Preplanned Studies

Longitudinal Analysis of Antibody Dynamics in Severe Fever with Thrombocytopenia Syndrome Patients — High-Incidence Regions of China, 2010–2023

Yanhan Wen[1,&]; Dongmei Song[2,&]; Aqian Li[1,&]; Lina Sun[1]; Shuhua Ma[2]; Tiezhu Liu[1]; Xiaoxia Huang[1]; Tao Huang[1]; Boyang Li[1]; Mengxuan Wang[1]; Jiandong Li[1]; Mifang Liang[1]; Dexin Li[1]; Wei Wu[1,#]; Xinxian Dai[2,#]; Shiwen Wang[1,#]

Summary

What is already known about this topic?

Severe fever with thrombocytopenia syndrome (SFTS) is a serious tick-borne disease in East Asia with high mortality, particularly affecting the elderly. Since its discovery in 2010, inconsistencies in small-scale studies and the lack of decade-long research on antibody levels in large population samples after natural infection, along with the absence of an effective vaccine, highlight the need for large-scale, long-term data in high-incidence regions of China.

What is added by this report?

This study of 1,410 serum samples from SFTS patients in high-incidence regions of China reveals that immunoglobulin M (IgM) levels peak at 8–14 days post-infection, declining to nearly undetectable levels by 180 days. Immunoglobulin G (IgG) and neutralizing antibodies (NAb) levels peak at 22–180 days, persisting up to 10 years. IgM levels correlate with viral load and various immune and coagulation parameters, with lower levels observed in fatal cases. During convalescence, elderly patients have lower IgG levels, whereas females exhibit higher IgG levels compared with males.

What are the implications for public health practice?

The study's findings on long-term antibody dynamics in SFTS patients can significantly improve vaccine development, optimize therapy scheduling, inform public health policies, and enhance diagnostic tools, leading to better disease management and prevention in high-incidence areas.

Severe fever with thrombocytopenia syndrome (SFTS) is a serious tick-borne disease in East Asia with high mortality, particularly among the elderly. Since its discovery in 2010, research on antibody levels in large populations following natural infection has been limited, and no effective vaccine is available, highlighting the need for comprehensive long-term data in high-incidence regions of China (*1*–*3*). To address this public health challenge, we conducted a retrospective study on the dynamics of nucleoprotein (NP)-specific immunoglobulin M (IgM), immunoglobulin G (IgG), and neutralizing antibodies (NAb) using 1,410 serum samples from SFTS patients in high-incidence areas across Shandong, Anhui, Hubei, Liaoning, Henan, and Jiangsu provinces from 2010 to 2023. Our findings reveal that IgM levels peak at 8–14 days post-infection and decline to nearly undetectable levels by 180 days. In contrast, IgG and NAb levels peak at 22–180 days and persist for up to 10 years. Public health practitioners should prioritize long-term surveillance and vaccine research to combat SFTS effectively.

The study enrolled confirmed SFTS cases with laboratory evidence, including severe fever with thrombocytopenia syndrome virus (SFTSV) nucleic acid detection or a fourfold increase in IgG antibody titers during recovery (*2*). Participants aged 5 to 90 years were included, and general epidemiological data were collected, categorizing them by age (under 50, 50–70, over 70) and disease onset time (≤7 days, 8–14 days, 15–21 days, 22–180 days, 181–365 days, 1–2 years, 2–3 years, 3–4 years, 4–5 years, 5–6 years, 6–7 years, 7–8 years, 8–9 years, 9–10 years). Disease phases were classified as acute (within 2 weeks) and recovery (after 2 weeks). Serum samples were subjected to quantitative reverse transcription polymerase chain reaction (qRT-PCR) targeting the S segment of SFTSV for nucleic acid detection and viral load quantification, IgM-capture enzyme-linked immunosorbent assay (ELISA) for NP-specific IgM, indirect ELISA for NP-specific IgG, and microneutralization assays to assess NAb titers. Case questionnaires were used to collect data on clinical parameters, prognosis, clinical outcomes, and disease

severity. Statistical analyses were performed using GraphPad Prism (version 10.1.2; La Jolla, CA). The Mann-Whitney *U*-test was used for group differences, the Wilcoxon signed-rank test for paired serum samples, the Kruskal-Wallis test for categorical variables, and Spearman's rank correlation for correlation analysis. Significance levels were defined as follows: ns (not significant), $P<0.05$, $P<0.01$, $P<0.001$, $P<0.0001$. This study was approved by the ethics committee of the National Institute for Viral Disease Control and Prevention, China CDC (IVDC2022-011).

In high-incidence areas of China, 1,410 serum samples from 1,233 SFTSV-infected patients were collected, primarily between April and October, peaking in July with 541 samples (38.37%). Of these, 64.47% (909/1,410) were from the acute phase, and 35.53% (501/1,410) were from the convalescent phase. The samples comprised 53.45% (596/1,115) females and 46.55% (519/1,115) males. Regarding age distribution, 57.48% (638/1,110), 22.88% (254/1,110), and 19.64% (218/1,110) were collected from patients older than 70 years, 50–70 years, and younger than 50 years, respectively. Farmers constituted the largest occupational group. Samples were predominantly collected within 14 days post-onset, while convalescent-phase samples, especially those collected more than one year post-onset, were relatively few (Supplementary Figure S1A–D, available at https://weekly.chinacdc.cn/). IgM positivity was initially 51.22% (293/572) at 1–7 days post-onset, peaking at 64.89% (146/225) at 8–14 days. It then decreased slightly to 60.94% (39/64) at 15–21 days and declined to nearly 0% after six months. IgG positivity peaked at 76.83% (189/246) at 22–180 days and remained above 60% (6/10) after 1–10 years. NAb positivity peaked at 76.53% (163/213) at 22–180 days and remained above 50% (5/10) after 1–10 years. Initially 100% among the 108 PCR-positive serum samples out of 325 tested, PCR positivity declined significantly and became undetectable after 28 days (Figures 1A–1C). IgM levels peaked at 8–14 days, declined at 15–21 days, and remained negligible after 180 days. IgG and NAb levels both rose significantly at 8–21 days, peaked at 22–180 days, declined slightly at 181–365 days, and remained relatively high after 1–10 years (Figures 1D–1H).

IgM levels positively correlated with viral load on days 1–7 ($r=0.2279$, $P<0.05$) but not on days 8–14, while IgG levels showed no significant correlation with viral load. IgM levels positively correlated with IgG only on days 1–7 ($r=0.7178$, $P<0.05$). IgM and NAb levels were positively correlated only during 22–180 days ($r=0.4254$, $P<0.0001$), whereas IgG and NAb were not significantly correlated in the acute phase but showed a moderate positive correlation during the recovery phase ($r=0.4$–0.6) (Figure 2A–D). IgM levels were positively correlated with platelets (PLT) ($r=0.3551$, $P<0.01$), white blood cells (WBC) ($r=0.2606$, $P<0.05$), lymphocyte percentage (LY%) ($r=0.2791$, $P<0.05$), monocyte percentage (MONO%) ($r=0.3389$, $P<0.01$) and fibrinogen (FIB) ($r=0.4694$, $P<0.0001$), and negatively correlated with activated partial thromboplastin time (APTT) ($r=-0.4706$, $P<0.0001$), thrombin time (TT) ($r=-0.4531$, $P<0.001$), prothrombin time (PT) ($r=0.4417$, $P<0.001$), C-reactive protein (CRP) ($r=-0.2860$, $P<0.05$), aspartate aminotransferase (AST) ($r=-0.2187$, $P<0.05$), total protein (TP) ($r=-0.2921$, $P<0.05$), blood urea nitrogen (BUN) ($r=-0.4838$, $P<0.0001$), creatine kinase (CK) ($r=-0.2419$, $P<0.05$), lactate dehydrogenase (LDH) ($r=-0.2468$, $P<0.05$) and procalcitonin (PCT) ($r=-0.4807$, $P<0.0001$) (Supplementary Figure S2, available at https://weekly.chinacdc.cn/). Patients in the <50 age group had higher IgM levels during 8–14 days ($P<0.01$) and 15–21 days ($P<0.05$) compared to those in the 50–70 and >70 age groups. Similarly, patients in the >70 age group had lower IgG levels during 22–180 days ($P<0.05$) and 181–365 days ($P<0.01$) compared to those in the <50 and 50–70 age groups (Supplementary Figure S3A–B, available at https://weekly.chinacdc.cn/). Gender did not influence IgM and NAb levels, but females exhibited significantly higher IgG levels during the 22–180 day period ($P<0.05$). IgM levels were significantly lower in fatal cases than in survivors ($P<0.05$), with no significant differences between mild and severe cases or between non-fatal and severe survivors, while IgG levels showed no significant differences across all groups (Supplementary Figure S3C–G).

This research also analyzed IgM, IgG, and NAb dynamics in 64 SFTS patients with paired acute and recovery phase serum samples. IgM levels increased significantly in 69.23% (9/13) and 55.56% (5/9) of patients at 15–21 and 22–30 days, respectively ($P<0.05$). However, IgM levels decreased or stabilized in 50.00% (9/18) and 58.33% (7/12) of patients at 31–60 and 61–90 days, respectively (no significant difference), and decreased significantly in 66.67% (8/12) of patients after 90 days ($P<0.05$) (Figure 3A). IgG levels increased significantly in 84.62% (11/13) of

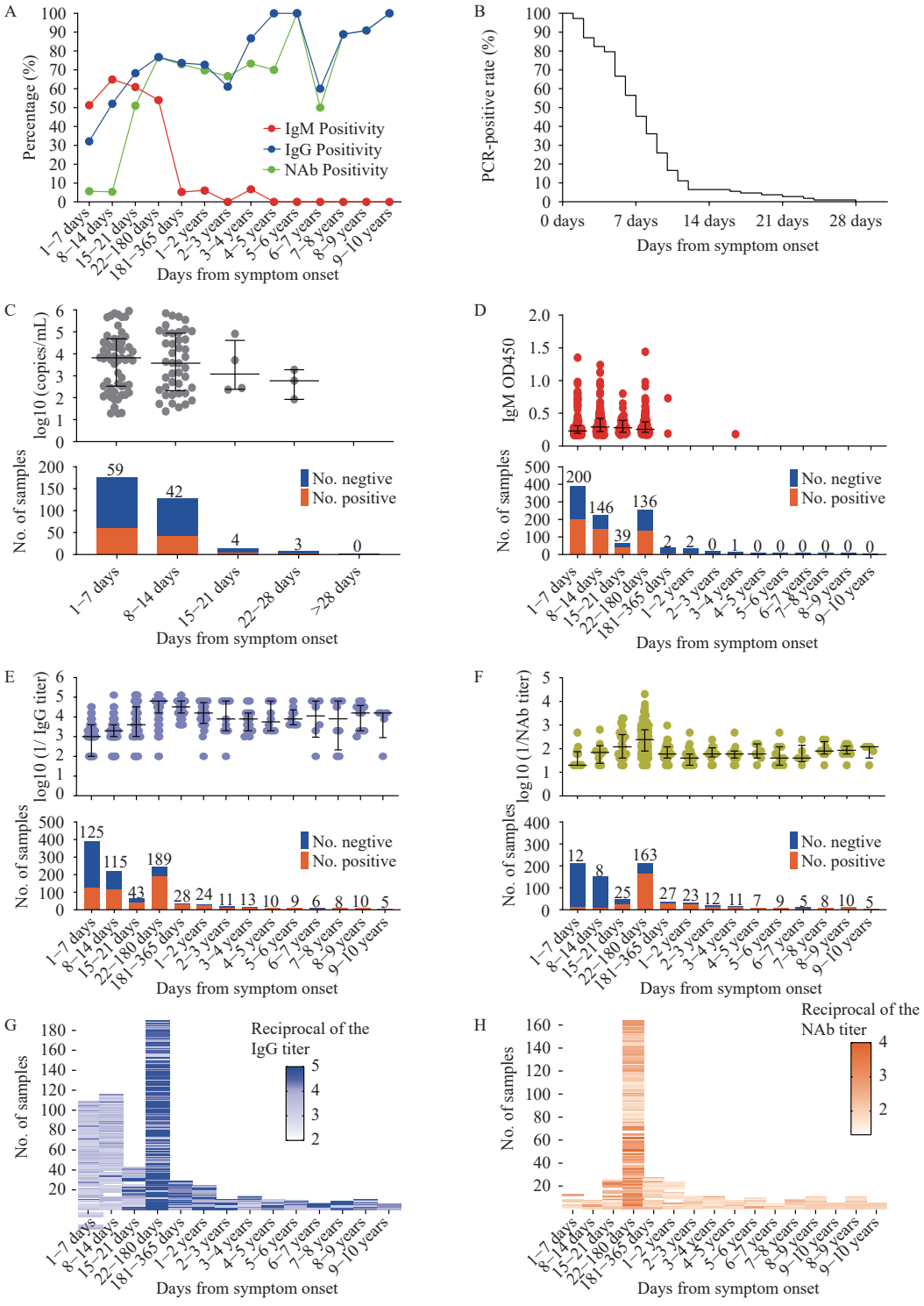

FIGURE 1. Temporal dynamics of antibody response and viral load post-symptom onset. (A) Percentage of patients testing positive for IgM, IgG, and NAb over time; (B) Decline in PCR positivity rates among the 108 patients who initially tested positive (out of 325 samples tested); (C) Changes in viral load over time post-symptom onset; (D) Temporal changes in IgM levels; (E) Temporal changes in IgG levels; (F) Temporal changes in NAb levels; (G) Temporal changes in IgG titers; (H) Temporal changes in NAb titers.

Note: For (C) to (F), the bar charts below each panel show the number of positive and negative samples for each interval, with positive sample counts noted above the bars. (G) and (H) heatmaps for IgG and NAb titers, respectively, displaying changes in titer concentrations over time post-symptom onset and offering a visual overview of antibody response dynamics.

Abbreviation: IgM=immunoglobulin M; IgG=immunoglobulin G; NAb=neutralizing antibody; PCR=polymerase chain reaction.

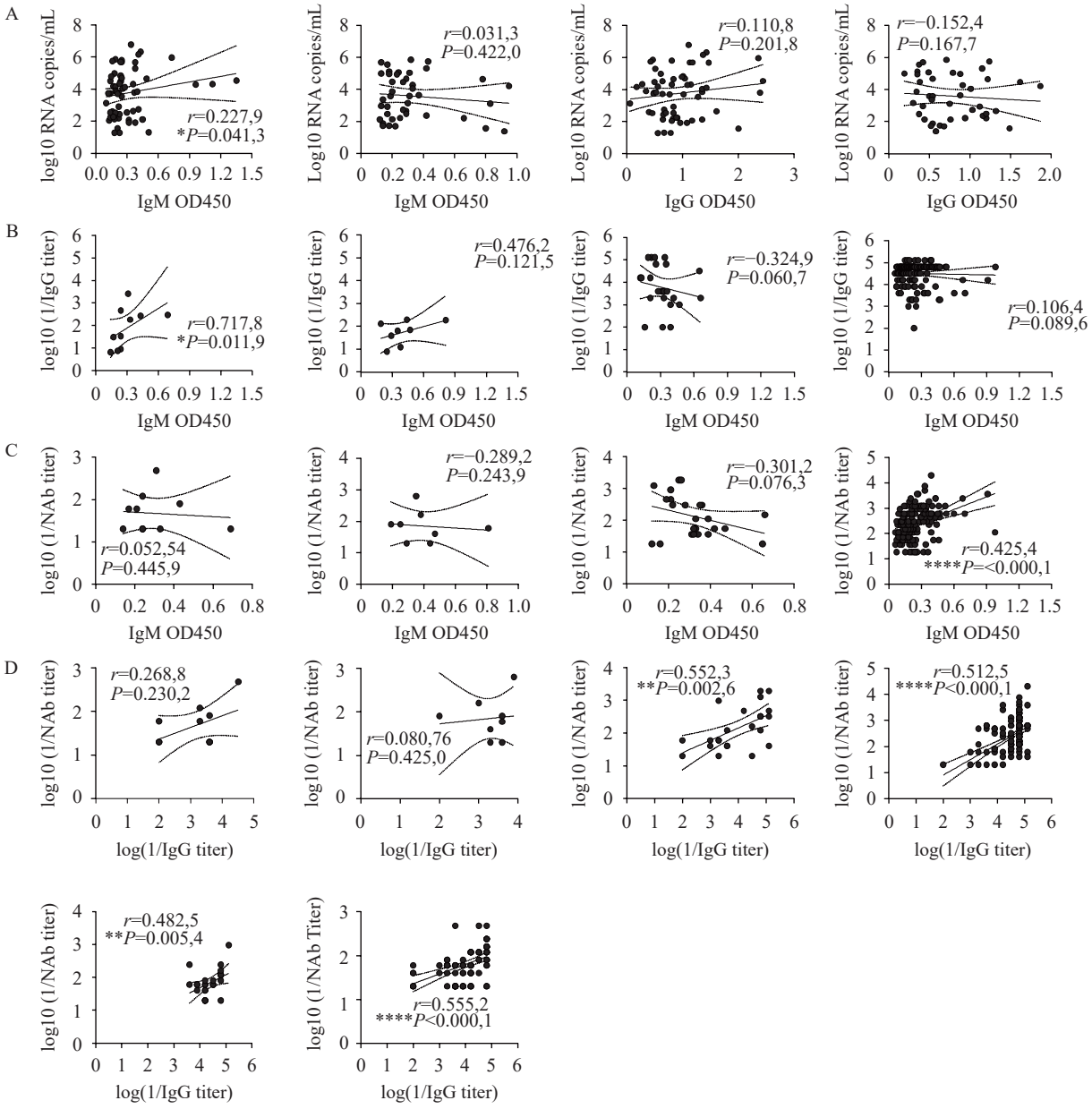

FIGURE 2. Correlations between IgM, IgG levels, and viral load, and among IgM, IgG, and NAb levels in SFTS patients. (A) Correlation of IgM and IgG levels with viral load during 1–7 and 8–14 days post-symptom onset; (B) Correlation between IgM and IgG levels at 1–7, 8–14, 15–21, and 22–180 days post-symptom onset; (C) Correlation of IgM with NAb levels at 1–7, 8–14, 15–21, and 22–180 days post-symptom onset; (D) Correlation of IgG with NAb levels at 1–7, 8–14, 15–21, 22–180, 181–365, and >365 days post-symptom onset.

Note: The correlation coefficient (r) denotes the strength of the correlation: very strong (0.8–1.0), strong (0.6–0.8), moderate (0.4–0.6), weak (0.2–0.4), or very weak/no correlation (0.0–0.2).

Abbreviation: IgM=immunoglobulin M; IgG=immunoglobulin G; NAb=neutralizing antibody; SFTS=severe fever with thrombocytopenia syndrome.

* P<0.05;

** P<0.01;

*** P<0.001;

**** P<0.0001.

patients at 15–21 days (P<0.001), 100% (9/9) at 22–30 days (P<0.01), 94.44% (17/18) at 31–60 days (P<0.0001), and 75.00% (9/12) at 61–90 days (P<0.01). Furthermore, 66.67% (8/12) of patients showed increases after 90 days, although this was not statistically significant. NAb levels increased

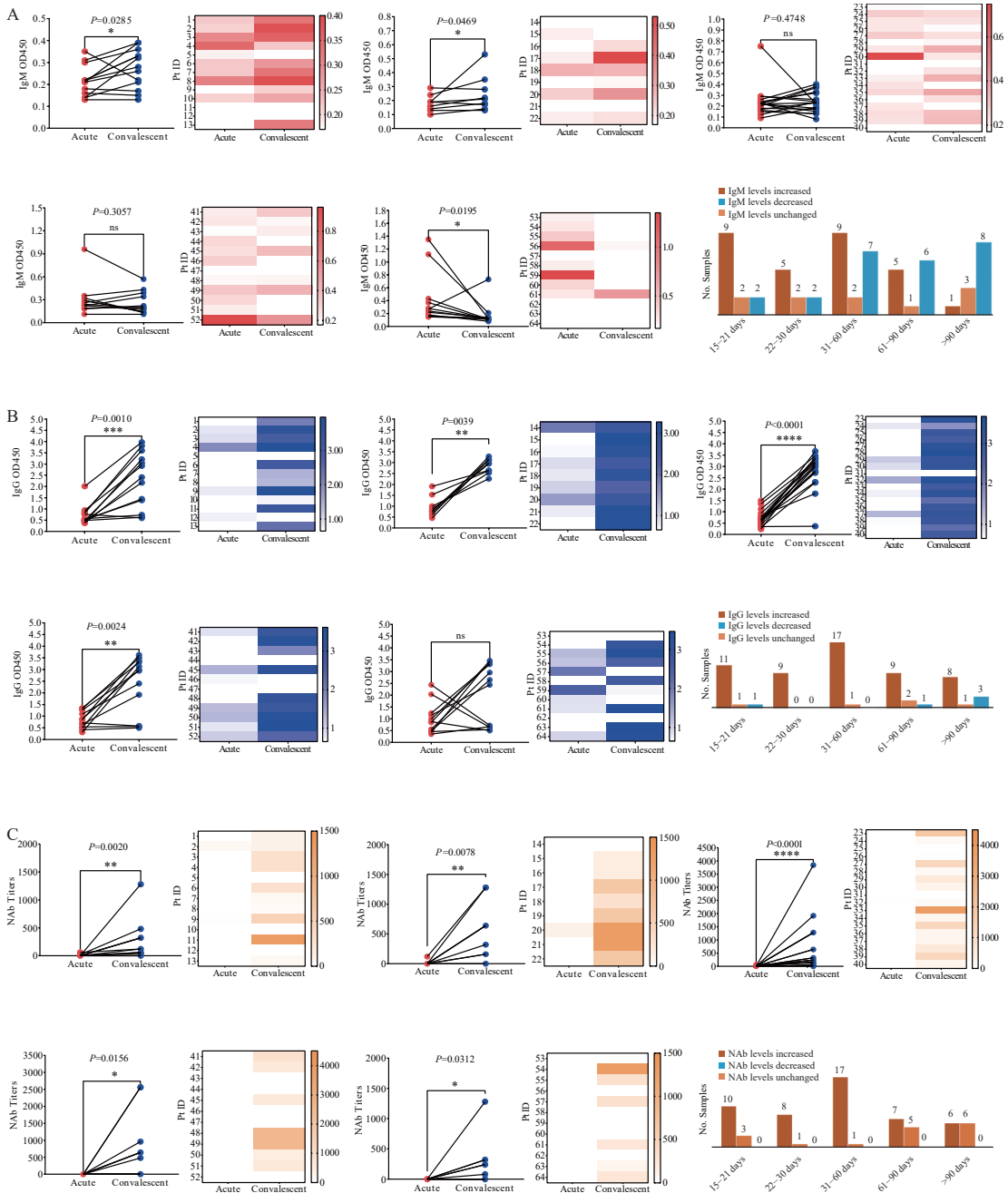

FIGURE 3. Comparative analysis of IgM, IgG, and NAb levels in paired serum sample of SFTS patients. (A), (B), and (C) show IgM, IgG, and NAb levels, respectively.

Note: This figure illustrates changes in IgM, IgG, and NAb levels in paired serum samples from 64 SFTSV-infected patients, comparing the acute phase with recovery stages at 15–21 days, 22–30 days, 31–60 days, 61–90 days, and beyond 90 days. The left sections depict individual patient changes, with red dots for the acute phase and blue dots for the recovery phase. The right sections present heatmaps displaying antibody level distributions at various stages, with darker shades indicating higher levels. Bar charts summarize antibody level changes: red bars for increases, orange bars for stable levels, and blue bars for decreases during recovery.

Abbreviation: IgM=immunoglobulin M; IgG=immunoglobulin G; NAb=neutralizing antibody; SFTS=severe fever with thrombocytopenia syndrome; SFTSV=severe fever with thrombocytopenia syndrome virus.

* $P<0.05$;

** $P<0.01$;

*** $P<0.001$;

**** $P<0.0001$.

significantly in 76.92% (10/13) of patients at 15–21 days (P<0.001), 88.89% (8/9) at 22–30 days (P<0.001), and 94.44% (17/18) at 31–60 days (P<0.0001). Moreover, 58.33% (7/12) of patients showed increases at 61–90 days (P<0.05), and 50.00% (6/12) after 90 days (P<0.05) (Figure 3B and 3C).

DISCUSSION

This study presents essential longitudinal insights into the dynamics of SFTS antibodies in high-incidence areas of East Asia, particularly China, spanning a 10-year period. The temporal profile of IgM highlighted its value as an early diagnostic marker, with a rapid rise post-infection and becoming nearly undetectable after 180 days, reflecting its role in the acute immune response. In contrast, both IgG and NAb remained detectable for up to 10 years post-infection. Importantly, NAb retained functional neutralizing activity throughout this period, underscoring their critical role in long-term immune defense against SFTS. The comprehensive analysis of paired serum samples further reinforces these findings, providing a detailed trajectory of antibody responses over time. This study addresses the limitations of prior research, which often involved smaller cohorts and primarily focused on seroconversion without fully capturing the extended dynamics of antibody persistence and activity (*3*–*9*).

Our analysis revealed that acute-phase IgM levels positively correlate with viral load and IgG levels, underscoring IgM's pivotal role in early diagnosis and immune response. In the recovery phase, IgG and NAb levels exhibited a moderately positive correlation, suggesting that the IgG immune response corresponds with NAb production, thereby contributing to long-term immunity. IgM levels are significantly associated with both immune response and coagulation dysfunction, underscoring their value in identifying high-risk SFTS patients. The observed correlations with various clinical parameters reflect IgM's role in pathophysiological processes, such as prolonged coagulation and potential liver impairment, reinforcing its utility in guiding diagnosis and therapeutic interventions. Additionally, elderly patients exhibited lower IgG levels during convalescence, likely attributable to age-related immune decline. In contrast, females showed higher IgG levels during days 22–180 compared to males, consistent with reports of higher antibody production in females (*10*). This highlighted age-related immune compromise and a more robust female immune response. Furthermore, lower IgM levels observed in fatal cases suggest a link with increased mortality, though whether this represents a causal relationship or a consequence of severe disease remains unclear.

However, limitations include insufficient long-term samples and uneven group sizes, highlighting the need for future research with diverse populations and standardized protocols to validate these findings and improve SFTS management and prevention.

Despite these limitations, this study examined a large cohort of naturally infected individuals over a 10-year period, offering key insights into SFTS immune response dynamics. The extensive sample analysis enhances our understanding of SFTS pathogenesis and provides a basis for refining diagnostic and therapeutic strategies.

Conflicts of interest: No conflicts of interest.

Acknowledgements: The authors gratefully acknowledge the invaluable support of the local Centers for Disease Control and Prevention in high-incidence regions, as well as the technical teams at the National Key Laboratory of Intelligent Tracking and Forecasting for Infectious Diseases (NITFID), the NHC Key Laboratory of Medical Virology and Viral Diseases, and the National Vaccine and Serum Institute, for their contributions to sample collection, data management, and laboratory analyses essential to this study.

Funding: Supported by the National Key R&D Program of China (ZDYF-2022YFC2303402), the Young Scientists Fund of China CDC (CCDC-2022A104), and the National Major Science and Technology Project of China (2018ZX10711001).

doi: 10.46234/ccdcw2024.244

[#] Corresponding authors: Wei Wu, wuwei@ivdc.chinacdc.cn; Xinxian Dai, daixinxian@sinopharm.com; Shiwen Wang, wangsw@ivdc.chinacdc.cn.

[1] National Key Laboratory of Intelligent Tracking and Forecasting for Infectious Diseases, NHC Key Laboratory of Medical Virology and Viral Diseases, National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, China; [2] National Vaccine and Serum Institute, Beijing, China.

[&] Joint first authors.

Submitted: July 29, 2024; Accepted: November 10, 2024

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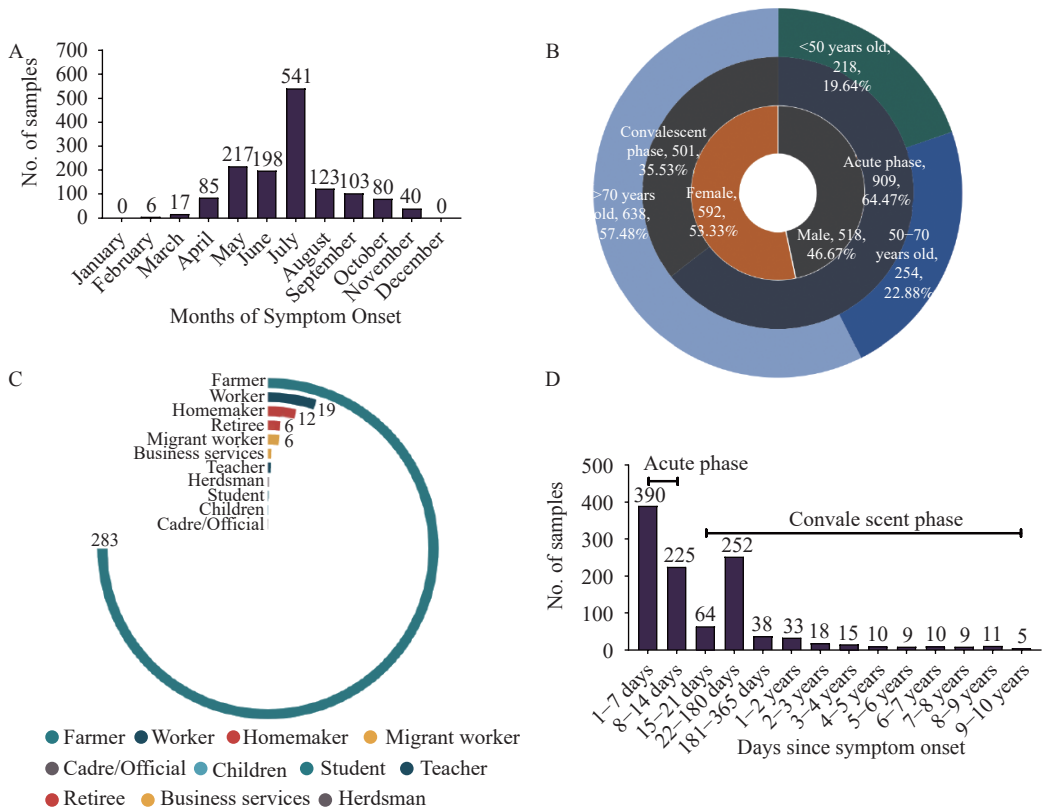
SUPPLEMENTARY MATERIAL

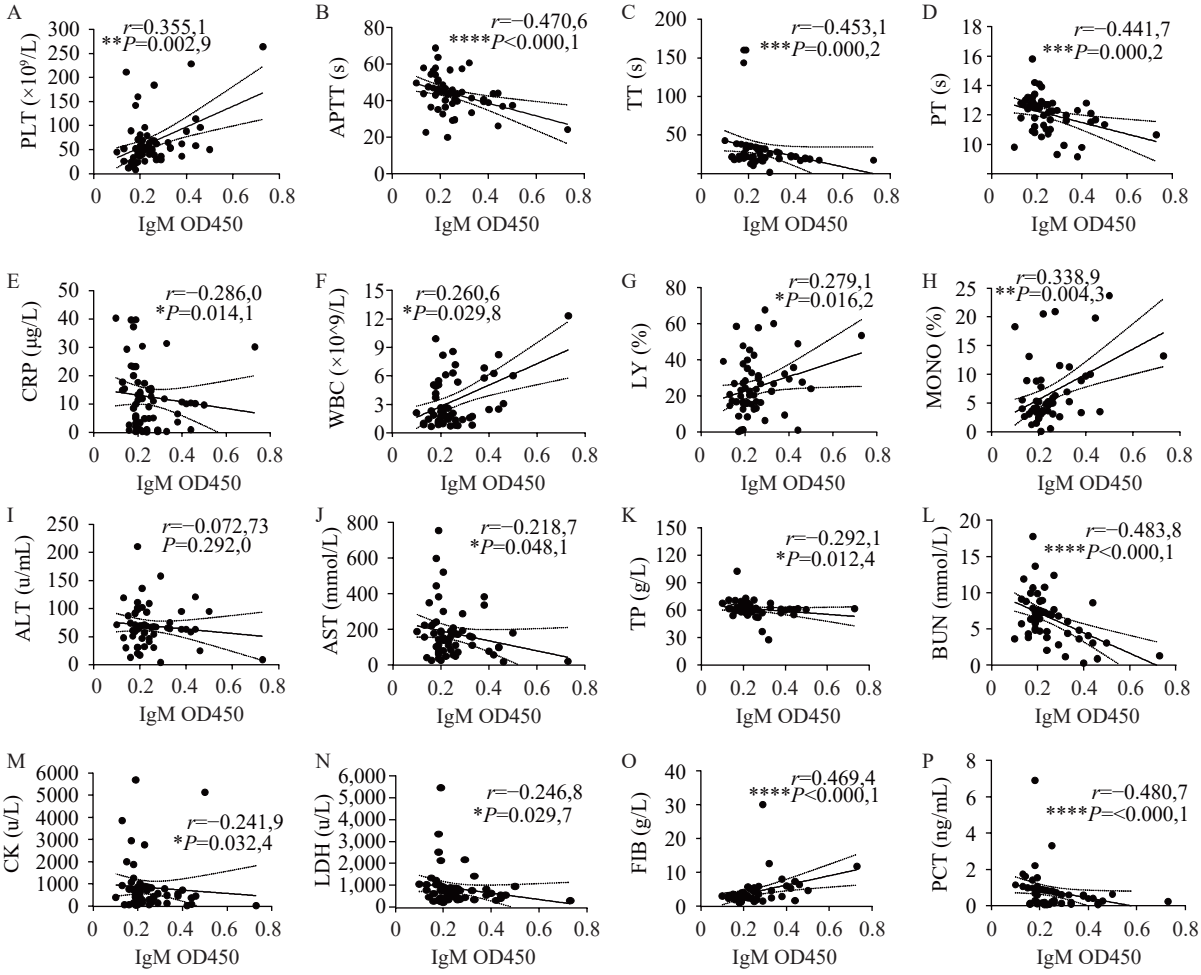
Methods Study Design and Participants

The study enrolled confirmed severe fever with thrombocytopenia syndrome (SFTS) cases with laboratory evidence, comprising either severe fever with thrombocytopenia syndrome virus (SFTSV) nucleic acid detection or a fourfold increase in immunoglobulin G (IgG) antibody titers during recovery. Participants aged 5 to 90 years were recruited from high-incidence regions, defined by national surveillance data as areas with consistently elevated SFTS case numbers and significant spatial clustering. These regions were predominantly located in Shandong, Anhui, Hubei, Liaoning, Henan, and Jiangsu provinces. We collected 1,410 serum samples from SFTS patients in these areas from 2010 to 2023. Participants were categorized by age (under 50, 50–70, over 70) and disease onset time (≤7 days, 8–14 days, 15–21 days, 22–180 days, 181–365 days, 1–2 years, 2–3 years, 3–4 years, 4–5 years, 5–6 years, 6-7 years, 7–8 years, 8–9 years, 9–10 years), and classified into acute (within 2 weeks) and recovery (after 2 weeks) phases. Serum samples from healthy individuals without SFTS infection history or tick exposure were included as negative controls for both immunoglobulin M (IgM) and IgG enzyme-linked immunosorbent assays (ELISA). Due to data availability constraints, not all patients had complete data for specific variables, resulting in varying sample sizes across analyses. For instance, 1,115 patients had complete gender data, and 1,110 patients had complete age data, accounting for the differences in sample sizes for these variables.

Serum samples underwent analysis for SFTSV nucleic acid and viral load quantification using (qRT-PCR quantitative reverse transcription polymerase chain reaction), targeting the S segment of the SFTSV genome. ELISA assays measured nucleoprotein (NP)-specific IgM and IgG levels, while microneutralization assays assessed neutralizing antibody (NAb) titers. Clinical data on prognosis, outcomes, and disease severity were collected through case questionnaires. The study examined correlations between antibody levels and viral load, as well as various hematologic and biochemical markers: platelet count (PLT), white blood cell count (WBC), lymphocyte percentage



SUPPLEMENTARY FIGURE S1. Demographic profile and serum sample distribution of SFTSV-infected patients. (A) The monthly distribution of symptom onset; (B) The distribution by infection phase, gender, and age; (C) The occupational distribution of patients; (D) The timing of sample collection relative to symptom onset.



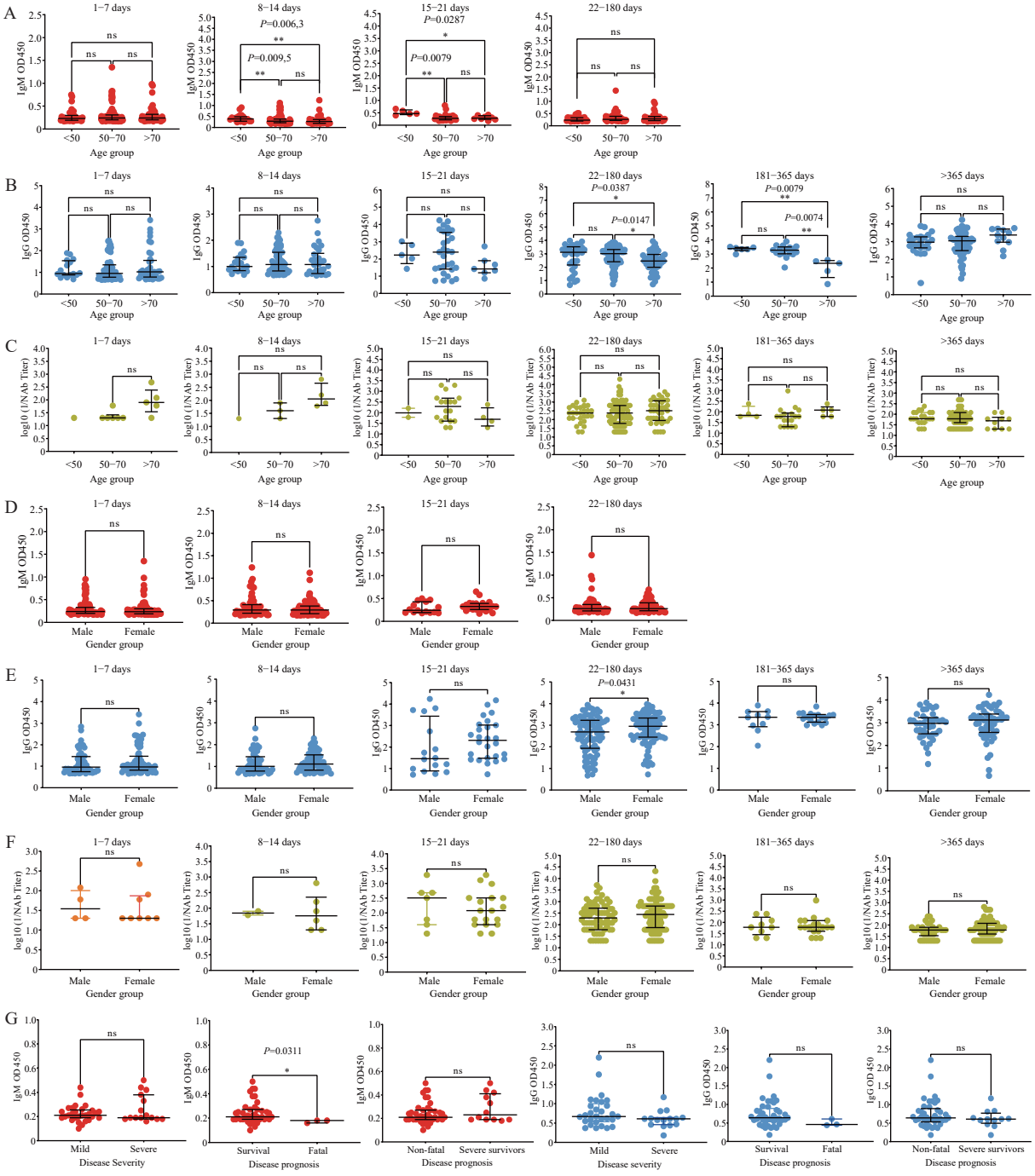
SUPPLEMENTARY FIGURE S2. Correlation between IgM levels and various biochemical and hematological indicators in SFTSV-infected patients, illustrating the correlation between IgM levels and various biochemical and hematological parameters in 59 SFTSV-infected patients. (A) PLT; (B) APTT; (C) TT; (D) PT; (E) CRP; (F) WBC; (G) LY%; (H) MONO%; (I) ALT; (J) AST; (K) TP; (L) BUN; (M) CK; (N) LDH; (O) FIB; (P) PCT.

Note: The correlation coefficient (r) indicates the strength of the correlation: very strong (0.8–1.0), strong (0.6–0.8), moderate (0.4–0.6), weak (0.2–0.4), or very weak/no correlation (0.0–0.2).

Abbreviation: IgM=immunoglobulin M; SFTSV=severe fever with thrombocytopenia syndrome virus; PLT=platelet count; APTT=activated partial thromboplastin time; TT=thrombin time; PT=prothrombin time; CRP=C-reactive protein; WBC=white blood cells; LY%=lymphocyte percentage; MONO%=monocyte percentage; ALT=alanine aminotransferase; AST=aspartate aminotransferase; TP=total protein; BUN=blood urea nitrogen; CK=creatine kinase; LDH=lactate dehydrogenase; FIB=fibrinogen; PCT=procalcitonin.

* $P<0.05$;
** $P<0.01$;
*** $P<0.001$;
**** $P<0.0001$.

(LY%), monocyte percentage (MONO%), fibrinogen (FIB), activated partial thromboplastin time (APTT), thrombin time (TT), prothrombin time (PT), C-reactive protein (CRP), aspartate aminotransferase (AST), total protein (TP), blood urea nitrogen (BUN), creatine kinase (CK), lactate dehydrogenase (LDH), and procalcitonin (PCT). Antibody levels were compared across genders, age groups, and disease severity and prognosis, including comparisons between mild and severe cases, survival and fatal outcomes, and non-fatal (encompassing both mild and severe survivors) versus severe survivors. Analysis of paired serum samples from acute and recovery phases enabled tracking of longitudinal antibody level changes within individuals, minimizing the impact of inter-individual variability.



SUPPLEMENTARY FIGURE S3. Comparative analysis of antibody levels in SFTSV-infected patients by age, gender, disease severity and clinical outcomes. (A–C) show IgM, IgG, and NAb levels across age groups (<50, 50–70, >70 years) at various disease phases (1–7, 8–14, 15–21, 22–180, 181–365, >365 days), with availability of data varying by phase and antibody type. (D–F) compare IgM, IgG, and NAb levels between male and female patients, with some time points missing due to data limitations. (G) presents a comparative analysis of acute-phase IgM and IgG levels among 44 patients with comprehensive severity and prognosis data, examining differences across mild *vs*. severe cases, survival *vs*. fatal outcomes, and a comparison between non-fatal (including both mild and severe survivors) *vs*. severe survivors.

Abbreviation: IgM=immunoglobulin M; IgG=immunoglobulin G; NAb=neutralizing antibody; SFTSV=severe fever with thrombocytopenia syndrome virus.

* $P<0.05$;
** $P<0.01$;
*** $P<0.001$;
**** $P<0.0001$.

Sample Collection and Processing

Serum samples were collected through local Centers for Disease Control and Prevention (CDC) or partner hospitals following standardized procedures. After obtaining informed consent and verifying patient identification, 10 mL of venous blood was drawn into ethylenediaminetetraacetic acid (EDTA) anticoagulant tubes under sterile conditions. Following site disinfection, tubes were gently inverted to prevent coagulation. Blood samples were centrifuged at 3,000 rpm for 10 minutes at 4 °C to separate serum, which was then aliquoted into cryovials and stored at −80 °C until analysis. All collection materials were disposed of following biohazard protocols, with comprehensive documentation maintained for complete sample traceability.

Nucleic Acid Detection and Viral Load Quantification by qRT-PCR

SFTSV RNA detection and quantification were performed using a validated qRT-PCR protocol previously established in our laboratory. RNA was extracted from 140 μL of serum using the RNeasy Mini Kit (Qiagen, Germany). qRT-PCR was performed using the SuperScript III Platinum One-Step Quantitative RT-PCR System Kit (Invitrogen, USA) according to manufacturer's instructions. The reaction conditions were: reverse transcription at 50 °C for 30 minutes, initial denaturation at 95 °C for 2 minutes, followed by 40 cycles of amplification (95 °C for 15 seconds, 60 °C for 30 seconds). Previously described primers and probes targeting the SFTSV S segment were used (*1*). The cycle threshold (Ct) cut-off value was set at 35 cycles.

NP-Specific IgM Detection by IgM-Capture ELISA

NP-specific IgM antibodies were detected using a validated IgM-capture ELISA protocol, previously developed and validated in this laboratory, incorporating healthy control sera as negative controls. Ninety-six-well plates were coated with anti-human IgM (μ-chain specific) antibody (100 μL/well) and incubated overnight at 4 °C. After five phosphate-buffered saline with Tween-20 (PBST) washes, plates were blocked with ELISA-specific BSA blocking solution (Beijing Biodragon Immunotechnologies Co., Ltd., China) at 37 °C for 1 hour. Following five washes, 100 μL of 1:100 diluted patient serum and controls were added and incubated at 37 °C for 30 minutes. After washing, 100 μL of horseradish peroxidase (HRP)-conjugated SFTSV NP antigen (1:500) was added and incubated for 30 minutes. The wells were washed five times again, and 100 μL of 3,3',5,5'-tetramethylbenzidine (TMB) substrate was added for color development in the dark. After 5–10 minutes, the reaction was stopped with a stop solution, and optical density (OD) was measured at 450 nm. Samples with OD≥2.1 times the mean negative control OD were considered positive.

NP-Specific IgG Detection by Indirect ELISA

NP-specific IgG antibodies against SFTSV were detected using a validated indirect ELISA protocol, with healthy control sera serving as negative controls. Ninety-six-well plates were coated with SFTSV-NP protein antigen (500 ng/well) and incubated overnight at 4 °C. After five PBST washes, plates were blocked with BSA blocking solution (Beijing Biodragon Immunotechnologies Co., Ltd., China) at 37 °C for 1 hour. Following blocking, 100 μL of 1:100 diluted patient serum and controls were added and incubated at 37 °C for 30 minutes. After washing, 100 μL of anti-human IgG HRP-conjugated antibody (1:5,000) was added and incubated for 30 minutes. Following washing, TMB substrate was added for color development. The reaction was stopped after 5–10 minutes, and OD was measured at 450 nm. Samples with OD≥2.1 times the mean negative control OD were considered positive.

Indirect ELISA for Quantification of IgG Titers

A well-established indirect ELISA protocol, developed and validated in this laboratory, was employed to quantify NP-specific IgG titers against SFTSV in the serum of SFTS patients. Ninety-six-well plates were coated with SFTSV-NP protein antigen (500 ng/well) and incubated overnight at 4 °C. Following five washes with PBST, plates were blocked with BSA blocking solution (Beijing Biodragon Immunotechnologies Co., Ltd., China) at 37 °C for 1 hour. After blocking, patient serum was added to each column in the first row at a 1:1,000 dilution and subjected to two-fold serial dilutions across the remaining rows (1:2,000, 1:4,000, 1:8,000, 1:16,000, 1:32,000, 1:64,000, and 1:128,000) with positive and negative controls included. The plates were incubated at 37 °C for 30 minutes.

Following another five washes, 100 μL of anti-human IgG HRP-conjugated antibody (1∶5,000 dilution) was added and incubated for 30 minutes. The wells were washed five times again, and 100 μL of TMB substrate was added for color development in the dark. After 5–10 minutes, the reaction was stopped with a stop solution, and optical density (OD) values were measured at 450 nm. Samples were considered positive if the OD value was ≥2.1 times the mean OD of the negative controls.

Microneutralization Assay

A well-established microneutralization assay protocol, developed and validated in this laboratory, was used to determine neutralizing antibody titers in patient serum against SFTSV, utilizing the HB29 strain in Vero cells. After heat-inactivating patient serum samples at 56 °C for 30 minutes to eliminate complement activity, a starting dilution of 1:20 was prepared, followed by 12 two-fold serial dilutions in a 96-well plate, with each dilution tested in duplicate. Subsequently, 50 μL of 100 $CCID_{50}$ SFTSV virus solution was added to each well and incubated at 37 °C with 5% CO_2 for 2 hours. Next, 100 μL of a Vero cell suspension (1×10^5 cells/mL) was added to each well, and the plates were incubated for 7 days under the same conditions. The neutralization titer was defined as the highest serum dilution that inhibited virus-induced cytopathic effects (CPE) by 50%, expressed as the reciprocal of this dilution. Assay validation included positive serum, virus-only, and cell-only controls, with virus back-titration confirming consistent viral input.

Ethical Review and Funding Sources

This study received approval from the ethics committee of the National Institute for Viral Disease Control and Prevention, Chinese 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. This research was supported by the National Key R&D Program of China (ZDYF-2022YFC2303402), the Young Scientists Fund of the China CDC (CCDC-2022A104), and the National Major Science and Technology Project of China (2018ZX10711001).

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