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Efficacy and safety of sofosbuvir/velpatasvir versus the standard of care in adults hospitalized with COVID-19: a single-centre, randomized controlled trial

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Objectives: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for the COVID-19 pandemic. The majority of patients experience asymptomatic to mild self-limited disease, but some cases progress to respiratory and multi-organ failure. However, so far, no approved antiviral therapy has been available for treatment of COVID-19. Sofosbuvir/velpatasvir (SOF/VEL) is an approved anti-HCV drug that is capable of suppressing other families of positive-sense RNA viruses with conserved polymerase and may be effective against SARS-CoV-2. This study was conducted to evaluate the efficacy of the SOF/VEL combination in addition to the national standard of care versus the national standard of care alone (hydroxychloroquine and lopinavir/ritonavir as well as supportive care) in patients with moderate to severe COVID-19 infection.

Methods: This single-centre, randomized, open-labelled, prospective clinical trial was done in patients with moderate to severe COVID-19 admitted to Farabi Hospital in Kermanshah Province, Iran. Eligible patients were randomly assigned in a 1:1 ratio to the SOF/VEL arm (SOF/VEL plus the national standard of care) or the control arm (the national standard of care alone). The main outcome of the study was the mortality on Day 28 after randomization. Secondary outcomes were time from the start of medication to clinical improvement, hospital length of stay, need for mechanical ventilation, duration of mechanical ventilation and conversion of RT–PCR results from positive to negative from the time of randomization to discharge. Adverse events were evaluated in all patients who started their assigned treatment.

Results: Between 11 April and 8 June 2020, 80 patients were recruited and randomly assigned into the SOF/VEL ($n = 40$) and control ($n = 40$) arms. The primary outcome was not significantly different between the two arms ($P = 1.00$). Secondary outcomes, including time to clinical improvement, hospital length of stay, need for mechanical ventilation, duration of mechanical ventilation and RT–PCR conversion, were not significantly different between arms either ($P > 0.05$). SOF/VEL treatment and the national standard of care were tolerated similarly.

Conclusions: Although treatment with SOF/VEL was safe, adding SOF/VEL to the standard of care did not improve the clinical status or reduce mortality in patients with moderate to severe COVID-19. However, larger randomized clinical trials including more parameters are needed for accurate estimation of the efficacy of SOF/VEL.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first reported in Wuhan, China on 31 December 2019. Since then, a significant number of people have been infected with the virus. However, extensive studies have shown that none of the potential drugs are effective for treatment of COVID-19.[1] A rapid increase in the prevalence of SARS-CoV-2 has prompted countries around the world to take immediate action to design therapeutic interventions or develop effective vaccines to prevent or treat COVID-19. The treatments that are currently in use include repurposed drugs, like antiviral drugs, antiparasitic drugs and anti-inflammatory drugs, as well as monoclonal antibodies.[2,3]

SARS-CoV-2 belongs to the family of betacoronaviruses. This family has other members, including Middle East respiratory syndrome human coronavirus (MERS-HCoV) and severe acute respiratory syndrome human coronavirus (SARS-HCoV).[4,5] HCoVs are a group of positive-sense single-stranded RNA (+ ssRNA) viruses that are very long (30 000 bp), characterized by two groups of proteins: non-structural proteins like RNA-dependent RNA polymerase (RdRp) (nsp12) and structural proteins, like matrix, envelope, spike and nucleocapsid.[5–9] RdRp is an enzyme with a significant effect on the life cycle of RNA viruses, including coronaviruses.[6,10]

HCV and Flaviviridae, like MERS and SARS-CoV-2 coronaviruses, are + ssRNA viruses that have similar replication mechanisms requiring RdRp. Therefore, it is possible that HIV or HCV and other Flaviviridae nucleoside/nucleotide analogues such as azithromycin, remdesivir and sofosbuvir could bind strongly to SARS-CoV-2 RdRp. A recent preliminary *in silico* study developed a SARS-CoV-2 RdRp model using homology modelling and sequence analysis. It was targeted using antipolymerase drugs, such as ribavirin and sofosbuvir. The available data indicated the theoretical efficacy of ribavirin and sofosbuvir for treatment of the new coronavirus.[6,11–13]

Velpatasvir is also an inhibitor that targets the HCV NS5A protein. There are recent reports of the inhibitory activity of velpatasvir tailored to A chain and B chain active sites of the coronavirus 3C-like protease (3CLpro).[11] Two-component HCV drugs (sofosbuvir/velpatasvir) may be well-favoured candidates for a repurposing application because they may inhibit two coronavirus enzymes. This combined drug targeting two viral proteins decreases development of resistance by the virus. Direct-acting antiviral drugs are also associated with very few side effects and are easily administered orally.[12,14] This study was conducted to evaluate the efficacy of the sofosbuvir/velpatasvir combination plus the national standard of care in adult patients hospitalized with moderate to severe COVID-19 infection.

Materials and methods

Study design and patients

This single-centre, open-labelled, randomized clinical trial was conducted to evaluate the efficacy and safety of oral sofosbuvir/velpatasvir as a fixed-dose combination in adult patients hospitalized with moderate to severe COVID-19 in Farabi Hospital, Kermanshah, Iran from 11 April to 8 June 2020. Because of the emergency nature of the trial, placebos of sofosbuvir/velpatasvir were not prepared. The patients were evaluated for eligibility based on a positive RT–PCR test for SARS-CoV-2 on a nasopharyngeal swab and/or a compatible chest CT scan. Individuals 18 years of age or older were eligible if they had an oxygen saturation (SaO_2) of 93% or less in ambient air and/or an absolute lymphocyte count of $<1.1 \times 10^9$ cells/L. Exclusion criteria were pregnancy and breastfeeding, a physician's decision against enrolment, conditions that did not allow complete implementation of the protocol, allergy or hypersensitivity to the drugs used in this trial, severe liver disease (e.g. cirrhosis or an ALT or AST level >5 times the upper limit of the normal range), use of medications that are contraindicated with the drugs used in this trial, known HIV infection (due to concerns about resistance to lopinavir/ritonavir if used without combination with other antiviral agents) and known HCV infection (due to concerns about resistance to sofosbuvir/velpatasvir due to short-term use of this drug). Patients who were unable to swallow received oral medications through a nasogastric tube.

Sample size

Although there were different outcomes in which the researchers were interested, it was decided to calculate the sample size based on the length of hospital stay. It was postulated that the real difference between the two groups was only 1 day. Therefore, the sample size was calculated assuming a 1 day difference between the two study arms with an SD of 1.5 days. To reject the null hypothesis that the population means of the sofosbuvir/velpatasvir and national standard of care arms were equal with a probability of 0.8, the number of patients was estimated as 36 for each arm. The probability of type I error associated with the test for the null hypothesis was 0.05.

Randomization and allocation

The participants were randomly assigned in a 1:1 ratio. In order to generate an allocation sequence, simple random allocation was applied using an Excel file; 80 eligible patients were enrolled in the study (40 individuals in each group). For allocation sequence concealment, the study arm for each patient was contained in a sealed envelope labelled with a number from 1 to 80.

Procedures

The intervention arm received a fixed-dose combination tablet containing 400 mg sofosbuvir and 100 mg velpatasvir (Shari Pharmaceutical Industry Co., Tehran, Iran) orally once daily for 10 days plus the national standard of care. The comparator arm only received the national standard of care including 400 mg hydroxychloroquine as a single dose and lopinavir/ritonavir (400 mg/100 mg) orally twice daily for 10 days as well as supplemental oxygen, non-invasive and invasive ventilation, antimicrobials, vasopressors and corticosteroids, if needed.

Ethics

Written informed consent was obtained from all patients, or their legal representatives if they were unable to provide consent. The protocol was approved by the Ethics Committee of Kermanshah University of Medical Sciences, Iran on 3 March 2020 (reference IR.KUMS.REC.1399.044). The study has been registered in the Iranian Registry of Clinical Trials (IRCT ID: IRCT20130812014333N145; https://www.irct.ir/trial/46790).

Clinical and laboratory monitoring

Nasopharyngeal swab samples were obtained on Day 1 (before the drugs were administered) and at the time of discharge for qualitative SARS-CoV-2 RT–PCR. The patients were visited daily by an infectious disease specialist and their treatment was carefully managed. Moreover, they were assessed every day by trained nurses using daily record cards and flowsheets that captured data on six-stage saturation status and safety from Day 1 to hospital discharge or death. If a patient was discharged before 28 days from enrolment, the patient's health status was monitored by phone on

the twenty-eighth day in terms of survival or death. The six-stage saturation status consisted of the following stages: (1) $SaO_2 > 93\%$, not requiring supplemental oxygen; (2) SaO_2 88%–93%, requiring supplemental oxygen at 4 L/min using a nasal cannula; (3) SaO_2 85%–87%, requiring supplemental oxygen at 6–10 L/min using a face mask; (4) SaO_2 80%–84%, requiring supplemental oxygen at 10–15 L/min with a reservoir bag; (5) $SaO_2 < 80\%$, requiring non-invasive mechanical ventilation; and (6) $SaO_2 < 80\%$, requiring invasive mechanical ventilation. Other clinical and laboratory data were recorded on paper files and then re-entered into an Excel file by the trial staff. Safety was monitored and recorded according to Good Clinical Practice guidelines.

Outcomes

The main outcome of the study was the mortality on Day 28 after randomization. Secondary outcomes were: time from enrolment to clinical improvement, defined as a decline of two stages in the six-stage saturation status, or hospital discharge, whichever occurred earlier; hospital length of stay; need for mechanical ventilation; duration of mechanical ventilation; and conversion of RT–PCR results from positive to negative from the time of randomization to discharge. Adverse events were evaluated in all patients who started their assigned treatment. Safety outcomes included adverse events during treatment, serious adverse events and early discontinuation of treatment.

Statistical analysis

Continuous variables, expressed as mean (SD) and median (IQR), were compared using the independent samples *t*-test and the Mann–Whitney test. The Shapiro–Wilk test was applied to evaluate the normal distribution of the data in each group. Categorical variables, presented as number (%), were compared using the chi-squared test. Moreover, Fisher's exact test was applied in the case of data sparsity. Using the Cox proportional hazards model, adjusted for time from starting symptoms to admission time, HRs with 95% CIs were calculated for time to clinical improvement and length of hospital stay. Stata software version 14 was used for statistical analysis. In the course of analysis, impossible range data and outliers were taken into consideration. $P < 0.05$ was considered as statistically significant. The proportional hazards assumption was confirmed.

Results

Patients

As Figure 1 shows, 59 of 139 patients with COVID-19 were excluded from the study for various reasons. Eighty patients were assigned to sofosbuvir/velpatasvir (sofosbuvir/velpatasvir plus the national standard of care) and control (only the national standard of care) arms in a 1:1 ratio. After randomization, one patient in the sofosbuvir/velpatasvir arm died before receiving the sofosbuvir/velpatasvir treatment and three patients in the control arm received sofosbuvir/velpatasvir at the physician's discretion. All of the hospitalized patients with SARS-CoV-2 infection were diagnosed according to the clinical signs/symptoms along with the radiographic findings. In addition, 59 (73.7%) patients had a positive RT–PCR test for SARS-CoV-2.

The mean of age of the participants was 54.1 ± 17.8 years. The male to female ratio was 1:1 and 1.2:1 in the sofosbuvir/velpatasvir and control arms, respectively; however, no statistically significant difference was observed ($P = 0.36$). The median time from the onset of symptoms to the start of the study treatment in the intervention and control groups was 7 (IQR 4–10) and 6 (IQR 4–10) days, respectively ($P = 0.22$). Hypertension (30%), diabetes mellitus (20%), cardiovascular diseases (17.5%) and pulmonary disorders (10%) were the most common comorbidities, respectively. No significant difference was observed between the groups in other treatments received (including antibiotics or corticosteroids). Also, there was no significant difference in terms of other demographic and clinical characteristics between the arms, except for the rate of anorexia ($P = 0.02$), the median of WBC count ($P = 0.05$), AST ($P = 0.04$) and creatinine levels ($P = 0.02$).

The demographic and clinical characteristics of the participants are represented in Table 1.

Primary outcome

As a primary outcome, for the intention-to-treat (ITT) population, the 28 day mortality rate in the sofosbuvir/velpatasvir and control arms was the same (three cases in each arm, 7.5%). For the modified ITT population, the mortality rate was numerically lower in the sofosbuvir/velpatasvir arm (5.1%) compared with the control arm (7.5%); however, the difference was not statistically significant. There were two cases of out-of-hospital deaths during the 28 day follow-up (one case in each arm). Besides, all deaths occurred among male patients and the mean age of the deceased was 61.3 ± 6.3 years. All of the cases had at least one pre-existing condition and comorbidities.

Secondary outcomes

The median time to clinical improvement within 28 days was shorter in the sofosbuvir/velpatasvir arm compared with the control arm [6 (IQR 4–8) versus 7 (IQR 4–11) days]. The HR for clinical improvement was estimated to be 1.2 (95% CI 0.6–2.2); however, the difference was not statistically significant ($P = 0.30$). Furthermore, results for the modified ITT population were not statistically different. Figure 2 shows the probability of clinical improvement within 28 days for the two trial arms.

Another secondary outcome was the length of hospital stay. The median length of hospital stay was 6 (IQR 5–8.5) days for the ITT population in the sofosbuvir/velpatasvir arm, which was shorter than the 7 (IQR 5–13) days hospital stay in the control arm (Figure 3). The HR was 1.6 (95% CI 0.9–2.5); $P = 0.25$. For the modified ITT population, the median length of hospital stay was 6 (IQR 5–9) days in the sofosbuvir/velpatasvir arm and 7 (IQR 5–13) days in the control arm (HR 1.6; 95% CI 0.9–2.5; $P = 0.89$). For the ITT population, one patient in the sofosbuvir/velpatasvir arm (2.4%) and three patients in the control arm (8.1%) needed invasive mechanical ventilation ($P = 0.61$). Other secondary outcomes, such as duration of mechanical ventilation and conversion of nasopharyngeal swab RT–PCR result were not significantly different between the two arms; $P = 0.51$ and 0.49, respectively (Table 2).

In the ITT population, the median time from randomization to death was shorter in the sofosbuvir/velpatasvir arm compared with the control arm [6 (IQR 2–9) versus 7 (IQR 7–30) days; $P = 0.38$]. The median time to death was 7.5 (IQR 6–9) and 7 (IQR 7–30) days for the modified ITT population in the sofosbuvir/velpatasvir arm and the control arm, respectively.

For subgroup analysis, the patients in both arms were divided into two groups: those who received treatment within 7 days of the onset of symptoms and those who received it after 7 days. Based on the results, in patients who received treatment within

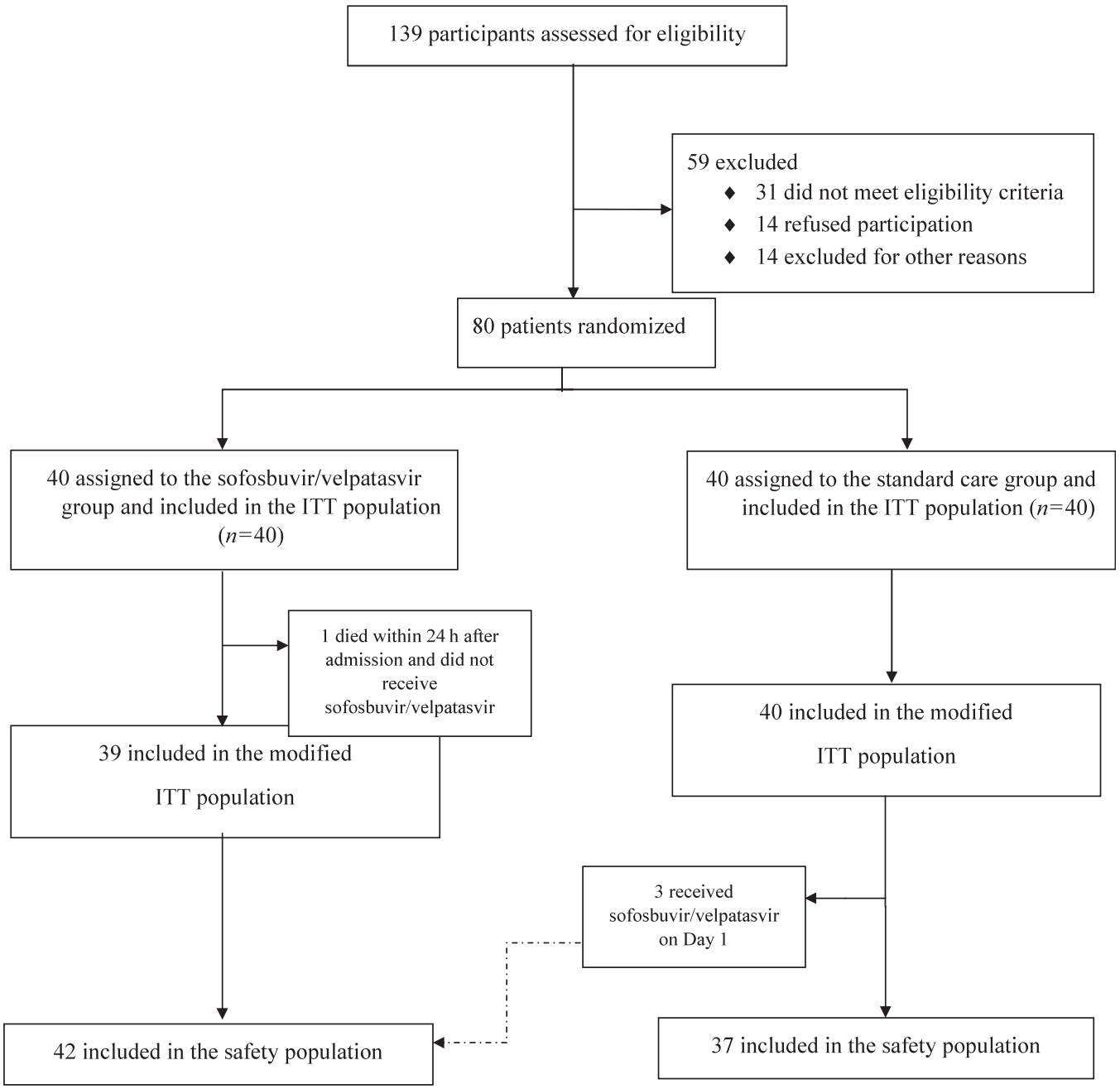

Figure 1. Randomization and treatment assignment.

7 days of the onset of symptoms, the median time to clinical improvement was 6 (IQR 5–9) days in the sofosbuvir/velpatasvir arm and 6 (IQR 4–10) days in the control arm, indicating no significant difference ($P = 0.85$). Moreover, the median time to clinical improvement was 5.5 (IQR 4–8) and 8 (IQR 6–11) days in patients who received sofosbuvir/velpatasvir and patients who received only the national standard of care after 7 days of the onset of symptoms, respectively, but the difference was not statistically significant ($P = 0.20$). These analyses were repeated for hospital stay and time to death, but no significant differences were observed ($P = 0.20$).

Safety evaluation

Adverse events were not reported by 12 of 42 patients (27.9%) in the sofosbuvir/velpatasvir arm and 14 of 37 patients (37.8%) in the control arm during the study (Table 3). The most common adverse events in the sofosbuvir/velpatasvir arm were nausea and vomiting (18.6%), diarrhoea (9.3%) and headache (4.7%), compared with nausea and vomiting (18.9%), headache (8.1%) and diarrhoea (2.7%) in the control arm. A significantly higher incidence of diarrhoea was observed in the sofosbuvir/velpatasvir arm compared with the control arm [4 of 42 (9.3%) versus 1 of 37 (2.7%);

Table 1. Demographic, clinical and laboratory characteristics of the patients at baseline in the ITT population

Characteristics	All patients	Sofosbuvir/velpatasvir ($n = 40$)	Control ($n = 40$)	*P* value[a]
Age (years), mean ± SD	54.1 ± 17.8	53.6 ± 16.3	54.6 ± 19.4	0.81
Male sex, *n* (%)	44 (55)	20 (50)	24 (60.0)	0.36
Had contact history, *n* (%)	15 (18.8)	7 (17.5)	8 (20)	0.96
Positive RT–PCR, *n* (%)	59 (73.7)	30 (75.0)	29 (72.5)	0.94
Time from starting symptoms to admission (days), median (IQR)	7 (4–10)	7 (4–10)	6 (4–10)	0.22
Corticosteroid use, *n* (%)	10 (12.5)	3 (7.5)	7 (17.5)	0.31
Antibiotic use, *n* (%)	75 (95.0)	37 (92.5)	39 (97.5)	0.30
Comorbidities, *n* (%)				
Hypertension	24 (30)	9 (22.5)	15 (37.5)	0.22
Diabetes	16 (20)	10 (25)	6 (15)	0.40
Cardiovascular disease	14 (17.5)	4 (10)	10 (25)	0.06
Pulmonary disorders	8 (10)	4 (10)	4 (10)	1.00
Others	11 (13.7)	6 (15.0)	5 (12.5)	0.74
Chief complaint, *n* (%)				
Anorexia	58 (72.5)	34 (85)	24 (60)	0.02
Cough	57 (71.2)	27 (67.5)	30 (75)	0.62
Chills	54 (67.5)	29 (72.5)	25 (62.5)	0.47
Fever	52 (65)	27 (67)	25 (62.5)	0.81
Vital signs, median (IQR)				
Temperature (°C)	37 (36.5–37.4)	37 (36.5–37.3)	37 (36.6–37.4)	0.80
Systolic blood pressure (mmHg)	120 (110–130)	120 (110–130)	120 (110–130)	0.98
Respiratory rate (breaths/min)	18 (18–19)	18 (18–19)	18 (18–19)	0.79
Pulse rate (beats/min)	87 (80–93)	84 (80–94)	87.5 (84–93)	0.80
O_2 saturation (%)	92 (88–94)	93 (90–94)	91 (86.5–93)	0.09
Complete blood count				
WBC count (cells/L), median (IQR)	6.8 (4.9–11.6) $\times 10^9$	5.65 (4.1–8.6) $\times 10^9$	7.5 (6.5–12) $\times 10^9$	0.05[b]
$>10 \times 10^9$, *n* (%)	27 (33.7)	10 (25.0)	17 (42.5)	
$4–10 \times 10^9$, *n* (%)	41 (51.3)	21 (52.5)	20 (50.0)	
$<4 \times 10^9$, *n* (%)	12 (15.0)	9 (22.5)	3 (7.5)	
Lymphocyte count (cells/L), median (IQR)	1.2 (0.9–1.7) $\times 10^9$	1.1 (0.9–1.6) $\times 10^9$	1.3 (1.0–2.0) $\times 10^9$	0.61[b]
$\geq 1.1 \times 10^9$, *n* (%)	53 (66.3)	21 (52.5)	32 (80.0)	
$<1.1 \times 10^9$, *n* (%)	27 (33.7)	19 (47.5)	8 (20.0)	
Platelet count (cells/L), median (IQR)	193 (135–227) $\times 10^9$	185.5 (132–219) $\times 10^9$	206 (160–284) $\times 10^9$	0.20[b]
$\geq 100 \times 10^9$, *n* (%)	73 (91.2)	36 (90.0)	37 (92.5)	
$<100 \times 10^9$, *n* (%)	7 (8.8)	4 (10.0)	3 (7.5)	
Haemoglobin (g/dL), median (IQR)	14 (12.9–15.8)	13.75 (13–15.2)	14.1 (12.8–16.3)	0.32
Biochemical parameters				
Fasting blood sugar (mg/dL), median (IQR)	116 (101–142.5)	114.5 (102–141.5)	120 (99.5–146.5)	1.00[b]
≥126, *n* (%)	32 (40.0)	14 (35.0)	18 (45.0)	
<126, *n* (%)	48 (60.0)	26 (65.0)	22 (55.0)	
Creatinine (mg/dL), median (IQR)	1 (0.9–1.1)	1 (0.8–1)	1.04 (1–1.2)	0.02[$]
≥1.5, *n* (%)	9 (11.2)	4 (10.0)	5 (12.5)	
<1.5, *n* (%)	71 (88.8)	36 (90.0)	35 (87.5)	
AST (U/L), median (IQR)	29 (22–39)	25 (19–41)	26.5 (22–37)	0.04[b]
≤30, *n* (%)	40 (50.0)	32 (80.0)	16 (40.0)	
>30, *n* (%)	22 (50.0)	8 (20.0)	24 (60.0)	
ALT (U/L), median (IQR)	25 (19–41)	32 (22–43)	24.5 (18.5–42.5)	0.90[b]
≤30, *n* (%)	61 (76.3)	24(60.0)	29 (72.5)	
>30, *n* (%)	19 (23.2)	16 (40.0)	11 (27.5)	
Creatine kinase (U/L), median (IQR)	102 (69–182)	123 (70–169)	94 (46.5–193.5)	0.96[b]
≥185, *n* (%)	28 (35.0)	11 (27.5)	17 (42.5)	

Continued

Table 1. *Continued*

Characteristics	All patients	Sofosbuvir/velpatasvir ($n=40$)	Control ($n=40$)	*P* value[a]
<185, *n* (%)	52 (65.0)	29 (72.5)	23 (57.5)	
Lactate dehydrogenase (U/L), median (IQR)	429 (321–574)	413 (315–533)	458.5 (360–624)	0.40[b]
≥245, *n* (%)	78 (97.5)	38 (95.0)	40 (100.0)	
<245, *n* (%)	2 (2.5)	2 (5.0)	0 (0.0)	
Triglycerides (mg/dL), median (IQR)	118.5 (79.5–157)	117 (77–171)	120.5 (80–146)	0.80[b]
≥200, *n* (%)	23 (28.8)	11 (27.5)	12 (30.0)	
150–200, *n* (%)	7 (8.7)	6 (15.0)	1 (2.5)	
<150, *n* (%)	50 (62.5)	23 (57.5)	27 (67.5)	
Ferritin (ng/mL), median (IQR)	412 (170–674)	444.2 (244.5–595)	356 (148.1–698)	0.31[b]
≥300, *n* (%)	62 (77.5)	32 (80.0)	30 (75.0)	
<300, *n* (%)	18 (22.5)	8 (20.0)	10 (25.0)	
D-dimer (ng/mL), median (IQR)	891 (669–2288)	860 (579–2280)	1100 (701–2546)	0.67[b]
≥250, *n* (%)	75 (93.8)	35 (87.5)	40 (100.0)	
<250, *n* (%)	5 (6.2)	5 (12.5)	0 (0.0)	
Erythrocyte sedimentation rate (ng/mL), median (IQR)	30 (17–45)	27 (18–45)	32 (15–42)	0.81[b]
≥30, *n* (%)	47 (58.8)	21 (52.5)	26 (65.0)	
<30, *n* (%)	33 (41.2)	19 (47.5)	14 (35.0)	

[a]*P* value less than 0.05 (typically ≤0.05) is statistically significant.
[b]*P* values are expressed to compare statistical differences between the medians of two groups.

$P < 0.001$]. There were no significant differences in the incidence of other adverse events between the arms. In addition, no apparent differences in biochemistry laboratory abnormalities were seen between the arms (Table 3).

Discussion

As stated earlier, there are no specific treatments for COVID-19 as yet, though a number are under evaluation, including experimental antivirals. SARS-CoV-2 RdRp especially is very likely to be effectively inhibited by sofosbuvir (which is capable of suppressing other families of positive-strand RNA viruses: Flaviviridae and Togaviridae). More importantly, sofosbuvir is safe and well tolerated at 400 mg (and even at 1200 mg) daily in a 24 week therapeutic regimen.[12] The active metabolite of sofosbuvir, however, shows an extremely high intracellular stability, so it is hypothesized that SARS-CoV-2 infection *in vivo* could also be susceptible to sofosbuvir;[12] on the other hand, the inhibitory activity of velpatasvir tailored to A chain and B chain active sites of the coronavirus 3CLpro has been reported, so we were convinced to design and run the current clinical trial study to evaluate the efficacy of a combination of sofosbuvir and velpatasvir plus the national standard of care in adult patients hospitalized with moderate to severe COVID-19 infection.

In our trial, the addition of sofosbuvir/velpatasvir to the current national standard of care did not result in a significant reduction in the 28 day mortality rate in patients admitted to hospital with moderate to severe COVID-19. Furthermore, patients in the sofosbuvir/velpatasvir arm had an almost similar mortality, time to clinical improvement, duration of hospital stay, RT–PCR conversion, need for mechanical ventilation and time free from mechanical ventilation compared with patients in the control arm.

We used the current FDA-approved dosing for HCV treatment (400 mg sofosbuvir and 100 mg velpatasvir orally once daily for 10 days). Though the fixed-dose combination sofosbuvir/velpatasvir for treatment of a hepatotropic virus has been designed/developed to facilitate intracellular penetration in liver tissue, the presence of kinase/esterase enzymes in the lung can, in part, explain the successful uptake and intracellular activation of sofosbuvir in alveolar epithelial cells, as a viral reservoir. We may also assume that cellular uptake of the pro-drug (sofosbuvir) as well as intracellular concentrations of biologically active triphosphate metabolites within lung epithelial cells are low, compared with those in hepatocytes.[12] Also, the estimated level of the triphosphate metabolites are greater than the inhibition constant for the HCV NS5B polymerase.[15] Although sofosbuvir/velpatasvir may effectively inhibit the SARS-CoV-2 RdRp, our results suggest that higher treatment doses against SARS-CoV-2 in respiratory epithelial cells may be needed than those recommended for HCV. In addition, the sample size we used was relatively small and sofosbuvir/velpatasvir administration along with the standard of care at early stages of disease may be more effective. These limitations may have influenced the effectiveness of sofosbuvir/velpatasvir versus the standard of care; therefore, the results should be interpreted with caution.

By the time of this study, three randomized clinical trials had compared the efficacy of oral sofosbuvir/daclatasvir and the standard of care in COVID-19 treatment and reported inconsistent results.[16–18] However, Simmons *et al.*[19] conducted a meta-analysis of these three studies with 176 patients and concluded that

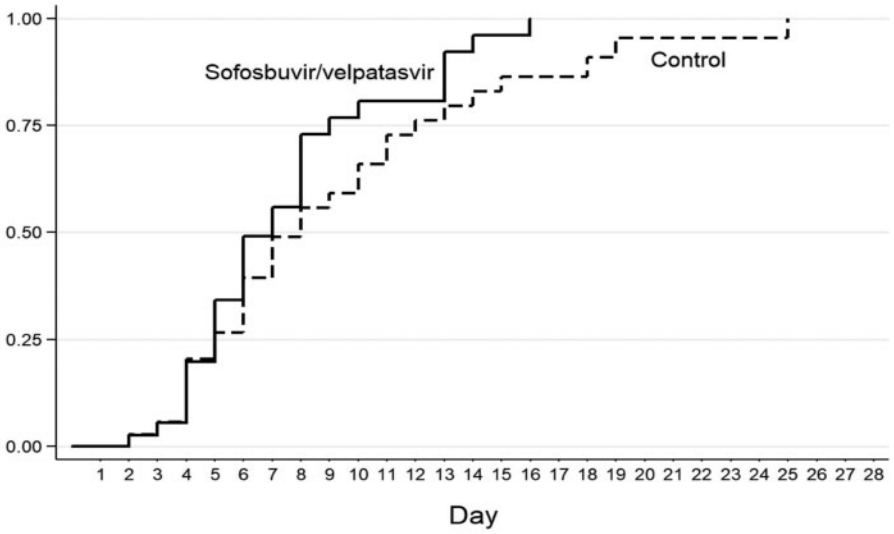

Figure 2. Probability of clinical improvement in the ITT population.

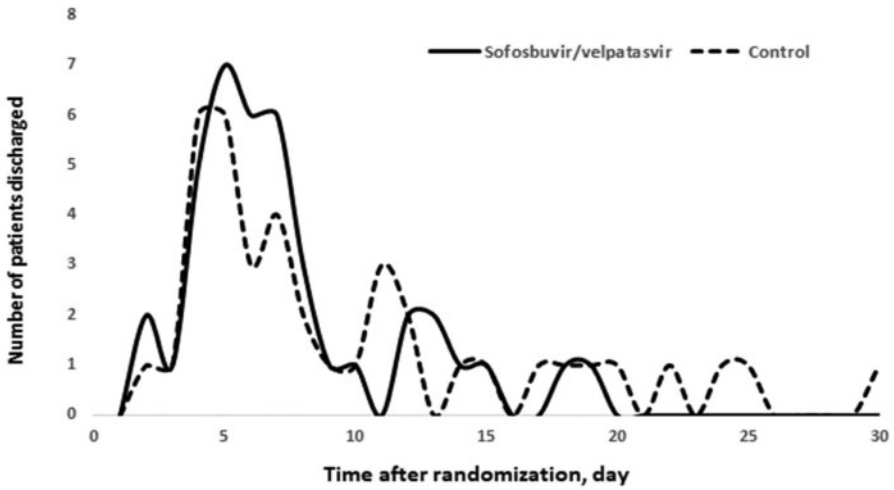

Figure 3. Number of patients discharged by time in the ITT population.

Table 2. Outcomes in the ITT population

Outcome of interest	Total	Sofosbuvir/velpatasvir ($n = 40$)	Control ($n = 40$)	*P* value	HR[a]
All-cause mortality, *n* (%)	6 (7.5)	3(7.5)	3 (7.5)	1.00	
Time to clinical improvement (days), median (IQR)	6 (4–9.5)	6 (4–8)	7 (4–11)	0.30	1.2 (0.6–2.2)
Clinical improvement, *n* (%)					
Day 3	10 (12.5)	4 (10)	6(15)	0.49	
Day 5	22 (27.5)	12 (30)	10 (25)	0.61	
Day 7	44 (55)	23 (57.5)	21 (52.5)	0.65	
Duration of hospital stay (days), median (IQR)	7 (5–11.5)	6 (5–8.5)	7 (5–13)	0.25	1.6 (0.9–2.5)
Time from randomization to death (days), median (IQR)	6 (7–9)	6 (2–9)	7 (7–30)	0.38	
Need for mechanical ventilation, *n* (%)	4 (5.0)	1 (2.4)	3 (8.1)	0.61	
Duration of mechanical ventilation (days), median (IQR)	2 (1–3)	3 (3–3)	1 (1–1)	0.51	
RT–PCR conversion (positive to negative), *n* (%)	10 (12.5)	6 (15)	4 (10)	0.49	

[a]The HR was estimated by the Cox proportional-risk model adjusted for time from starting symptoms to admission time.

Table 3. Adverse events in the modified ITT population

Variable	Total	Sofosbuvir/velpatasvir ($n = 42$)	Control ($n = 37$)	*P* value[a]
Adverse events, *n* (%)				
No adverse drug reaction	26 (32.5)	12 (27.9)	14 (37.8)	0.34
Nausea and vomiting	15 (18.7)	8 (18.6)	7 (18.9)	0.55
Diarrhoea	5 (6.25)	4 (9.3)	1 (2.7)	<0.001
Headache	5 (6.2)	2 (4.7)	3 (8.1)	0.16
Discontinued drug	1 (1.2)	0 (0.0)	1 (2.7)	0.31
More than one drug reaction	28 (35.0)	17 (39.5)	11 (29.7)	0.16
Haematological adverse events, *n* (%)				
Leucopenia ($<4 \times 10^9$ cells/L)	10 (12.5)	7 (16.3)	3 (8.1)	0.31
Lymphopenia ($<1.1 \times 10^9$ cells/L)	21 (26.3)	11 (29.7)	10 (23.3)	0.79
Thrombocytopenia ($<100 \times 10^9$ cells/L)	9 (11.2)	4 (9.3)	5 (13.5)	1.00
Haemoglobin decreased by >1 g/dL	33 (38.8)	14 (35.0)	19 (42.2)	0.49
Biochemical laboratory abnormalities, *n* (%)				
Increased AST (>60 U/L)	18 (22.5)	10 (23.3)	8 (21.7)	0.86
Increased ALT (>60 U/L)	11 (13.7)	9 (21.0)	8 (21.6)	0.94
Increased triglycerides (increase of >100 mg/dL relative to last measurement)	7 (8.8)	2 (5.0)	5 (12.5)	0.37
Increased creatinine (increase of >0.5 mg/dL relative to last measurement)	1 (1.2)	0 (0.0)	1 (2.5)	—

[a]*P* value less than 0.05 (typically $\leq$0.05) is statistically significant.

sofosbuvir/daclatasvir improved survival and clinical recovery in patients with moderate to severe COVID-19.

During the COVID-19 pandemic, with the increase in the number of hospitalized patients, hospitals may increase staff and equipment or cancel non-essential elective surgery in order to increase the number of beds available for additional patients; however, spare capacity for COVID-19 patients is limited at the time of peak demand. According to our findings, treatment with sofosbuvir/velpatasvir might have reduced the length of hospital stay and shortened the time to clinical improvement. So, perhaps it can increase the availability of hospital beds during the COVID-19 pandemic.

In the present study, the male gender was dominant (55%), which was similar to the results of other studies in this regard. Interestingly, all deaths occurred in male patients and the mean age of the deceased was 61.3 ± 6.3 years. Several studies have found that male patients with COVID-19 are at higher risk of more severe clinical outcomes and mortality, as observed in SARS and MERS infections.[20,21] This difference may be related to the immune system and sociocultural factors.[22] The determinants of these gender-dependent differences should be confirmed in future studies. However, it was not possible to perform subgroup analysis for sex and age because of the small sample size.

In a pooled analysis of the ASTRAL trials, in order to evaluate the safety and efficacy of sofosbuvir/velpatasvir for chronic HCV infection, headache, fatigue, nausea and nasopharyngitis were the most common adverse events in individuals receiving sofosbuvir/velpatasvir.[23] The results showed that in the modified ITT population the incidence of diarrhoea was significantly higher in the sofosbuvir/velpatasvir arm compared with the control arm ($P < 0.001$). Considering that diarrhoea is not a typical adverse reaction of sofosbuvir/velpatasvir, it was most likely due to the synergistic antiviral affect of sofosbuvir/velpatasvir plus lopinavir/ritonavir and hydroxychloroquine. However, the rates of other adverse events were similar in the patients receiving sofosbuvir/velpatasvir and the national standard of care. No patient interrupted treatment or discontinued treatment early because of these events.

The results of the second RT–PCR test for SARS-CoV-2 were not available in 41 patients (51.25%). Nevertheless, of 39 patients who had a second PCR test, 16 patients (6 in the sofosbuvir/velpatasvir arm and 11 in the control arm) had RT–PCR conversion at discharge from the hospital. Zhou *et al.*[24] found the median duration of viral shedding was 20 days in patients with severe COVID-19 and could be as long as 37 days. The highest temperature at admission and time from symptom onset to admission are factors associated with a prolonged duration of viral shedding.[25] Therefore, the low rate of RT–PCR conversion might be due to the earlier discharge of patients from the hospital. Furthermore, quantitative viral load data were not available; therefore, it cannot be assessed whether adding sofosbuvir/velpatasvir treatment could reduce the duration and quantity of virus shedding compared with the control arm.

In the hepatoma cell line HuH-7 and type II pneumocytes (Calu-3), sofosbuvir inhibited SARS-CoV-2 with an EC_{50} of 6.2 and 9.5 μM, respectively.[26] In addition, the bioavailability of sofosbuvir is high and maximum plasma concentrations (C_{max}) occurred about 0.5–2 h after administration.[27] In this trial, only one dosing regimen of sofosbuvir was studied; this regimen was selected based on previous studies and the doses commonly used for hepatitis treatment in Iran. However, the concentration of sofosbuvir

was not monitored and it is not clear whether these concentrations are effective against SARS-CoV-2 in the body.

It is worth noting that the patients were heterogeneous in terms of disease duration and the median time from the onset of symptoms to the start of the study treatment; therefore, a combination of sofosbuvir/velpatasvir may be more effective if it is started as soon as possible after the onset of symptoms of a probable COVID-19 infection. It should be mentioned that the efficacy of oseltamivir, an antiviral agent used for treatment of influenza, is greatest when administered within 48 h of the onset of symptoms.[28] Similar arguments are proposed for early treatment of COVID-19. In this regard, we also registered a clinical trial (https://www.irct.ir/) to evaluate the safety and efficacy of sofosbuvir/daclatasvir versus the standard of care alone in non-hospitalized adults with early COVID-19.

To the best of our knowledge, this study is the first published clinical trial of the effect of a sofosbuvir/velpatasvir combination on treatment of patients with COVID-19. It was performed in a public hospital and these hospitals manage most of the patients in Iran. However, the study also had several limitations, such as not assessing the viral load, the small sample size and an open-label design. Furthermore, in order to facilitate and speed up the study in the critical conditions of pandemic occurrence, we did not want to use a placebo in this clinical trial. Larger randomized clinical trials in more homogeneous study populations, considering more parameters, are needed for accurate estimation of the efficacy of sofosbuvir/velpatasvir.

To summarize the above-mentioned data, adding sofosbuvir/velpatasvir to the standard of care did not improve the clinical status or reduce mortality in patients with moderate to severe COVID-19. The data of this well-designed trial is of great importance because, as previously mentioned, in a recently published report Simmons *et al.*[19] concluded that sofosbuvir/daclatasvir improves survival and clinical recovery in patients with moderate to severe SARS-CoV-2 infection. However, it is noteworthy that, contrary to our well-designed clinical trial, in the mentioned study:[18] (a) the sample size for analysis was relatively small; (b) one of the trials was not randomized; and (c) the designs were not standardized, and the results need to be confirmed in larger randomized controlled trials. We recommend that a change in the route of drug administration (for instance as a rectal formulation), increasing the duration of the intervention or increasing drug dosage (e.g. 800 mg sofosbuvir daily) be considered in future clinical trials.

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Transparency declarations

The authors declare that they have no competing interests.

Author contributions

B.S. and R.K. designed the study. Recruitment and care of patients were done by B.S., R.M., Z.M.A., F.M., M. Shirvani., S.V., A.J., M.H.Z., M.A. and M. Salimi. F.N. and F.K.S. were involved in statistical analysis, data management and randomization. B.S., R.K., Z.R. and A.B. drafted the paper. All authors were involved in critical revision of the manuscript and final approval of the published version.

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