HUMAN VACCINES & IMMUNOTHERAPEUTICS
2019, VOL. 15, NO. 10, 2249–2257
https://doi.org/10.1080/21645515.2019.1633875

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REVIEW

Severe fever and thrombocytopenia syndrome virus infection: Considerations for vaccine evaluation of a rare disease

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ABSTRACT

Infection caused by the severe fever and thrombocytopenia syndrome virus (SFTSV) causes a hemorrhagic illness with a mortality between 20% and 40%. Initially recognized in 2009 in China, cases have additionally been documented in Japan and Korea although retrospective studies have documented seroprevalence since 1996. Although case rates have increased due to increased awareness and more widely available diagnostics, SFTSV infection remains rare with the highest rates documented in Korea for Jeju Province (3.5 cases per 100,000 population) and the Inje-gun region (66.2 cases per 100,000). Because of the very low incidence of infection, a placebo-controlled study with 1:1 randomization to evaluate an SFTSV vaccine would require a sample size that is 25% greater than the region of study. We discuss alternatives to licensure. Vaccine effectiveness may be assessed through a registry, comparing rates of infection over time between vaccine recipients versus regional populations. Modeled data can be updated based on actual case rates and population changes over the years of follow-up. Using one model, statistically significant differences are seen after 10 years in Inje-gun and 15 years of follow-up in Jeju. This approach may be applicable to other uncommon infectious diseases for which a standard study design is difficult.

ARTICLE HISTORY
Received 1 April 2019
Revised 29 May 2019
Accepted 15 June 2019

KEYWORDS
SFTS virus; severe fever and thrombocytopenia virus; emerging infectious disease; rare disease; clinical trial design; sample size estimate; seroprevalence

Introduction

Over the past decade, a number of severe infectious diseases have been identified that remain limited in scope and case number. Mayaro virus in South America, Powassan virus in North America, severe fever and thrombocytopenia syndrome virus (SFTSV) in the Far East, and Heartland virus in the US Midwest represent examples. Epidemiologic mapping, including use of geographic information systems, may be insufficient to identify sub-regions with consistent yearly attack rates to allow planning of a clinical trial that has acceptable statistical power for confirmation of efficacy. Field trials of vaccines against infectious diseases with very low attack rates thus present a significant challenge.

Rare genetic diseases represent the paradigm for illnesses of very low-incidence. In contrast to studies of infectious diseases for which incident cases are identified after diagnosis, studies of therapeutics for rare disease are reliant on prevalent (already diagnosed) cases. Registration trials for rare diseases, in many cases, involve small cohorts of patients as part of non-comparative, open-label studies as placebo-controls may not be considered ethical.

Clinical trial design to evaluate the efficacy of any new vaccine is critically dependent upon the attack rate of the disease in question. For diseases such as influenza and respiratory syncytial virus, outbreaks during the winter months are typical, providing opportunity for standard placebo-controlled trials to assess new vaccine candidates. In contrast, infectious diseases of very low incidence or those occurring in geographically isolated regions create a need for alternative approaches to assess vaccine effectiveness.

In this manuscript, we analyze the epidemiology of SFTSV with regard to vaccine development. The challenges of standard clinical trial design, and the use of post-approval follow-up as a means to evaluate vaccine effectiveness are discussed.

Clinical illness and epidemiology of SFTSV infection

SFTS clinical disease

The first report of SFTS detailed a cluster of cases in China of febrile illness associated with thrombocytopenia, leukopenia, and organ failure leading to death in as many as 40%.[1] Although initially suspected as caused by Anaplasma infection, a novel phlebovirus recognized as a member of the *Bunyaviridae*, was isolated and identified as the causative agent. Additional clinical aspects of SFTSV infection include renal and liver failure, coma and mental confusion, and cytopenias including leukopenia and anemia.[1] Hemophagocytic lymphocytosis has also been identified as a complication of SFTSV infection diagnosed in four patients from Korea[2] and three from Japan.[3,4] An autopsy study from Japan additionally noted systemic necrotizing lymphadenitis in two patients and considered this a pathognomonic finding[5] that was documented in other studies.[6]

As infection with SFTSV has become increasingly recognized, case-fatality rates have declined from the original

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Table 1. Mortality rates of SFTSV infection in Korea, 2013–2017.

Year	# of reported cases [1]	# fatal cases [2]	Mortality rate (%)
2013	36	17	47.2
2014	55	16	29.1
2015	79	21	26.6
2016	165	19	11.5
2017	272	54	19.9
2018	259	N/A	N/A

[1] Yearly case data for SFTS infection are reported as part of the Korean CDC Weekly report at https://www.cdc.go.kr/CDC/eng/info/CdcKeDIDO.jsp?menuIds=HOME002-MNU0576-MNU0586

[2] Mortality data for SFTSV infection are reported by the Korean CDC at http://www.cdc.go.kr/npt/biz/npp/portal/nppPblctDtaMain.do. Mortality data for 2018 have not yet been reported by the Korean CDC.

reports primarily attributed to earlier identification and intervention, however, SFTSV infection remains highly fatal. The first report of SFTSV infection from China reported 21 deaths for the 171 identified cases (12% mortality rate).[1] A 56% mortality rate was noted in early reports from Japan while early studies in Korea reported mortalities of 38% and 63%.[6–8] Country-wide case tracking for Korea shows a decline in case-fatality rate from 43.7% in 2013 to 19.9% in 2017 (Table 1).

The kinetics of viral infection has been examined in one study of 11 patients.[9] Baseline (log_{10}) viral loads ranged between 2 and 5.5 for the 10 survivors with resolution of viremia by two to three weeks from admission. In contrast, for the single fatal case, the viral load was approximately 6.2 at admission, rising over the next few days and plateauing at approximately 8.5 until death on day 7.

Epidemiology of SFTSV infection

Cases of SFTSV infection have been documented in China, Japan, and South Korea and more recently in Vietnam. As outlined below, each endemic region has experienced increasing case rates since discovery due both to better diagnostic tools and increased recognition of disease.[10] As detailed below, retrospective analysis of stored specimens demonstrated that SFTSV has been endemic in East Asia for approximately two decades prior to discovery.

The prevalence of undiagnosed STFSV infection is unknown. Of the 866 cases diagnosed and reported to the Korean Centers for Disease Control (CDC), only four (0.46%) were asymptomatic. However, a seroprevalence study from rural Korea found that while symptoms of fever in the three years prior to serum collection represented a risk for SFTSV seropositivity (odds ratio of 4.09, 95% CI of 1.25–13.36), only four (8%) of the 50 seropositive individuals reported such symptoms.[11] These data suggest that subclinical infection may occur, the potential for which is explored in more detail below.

Risk factors for SFTSV infection

SFTSV was suspected as a tick-borne illness as a sylvatic study, performed as part of the investigation of the index Chinese series of cases, demonstrated the presence of SFTSV in approximately 5% of *Haemaphysalis longicornus* ticks but was not detected in mosquitoes.[1] Consistent with a tick-borne illness, cases were concentrated in late spring and summer, especially between May and July.[12] Other factors that were potential risks for infection included higher temperatures, greater rainfall, and increased relative humidity.[13] factors that would favor expansion of tick populations.

Documentation of a history of tick bite is variable among studies. Whereas a serosurvey in hunters documented that greater than 90% reported prior tick bites,[14] other studies found that a history of tick bite was uncommon. In the study by Han et al., only 5 of 50 seropositive individuals reported a recent tick bite and this factor was not significant as a risk for infection in multivariate analysis.[11] In the study by Choi et al., only 22% of case-patients reported tick bites.[7]

The initial report from China found that farmers living in hilly and wooded areas accounted for greater than 95% of cases of SFTSV infection.[1] Subsequent studies from China similarly identified work in agriculture or wooded areas as risk factors for infection,[12,15] which further support the potential for ticks (or other insects) to act as vectors. In contrast, a detailed epidemiologic analysis of 172 Korean cases showed significant geographic diversity, with fewer than 50% employed in agriculture including many who resided in urban areas.[7] Other Korean studies have similarly reported a significant fraction of cases in individuals residing in urban areas.[16]

Some case series examined co-morbid illness, age, or gender as risks for SFTSV infection or death, with most studies not finding any association[7,11,16,17] or only that very advanced age greater was a risk for mortality.[18] Alternatively, two studies from China documented an association between SFTSV infection and older age;[1,12] whether this relates to societal factors (i.e., that those working in agriculture were typically older) is unknown. And an additional study from China found that a history of diabetes mellitus (odds ratio (OR) 2.30, 95% confidence interval (CI) 1.52–3.49), chronic viral hepatitis (OR 1.55, 95% CI 1.05–2.29), and chronic obstructive lung disease (OR 2.17, 95% CI 1.22–3.87) were independently associated with a greater risk for mortality.[19]

SFTSV epidemiology China

SFTSV has been circulating in China for more than 20 years and has been seroprevalent at least since 1996.[20] Geographically, cases of SFTSV infection were initially identified between 2009 and 2010 in six eastern and northeastern provinces of Liaoning, Shandong, Jiangsu, Anhui, Henan, and Hubei.[1] Subsequent studies showed a steady increase in persons diagnosed as infected with SFTSV infection over a larger geographic region with 1,768 cases reported in 14 provinces over the first four years of the epidemic.[12] In this report,[12] case rates ranged from 0.12 per 100,000 persons in Zhejiang Province to 0.53 and 0.78 per 100,000 for Shandong and Henan Provinces, and were highest for the six provinces in the seminal report from Yu et al.[1] Spatial localization identified three high-incidence "hot-spots" located in agricultural and wooded regions in southeastern Henan Province, eastern Lianjiang Province, and central Shandong Province.[12]

Seroprevalence studies provide a complementary assessment of the clinical impact and extent of SFTS in China. A recent review and meta-analysis of 21 published English and Chinese language studies examined the seroprevalence of SFTSV infection in China.[15] Diagnosis was based on reactivity

on a double-sandwich enzyme-linked immunosorbent assay (ELISA) or indirect ELISA. Seroprevalence was highest for five provinces: Hubei (6.9%), Zhejiang (5.9%), Anhui (4.9%), Shandong (3.9%), and Jiangsu (0.7%) with cases more common among farmers and those residing in rural regions.[15]

SFTSV epidemiology Korea

SFTSV infection was first documented in Korea for a patient admitted in 2012 to a hospital in Chuncheon, Gangwon Province in north-central Korea.[8] Later studies documented that SFTSV has been present since 2008, with a case identified retrospectively at Pusan Hospital located in the southeastern coastal city of Busan[2] and two cases dating to 2010 from Jeju island, located off of the southern coast of Korea.[21] SFTSV infection is a reportable disease to the Korean CDC and has been identified countrywide in all provinces with increasing case numbers since 2013 (Table 2). For 2017, case rates ranged from 0.45 per 100,000 population for Gyeonggi province to a high of 2.48 and 3.50 per 100,000 for Gangwon and Jeju provinces, respectively. Gangwon province is located in the far north and Jeju province in the far southern part of Korea. Of note, 2018 case numbers were slightly decreased from the prior year (Table 2); whether this is indicative of a peak in diagnosed cases is unknown.

A 2018 seroprevalence study in Korea analyzed blood samples collected from 1,228 residents in three rural locales endemic for SFTSV.[11] Seroreactivity by indirect immunofluorescence assay (IFA) was found for 2.7%, 4.0%, and 5.5% samples from Chungcheong, Gyeongsangnam, and Jeolla provinces, respectively.[11] An earlier cross-sectional serosurvey was of 1,069 patients who presented for medical care at Busan hospital or regional outpatient clinics on two days in May 2015.[16] Testing found 22 (2.1%) reactive by double-sandwich ELISA against the SFTSV nucleoprotein (NP), including 18 who resided in Busan and four from Gyeongnam Province. All ELISA-positive samples were tested for SFTSV RNA, with no samples positive.

SFTS epidemiology Japan

SFTSV infection was first recognized in Japan in 2012[6] although the virus had been circulating since at least 2005.[22] Similar to the experience in Korea and China, the number of reported cases has steadily increased throughout Japan, with the highest case rates in prefectures in the south of Japan (Table 4).

A number of serologic studies from Japan have been published.[14,23–25]

Satoh et al. examined 464 samples from 222 patients who had been evaluated for possible rickettsial infection between 1999 and 2012.[25] A total of 16 samples were reactive by ELISA against an SFTSV lysate. Only one of the 16 samples, collected from a patient who resided in Yamaguchi prefecture in Southwestern Japan, was IFA positive against the SFTSV NP[6] and Vero cell plaque reduction neutralization test (NT).

To determine the extent of SFTSV infection in the region, Kimura et al.[23] evaluated samples from 694 individuals collected July through August 2015 from healthy individuals who presented for care at one of the two health centers in Ehime prefecture. Testing methodologies were as described above by Satoh. Screening ELISA was positive for eight individuals, of whom two were positive by IFA with only one IFA-positive sample additionally NT positive in a woman with no memory of clinical illness. Viral isolation was not performed.

Gokuden et al.[14] analyzed serum collected from 646 healthy individuals from Kagoshima prefecture, including 125 involved in hunting activities, 521 non-hunters, and 1,000 blinded samples from blood donors. Serologic methodologies were as described above. There were 18 ELISA-positive samples including five from hunters, seven from non-hunters, and six from blood donors. Five samples were reactive by NP-IFA (two hunters, three non-hunters) and of those, two were additionally positive by NT. Viral isolation was not performed.

Matsumoto et al.[24] examined 3990 samples collected at nine prefectures in the Chugoku-Shikoku region of Western Japan, that included the Hiroshima and Yamaguchi prefectures with one indeterminate sample by IFA, but negative by confirmatory luciferase immunoprecipitation.

Thus, of four sero-surveys of 5552 individuals residing in high-risk regions, 42 (0.76%) were positive by ELISA of which eight (0.14%) were IFA positive of which five were additionally positive by NT. Only two of the latter five samples had viral detection and/or isolation attempted and both were positive demonstrating a low level of seroprevalence in SFTSV-endemic regions of Japan.

SFTSV cases outside of China, Korea, and Japan

A recent study from Vietnam examined stored serum from patients admitted to hospital in 2017 with febrile illness.[26] SFTSV was identified by PCR for two patients, one of whom also had IgM antibodies present. The only reported case of SFTSV infection outside of the Far East was of a North

Table 2. SFTS provincial case numbers and case rates for South Korea 2013–2018.

Province	2013	2014	2015	2016	2017	2018	Total	Population (in millions)	Incidence per 100,000 population (2017)
Gyeonggi	0	8	7	28	56	47	146	12.24	0.45
Gangwon	3	4	15	29	39	35	125	1.57	2.48
Chungcheongbuk	0	2	0	11	12	12	37	1.58	0.76
Chungcheongnam	2	2	5	9	30	22	70	2.06	1.46
Jeollabuk	0	0	2	3	10	13	28	1.87	0.54
Jeollanam	5	1	9	9	18	16	58	1.90	0.95
Gyeongsangbuk	6	19	9	25	39	38	136	2.70	1.44
Gyeongsangnam	5	5	10	15	16	28	79	3.34	0.48
Jeju	6	7	9	8	21	15	66	0.6	3.50

Data as provided by the Korean CDC. Population data for 2018 were accessed at https://www.google.com/search?q=population+korea+provinces&ie=utf-8&oe=utf-8&client=firefox-b-1.

Korean, diagnosed by clinical presentation in 2011, who had been working in the United Arab Emirates (UAE) for 12 months without recent travel.[27] Since neither serologic nor virologic confirmation was performed, this case cannot be considered as definitive. No other known primary cases have been published.

Summary of SFTSV infection in Southeast Asia

Infection with SFTSV has been increasingly recognized since its identification a decade ago. Much of the increase in case rates has been attributed to greater appreciation of SFTSV as a cause of disease in patients presenting for medical care, as well as better and more widely available diagnostics.[10] SFTSV has not apparently expanded beyond its original endemic region, with concentrations in eastern Chinese provinces, South and West Japan, and throughout Korea. Despite the increase in case rates, the incidence of disease remains low. Seroprevalence studies in Japan are similarly low, whereas the seroprevalence in Korea is apparently higher. Correlation between serologic studies and incidence data will be explored in the next section and the implications as to the conduct of vaccine studies to follow.

Correlating SFTSV infection case rates based on incidence rates versus serosurveys

In this section, we analyze reported incidence and serologic surveys and whether these are mutually consistent or whether serologic assessments may predict a background of minimally symptomatic infection. Key considerations and plausible assumptions are discussed.

Cases confirmed to governmental CDCs may be over-represented by individuals who present to medical care and thus have greater likelihood to meet the clinical case definition of moderate or severe SFTSV infection. This assumption may be supported by the fact that of SFTSV cases reported to the Korean CDC, approximately 98% were symptomatic. Symptomatic cases would provide a lower bound of the true incidence of infection.

A crude estimate of the upper bound of the attack rate of SFTSV infection can be derived from serologic studies. Serosurveys have the potential to detect cases that are asymptomatic, minimally symptomatic, or have an atypical presentation. Based on studies of stored sera, SFTSV has been circulating in East Asia for at least 20 years. A first-order model assumes no change in true disease incidence or prevalence over this time period.

Secondly, serologic assessments are limited by potential non-specificity and cross-reactivity to other viruses, thus providing uncertainty as to the definition of a true positive sample in the absence of viral isolation or PCR identification. This limitation is highlighted by the four serologic studies from Japan: of 42 ELISA-positive samples, only 19% were IFA-positive, and 11.9% able to neutralize SFTSV infection of VERO cells.[14,23,25]

One assay reported as 100% specific and 98% sensitive was a double-sandwich IFA against the SFTSV NP[28] that was utilized in serologic studies from China and Korea.[11,28] This assay was initially qualified comparing reactivity of sera infection-naïve versus experimentally infected goats. Validation of the assay was based on 250 human and 304 animal sera samples. Human samples included convalescent sera from 35 individuals diagnosed with SFTSV infection by PCR as part of the 2010 outbreak in China and sera collected as part of a field study during the outbreak from 215 individuals residing in high-risk regions. Animal sera were similarly collected during the 2010 outbreak from the same geographic locales. All but two of the 189 ELISA-positive samples neutralized SFTSV infection of Vero cells; all 465 ELISA-negative samples did not express neutralizing antibodies. Definitive confirmation of infection by PCR and assessment of the ELISA against sera with a low pre-test likelihood of infection was not reported.

Using these observations, the four serologic studies from Japan provide an estimate of the prevalence of SFTSV seroreactivity in endemic regions as 90.1 cases per 100,000 population.[14,23–25] Disease incidence, as reported to the Japan CDC in 2018 ranged between 1.2 and 5.4 cases per 100,000 population (Table 4) and is assumed to capture almost all symptomatic infections as disease recognition has likely peaked. Second, assuming that SFTSV has been prevalent in Japan for 20 years, similar to China, seroprevalence data would suggest a crude incidence of approximately 4.5 to 9 cases per 100,000 population, dependent on whether serologic status is maintained for 20 vs 10 years in line with incidence rates.

For Korea, there have been fewer serologic surveys conducted, and they were not subjected to the same level of rigor regarding confirmation of diagnostic specificity. This notwithstanding, the reported seroprevalence ranged from 2.1% to 4.7% of samples (2,100 to 4,700 per 100,000 population) in high-risk, endemic regions versus rates of 0.95 to 3.5 diagnosed cases per 100,000 population in 2018 for the same Provinces (Table 2, 3). By applying a similar diagnostic confirmatory cascade as used in Japan (12% of ELISA positive samples are considered as true positives) and similarly assuming that SFTSV has been endemic in Korea 20 years, the seroprevalence data would predict incidence rates of about 10 to 2.5 per 100,000 population, about 10-fold higher than reported as part of disease surveillance, although as noted there are significant methodologic differences in ELISA methodology.

SFTSV vaccine development

There has been minimal work on development of a vaccine to prevent infection with SFTSV.[29] Two vaccine studies that include SFTSV challenge have been published. A protein subunit vaccine comprised of recombinant SFTSV non-structural (NS) proteins (100 μg in Freund's complete adjuvant) administered as a prime-boost generated a robust antibody response (titers > 1:2,560) in mice.[30] Following viral challenge, viral loads were similar in serum, liver, spleen, and kidneys for vaccinated and control animals although no animal-manifested illness. A second study detailed a recombinant vesicular stomatitis virus-vectored (rVSV) vaccine encoding for the SFTSV glycoproteins Gn/Gc.[31] A single immunization induced neutralizing antibodies with

Table 3. SFTSV infection case numbers and case rates for high-incidence regions, South Korea 2013–2018.

									Total		Total cases per 100,000 pop	Case rate per 100,000 pop[1]
Country	Province	Region	2013	2014	2015	2016	2017	2018	2013–18	Population		
Korea	Gyeonggi	Gapyeong-gun	0	0	4	10	7	5	26	63,157	41.2	7.9
		Namyangju-si	0	0	0	6	14	12	32	681,385	4.7	1.7
		Poncheon-si	0	1	0	2	9	3	15	151,495	9.9	2.0
	Gangwon	Wonju-si	0	0	5	5	3	8	21	343,367	6.1	2.3
		Inje-gun	0	2	3	4	6	5	20	32,179	62.2	15.6
		Chuncheon-si	2	1	0	7	12	5	27	280,582	9.6	1.8
	Gyeongsangbuk	Sangju-si	0	2	0	3	2	3	10	100,139	10.0	3.0
		Yeongju-si	0	0	0	0	3	5	8	107,136	7.5	4.7
		Uiseong-gun	1	0	0	0	0	5	6	52,929	11.3	9.5
		Pohang-si	2	2	0	1	5	5	15	510,401	2.9	1.0
	Jeju-do	Seogwipo-si	4	3	3	3	11	5	29	181,418	7.6	2.1
		Jeju-si	2	4	6	5	10	10	37	485,268	7.6	2.1

[1]Case rates were calculated based on data from 2018.
Data as supplied by the Korean CDC.

Table 4. SFTSV infection case numbers and case rates, Japanese prefectures 2013–2018.

							Total cases		Total cases per 100,000 pop	Case rate per 100,000 pop[a]
Prefecture	2013	2014	2015	2016	2017	2018	2013–18	Population		
Miyazaki	7	11	9	9	13	11	60	1,079,727	5.4	1.0
Kagoshima	5	4	6	4	11	9	39	1,613,969	2.4	0.6
Yamaguchi	3	4	6	4	12	8	37	1,368,495	2.6	0.6
Hiroshima	4	3	10	3	5	10	35	2,819,962	1.2	0.4
Kochi	3	11	3	7	5	5	34	705,880	4.7	0.7
Ehime	8	11	1	1	4	3	28	1,351,510	2.0	0.2
Nagasaki	5	2	2	2	11	4	26	1,339,438	1.9	0.3
Tokushima	2	7	3	8	4	1	26	736,475	3.3	0.1
Wakayama	0	2	1	5	3	5	16	934,051	1.7	0.5

[a]Case rates were calculated based on data from 2018.
SFTS case data were provided from the following sources: Data for Korea was kindly provided by the Korean CDC. Case rates for Japan were collated from individual Infectious Diseases Weekly Reports for the following calendar years:

- 2018: https://www.niid.go.jp/niid/en/survaillance-data-table-english.html
- 2017: https://www.niid.go.jp/niid/en/survaillance-data-table-english/7756-idwr-sokuho-data-e-1752.html
- 2016: https://www.niid.go.jp/niid/en/survaillance-data-table-english/6999-idwr-sokuho-data-e-1652.html
- 2015: https://www.niid.go.jp/niid/en/survaillance-data-table-english/6199-idwr-sokuho-data-e-1553.html
- 2014: https://www.niid.go.jp/niid/en/survaillance-data-table-english/5251-idwr-sokuho-data-e-14-52.html
- 2013: https://www.niid.go.jp/niid/en/survaillance-data-table-english/4180-idwr-sokuho-data-e-13-52.html

titers of approximately 1:500 in both immuncompetent and IFNAR -/- mice and was fully protective against lethal infection.

Sample size estimations and design of a placebo-controlled study of an SFTSV vaccine

Standard clinical trial design employs a double-blind, placebo-controlled study with 1:1 assignment between study arms. An underlying assumption of this methodology is that incident disease is random within the population studied. Ideally, a vaccine trial for SFTSV would be sited in regions of high prevalence to increase the chance of incident cases. Although person-to-person spread has been documented for SFTSV, this has occurred in nosocomial settings during invasive procedures[32] and thus a ring vaccination design, such as was performed for the rVSV-Ebola vaccine[33] is not feasible.

A key consideration for a vaccine study is a clear case definition. PCR detection of viral RNA is currently considered the gold-standard of infection, although studies have alternatively used a fourfold rise in antibody titers.[1] Individuals who become ill post-vaccination would be instructed to return should any symptoms consistent with infection occur, such as fever, gastrointestinal illness, or new bleeding disorder that would then prompt medical evaluation and collection of diagnostic specimens. SFTSV is detectable in serum for 14 days post-presentation for approximately 70% of individuals and clears by 21 days.[9] Urine may represent an alternative sample type to detect viral RNA as viral RNA has been detected to day 5 of hospitalization for one patient.[34]

For a standard trial design, estimates of sample size can be determined. Taking as example the Korean provinces and regions with the highest incidence, sample sizes can be calculated for a randomized, placebo-controlled study. For a study based in Jeju province (incidence of 3.5 cases per 100,000 population; Table 2), assuming a vaccine efficacy of 90%, a sample size of 730,536 would be required (Table 5) that is approximately 22% greater than the entire provincial population. Similarly, for the Inje-gun region of Gangwon Province (incidence of 62.2 cases per 100,000 population in 2018; Table 3), a study would require a sample size 28% greater than the entire population of that region. Thus, for both regions, sample size estimates significantly exceed the respective populations. Notwithstanding the challenging logistical barriers to enroll sufficient numbers of study subjects, study costs would be insurmountable: assuming per-

Table 5. Sample size for SFTSV vaccine study in Korea.

Category	Attack rate (per 100,000 population)	Vaccine effectiveness	Statistical Power	Estimated Sample size
Low	3.5	75%	80%	1,143,966
		90%	80%	730,536
Medium	22.5	75%	80%	177,930
		90%	80%	113,628
High	62.2	75%	80%	42,774
		90%	80%	41,102

Sample sizes are based a two-arm, placebo-controlled, double-blind study with 1:1 randomization. Korean estimates of SFTSV attack rates based on estimates derived from variable vaccine effectiveness, with a power of 80 at an α of 0.05. Attack rates are those from Jeju Province (low estimate) and Inje-gun region of Gangwon Province (high estimate).

subject costs of US$20,000 USD, a study would cost US$14B on Jeju Island and $820M in Inje-gun. Thus, an alternative strategy is needed to evaluate a vaccine against SFTSV.

Assessment of SFTSV vaccine effectiveness

As discussed above, an SFTSV vaccine cannot be evaluated using a standard clinical trial approach. As a rare, high mortality disease, licensure via the animal rule would be considered appropriate. Vaccine effectiveness can be assessed by means of a registry that would compare disease incidence in vaccine recipients to a comparator population. Specifics of this approach are presented below.

SFTSV licensure via the animal rule

Approval of a drug using the animal rule requires that standard methods to assess drug effectiveness are not feasible. Second, a drug should be shown as effective in two animal models. It has already been demonstrated above that a placebo-controlled study is not feasible due to impossibly large sample sizes required. Below is presented information regarding potential animal models to test a vaccine.

SFTSV is highly pathogenic for adult ferrets and immune-suppressed interferon α/β receptor-deficient (IFNAR -/-) mice causing lethal infection.[35–37] For both animal models, viral RNA is detectable in multiple organs, although histologic evidence of organ pathology was only found in ferrets. Golden Syrian hamsters were shown to be susceptible to SFTSV infection and developed lethal infection by one group of investigators,[38] whereas others have reported that adult and newborn hamsters are resistant to SFTSV infection.[35] Additionally, immune-competent C57BL/6 mice have been shown to develop transient viremia and transient organ pathology following SFTSV infection.[39] Rhesus macaques also develop self-limited illness including fever and thrombocytopenia, along with transient viremia and elevations in liver transaminases.[40] Thus, there are a number of lethal and non-lethal animal models that can be used to assess an SFTSV vaccine for immunoprotection.

Assessment of SFTSV vaccine effectiveness

Following regulatory approval, effectiveness of an SFTSV vaccine can be assessed by comparing rates of infection in vaccine recipients to non-vaccinated individuals in the population as a function of time. Follow-up of vaccine recipients would be best accomplished through establishment of a registry of all vaccinees; "controls" would constitute the entire population in respective regions and sub-regions. This approach is outlined below and discusses the underlying structure and challenges. Such a registry would require input and oversight from central agencies, ideally from the respective country's CDC, to assist with data collection and analysis.

Assuming that licensure can occur early after the completion of immunogenicity studies in humans and animal challenge experiments, it is likely that vaccine utilization will be greatest in regions with the highest disease incidence, i.e. targeting those at greatest risk for infection, via two mechanisms. First, physicians caring for residents living in endemic regions have a high incentive to offer potential protection to their patient base, especially those who may be at increased risk for tick exposure. Second, as continued cases are reported in the lay press, there are many who may deem themselves at increased risk of infection, particularly those who may travel to areas with reported cases, and who may thus seek pre-travel vaccination.

A vaccine registry could require prescribing physicians to collect baseline and follow-up information from vaccine recipients. This could either be linked to vaccine distribution through a central agency, such as the country's CDC, or as policy. Information collected at baseline would ideally include basic demographics (age, residential zip codes, vocation). Physicians would be prompted to provide yearly health updates. Vaccine recipients would be asked to report episodes of fever and gastrointestinal illnesses, possibly returning for blood draws and urine collection at the time of illness for diagnosis. Active follow-up would increase the likelihood that all or nearly all incident infections in vaccine recipients would be identified. As additional persons are vaccinated, both the group size and exposure years increases.

Incidence of SFTSV infection in vaccinees would be compared to non-vaccinated regional "controls". It is noteworthy to consider that cases reported to the respective CDC consist primarily of symptomatic individuals who present to medical care who are subsequently diagnosed with SFTSV infection. Not represented are persons diagnosed with infection, but not reported to the CDC and those with subclinical infection who do not present for care and are not tested. Missing cases of SFTSV infection in controls may be small since the disease is considered highly symptomatic and recognition has significantly increased over time.[10] Thus, while the true incidence of infection in the control group may be underrepresented, underrepresentation may be small.

Therefore, such an analysis is skewed against finding differences between vaccine recipients and population control. Second, vaccination is assumed to provide multi-year immune-protection and/or booster vaccinations would be performed episodically as needed. If booster vaccinations are necessary and not performed periodically, it may reduce the expected difference in disease incidence between the two groups. Third, it is expected that the number of vaccine recipients represents only a very small fraction of the population as a whole and will not materially alter population numbers. Similarly, there is not the need for censoring for cases of incident infection with case rates

of 3.5 per 100,000 population (taking the example of Jeju Province). And fourth, infection risk is assumed similar for vaccine recipients and non-vaccinated individuals, although actual risk may be greater for vaccinees as discussed above.

For a range of predicted vaccine efficacy rates from 0% effective (no benefit) to 90% effective, it is possible to estimate expected case numbers predicted for two high-incidence regions in Korea, Jeju Province and Inje-gun (Table 6). Case rates for the vaccine group are based on the total number of persons "at-risk" in each respective years, summing the entirety of cases that could have been expected through the end of follow-up for each follow-up year. These estimates can be refined utilizing actual incidence rates and actual population statistics. To observe a total of five or more cases in the vaccine group in Jeju Province, assuming 2,500 individuals vaccinated yearly, would require 15 years of observation for a vaccine that is 50% effective and 20 years if vaccine efficacy is 75% (Table 6). And for Inje-gun region, assuming 250 individuals vaccinated yearly, it would require 10 years or 15 years to accrue five or more cases for a vaccine that is 50% or 75% effective, respectively. By calculating the number of cases expected over the years for various vaccination efficacies, we can estimate when sufficient evidence of efficacy would become statistically significant. Using continuity-corrected chi-square analysis of expected cumulative cases, we see that statistically significant case differences would emerge in approximately 15 years in Jeju Province and in approximately 10 years in the Inje-gun region if the vaccine has either 75% or 90% efficacy.

Mass vaccination programs

Because of the high mortality of infection, and the time and difficulty required to demonstrate efficacy, health agencies may elect to offer vaccination to those residing in or who travel to endemic regions for a vaccine that has demonstrated safety and also shown to be effective in animal models of infection. Any consideration of large-scale vaccination programs would necessarily require significant input and discussion from public health officials and physicians in those regions affected. Educational and informational campaigns are similarly necessary to understand the concerns of those targeted for vaccination. Even if an effective anti-viral drug is developed, SFTSV vaccination programs are beneficial to avoid excess mortality because of the delays in disease recognition, diagnosis, and inconsistent drug availability.

Caution is needed when considering large-scale vaccination, to avoid a false sense of expectations without knowledge of true vaccine effectiveness. The current experience in Africa surrounding Ebola campaigns is a sobering outcome when cultural fears have not been adequately addressed. Additionally, while antibody-dependent enhancement of infection has not yet been described for SFTSV or other bunyaviruses, one must be cognizant of these theoretical possibility.

Limitations of the study and consideration for mass vaccination

As discussed above, any estimate of vaccine efficacy and the presented analyses are limited in a number of ways. First, SFTSV disease incidence may change as better and more widely available diagnostic tests are developed. Importantly, disease efficacy is dependent on case identification and disease reporting and may miss cases with lower levels of symptomatology. Moreover, the validity of disease modeling will require continuous updating.

Conclusions

For tick-borne illnesses such as SFTS, while one might assume a regional concentration of cases that may make clinical trial design possible, the current knowledge of the epidemiology of

Table 6. Assessment of SFTSV vaccine effectiveness using a case-control design as a function of time; vaccine efficacy of 90%.

Region and incidence								
Jeju Province	Non-vaccinated		Vaccinated					
Time of follow-up	Person-years	Expected case #	Person-years	Expected case #				
Vaccine efficacy >				0%	25%	50%	75%	90%
1 year	600,000	21	2,500	0.1	0.1	0	0	0
3 years	1,800,000	63	15,000	0.5	0.4	0.3	0.1	0.1
5 years	3,000,000	105	37,500	1.3	1.0	0.7	0.3	0.1
10 years	6,000,000	210	137,500	4.8	3.6	2.4	1.2	0.5
15 years	9,000,000	315	300,000	10.5	7.9	5.3	2.6*	1.1 $^{\varphi}$
20 years	12,000,000	420	525,000	18.4	13.8	9.2*	4.6 $^{\varphi}$	1.8§
Inje-gun region	Non-vaccinated		Vaccinated					
Time of follow-up	Person-years	Expected case #	Person-years	Expected case #				
Vaccine efficacy >				0%	25%	50%	75%	90%
1 year	32,000	21.2	250	0.2	0.1	0.1	0	0
3 years	96,000	63.6	1500	1.0	0.7	0.5	0.2	0.1
5 years	160,000	105.9	3750	2.5	1.9	1.2	0.6	0.2
10 years	320,000	211.8	13,750	9.1	6.8	4.6	2.3*	0.9*
15 years	480,000	317.8	30,000	19.9	14.9	9.9*	5.0 $^{\varphi}$	2.0§
20 years	640,000	423.7	52,500	34.8	26.1	17.4 $^{\varphi}$	8.7§	3.5§

For each region (Jeju Province or Inje-gun), the population represents an estimate of the entire population and is assumed to remain constant each year with population increases to match vaccine recipients. Actual person-years can be adjusted based on actuals once the SFTSV vaccine is licensed and enters into clinical use. For the vaccine group, person-years are cumulative such that if 2,500 persons are vaccinated in year 1, this group represents 7,500 person-years at the end of year 2. For Jeju Province, the population is estimated as 600,000 and for Inje-gun 32,000 for the purposes of the table. Expected cases are based on the reported incidence of infection as reported for 2017, 3.5 and 66.2 cases per 100,000 population, respectively.

Differences in the number of cases would that become statistically significant under these conditions are denoted: * $p < 0.05$, $^{\varphi}$ $p < 0.01$, § $p < 0.001$. Comparisons were made using continuity-corrected Chi-Square analyses on the proportions of expected cases.

SFTS is more complex. SFTSV infection case rates have increased since the original discovery approximately 10 years ago; however, much of this increase is likely related to greater recognition of SFTSV and better and more available diagnostics. To date, there has been relatively limited research on the development of an SFTSV vaccine. Because of the extremely low incidence of disease, regulatory approval of vaccine candidates will rely on alternative pathways. Post-licensure evaluation of vaccine effectiveness is possible through a centralized registry of vaccine recipients.

Acknowledgements

Eun Hae Oh is acknowledged for editorial assistance. The library staff at Morristown Medical Center is also acknowledged for their assistance in researching SFTSV.

Disclaimer

GeneOne Life Science is a biotechnology company developing vaccines against multiple emerging infectious diseases including the Severe Fever and Thrombocytopenia Syndrome virus.

Disclosure of potential conflicts of interest

All authors receive a salary from GeneOne. GeneOne is developing a vaccine for SFTS.

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