework to delineate cytokine trajectories across distinct disease phases systematically. Drawing on a decade-long, multicenter dataset collected from high-incidence provinces in China (Shandong, Anhui, Hubei, and Jiangsu) between 2010 and 2023, the analysis identifies IL-10, IL-6, IFN-α, IL-8, IP-10, IFN-γ, and TNF-α as key regulators of the host immune response. Meanwhile, IL-12P70, IL-5, IL-2, IL-4, IL-17A, IL-1β, MCP-1, MIP-1α, MIP-1β, and G-CSF demonstrate more limited activity, revealing a functional hierarchy that reflects their differential contributions to immune regulation and disease pathogenesis. Furthermore, critical associations between cytokine activity and factors such as disease severity, sex, age, and antibody responses underscore the complex immunological interactions shaping SFTS progression and recovery.

The acute phase of SFTS is characterized by a hyperinflammatory state with elevated levels of IL-6, IFN-α, IL-8, and IP-10, which rapidly increase in positivity rates and concentrations, alongside the upregulation of IL-10, with IFN-α showing relatively moderate positivity compared to the others. IL-10, an anti-inflammatory cytokine, mitigates excessive immune activation to limit tissue damage but simultaneously suppresses antiviral immunity, promoting viral persistence and poor outcomes, as observed in dengue and Ebola virus infections [25-27]. IL-6, a pleiotropic cytokine, amplifies inflammatory responses, with its excessive production often linked to cytokine-release syndromes in severe infections like COVID-19 [28, 29]. IFN-α, a critical mediator of antiviral immunity, demonstrates moderate positivity, potentially reflecting both the natural attenuation of interferon production as viral load declines and SFTSV-mediated immune evasion, whereby non-structural proteins (NSs) disrupt type I interferon signaling by targeting TANK-binding kinase 1 (TBK1) and interferon regulatory factor 3 (IRF3), impairing downstream gene activation and facilitating viral replication [30, 31]. Elevated IL-8 and IP-10 further perpetuate hypercytokinemia by recruiting neutrophils and lymphocytes, exacerbating systemic inflammation and tissue damage [32, 33]. These cytokines form a tightly regulated network balancing antiviral defenses and immune-mediated pathology [25, 29, 34]. As the disease transitions into the recovery phase, cytokine concentrations and positivity rates, including IL-10, IL-6, IL-8, IP-10, and IFN-α, show a notable decline, as evidenced by paired serum analyses, indicating a shift from hyperinflammation to immune resolution and the restoration of homeostasis [26, 29, 35]. Nevertheless, the relatively prolonged positivity rates and elevated concentrations of IL-8 and IP-10 in certain patients during the recovery phase suggest their extended roles in immune activation, with IL-8 potentially continuing to drive neutrophil-mediated inflammation and IP-10 sustaining lymphocyte recruitment during this period, before both cytokines ultimately decline to negative levels, reflecting the resolution of inflammation and progression toward recovery [30, 36]. These findings highlight the heterogeneity in recovery pathways among patients and suggest that persistent elevation of these chemokines may represent potential therapeutic targets to accelerate immune resolution and promote recovery in SFTS.

Comparative stratification of cytokine levels between mild and severe SFTS cases highlights significant disparities in hypercytokinemia, revealing distinct immune profiles that probably drive disease severity, with IL-10, IL-6, IL-8, and IP-10 elevated and exceeding positivity thresholds in both groups, suggesting their fundamental roles in the pathophysiology of SFTS across disease severities [37]. However, their markedly higher levels in severe cases reflect a more pronounced dysregulated inflammatory response, with IL-6 as a central driver of hypercytokinemia and IL-10

playing a dual role. While it limits tissue damage through its anti-inflammatory effects, its overproduction may suppress antiviral immunity, contributing to viral persistence and indirectly intensifying systemic inflammation. Meanwhile, IL-6 amplifies inflammatory responses, consistent with its role in cytokine release syndromes observed in other severe infections [28, 29, 38]. Notably, both IL-8 and IP-10 remain positive in mild and severe cases. However, their significantly higher concentrations in severe cases are presumed to exacerbate immune-mediated tissue damage, with IL-8 driving neutrophil recruitment and systemic inflammation and IP-10 sustaining lymphocyte migration, thereby amplifying disease severity and hindering recovery. These elevated levels in severe cases suggest that the combined effects of these cytokines exacerbate inflammatory responses, potentially leading to more significant tissue injury and worse clinical outcomes [30, 31, 39].

In contrast, IFN-α and TNF-α exhibited distinct positivity patterns, being exclusively positive in severe cases and remaining negative in mild cases, with their elevation in severe cases underscoring their critical roles in disease progression. The pronounced elevation of IFN-α in severe cases reflects an intensified antiviral response essential for inhibiting viral replication and activating immune cells. However, its excessive production may lead to immune dysregulation and tissue damage, with the immune system's efforts to control viral load potentially causing pathological consequences. Similarly, the upregulation of TNF-α in severe cases amplifies systemic inflammation and disease progression by increasing vascular permeability, promoting immune cell recruitment to infection sites, and driving the inflammatory cascade. TNF-α is well known for its pro-inflammatory effects, inducing tissue leakage and potentially leading to platelet damage, either through reduced production from the bone marrow or through direct degradation of platelets. Studies have shown that TNF-α induces both apoptosis and tissue necrosis, contributing to its toxic effects. Furthermore, TNF-α levels have been shown to directly correlate with the degree of inflammation and with the presence and activation of certain types of cells involved in inflammation, thereby elevating the risk of severe complications such as disseminated intravascular coagulation (DIC) [19,40-43]. These findings highlight the critical role of cytokine dysregulation in the pathogenesis of SFTS, underscoring the potential utility of elevated cytokines as reliable biomarkers for disease stratification and as actionable therapeutic targets to mitigate hyperinflammation and improve clinical outcomes.

The analysis of sex-based and age-based differences further elucidates the complex immunological responses underpinning SFTS pathogenesis. In contrast to its absence in males, the observed IFN-γ positivity and elevated levels in females suggest a potential sex-related difference in immune responses, reflecting a more pronounced Th1 response in females critical for antiviral immunity. Similarly, the slight elevation of IL-8 in females, exceeding positivity thresholds in both sexes, aligns with observations in inflammatory and infectious diseases such as ulcerative colitis and COVID-19, where females exhibit a more robust immune response. These differences may be attributed to genetic predispositions and the effects of estrogen, which enhance B cell activation, antibody production, and cytokine regulation while also increasing susceptibility to immune dysregulation and autoimmune conditions [37, 44-48]. The age-related differences in IL-10, IFN-γ, and IFN-α levels reveal distinct immune dynamics, reflecting the interplay between aging and immune function. IL-10 was consistently positive across all age groups, with significantly higher levels observed in patients over 70 years, suggesting a compensatory mechanism to mitigate chronic

inflammation associated with aging. Meanwhile, IFN-γ and IFN-α, which were negative in the <50 years group but became positive in the 50–70 years and >70 years groups, with IFN-γ reaching its highest levels in the 50–70 years group and IFN-α relatively higher in the >70 years group, reflect an age-related shift toward adaptive immune compensation that may become less effective in advanced age due to immune system dysfunction or reduced capacity to respond to immunological challenges [28,49-51]. These findings elucidate the complex interplay of sex-based and age-related immune variations in SFTS, enhancing understanding of disease pathogenesis and providing a robust foundation for developing targeted therapeutic strategies tailored to vulnerable populations.

The correlations between antibody levels, cytokine concentrations, and inter-cytokine interactions during the acute and recovery phases of SFTS provide critical insights into the underlying immunological mechanisms. During the acute phase, all significant correlations observed among meaningful cytokines were positive, meaning that an increase in one cytokine was typically associated with an increase in others. For example, a robust positive correlation between IL-10 and IFN- γ may suggest a synergistic interplay, with IL-10 potentially modulating the Th1 response driven by elevated IFN- γ , balancing excessive inflammation suppression with antiviral immunity. Moderate positive correlations between IL-10 and IP-10, TNF-α, IFN-α, and IL-6 individually; IFN-γ and IP-10, TNF-α, IFN-α, and IL-6 individually; and IL-6 with TNF-α and IL-8 individually suggest the presence of coordinated feedback loops, where pro-inflammatory cytokines enhance downstream chemokines like IP-10, thereby sustaining immune cell recruitment and activation. Weaker positive correlations between IL-10 and IL-8; IFN-γ and IL-8; IL-6 with IP-10 and IFN-α; and IFN-α with TNF-α and IL-8 may indicate secondary or context-dependent interactions that contribute to the fine-tuning of the inflammatory milieu observed in severe SFTS cases. The moderate positive correlation between IgM and IgG during the acute phase indicates the initiation of humoral responses. However, these appear primarily independent of the immediate cytokine network, reflecting the delayed nature of antibody production in viral infections dominated by cellular immunity [19, 42, 52]. In the recovery phase, significant negative correlations between IgG levels and IL-10, IFN-γ, and IP-10 highlight a transition toward immune resolution, suggesting that increasing antibody levels may help suppress inflammation by downregulating pro-inflammatory cytokines and enhancing regulatory pathways [18, 53, 54]. Conversely, the positive correlations between IgM and IL-10, IFN-γ, IL-6, IFN-α, TNF-α, IL-8, and IP-10 indicate ongoing immune interactions likely to resolve inflammation and restore immune balance. Strong positive inter-cytokine correlations, including IL-10 with IL-6, TNF-α, IP-10, and IFN-γ; IFN-γ with TNF-α; IFN-α with both TNF-α and IL-8; and IL-6 with TNF-α, underscore the intricate connections within the immune network during SFTS. These correlations suggest a regulatory system that balances inflammation and tissue repair, which is essential for recovery. Additionally, moderate positive correlations between IL-10 and IL-8, IFN-γ with IL-6, IL-8, and IP-10 collectively highlight the dynamic nature of cytokine signaling, reflecting adaptive mechanisms employed by the immune system to facilitate resolution and recovery in viral infections [42, 55-57]. These findings underscore the pivotal role of cytokine interactions in balancing pro-inflammatory and regulatory responses, shaping SFTS progression and recovery, while further research is needed to determine if these correlations reflect direct regulatory mechanisms or coincidental recovery patterns.

In conclusion, this decade-long multicenter study provides valuable insights into the

immunopathogenesis of SFTS by identifying critical immune markers and delineating their dynamic roles in disease progression. Despite the inherent limitations of an observational design and the potential for sampling biases, the extensive dataset and rigorous methodology employed in the study enhance the reliability and relevance of the findings. These findings provide a robust foundation for future research on cytokine-targeted therapies and incorporating predictive biomarkers into clinical practice to improve patient outcomes through precision medicine.

Ethics statement

This study received approval from the National Institute for Viral Disease Control and Prevention ethics committee, China CDC (IVDC2022-011). The study adhered to the ethical standards of the institutional and national research committee and the 1964 Helsinki Declaration and its later amendments. All participants provided informed consent.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

Author contributions

Yanhan Wen: Writing - Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing - original draft, Writing - review & editing. **Yeqing Tong:** Investigation, Methodology, Validation. **Lei Gong:** Data curation, Formal analysis, Resources, Validation. **Aqian Li:** Formal analysis, Writing - original draft, Validation. **Xiaoxia Huang:** Methodology, Investigation, Validation. **Tingting Tian:** Data curation, Formal analysis, Resources, Validation. **Tiezhu Liu:** Formal analysis, Funding acquisition, Data curation, Investigation. **Lina Sun:** Formal analysis, Writing - original draft, Validation. **Jiandong Li:** Supervision, Writing - review & editing. **Dexin Li:** Conceptualization, Writing - review & editing. **Mifang Liang:** Conceptualization, Writing - review & editing. **Wei Wu:** Conceptualization, Supervision, Project administration, Writing - review & editing. **Jiabing Wu:** Conceptualization, Supervision, Project administration, Writing - review & editing. **Shiwen Wang:** Conceptualization, Supervision, Project administration,

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Supplementary data

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Fig. 1. Temporal dynamics of cytokine positivity rates in SFTS patients

This heatmap illustrates the temporal dynamics of positivity rates (%) for 17 cytokines in Severe Fever with Thrombocytopenia Syndrome (SFTS) patients across five time intervals post-onset: 1–7 days, 8–14 days, 15–21 days, 22–180 days, and >180 days. The y-axis lists cytokines, while the x-axis represents time intervals from symptom onset. Each cell contains the positivity rate (%) and the corresponding fraction (number of positive samples/total samples). The color gradient reflects positivity rates, with darker shades indicating higher positivity.

Fig. 2. Temporal dynamics of cytokine levels in SFTS patients

This figure presents the temporal dynamics of cytokine levels in Severe Fever with Thrombocytopenia Syndrome (SFTS) patients across five post-onset intervals: 1–7, 8–14, 15–21, 22–180, and >180 days. Each subfigure depicts the concentration of a specific cytokine (pg/mL) with the cutoff value for positivity indicated by a dashed line. Scatter plots represent individual serum samples, with median concentrations and interquartile ranges (IQRs) displayed. Red points denote cytokine levels above the positivity threshold ("positive"), while blue points indicate levels below the threshold ("negative").

Fig. 3. Comparison of cytokine levels by disease severity in acute-phase SFTS patients

This figure compares 17 cytokine concentrations (pg/mL) among SFTS patients with mild and severe disease, and healthy controls during the acute phase. Scatter plots represent individual serum samples, with median concentrations and interquartile ranges (IQRs) displayed. Dashed lines indicate cytokine positivity thresholds. Samples with cytokine levels exceeding the positivity threshold are marked as red points, while those below the threshold are marked as blue points. Statistical significance is marked as $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$; ns denotes non-significance.

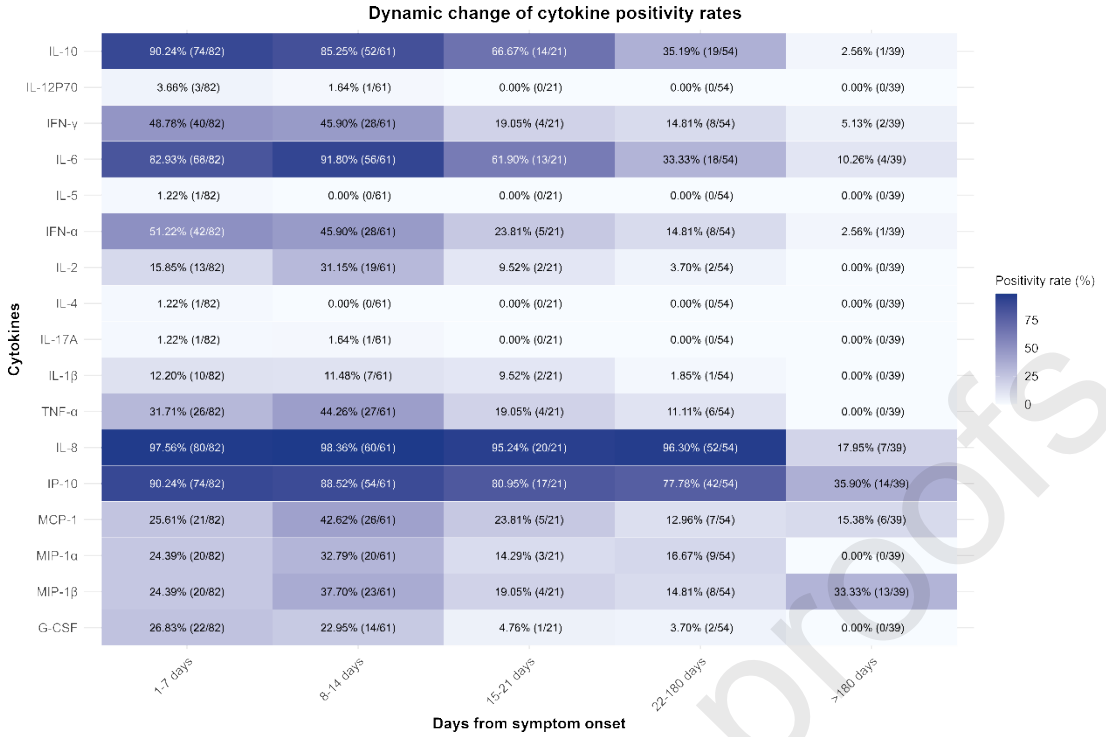
Fig. 4. Correlation matrix of antibody levels, cytokine concentrations, and inter-cytokine relationships in acute and recovery phases of SFTS patients

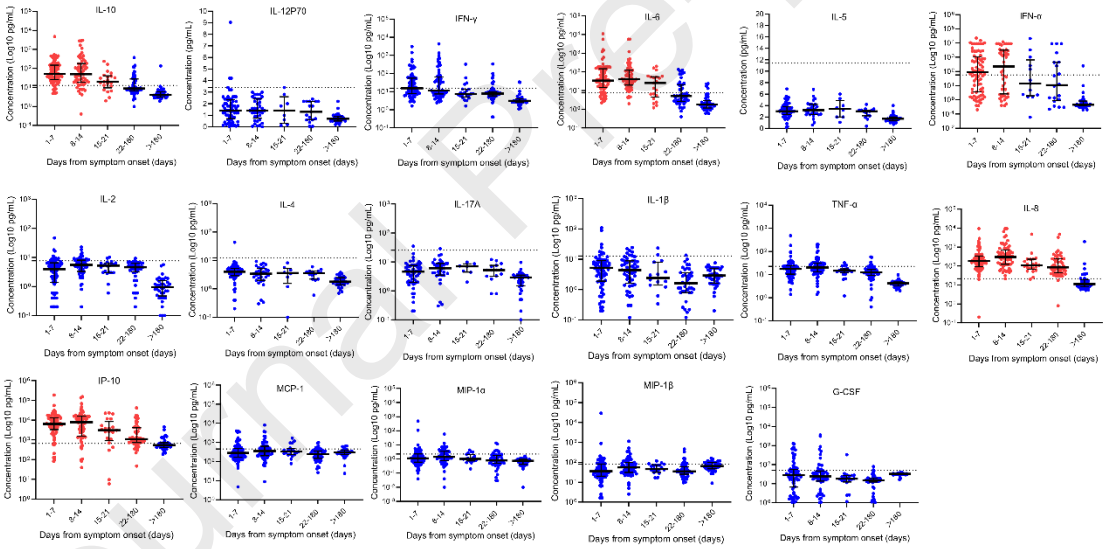
This figure presents the correlation analysis results for IgG, IgM, and the levels of 17 cytokines, illustrating their inter-relationships in SFTS patients during the acute phase (A) and recovery phase (B). Spearman's method was used to evaluate monotonic associations, yielding correlation coefficients (r) ranging from -1 to 1. Correlation strength is categorized as strong ($|r| \geq 0.6$), moderate ($0.4 \leq |r| < 0.6$), and weak ($|r| < 0.4$). A heatmap visually represents correlation strength using a color gradient: red indicates positive correlations, while blue indicates negative correlations. Each cell contains the correlation coefficient (r) and the corresponding significance level ($*P < 0.05$,

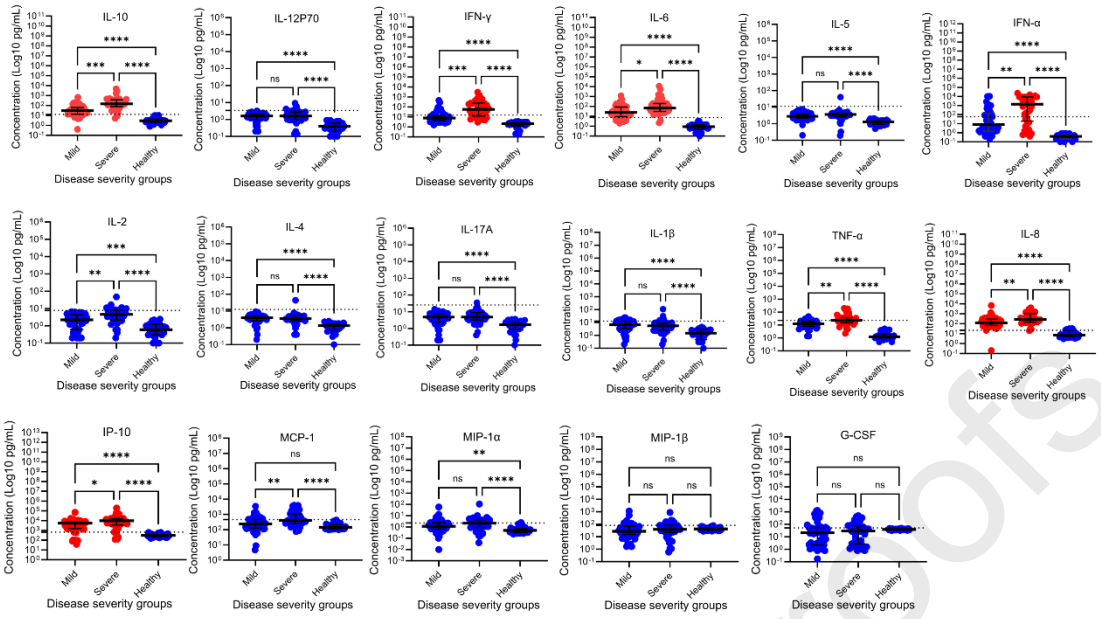
$^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$). Non-significant correlations are not labeled.

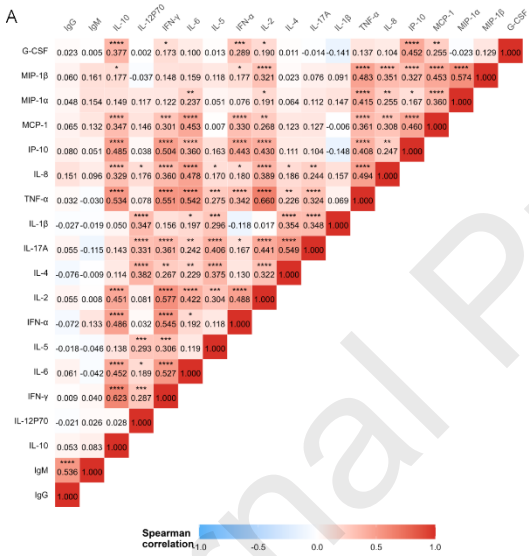
Fig. 5. Paired comparison of cytokine levels in acute and convalescent sera from SFTS patients

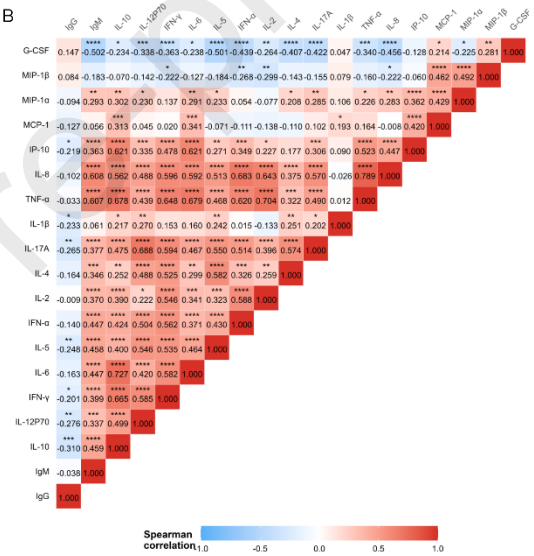
This figure illustrates the changes in 17 cytokines in paired serum samples from SFTS patients during the acute and convalescent phases. Cytokine concentrations (pg/mL) are shown on the vertical axis, with acute and convalescent phases on the horizontal axis. Dashed lines indicate positivity thresholds, and connecting lines illustrate paired changes for individual patients. Pie charts depict the proportions of increased concentration (IC, red), remain negative concentration (RNC, gray), and decreased concentration (DC, blue) between the two phases. Statistical significance is marked as $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$; "ns" indicates no significant change.

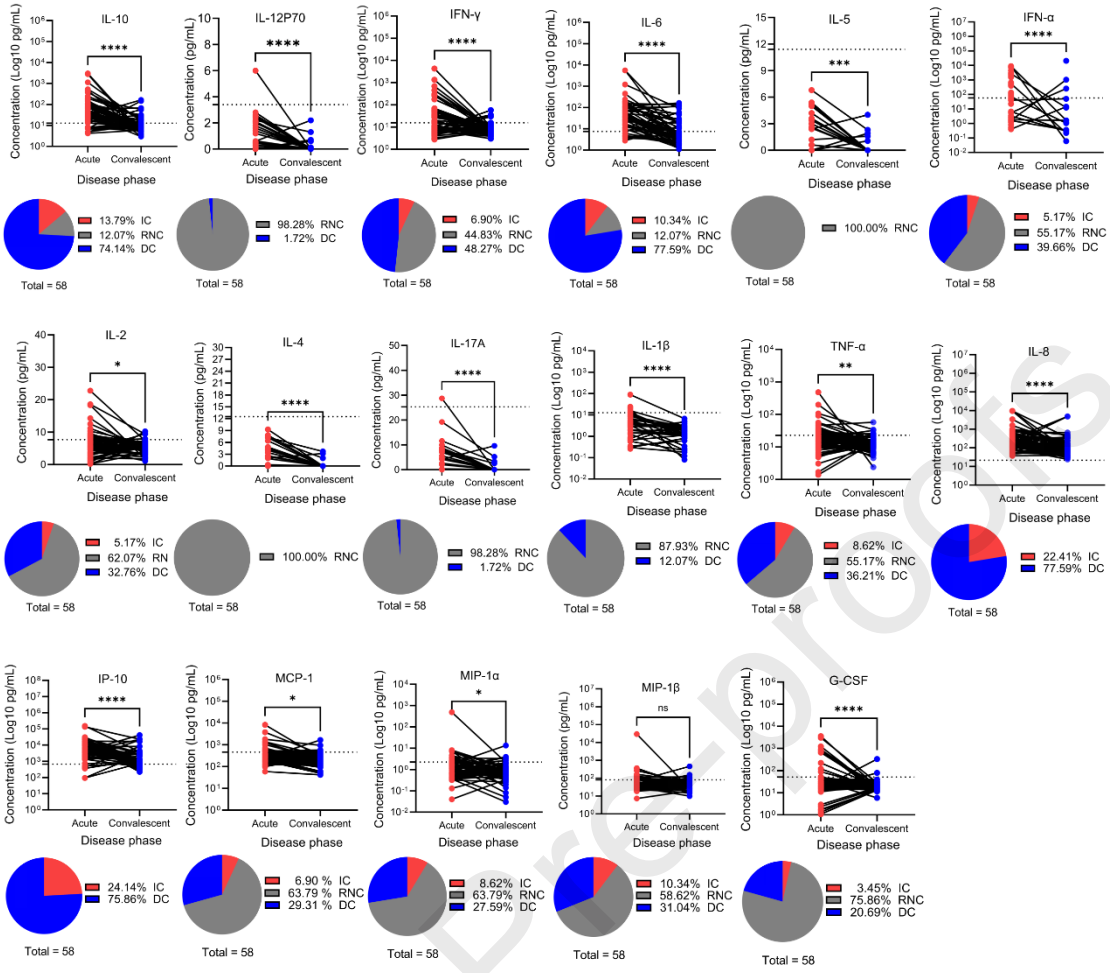












Highlights

Scientific questions

This study investigates the dynamic changes of 17 cytokines across acute and recovery phases of severe fever with thrombocytopenia syndrome (SFTS) and examines how these patterns correlate with disease severity, sex, age, and antibody responses.

Evidence before this study

Previous studies highlighted cytokine storms in SFTS pathogenesis but were constrained by small samples and cross-sectional designs, lacking longitudinal, multi-center data on cytokine trajectories and their links to disease severity and patient demographics.

New findings

Our multi-center retrospective analysis (2010–2023) of 287 serum samples from high-incidence

regions of China reveals that IL-6, IFN-α, IL-8, and IP-10 drive hyperinflammation during the acute phase, while IL-10 moderates immune activation and may promote viral persistence, with prolonged elevation of IL-8 and IP-10 in some patients suggesting heterogeneous recovery pathways. Severe cases exhibit significantly higher IL-10, IFN-γ, IL-6, IFN-α, TNF-α, IL-8, and IP-10, highlighting their potential as severity biomarkers. At the same time, sex-based differences show elevated IFN-γ and IL-8 levels in females, and older patients demonstrate increased IL-10, IFN-γ, and IFN-α. Significant inter-cytokine correlations were observed during both the acute and recovery phases of SFTS, with robust IL-10–IFN-γ interactions and limited antibody-cytokine correlations in the acute phase. In contrast, in the recovery phase, IgM positively correlated with IL-10, IFN-γ, IL-6, IFN-α, TNF-α, IL-8, and IP-10, and IgG negatively correlated with IL-10, IFN-γ, and IP-10, highlighting dynamic shifts in immune regulation.

Significance of the study

Our decade-long multicenter study provides a comprehensive understanding of the immunopathogenesis of SFTS by systematically delineating the temporal dynamics of cytokine profiles throughout the disease. This work establishes a theoretically robust foundation for further investigations into cytokine-targeted therapeutic strategies and the incorporation of predictive biomarkers into clinical practice, ultimately aiming to refine diagnostic methodologies and enhance clinical management.