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Clinical efficacy and safety evaluation of favipiravir in treating patients with severe fever with thrombocytopenia syndrome

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ABSTRACT

Background: Severe fever with thrombocytopenia syndrome (SFTS) is an emerging infectious disease with high mortality, however with no effective therapy available.
Methods: The effect of favipiravir (FPV) in treating SFTS was evaluated by an integrated analysis on data collected from a single-arm study (n=428), a surveillance study (n=2350) and published data from a randomized controlled trial study (n=145). A 1:1 propensity score matching was performed to include 780 patients: 390 received FPV and 390 received supportive therapy only. Case fatality rates (CFRs), clinical progress, and adverse effects were compared.
Findings: FPV treatment had significantly reduced CFR from 20.0% to 9.0% (odds ratio 0.38, 95% confidence interval 0.23-0.65), however showing heterogeneity when patients were grouped by age, onset-to-admission interval, initial viral load and therapy duration. The effect of FPV was significant only among patients aged ≤70 years, with onset-to-admission interval ≤5 days, therapy duration ≥5 days or baseline viral load $\leq 1 \times 10^6$ copies/mL. Age-stratified analysis revealed no benefit in the aging group >70 years, regardless of their sex, onset-to-admission interval, therapy duration or baseline viral load. However, for both ≤60 and 60-70 years groups, therapy duration and baseline viral load differentially affected FPV therapy efficiency. Hyperuricemia and thrombocytopenia, as the major adverse response of FPV usage, were observed in >70 years patients.
Interpretation: FPV was safe in treating SFTS patients but showed no benefit for those aged >70 years. Instant FPV therapy could highly benefit SFTS patients aged 60-70 years.
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1. Introduction

Severe fever with thrombocytopenia syndrome (SFTS) is a viral hemorrhagic fever (VHF) that was first reported in China in 2009 [1], which was later reported in Japan, Korea and other Asian countries [2–6]. The causative agent of SFTS is a novel *Bandavirus* of the *Phenuiviridae* family, *Dabie bandavirus*, also named SFTS virus (SFTSV). Novel SFTSV-like viruses continued to be detected or isolated around the world. The major clinical symptoms and laboratory findings of SFTS include fever, gastrointestinal symptoms, thrombocytopenia, leukopenia, and elevated serum hepatic enzyme levels [7]. Severe complications were reported in critically ill patients, eventually leading to an average case fatality rate (CFR) of 12% to 50% [1–3,8]. However, there are currently no approved effective antiviral agents for either treating SFTS or other SFTS-like diseases.

Favipiravir (FPV) is a novel RNA-dependent RNA polymerase (RdRp) inhibitor, which has been initially licensed as an anti-influenza drug in Japan [9]. In the fight against the coronavirus disease

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Research in context

Evidence before this study

We performed a search on PubMed for articles published from April 1, 2011 to June 1, 2021 using the search terms "favipiravir" or "T-705" and "severe fever with thrombocytopenia syndrome" or "SFTS" or "bunyavirus" or "SFTSV", with no language restrictions. Altogether 21 papers were retrieved, among which five were review papers and seven were unrelated to the study topic. Six studies identified the efficacy of favipiravir (FPV) in treating SFTSV infection by *in vitro* or animal models. Three studies reported the clinical efficiency of favipiravir in treating clinical patients, which were case report, single-arm study and RCT study, respectively. But all of them were of small case number no more than 145 patients (with the RCT study reporting data from 74 cases and 71 controls). None of the studies have ever reported the clinical efficiency among subgroups of patients (e.g., age, sex, underlying condition, etc.), drug regimens or timing. The differential effect from heterogenous patients have not been explored.

Added value of this study

Here by performing an integrated analysis on multiple data sources from an RCT study, a single-arm study and a surveillance study, we performed a comprehensive evaluation of clinical efficacy and safety of FPV in treating SFTS patients. The current study employed broad eligibility criteria to recruit large sample size, heterogeneous populations of SFTS patients. This allowed an opportunity to evaluate the effect in treating patients who might have been excluded from the RCT study, when the rigorous protocol was administered. We confirmed that FPV treatment could significantly reduce the case fatality rate of SFTS, however showing heterogeneous effects when patients were grouped by age, onset-to-admission interval, initial viral load and therapy duration. The effect of FPV was significant only among patients aged $\leq$70 years, with onset-to-admission interval $\leq$5 days, therapy duration $\geq$5 days or baseline viral load $\leq 1 \times 10^6$ copies/mL. Further age stratified analysis revealed no benefit in the aging group $>$70 years, regardless of their sex, onset-to-admission interval, therapy duration or baseline viral load. However, for both $\leq$60 and 60-70 years groups, therapy duration and baseline viral load affected FPV therapy efficiency differentially. Hyperuricemia and thrombocytopenia, as major adverse responses of FPV usage, was observed in patients $>$70 years. A better effect could be obtained when administered at early phase of illness and/or with adequate therapy duration, especially for patients $\leq$70 years old. The drug should be prescribed with high caution for patients aged $>$70 years, since there is no therapy benefit anyway.

Implications of all the available evidence

The current study verified the safety of FPV in treating patients with SFTS, provided therapeutic insights to target the specific population with the highest benefit from FPV treatment. These findings might also contribute to precision medical interventions for other diseases with similar pre-clinical benefit from FPV, such as Ebola and other viral hemorrhagic fever.

2019 (COVID-19), FPV has quickly been approved for usage as an oral medication in several countries, such as China, Russia and India [10]. However, limited data are available regarding safety and efficacy of FPV [11,12]. In addition to influenza virus and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), FPV has shown antiviral activities against a broad spectrum of other RNA virus, encompassing arena-, bunya-, flavi-, and alphaviruses, which can likewise cause VHF and/or encephalitis with high mortalities. This has mostly been displayed on Ebola virus, Lassa virus, rabies virus, norovirus and SFTSV by *in vitro* or animal studies [9]. FPV treated mice with SFTSV infection had seen no deaths, demonstrating robust protective effect of FPV against SFTSV when compared with ribavirin treated group which suffered many deaths [13]. Inspired by the potential therapy effect of FPV, we have performed the first randomized controlled trial (RCT) in the context of good quality supportive care, confirmed the therapeutic benefit of FPV in SFTS [14]. A multicenter non-randomized, uncontrolled single arm trial that was performed in Japan also supported the effectiveness of favipiravir for patients with SFTS [15]. But due to the small case number and population limitations of the available studies, the comparison of therapy effect and adverse response from population subgroups, different dose, regimens, or timing was lacking, the robustness of the results needs to be verified by a large-scale clinical study.

Here by using an integrated analysis on multiple data sources from a single-arm study, a surveillance study and a previous RCT study [14], we evaluated the clinical efficacy and safety of FPV treatment for SFTS patients, with the purpose of examining the generalizability of RCT findings to the overall disease population.

2. Methods

2.1. Study design and participants

This study presents an integrated analysis using data collected from three studies: a RCT study [14] (clinical trial registry number: ChiCTR1900023350), a single-arm study (clinical trial registry number: ChiCTR2100043342) on the therapy effect of FPV in treating SFTS, and a hospital-based sentinel surveillance study on SFTS patients. All studies had been conducted in the 990th Hospital, a designated hospital for SFTS therapy in Xinyang city, Henan province, China. Briefly, all the studied patients were laboratory-confirmed SFTS defined and treated as guided by National Health Commission of China [16].

Prior to data collection for the current research, we established an inclusion and exclusion protocol, which was utilized to screen patients and retrieve clinical data from the medical records of each patient (Fig. 1). For the patients recruited in the RCT study and single-arm study, similar exclusion criteria had been applied, i.e., those aged $<$18 years, with contraindications to FPV (i.e., pregnant/lactating women, having a history of gout or hyperuricemia, having a history of hypersensitivity to an antiviral nucleoside-analog drug targeting a viral RNA polymerase), and with other vector-borne diseases. For the studied participants in RCT, those having chronic diseases, currently using adrenocorticosteroids or immunosuppressive drugs were additionally excluded. For patients in the surveillance study, no predesigned therapy with FPV or other antiviral therapy were administered, thus no contraindications to FPV were applied. For the current analysis purpose, patients who received FPV therapy $\geq$3 days from the RCT study and single-arm study were included into the FPV treated group, patients who did not receive FPV therapy from the RCT study and surveillance study were included into the non-FPV group.

2.2. Treatment

All patients in FPV group received therapy at the first day of laboratory diagnosis of SFTS due to the emergency of the disease. FPV tablets were prescribed 1800 mg orally twice in the first day (3600 mg in total), and 1000 mg twice on day 2 which last at least 5 days or until to SFTSV RNA was reduced below the detection limit or until their

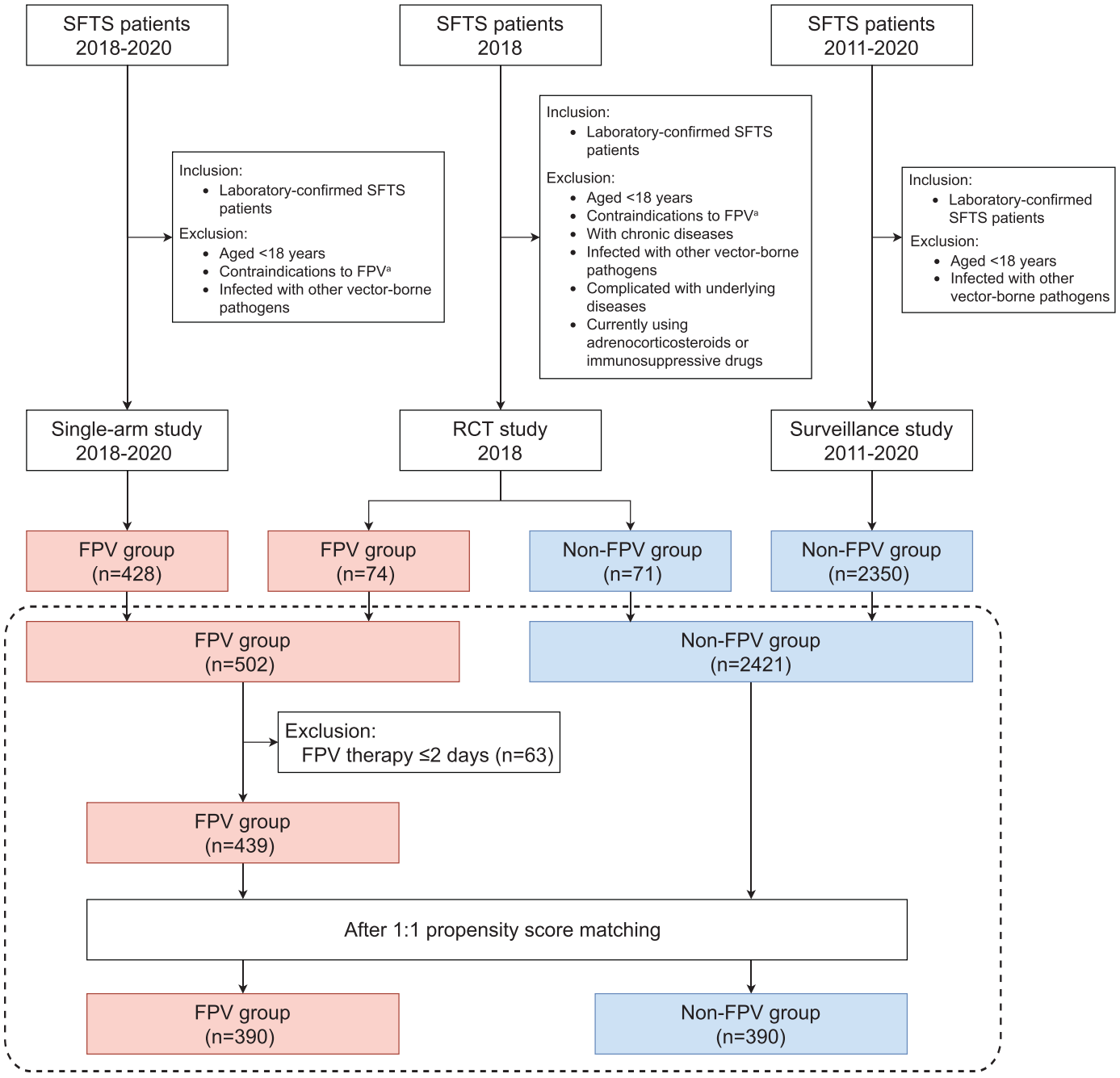


Fig. 1. Flowchart of the study design. [a]Contraindications to FPV referred to either of the following: pregnant/lactating women, having a history of gout or hyperuricemia and having a history of hypersensitivity to an antiviral nucleoside-analog drug targeting a viral RNA polymerase. FPV, favipiravir.

discharge from hospital, with administration duration no longer than 14 days. All other therapeutic decisions were at the discretion of the primary physician but were constrained to standard supportive care that was issued by the National Health Commission of China.

2.3. Ethics

The study protocol was approved by the hospital's ethics committee (NO. 154YY-LL-2018-02, 154YY-LL-2018-03). All patients provided written informed consent.

2.4. Data collection

Medical records of all the hospitalized patients had been extracted and sorted in a standard database with logic error correction function, thus ensuring the credibility of the data [8]. For the current research purpose, all the data regarding demography, preexisting comorbidities, clinical information, laboratory test results, and treatment regimens during the entire hospitalization were drawn from the database by a group of trained physicians using a standardized format and entered into an EpiData database. The data were further reviewed for accuracy and consistency by a second group of epidemiologists. For the patients who had demographic or medical history information missing, a trained study staff interviewed the patients or their family using a standardized supplemental questionnaire. Serum samples were collected from clinically diagnosed patients at admission and during hospitalization. Quantitation of virus was performed using Real-time RT-PCR targeting the same gene segments, which were expressed as copies/mL (detailed in Supplementary Appendix).

2.5. Outcome

The primary outcome was case fatality, which was firstly retrieved from medical records, and further verified by performing a follow-up visit required for all patients who discontinued therapy or had been discharged from hospital because of adverse clinical progression. The subgroup specific CFR were further calculated stratified by age (≤60, 60-70, >70 years), sex, interval from onset to hospital admission (1-5, ≥6 days), level of viral load on admission (≤1 × 10^6, >1 × 10^6 copies/mL) and duration of FPV therapy (3-4, ≥5 days). The secondary outcome was viral loads that were prospectively evaluated during hospitalization.

2.6. Safety analysis

The commonly seen adverse effect caused by FPV administration were recorded during the whole hospitalization, mainly involving gastrointestinal symptoms and abnormal measurements of laboratory parameters.

2.7. Statistical analysis

Descriptive statistics were performed to estimate frequency, proportion or rate for categorical variables, and to estimate median and interquartile range (IQR) for continuous variables. Categorical variables among groups were analyzed by chi-square test or Fisher exact test where appropriate. A Mann-Whitney U test was used to analyze continuous nonparametric data. To attain a comparison between FPV and non-FPV group with confounding controlled, we performed an optimal propensity score matching (PSM) in a 1:1 ratio to account for the inequality on the baseline characteristics (age, sex, and onset-to-admission interval). Briefly, a logistic regression model was used to calculate propensity scores predicting the probability of receiving FPV therapy. The nearest-neighbor matching algorithm with caliper widths equal to 0 was applied and a well-performed match was assessed by computing the standardized mean difference (SMD) of each covariate within perfect agreement. Based on the matched group, univariable and multivariable conditional logistic regression models were applied to estimate the effect from FPV therapy on CFR with odds ratio (OR) and 95% confidence interval (CI) estimated. Kaplan-Meier method was used to assess the survival rates, and the differences between groups with and without FPV treatment were analyzed by the log-rank test. The generalized estimating equation (GEE) was constructed to compare the inter-group difference in viral loads and laboratory parameters that were continuously evaluated over time. A two-sided P value of less than 0.05 was considered statistically significant. Statistical analysis was performed using R version 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria).

2.8. Role of funders

The funders did not have any role in the study design, data collection, analysis, interpretation, writing or submission of the manuscript. The corresponding author had complete access to the data and hold final responsibility for the decision to submit for publication.

3. Results

3.1. Patients and baseline analysis

A total of 2860 laboratory-confirmed SFTS patients, comprised of 439 (15.3%) receiving FPV therapy and 2421 (84.7%) without FPV therapy, met the current inclusion criteria (Fig. 1). By performing one-to-one greedy matching of PSM (Table 1), 390 patients in the FPV group and 390 in the non-FPV group who were comparable for age, sex and onset-to-admission interval, were used for the final analysis. Most of the clinical manifestations that were recorded on admission were comparable between two groups (Table 1). Most of the key laboratory parameters that were evaluated on admission were comparable between two groups, except for different blood urea nitrogen (BUN) levels in patients aged ≤60 years and 60-70 years (Supplementary Table 1). These data indicated the post-matching groups were well-balanced before therapy was initiated.

Overall, FPV therapy was administered in 234 patients with a duration ≥5 days and in 156 patients with a duration of 3-4 days, resulting in a median duration of five (IQR 4-5) days. Among all prescribed supportive care, plasma transfusion, platelet transfusion and xuebijing (Chinese medicine for immunoregulation [17]) were administered with significantly higher frequencies in non-FPV group than in FPV group, while granulocyte colony-stimulating factor (G-CSF) was more frequently administered in FPV group (Supplementary Table 2). All of these therapy choices have not been proved for their efficiency in improving disease outcome, thus was considered as less likely to affect the therapy effect of FPV in the current study.

3.2. Effect of FPV administration on CFR

The overall CFR of the analyzed patients was 14.5% (113/780). The survival curves showed significantly lower CFR in FPV group than non-FPV group (9.0% [35/390] *vs.* 20.0% [78/390]; P<0.001; Fig. 2a). Age subgroup analysis revealed significantly reduced fatality among the patients aged ≤60 years and 60-70 years in the FPV treatment group (both P<0.001), but not among the >70 years group (P=0.461; Fig. 2b). Multivariable conditional logistic regression also disclosed a significant reduction of CFR associated with FPV therapy (adjusted OR, 0.38, 95% CI 0.23-0.65, P<0.001; Fig. 2c, Supplementary Table 3). Subgroup analysis by sex revealed significantly reduced CFR due to FPV therapy for both male (8.3% *vs.* 17.5%, OR=0.45, 95% CI 0.22-0.91, P=0.026) and female (9.9% *vs.* 23.5%, OR=0.23, 95% CI 0.08-0.62, P=0.004; Supplementary Table 3). For patients with short interval of admission (interval of 1-5 days from symptom onset to hospital admission), FPV treatment had significantly reduced CFR from 19.3% to 7.8% (OR=0.32, 95% CI 0.16-0.62, P=0.001), by contrast, no such effect was observed for those with delayed entrance (onset-to-admission interval ≥6 days). Based on the median viral load value (1 × 10^6 copies/mL) on admission, the patients were divided into LVL group (viral load ≤1 × 10^6 copies/mL) and HVL group (viral load >1 × 10^6 copies/mL). Within LVL group, FPV treatment was associated with a significantly reduced CFR compared to non-FPV group (0.5% *vs.* 13.0%, OR=0.03, 95% CI 0.004-0.27, P=0.001). Within HVL group, comparable CFR was observed between two groups. The FPV group was further compared for the effect from therapy duration, which revealed significantly reduced CFR in those receiving FPV therapy ≥5 days *vs.* non-FPV (5.1% *vs.* 21.4%, OR=0.12, 95% CI 0.04-0.37, P<0.001), while no significant reduce of CFR was observed in patients receiving FPV therapy for 3-4 days (Supplementary Table 3).

Survival analysis that based on stratification of sex, viral load, and therapy duration, were in line with those obtained from multivariable analysis. The only difference was seen for patients with longer onset-to-admission intervals ≥6 days, when a significantly increased survival was observed for FPV *vs.* non-FPV (P=0.035) (Fig. S1).

3.3. Age-stratified analysis on the therapy effect of FPV

Considering that older age was the most significant risk factor for death, we made an age-stratified analysis to explore whether other factors, i.e., sex, onset-to-admission interval, viral loads and therapy duration were modified by age (Fig. 3). Three age groups were classified (≤60 years, 60-70 years and >70 years group) and within each age groups, FPV treated and non-treated patients were well matched for the baseline characteristics before therapy was administered (Supplemental Table 4). Resembling results for age subgroup analysis, no significant effect of FPV treatment was identified among patients >70 years old,

Table 1
Baseline characteristics of SFTSV patients on admission in the current study

	Before Propensity Score Matching			After Propensity Score Matching		
	Non-FPV (N=2421)	FPV (N=439)	P value	Non-FPV (N=390)	FPV (N=390)	P value
Age, median (IQR)	63 (53-70)	65 (55-71)	0.038	64 (56-70)	64 (56-70)	1.000
≤60 years	997 (41.2)	160 (36.4)	0.176	141 (36.2)	141 (36.2)	1.000
60-70 years	831 (34.3)	164 (37.4)		155 (39.7)	155 (39.7)	
>70 years	593 (24.5)	115 (26.2)		94 (24.1)	94 (24.1)	
Sex, male, n (%)	985 (40.7)	191 (43.5)	0.269	162 (41.5)	162 (41.5)	1.000
Days from disease onset to clinic visit, median (IQR)	5 (4-7)	5 (4-6)	<0.001	5 (4-6)	5 (4-6)	1.000
Comorbidity, n (%)	811 (33.5)	178 (40.5)	0.004	154 (39.5)	162 (41.5)	0.560
Hypertension	269 (11.1)	84 (19.1)	<0.001	44 (11.3)	76 (19.5)	0.001
COPD	218 (9.0)	32 (7.3)	0.242	52 (13.3)	30 (7.7)	0.010
DM	148 (6.1)	33 (7.5)	0.266	23 (5.9)	30 (7.7)	0.319
CVH	230 (9.5)	41 (9.3)	0.916	40 (10.3)	34 (8.7)	0.463
CVD	84 (3.5)	16 (3.6)	0.854	24 (6.2)	13 (3.3)	0.064
CHD	73 (3.0)	27 (6.2)	0.001	13 (3.3)	24 (6.2)	0.064
TB	21 (0.9)	5 (1.1)	0.781	4 (1.0)	5 (1.3)	1.000
Malignancy	16 (0.7)	5 (1.1)	0.438	3 (0.8)	5 (1.3)	0.722
Nonspecific symptoms, n (%)						
Fever	2411 (99.6)	438 (99.8)	0.875	390 (100.0)	389 (99.7)	1.000
Chills	297 (12.3)	57 (13.0)	0.675	60 (15.4)	54 (13.8)	0.543
Headache	337 (13.9)	64 (14.6)	0.715	64 (16.4)	51 (13.1)	0.189
Dizziness	500 (20.7)	74 (16.9)	0.068	100 (25.6)	62 (15.9)	0.001
Feeble	2319 (95.8)	391 (89.1)	<0.001	380 (97.4)	348 (89.2)	<0.001
Myalgia	1971 (81.4)	336 (76.5)	0.017	311 (79.7)	299 (76.7)	0.298
Lymphadenectasis	1313 (54.2)	262 (59.7)	0.035	199 (51.0)	233 (59.7)	0.014
Rash	26 (1.1)	5 (1.1)	1.000	1 (0.3)	5 (1.3)	0.219
Respiratory symptoms, n (%)						
Cough	1229 (50.8)	215 (49.0)	0.490	214 (54.9)	185 (47.4)	0.038
Expectoration	953 (39.4)	155 (35.3)	0.108	176 (45.1)	132 (33.8)	0.001
Gastrointestinal symptoms, n (%)						
Nausea	1429 (59.0)	286 (65.1)	0.016	208 (53.3)	255 (65.4)	0.001
Vomiting	660 (27.3)	139 (31.7)	0.059	118 (30.3)	119 (30.5)	0.938
Diarrhea	496 (20.5)	114 (26.0)	0.010	84 (21.5)	97 (24.9)	0.270
Bleeding symptoms, n (%)						
Melena	25 (1.0)	7 (1.6)	0.433	5 (1.3)	5 (1.3)	1.000
Hemoptysis	11 (0.5)	2 (0.5)	1.000	1 (0.3)	2 (0.5)	1.000
Haematemesis	9 (0.4)	0 (0)	0.414	2 (0.5)	0 (0)	0.479
Macroscopic haematuria	31 (1.3)	0 (0)	0.033	11 (2.8)	0 (0)	0.001
Gingival bleeding	26 (1.1)	6 (1.4)	0.772	5 (1.3)	5 (1.3)	1.000
Neurological symptoms, n (%)						
Dysphoric	48 (2.0)	2 (0.5)	0.025	5 (1.3)	2 (0.5)	0.448
Convulsion	131 (5.4)	11 (2.5)	0.010	17 (4.4)	11 (2.8)	0.248
Confusion	127 (5.2)	9 (2.1)	0.004	19 (4.9)	8 (2.1)	0.031
Coma	26 (1.1)	0 (0)	0.056	4 (1.0)	0 (0)	0.133
Lethargy	75 (3.1)	14 (3.2)	0.919	14 (3.6)	11 (2.8)	0.542

Values are median (IQR) or n (%). IQR, interquartile range. COPD, chronic obstructive pulmonary diseases; DM, diabetes mellitus; CVH, chronic viral hepatitis; CVD, cerebrovascular diseases; CHD, chronic heart diseases; TB, tuberculosis.

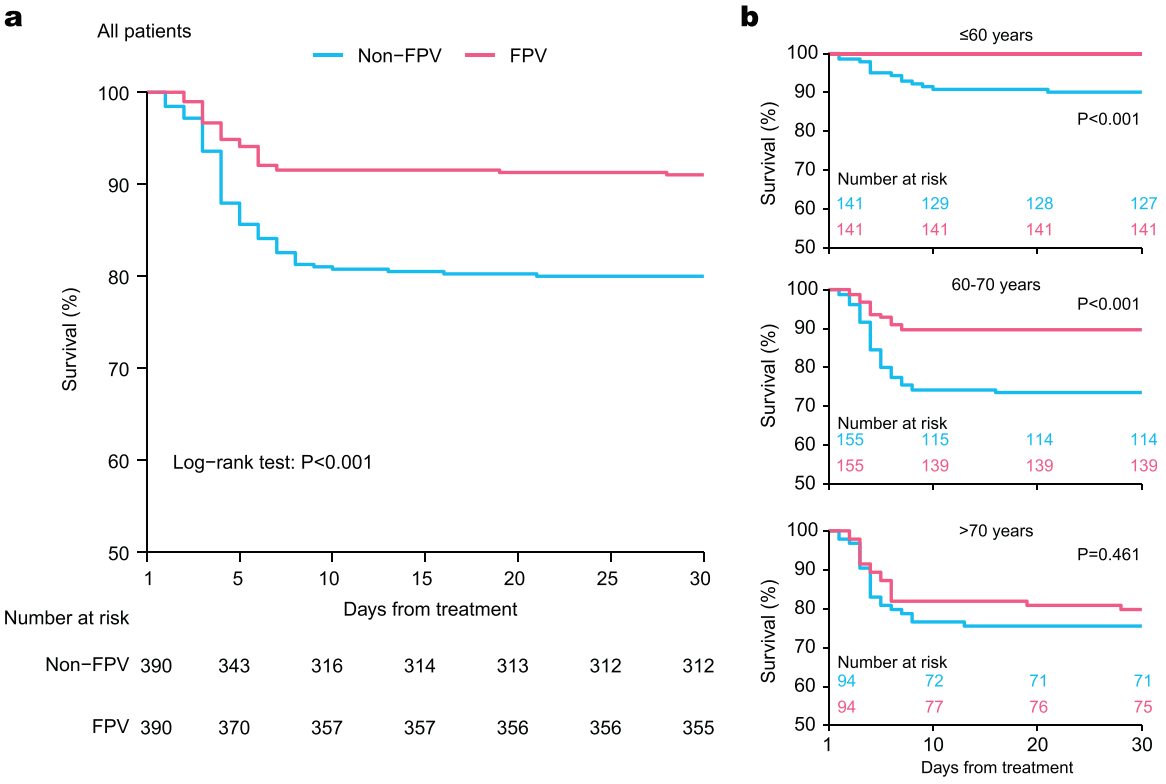
regardless of their further grouping for sex, onset-to-admission interval, initial viral load or duration of therapy. Sex showed no modifying effect, since significant effect of FPV treatment was observed for both sex when they were either ≤60 years or 60-70 years (Fig. 3a, Supplemental Table 5). Onset-to-admission interval affected the FPV efficiency in a similar manner for either ≤60 or 60-70 years group, i.e., significantly reduced CFR in patients who received FPV treatment within 5 days post symptom onset, while no longer significant for those treated ≥6 days post symptom onset (Fig. 3b, Supplemental Table 6). In contrast, therapy duration affected FPV efficiency differently between ≤60 and 60-70 years group. For patients ≤60 years old, FPV treatment was associated with a reduced CFR regardless of the therapy duration, while for patients aged 60-70 years, only those receiving FPV treatment ≥5 days showed a reduced CFR (Fig. 3d, Supplemental Table 7). Among patients aged 60-70 years old, FPV treatment was associated with reducing CFR only in the LVL group, while not in the HVL group. Unexpectedly, among the ≤60 years group, a significant effect of FPV treatment in reducing CFR was observed in the HVL group, while not in the LVL group. We postulated that the extremely low death number in the younger patients with LVL had hindered the inference of significance (Fig. 3c, Supplemental Table 8).

3.4. Profile of viral load in relate to FPV treatment

In the premise of comparable viral loads between FPV and non-FPV groups before therapy (median $\log_{10}$ copies/mL with IQR: 6.0 [5.2-6.8] *vs.* 6.1 [5.4-7.0]; Fig. 4a), differential viral patterns that were attributed to treatment had been displayed. For non-FPV group, viral load remained stably high till to the 5th day post admission, when gradual decrease was observed. For FPV group, an obvious decline in viral load was observed at day five post treatment. GEE analysis also verified rapid decrease in viral load in FPV group than non-FPV group ($P<0.001$). When the patients were further disseminated for baseline viral load, more rapid decrease in viral load was associated with FPV treatment in both LVL and HVL groups, yet with a higher decay rate from the LVL (61.0% *vs.* 47.5%; Fig. 4B).

3.5. Adverse effect of FPV therapy

Altogether three clinical manifestations which were probably related to adverse effect of FPV were evaluated. Among them, higher frequency of vomiting (14.9% *vs.* 9.0%, P=0.011; Supplementary Table 9), earlier development of nausea and diarrhea (Fig. S2a and c), were



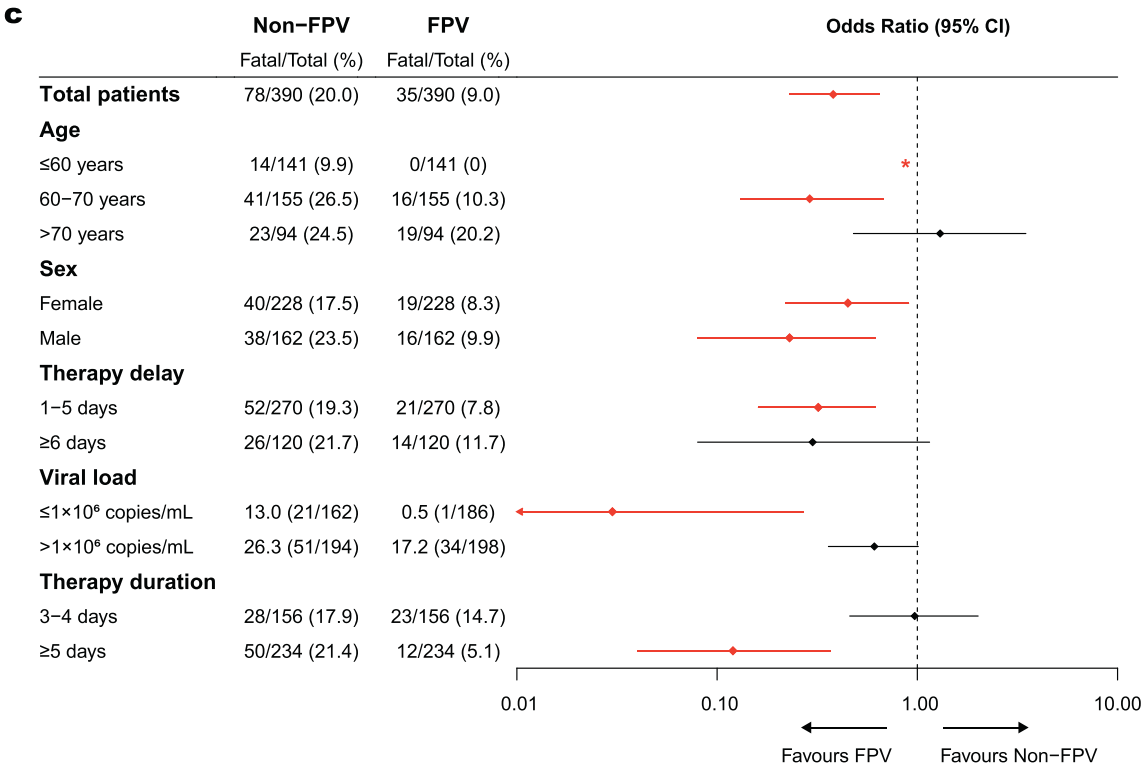


Fig. 2. The effect of FPV treatment in reducing case fatality rate of SFTS patients. a: Kaplan-Meier curves for FPV administration on the probability of survival for all SFTS patients. The numbers of at-risk patients at each time point were shown under the x-axis. P values were calculated by log-rank test. b: Kaplan-Meier curves for FPV administration on the probability of survival in three age groups. The numbers of at-risk patients at each time point were shown above the x-axis. P values were calculated by log-rank test. c: Forest plot in all patients and subgroups. Datapoints show odds ratios and error bars show 95% confidence interval. The red color represents $P<0.05$ and the black color represents $P\geq0.05$. In the subgroups of sex, therapy delay and duration, P values were calculated by multivariable conditional logistic regression model, after adjustment for comorbidities. In the subgroup of viral load, P values were calculated by multivariable logistic regression model, after adjustment for age, sex, delay from symptom onset to admission, and comorbidities. *P values were calculated by Pearson chi-square test. The odds ratio in the group of low viral load ($\leq1\times10^6$ copies/mL) on admission was 0.033 (0.004-0.265). FPV, favipiravir.

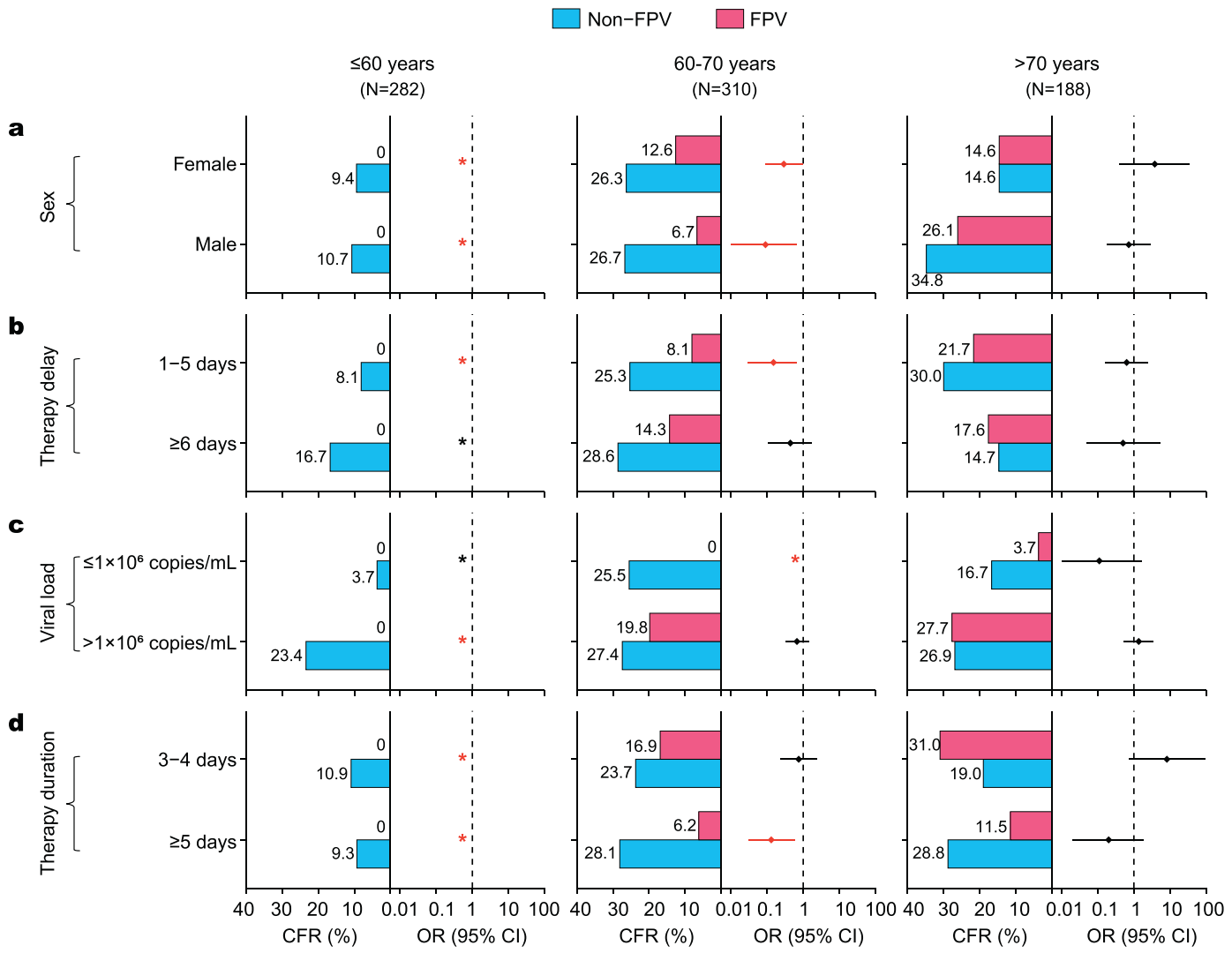


Fig. 3. Age-based subgroup analysis of the effect of FPV treatment in reducing case fatality rate of SFTS patients. The effect of FPV usage in reducing case fatality rate compared for sex (a), therapy delay (b), viral load (c) and therapy duration (d) in three age groups of SFTS patients. Datapoints show odds ratios and error bars show 95% confidence interval. The red color represents $P<0.05$ and the black color represents $P\geq0.05$. In the subgroups of sex, therapy delay and duration, P values were calculated by multivariable conditional logistic regression model, after adjustment for comorbidities. In the subgroup of viral load, P values were calculated by multivariable logistic regression model, after adjustment for age, sex, delay from symptom onset to admission, and comorbidities. *P values were calculated by Pearson chi-square test. CFR, case fatality rate; OR, odds ratio.

present in FPV group than non-FPV group. Higher frequency of vomiting was also seen for FPV treated patients aged ≤60 years old (19.1% *vs.* 9.2%, P=0.017; Supplementary Table 9) and onset time of nausea and diarrhea were earlier in FPV receivers ≤60 years and 60-70 years, respectively (P=0.014 and 0.004, respectively). FPV treatment did not have to be discontinued in any patients.

Selected laboratory parameters that might be altered due to FPV therapy were compared for their dynamic pattern between two groups. Lymphocytes%, neutrophils% and BUN levels recovered more quickly in FPV group (all $P<0.05$; Fig. S3a, b and d), although the median of BUN level was kept within normal range during the hospitalization. Hyperuricemia, a known adverse response of FPV usage was not seen. Despite of higher uric acid (UA) level in FPV than in non-FPV ($P<0.001$; Fig. S3h), the median level kept within normal range during the hospitalization. Aspartate aminotransferase (AST) was slightly increased in FPV group with significance. When further stratified by age, significantly decreased PLT and elevated UA were observed for >70 years group, with peaking median value seen at five days after commencement of treatment (Fig. S4c and h). For the other two age groups, the difference between FPV and non-FPV group were similarly observed for lymphocytes%, neutrophils%, BUN and UA levels, while the differences in AST level were not significant anymore (Fig. S5-6). Considering the adverse effect of liver damage from FPV therapy, we made additional analysis on patients with preexisting hepatitis (HBV or HCV). No severe laboratory abnormalities were observed in the hepatitis-SFTV patients receiving FPV therapy, compared with non-FPV receivers (Fig. S7).

4. Discussion

In clinical practice, FPV has been reported for treatment of human infections with life-threatening hemorrhagic fever virus, such as Ebola virus [18], Lassa virus [19], Arenavirus [20] and SFTSV [15], but only limited to case report or case series report with very small case number. Despite of FPV treatment benefit shown in the most recent RCT of SFTS [14], the result was obtained under near-ideal test conditions in highly selected patient populations, for whom the behavior of patients and investigators are highly compliant and adherent, and non-representative of routine clinical practice. Here by integrating three lines of data to enable the largest study on FPV therapy evaluation to date, we have displayed that FPV had a significant effect on decreasing SFTS related CFR (9.0% *vs.* 20.0%), which corresponded to a

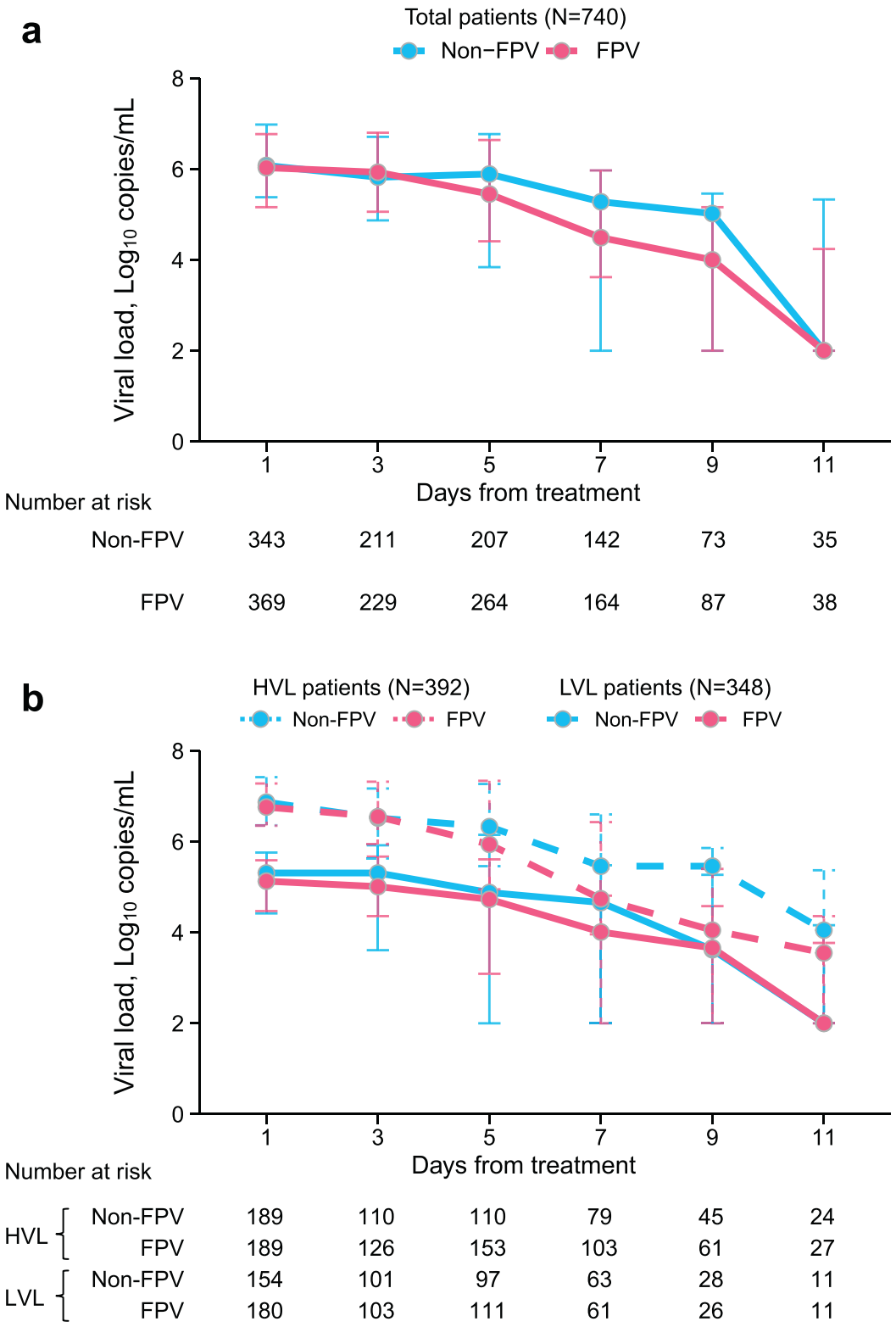


Fig. 4. Kinetics of viral loads in SFTS patients with or without FPV treatment. a: All patients; b: stratified by initial viral load on admission. Datapoints are median values and error bars show 95% confidence interval. LVL: low viral load ($\leq 1 \times 10^6$ copies/mL) on admission; HVL: high viral load ($>1 \times 10^6$ copies/mL) on admission. The numbers of patients who contributed to the at-risk population at each time point are shown under the x-axis.

55.0% (95% CI 23.2%-66.8%) reduced OR of fatal within non-FPV group. However, there was an inequity of FPV treatment effect when data were disseminated for analysis. The FPV treatment benefit was observed in patients aged ≤60 years and 60-70 years, with onset-to-admission interval ≤5 days, FPV therapy duration ≥5 days or baseline viral load $\leq 1 \times 10^6$ copies/mL, whereas no sex heterogeneity was observed.

Compared with the previous RCT study [14], we have displayed for the first time that the effect of FPV was remarkably determined by occasion and duration of drug use. This were in line with previous

animal studies showing that SFTSV infected mice had a higher survival rate when the FPV treatment was initiated on or earlier than three days post infection [13]. In a similar way, Oestereich et al. showed that initiation of FPV administration at day 6 post infection prevented a lethal outcome in 100% of the Ebola-infected mice, however, the delayed administration of FPV from 8 to 14 days, although prolonged the survival duration, only saved one of five mice [21]. Therefore, prompt diagnosis and timely initiation of treatment are critical for the intervention of SFTS.

The discrepancy owing to occasion of drug use was largely functional through level of viral loads, as the early administration obviously induced rapid virus clearance, indirectly leading to reduced organ damage and immunopathogenesis. In a non-randomized single-arm study of Ebola-infected patients in Guinea, FPV treatment reduced the mortality rate in the low viral load group [22]. The effect of FPV in SFTS patients with low viral loads on admission was revealed, in both reducing death rate and viremia, which was consistent with the underlying mechanism of FPV in viral clearance through inducing SFTSV mutations that are detrimental to viral proliferation [23−25].

We also revealed a critical role of aging in affecting the FPV effect. A notable finding was that when administered on patients ≤60 years, significant effect in reducing CFR was anyway observable independent of the therapy duration. By contrast, when administered on patients >70 years, no effect could be seen even for those receiving timely and long duration of therapy. For patients in the intermediate age group of 60-70 years, the effect of FPV was dependent on therapy delay, duration, and initial viral load. Approximately 26.5% of this age group succumbed to the disease, significantly higher than that of the ≤60 years old (9.9%) if given no antiviral therapy, therefore, a high vigilance should be given to this age group for an instant FPV therapy when the diagnosis was made and before the viremia soared. Moreover, a full course antiviral therapy should be advocated to reduce death in this age group.

The current study employed broad eligibility criteria to recruit large sample size, heterogeneous populations of SFTS patients. This allowed an opportunity to evaluate the effect in treating patients who might have been excluded from the RCT study, when the rigorous protocol was administered. We observed no more severe side effect that can aggravate the disease outcome owing to FPV therapy. The typical adverse response of hyperuricemia was indeed observed from FPV group, but limited in the >70 years patients, from which group a more rapid decrease in platelet counts was also observed. Therefore, the drug should be prescribed with high caution for patients aged >70 years, since there is no therapy benefit anyway. For patients with pre-existing hepatitis, no more severe laboratory abnormalities were found to be causative of drug usage. All these data, taken together, verified the safety of FPV.

One limitation of the study is that no dose effect was evaluated. Given that FPV dose of 1800/1000 mg BID was higher than the recommended 1600/600 mg BID that has been administered for severe influenza illness [26], it would seem that even the 1800/1000 mg BID dose was insufficient to generate a clinically meaningful antiviral effect for patient who failed to respond to the drug. On the other hand, this high dose was also well tolerated in treating SFTS, thus giving a reference as to the appropriate dose in the clinical practice. Another limitation is that only three variables were applied for matching, potential effect from other variables, such as the month of hospitalization, underlying diseases, etc, were not matched in the current analysis.

Overall, this current study that combined multiple data sources, in a clear way, showed the efficiency of FPV in treating SFTS patients. A better effect could be obtained when administered at early phase of illness and/or with adequate therapy duration, especially for patients ≤70 years old. No benefit was obtained for the >70 years old, for which population other therapy choice should be further investigated. These findings provide therapeutic insights to target the specific population with the highest benefit from FPV treatment and contribute to precision medical interventions for other diseases with similar pre-clinical benefit from FPV, such as Ebola and other viral hemorrhagic fever.

Declaration of Competing Interest

The authors declare that no conflict of interest exists.

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Data Sharing Statement

The study design, protocol and statistical analysis are provided in the main manuscript and the supplementary data files. The access to the data generated and analyzed in this study will be provided upon reasonable request to the corresponding author.

Contribution

J.Z., N.C., C.Y., Z-D.Y., W-S.Y., T.Y., X-F.P., S-M.L. and J-C.L. collected the epidemiological data and conducted laboratory tests. W.L., H.L., Q-B.L. and Y.Y. provided conception and designed the study. W.L., H. L., Q-B.L., Y.Y. and J.Z. cleaned, analyzed and interpreted the data. W. L., H-Q.W., X-A.Z., L-Q.F., Y-B.S. and D-N.Z. provided administrative, technical, or logistic support. W.L., Q-B.L. and Y.Y. drafted the manuscript. W.L., Q-B.L. and H.L. provided critical revision of the article for important intellectual content. All authors read and approved the final report.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2021.103591.

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