Review

Advancements in the Worldwide Detection of Severe Fever with Thrombocytopenia Syndrome Virus Infection from 2009 to 2023

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ABSTRACT

Severe fever with thrombocytopenia syndrome (SFTS) is a growing concern as an emerging tick-borne infectious disease originating from the SFTS virus (SFTSV), a recent addition to the *Phlebovirus* genus under the family of bunyaviruses. SFTS is typically identified by symptoms such as fever, thrombocytopenia, leukopenia, and gastrointestinal problems, accompanied by a potentially high case fatality rate. Thus, early and accurate diagnosis is essential for effective treatment and disease management. This review delves into the existing methodologies for SFTS detection, including pathogenic, molecular, and immunological technologies.

SEVERE FEVER WITH THROMBOCYTOPENIA SYNDROME

The severe fever with thrombocytopenia syndrome virus (SFTSV) is an emergent tick-borne virus causing severe fever and thrombocytopenia, accompanied by high mortality rates (*1*–*2*). Identified initially in 2009 within the Hubei Province, China, this virus fits into the *Bandavirus* genus of the *Phenuiviridae* family (*3*). It was later detected in Taiwan, China (*4*), Japan (*5*), the Republic of Korea (*6*), and Vietnam (*7*). Transmission of the SFTS disease is primarily via the arthropod vector, notably through tick bites (*8*). Transmission through animals such as cats (*9*), dogs (*10*), and cheetahs (*11*) is also reported. Cases of human-to-human transmission of SFTSV have been noted, involving contact with blood and bodily fluids, even in hospital settings (*12*–*13*). The potential for SFTSV transmission from pets to humans presents a risk to pet owners and veterinary professionals alike (*9*,*14*).

The clinical manifestation of SFTS typically presents with fever, thrombocytopenia, and leukocytopenia. Patients may also experience fatigue, chills, headaches, lymphadenopathy, and gastrointestinal symptoms, among other systemic manifestations like muscular symptoms and coagulopathy (*15*). The case fatality rate of SFTS patients varies across China, the Republic of Korea, and Japan, ranging from approximately 6% to over 40% (*2*,*16*–*17*). The case fatality rate can escalate to 75% in cases complicated by hemophagocytic syndrome (*18*). The world is yet without an effective clinical treatment for this condition, and work on the development of an inactivated vaccine against SFTS is still in progress. Consequently, the World Health Organization has designated SFTSV as a priority pathogen that requires urgent attention (*19*).

A variety of detection methods for SFTSV have been developed, encompassing pathogenic, molecular, and immunological approaches. Pathogenic detection includes virus isolation via cell culture and electron microscopy techniques (*3*,*20*–*21*). Nucleic acid amplification techniques such as reverse transcription-polymerase chain reaction (RT-PCR) (*22*–*23*), loop-mediated isothermal amplification (LAMP) (*24*), and recombinase polymerase amplification (RPA) (*25*). Rapid diagnostic tests, such as lateral flow assays, provide prompt results and prove beneficial in resource-limited environments (*3*). Serological assays, including enzyme-linked immunosorbent assays (ELISAs) (*26*), indirect immunofluorescence assays (IFAs) (*27*), and immunochromatographic tests (ICTs) (*28*), remain the most extensively used methods for identifying SFTSV-specific antibodies in patient serum or plasma.

This review will summarize the current landscape of SFTSV detection methods, The critical importance of prompt and precise diagnosis of SFTSV infection in patient management and disease control underscores the necessity for the development of rapid, sensitive, and specific diagnostic methods.

PATHOGENIC CHARACTERISTICS OF SFTSV

Structural and Genetic Analysis of SFTSV

SFTSV, a negative-sense RNA virus from the

Phenuiviridae family, typically possesses a spherical or pleomorphic structure, with a diameter measuring between 80 and 120 nm. It is an enveloped virus, characterized by a lipid bilayer and surface glycoproteins that form spike-like features (*20*–*21*). The genome of SFTSV is segmented into three distinct negative-sense RNA strands, specifically designated as small (S), medium (M), and large (L) segments (*20*). The S segment, containing 1,744 nucleotides, codes for the nucleocapsid protein (N) (*29*–*30*). The M segment, made up of 3,378 nucleotides, is responsible for coding the glycoprotein precursor (GPC) (*31*). Lastly, the L segment, with 6,368 nucleotides, codes for the RNA-dependent RNA polymerase (RdRp) (*32*).

The SFTSV N protein is a highly conserved 116-amino acid protein that forms the nucleocapsid through its interaction with the viral RNA. The N protein is composed of two domains: the N-terminal domain that interacts with the RNA, and the C-terminal domain that is involved in oligomerization and protein-protein interactions (*30*).

The glycoprotein precursor of SFTSV undergoes post-translational cleavage, forming spikes on the surface of the virus. The Gn protein plays a critical role in attaching to the receptors of the host cell, while the Gc protein facilitates fusion with the membranes of the host cell (*31*).

The SFTSV RdRp is a large protein with multiple domains. The N-terminal region contains the RNA-binding and capping domains, while the C-terminal region contains the polymerase domain, responsible for catalyzing the RNA replication and transcription (*32*).

Electron Microscopy (EM) for SFTSV

SFTSV presents as spherical or pleomorphic particles, with diameters ranging between 80 and 120 nm. The virus also features a lipid envelope with prominent surface spikes, approximately 12–20 nm in length, and houses a dense core protecting the vital genomic material, which was conducted by EM analysis (*3*). Then, the full-length structure and 3D model of SFTSV L protein by cryogenic EM were reported (*28*,*33*–*34*).

Virus Isolation

This process of isolating SFTSV often includes the introduction of clinical samples or cell culture supernatants into susceptible cell lines. This is then followed by observing cytopathic effects (CPE) and the verification of viral replication via molecular techniques (*3*). Numerous studies have reported success in isolating SFTSV from a range of sources, including patient samples, ticks, and animals that have been experimentally infected (*3*,*35*–*39*).

It's important to note that SFTSV can infect a variety of cells, including L929, Vero E6, Vero, and DH82 cells. However, CPEs were only identified in DH82 cells (*3*). Furthermore, Vero cells were employed to isolate SFTS at temperatures of 37 °C and 39 °C, suggesting that the SFTSV strain ZJ2013-06 from a patient demonstrated limited replication at 39 °C as per the research conducted by Feng et al. (*35*).

Ten infective SFTSVs were isolated successfully from various tick species in one 2021 study (*38*). Moreover, the viral sequences extracted from the ticks demonstrated remarkable homology to the sequences previously isolated from SFTS patients from the same region of sample collection.

Wei et al. (*39*) conducted a study on the ability of SFTSV to infect BEAS-2B cells. Utilizing cell culture techniques, they assessed the overall antibody production in the serum as well as the viral load in the tissue of mice infected with SFTSV via aerosol exposure.

Virus isolation has been utilized in the SFTSV transmission cycle. According to a study by Jiao et al., goats inoculated with SFTSV showed no disease signs and did not expel the virus through either respiratory or digestive routes. This finding suggests that without specific arthropod species as carriers, an efficacious viral transmission cycle cannot be established in natural conditions (*36*).

NUCLEIC ACID DETECTION

Detection of SFTSV genome could be achieved by different nucleic acid detection techniques such as RT-PCR (*13*,*40*–*43*), real-time RT-PCR (*23*,*28*,*41*, *44*–*48*), LAMP (*24*,*49*–*50*), as well as RPA (*25*,*51*–*52*).

Conventional Nucleic Acid Detection

The S segment codes for the nucleocapsid protein — a crucial element for the processes of viral assembly and replication (*29*). A two-tube multiplex real-time RT-PCR assay, designed for the identification of four hemorrhagic fever viruses: SFTSV, Hantaan virus, Seoul virus, and the dengue virus. It targets the nucleocapsid protein in the SFTSV genome (*47*).

The ability to differentiate between SFTSV strains can be facilitated by the M segment. A one-step RT-PCR assay targeting this M segment was developed by Sun et al. (*23*), which exhibited high specificity and sensitivity and was capable of detecting as few as 10 copies of the viral RNA per reaction.

The L segment — responsible for encoding the RNA-dependent RNA polymerase — is frequently targeted in SFTSV RT-PCR assays due to its relatively preserved characteristics. This focus on the L segment affords significant specificity in the detection of SFTSV (*32*).

RT-PCR and real-time RT-PCR assays are widely used for the detection and quantification of SFTSV in clinical samples, such as blood, serum, and cerebrospinal fluid (*43*,*53*–*55*). They are also employed in epidemiological investigations, such as tick and animal infected surveillance, analysis of viral genetic diversity, as well as a crucial role in the evaluation of antiviral drugs and vaccines against SFTSV (*9*,*36*,*56*–*61*).

Rapid Nucleic Acid Detection

The method of LAMP exhibits considerable potential for SFTSV detection given its efficiency, rapidity, and economic feasibility (*62*–*63*). The one-step, single-tube reverse transcription LAMP assay for rapid identification of RNA from SFTSV with a detection limit of 10×50% tissue culture infective dose ($TCID_{50}$) per mL, demonstrated high specificity and sensitivity. After combining with the fluorescent detection reagent (FDR) method, results could be determined by observing a color change within 30 min (*64*). Jang et al. developed a multiplex RT-LAMP to identify larger segments and GroES genes for SFTSV and *Orientia tsutsugamushi* (OT) (*24*). The sensitivity of the multiplex SFTSV/OT/Internal control (IC) RT-LAMP assay proved comparable to that of the commercial PowerChek™ SFTSV Real-time PCR (91.3% *vs*. 95.6%). Moreover, it displayed a higher sensitivity (91.6%) than the LiliF™ TSUTSU nested PCR (75%), with the multiplex SFTSV/OT RT-LAMP assay exhibited 100% specificity. The LAMP assay has been successfully implemented in clinical specimens from both humans (*50*,*64*–*66*) and cats (*67*), indicating promising applications.

RPA is a novel isothermal nucleic acid amplification technique that offers rapid, sensitive, and specific detection of SFTSV with constant temperature between 37 and 42 °C as well as eliminates the need for thermal cycling equipment (*68*–*70*). RPA assays can be combined with various detection methods, such as fluorescence, lateral flow, or colorimetric detection, to facilitate rapid and straightforward readouts (*71*–*72*).

Zhou et al. implemented the RT-RPA assay to detect SFTSV in serum samples (*25*). The detection limit was illustrated to be 241 copies per reaction at a 95% probability, with a sensitivity and specificity rate of approximately 96.00% and 98.95% respectively. Thus, the rapid RT-RPA assay presents itself as a promising candidate for point-of-care detection methods of SFTSV.

The advent of molecular technology has facilitated the development of novel detection methods for SFTSV, utilizing CRISPR-Cas13a (*73*). Huang et al. (*52*) and Park et al. (*74*) applied CRISPR-Cas12a system combined with RT-RPA to detect SFTS. In Huang et al.'s report, three copies of the L gene from the SFTSV genome per reaction were enough to ensure stable detection within 40 min. In Park et al. research, it successfully diagnosed SFTSV infections with the reaction time of 50 min from blood plasma without cross-reactivity to other viruses.

IMMUNOLOGICAL TEST

Serological assays, which detect SFTSV-specific antibodies in patients' or animals' serum or plasma, have been extensively utilized. These assays comprise ELISAs (*26*,*35*,*75*–*78*), IFAs (*45*,*78*), and ICTs (*28*).

ELISAs for SFTSV Detection

Various SFTSV-specific antigens have been employed in ELISAs, encompassing SFTSV nucleocapsid protein (NP), glycoprotein (GP), and non-structural protein (NSs). Predominantly, NP-based ELISA is utilized and has demonstrated superior diagnostic precision for SFTSV serodiagnosis (*26*,*79*).

A sandwich ELISA predicated on recombinant N protein for the detection of total antibodies targeting this virus in humans and animals (*36*). SFTSV-specific IgM antibodies detectable in patient serum merely three days post-symptom onset, peaking approximately two weeks later, have also been revealed (*78*). Furthermore, SFTSV-specific IgG antibodies became detectable about six days post-symptom onset, persisting up to six months. In a report by Yu, recombinant SFTSV-N (rSFTSV-N) protein was produced using an *Escherichia coli* expression system and purified (*80*). Additionally, Yu established

rSFTSV-N protein-based IgG ELISA and IgM ELISA systems.

ELISA methods are currently being extensively utilized to monitor SFTSV infection in humans as well as animals. According to a report by Tran et al. (*78*), the seroprevalence of anti-SFTSV IgM or IgG was recorded at 3.64% (26 out of 714) with a high IgM antibodies positivity titer >80 (0.28%, 2 out of 714). Lee et al. (*75*) developed a competitive ELISA for diagnosing STFV in bovine sera using a monoclonal antibody where lab-immunized positive sera exhibited a 98.1% consistency with IFA results. A 2020 study by Duan et al. (*81*) introduced enzyme-antibody-modified gold nanoparticle probes for the ultrasensitive detection of the nucleocapsid protein in SFTSV, where the detection limit for NP was 0.9 pg/mL, demonstrating good specificity and reproducibility.

Utilizing IFAs for the Detection of SFTSV

The IFA technique, which is recombinant antigen-based, utilizes recombinant viral proteins from a heterologous system as the source of the antigen. A case in point is the research conducted by Tran et al. whereby serum samples from 714 healthy Vietnamese residents were collected (*78*). To assess the SFTSV seroprevalence, the samples underwent IFA, ELISA, and the 50% focus reduction neutralization test (FRNT50) assay. The neutralizing antibodies against SFTSV recorded a range of 15.5 to 55.9 in terms of titer.

Utilizing ICAs for SFTSV Detection

Upholding the principle of antigen-antibody interaction, immunochromatographic tests employ capillary action to transport the sample along the strip, where either antibodies or antigens are immobilized and labeled. (*28*).

Wang et al. (*28*) implemented the ICA method, which involves the use of gold nanoparticles coated with recombinant SFTSV to simultaneously detect both IgG and IgM antibodies to SFTSV. This method was developed and assessed using 245 positive serum samples from China CDC of SFTSV infection. The ensuing results revealed positive and negative coincidence rates of 98.4% and 100% for IgM, as well as 96.7% and 98.6% for IgG, respectively.

DISCUSSION

In conclusion, a myriad of diagnostic methods have emerged and have been implemented for recognizing SFTSV infection. This includes etiological, immunological, and molecular methodologies. While strides have been made in detecting SFTSV, substantial efforts remain regarding standardization and automation, along with the cultivation of multiplex assays for enhancing detection efficiency and accuracy. As a result, forthcoming research should prioritize resolving these challenges, whilst seeking novel diagnostic approaches that will aid us in battling this lethal disease.

For consistent and trustworthy results vital for patient care, it is essential that all labs adopt uniform methodologies and procedures for detecting SFTSV. Without such standardization, the validity and reliability of SFTSV detection can fluctuate across different labs, impeding effective identification and containment of virus outbreaks. Automating these techniques could enhance efficiency, minimize costs, and allow labs to tackle larger volumes of samples in less time. Moreover, automation mitigates the risk of human errors, thereby enhancing the accuracy and reliability of the results obtained. Implementing multiplex assays could notably enhance the effectiveness and accuracy of SFTSV detection. These assays allow for the simultaneous detection of multiple pathogens in a single sample. Consequently, labs could identify SFTSV as well as other tick-borne diseases with similar symptoms, such as *Anaplasma phagocytophilum* and *Borrelia burgdorferi*. Multiplex assays would be especially beneficial in environments where multiple tick-borne diseases are prevalent.

SFTSV represents a significant health threat that necessitates prompt and precise identification to facilitate appropriate treatment and manage potential outbreaks. It is recommended that standardization and automation be prioritized in conjunction with the development of multiplex assays to enhance the detection effectiveness and precision. The introduction of innovative diagnostic approaches, such as next-generation sequencing and biomarker recognition, could potentially yield more meticulous and sensitive detection methods for SFTSV. Undertaking these challenges is integral to the effective containment and prevention of this virus' spread.

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