**Journal of
Antimicrobial
Chemotherapy**

Sofosbuvir/daclatasvir regimens for the treatment of COVID-19: an individual patient data meta-analysis

Bryony Simmons[1]†, Hannah Wentzel[2]†, Sara Mobarak[3], Gholamali Eslami[3], Anahita Sadeghi [4], Ali Ali Asgari[4],
Hamideh Abbaspour Kasgari[5], Hafez Tirgar Fakheri[6], Shahin Merat [4] and Andrew Hill[7]*

[1]Department of Infectious Diseases, Imperial College London, London, UK; [2]School of Public Health, Imperial College London, London, UK; [3]Abadan Faculty of Medical Sciences, Abadan, Iran; [4]Digestive Disease Research Institute, Tehran University of Medical Sciences, Tehran, Iran; [5]Department of Clinical Pharmacy, School of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran; [6]Gut and Liver Research Centre, Mazandaran University of Medical Sciences, Sari, Iran; [7]Department of Translational Medicine, University of Liverpool, UK

*Corresponding author. E-mail: microhaart@aol.com
†These authors contributed equally.

Background: The combination of sofosbuvir and daclatasvir has a well-established safety profile and improves clinical outcomes in HCV patients. *In silico* and *in vitro* studies suggest that sofosbuvir/daclatasvir may show antiviral activity against SARS-CoV-2.

Methods: Three clinical trials comparing sofosbuvir/daclatasvir-based regimens with a comparator in hospitalized COVID-19 patients were combined in a meta-analysis. The primary outcomes measured were clinical recovery within 14 days of randomization, time to clinical recovery and all-cause mortality. A two-step approach was used to analyse individual-level patient data. The individual trial statistics were pooled using the random-effects inverse-variance model.

Results: Our search identified eight studies of which three met the inclusion criteria ($n = 176$ patients); two studies were randomized and one was non-randomized. Baseline characteristics were similar across treatment arms. Clinical recovery within 14 days of randomization was higher in the sofosbuvir/daclatasvir arms compared with control arms [risk ratio = 1.34 (95% CI = 1.05–1.71), $P = 0.020$]. Sofosbuvir/daclatasvir improves time to clinical recovery [HR = 2.04 (95% CI = 1.25–3.32), $P = 0.004$]. The pooled risk of all-cause mortality was significantly lower in the sofosbuvir/daclatasvir arms compared with control arms [risk ratio = 0.31 (95% CI = 0.12–0.78), $P = 0.013$].

Conclusions: Available evidence suggests that sofosbuvir/daclatasvir improves survival and clinical recovery in patients with moderate to severe COVID-19. However, the sample size for analysis was relatively small, one of the trials was not randomized and the designs were not standardized. These results need to be confirmed in larger randomized controlled trials.

Introduction

Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), a positive-sense RNA virus. Repurposing existing pharmaceuticals that are effective against viruses with similar replication mechanisms to SARS-CoV-2 is an attractive short-term treatment strategy. Current clinical trials investigating treatments, such as remdesivir,[1] hydroxychloroquine,[2] chloroquine and lopinavir/ritonavir,[3] are yet to show clear survival benefits.

Sofosbuvir and daclatasvir are well tolerated and effective direct-acting antivirals (DAAs) against HCV.[4] Sofosbuvir has broad antiviral activity against other viruses including Zika[5] and Dengue.[6] Evidence from some *in silico* and *in vitro* studies suggests that sofosbuvir/daclatasvir and ribavirin bind to SARS-CoV-2 RNA-dependent RNA polymerase ('RdRp').[7–9] Molecular docking experiments predicts that sofosbuvir and ribavirin bind to SARS-CoV-2 with similar, high binding energies (-7.5 and -7.8 kcal/mol, respectively).[10] However, sofosbuvir and daclatasvir[11] showed less promise in another *in vitro* study with little antiviral activity against SARS-CoV-2. In another study, daclatasvir exhibited low binding affinity for SARS-CoV-2 main protease (molecular docking score = -45.44).[12] Sofosbuvir/daclatasvir is widely available in generic

formulations costing $6 per 14 day treatment course of 400/60 mg (India).[13]

Given the potential for sofosbuvir/daclatasvir as a therapeutic option for COVID-19, the combination has been evaluated in several small clinical trials. We, therefore, carried out this meta-analysis to determine whether sofosbuvir/daclatasvir-based regimens improve clinical outcomes of patients with moderate or severe COVID-19.

Methods

Search strategy and selection criteria

Trials were eligible for inclusion if they compared a sofosbuvir/daclatasvir-based regimen with a comparator for the treatment of hospitalized individuals diagnosed with COVID-19. Given the anticipated small number of trials, we included both randomized and non-randomized trials. We systematically identified trials by reviewing the following clinical trials databases (searched 1 June 2020): clinicaltrials.gov, Cochrane Central Register of Controlled Trials (CENTRAL), WHO International Clinical Trials Registry Platform (ICTRP), and the Iranian Registry of Clinical Trials (IRCT). For relevant and completed studies, we obtained individual patient data from trial investigators.

The primary outcomes were (i) clinical recovery within 14 days of randomization, (ii) time to clinical recovery and (iii) all-cause mortality from randomization to end of follow-up. Clinical recovery was defined as the point at which the individual was deemed eligible for discharge from hospital as per the study-specific criteria; in studies where recovery was not

defined, clinical recovery was assumed to be the day of hospital discharge. Secondary outcomes were duration of hospitalization (days) and a composite outcome of ICU admission or requirement for invasive mechanical ventilation (IMV).

Data analysis

Statistical analyses for all outcomes were conducted with individual patient data, on the ITT population. The analysis used a two-step method for all outcomes, first producing trial-specific estimates and then combining these to provide a pooled estimate of effect using standard meta-analysis techniques. Treatment effects were expressed as risk ratios (RRs) for binary outcomes and mean differences for continuous outcomes. For the survival outcome (time to recovery) we estimated (i) the cause-specific HRs for recovery using Cox proportional hazards models and (ii) the subdistribution HR (SHR) using the Fine and Gray[14] competing risks model to account for death as a competing risk. For each outcome we pooled the individual trial statistics using the random-effects inverse-variance model; a continuity correction of 0.5 was applied to studies with zero cells. Heterogeneity was evaluated by I^2. We planned a sensitivity analysis for the primary outcomes excluding non-randomized trials to account for the risk of bias. A second sensitivity analysis for the primary binary outcomes imputed the worst outcome for any individual randomized but not included in the ITT analyses of the respective studies (i.e. no clinical recovery and death). In a final sensitivity analysis to study the effects of non-random treatment assignment, we estimated the average treatment effect of sofosbuvir/daclatasvir on the binary outcomes (clinical recovery and death), using the inverse probability weighting (IPW) estimator and adjusting for age, sex and comorbidities

Table 1. Characteristics of included studies

Trial ID	Location	Design	n	Intervention	Control	Key inclusion criteria	Primary outcome
IRCT20200324 046850N2[17]	Abadan, Iran	non-randomized (single centre)	62	SOF/DCV +HCQ (n = 35)	HCQ+LPV/r +RBV (n = 27)	(i) real-time PCR confirmed or abnormal chest CT (ii) hospitalized (iii) severe disease (O2 sat <94% or respiratory rate >24/min or decreased consciousness)	time to hospital discharge
IRCT20200328 046886N1[15]	Sari, Iran	randomized (single centre)	48	SOF/DCV+RBV (n = 24)	HCQ+LPV/r±RBV (n = 24)	(i) real-time PCR confirmed or abnormal chest CT (ii) hospitalized (iii) mild/moderate disease [fever (≥37.8°C) and at least one of respiratory rate <24/min, O2 sat >94%] (iv) ≤8 days since symptom onset	duration of hospitalization
IRCT20200128 046294N2[16]	Tehran, Iran	randomized (multi centre)	66	SOF/DCV +HCQ±LPV/r (n = 33)	HCQ±LPV/r (n = 33)	(i) real-time PCR confirmed and abnormal chest CT (ii) hospitalized (iii) severe disease [fever (≥37.8°C) and at least one of respiratory rate >24/min, O2 sat <94% or PaO2/FiO2 ratio <300 mgHg] (iv) ≤8 days since symptom onset	clinical recovery within 14 days

DCV, daclatasvir; HCQ, hydroxychloroquine; LPV/r, lopinavir/ritonavir; O2 sat, O2 saturation; RBV, ribavirin; SOF, sofosbuvir.

Table 2. Combined baseline characteristics from the three clinical trials and outcomes in the ITT population

	Sofosbuvir/daclatasvir (n = 92)	Control (n = 84)	Pooled effect (95% CI)[b]
Age (years), median (IQR)	57 (42–68)	61 (48–71)	
Male, n (%)	48 (52)	35 (42)	
Coexisting conditions, n (%)			
diabetes	31 (34)	32 (38)	
hypertension	24 (26)	29 (35)	
chronic pulmonary disease	8 (9)	11 (13)	
Vitals on admission, median (IQR)[a]			
O2 saturation (%)	92 (90–93)	91 (90–92)	
respiratory rate (breaths/min)	22 (20–26)	22 (20–26)	
Laboratory findings, median (IQR)			
haemoglobin (g/dL)	12 (11–14)	12 (11–13)	
WBCs ($\times 10^9$/L)	7 (5–9)	8 (6–12)	
lymphocyte count ($\times 10^9$/L)	14 (10–25)	15 (7–25)	
AST (U/L)	28 (21–43)	32 (20–50)	
ALT (U/L)	28 (16–35)	27 (20–40)	
creatinine (mg/dL)	1 (0.8–1.2)	1 (0.8–1.2)	
Primary outcomes			
clinical recovery within 14 days	86/92 (93%)	57/84 (68%)	1.34 (1.05–1.71)[c]
time to clinical recovery (days), median (IQR)	6 (5–8)	8 (6–19)	2.04 (1.25–3.32)[d]
all-cause mortality	5/92 (5%)	17/84 (20%)	0.31 (0.12–0.78)[c]
Secondary outcomes			
duration of hospitalization (days), median (IQR)	6 (5–7)	8 (6–11)	−0.56 (−0.86 to −0.26)[e]
ICU admission and/or IMV	9 (10%)	24 (29%)	0.35 (0.18–0.69)[c]

Percentages are calculated from non-missing values.
[a]Vitals on admission were not available for the Sari study.
[b]Pooled estimate from two-step meta-analysis (random effects).
[c]RR (binary outcomes).
[d]Cause-specific HR (time-to-event outcome).
[e]Mean difference (continuous outcomes).

(diabetes, hypertension and chronic pulmonary disease). The significance threshold was set at 5% (two-sided) and all analyses were conducted using Stata (version 14.2, StataCorp) and RStudio (version 3.5.3, R Foundation).
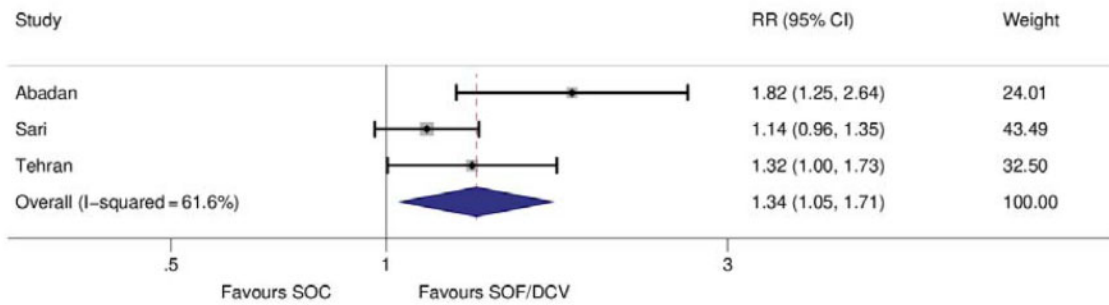
Results

Overall, eight studies were considered for inclusion; five did not meet the entry criteria [three were ongoing and use sofosbuvir either alone or in combination with alternative DAAs (velpatasvir or ledipasvir) and two were ongoing and enrolled outpatient populations]. Three studies met the inclusion criteria and all provided data for individual patients [n = 176 (92 intervention patients and 84 control patients)].[15–17] All studies were conducted in Iran; two were randomized[15,16] and one was non-randomized.[17] Two assessed patients with severe disease and one assessed mild/moderate disease. The intervention arms in each trial received (i) sofosbuvir/daclatasvir + standard of care (SOC) (hydroxychloroquine ± lopinavir/ritonavir), (ii) sofosbuvir/daclatasvir + ribavirin and (iii) sofosbuvir/daclatasvir + hydroxychloroquine. The control groups received SOC at the time of the trial, which was (i) hydroxychloroquine ± lopinavir/ritonavir, (ii) hydroxychloroquine + lopinavir/ritonavir ± ribavirin and (iii) hydroxychloroquine + lopinavir/ritonavir + ribavirin, respectively (Table 1). Age and sex were generally balanced between arms. There was a tendency for a higher frequency of comorbidities in the control arms (however, this was not significant); vitals and laboratory findings were balanced (Table 2).
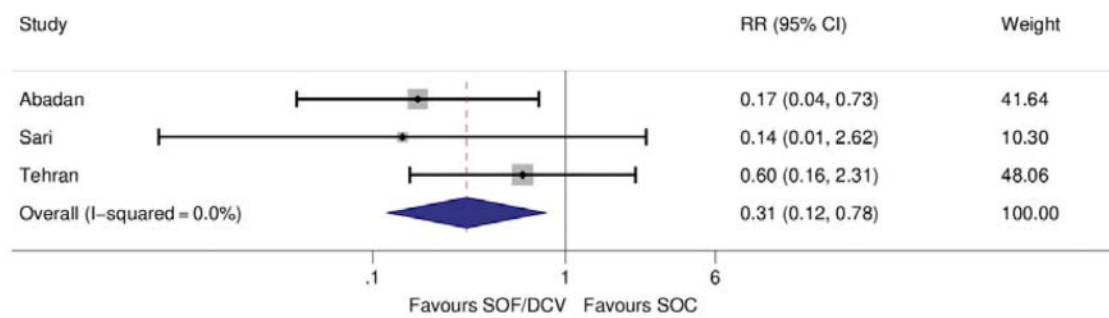
Overall, 86 (93%) of 92 patients in the sofosbuvir/daclatasvir arms and 57 (68%) of 84 patients in the control arms achieved clinical recovery within 14 days of randomization [RR = 1.34 (95% CI = 1.05–1.71), P = 0.020, I^2 = 62%; Figure 1a]. Figure 1(c) shows the cumulative incidence of recovery by treatment group. The combined recovery-specific HR was 2.04 (95% CI = 1.25–3.32) (P = 0.004, I^2 = 52%); similar results were observed in the competing risk analysis [SHR = 2.03 (95% CI = 1.33–3.08), P = 0.001, I^2 = 51%]. Considering all-cause mortality, 5/92 (5%) in the sofosbuvir/daclatasvir arms and 17/84 (20%) in the control arms died whilst enrolled in a trial. The pooled risk of all-cause mortality was significantly lower in the sofosbuvir/daclatasvir arms compared with control arms [RR = 0.31 (95% CI = 0.12–0.78), P = 0.013, I^2 = 0%; Figure 1b]. There were significant between-group differences in favour of sofosbuvir/daclatasvir for the secondary outcomes, duration of hospitalization and the composite of ICU admission and/or requirement for IMV (Table 1).

In sensitivity analyses, excluding non-randomized trials, the pooled RR for clinical recovery was 1.19 (95% CI = 1.03–1.37)
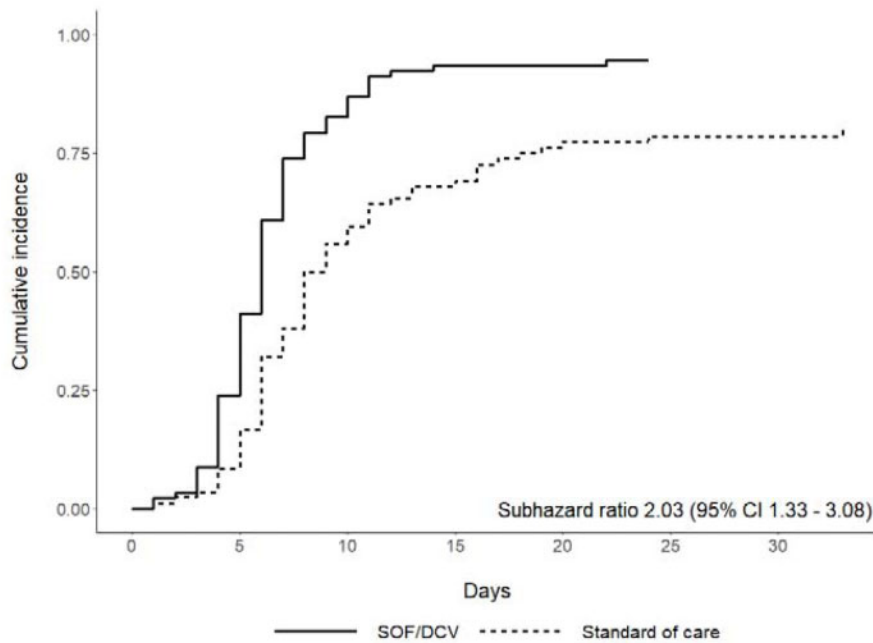
(a) Clinical recovery within 14 days

Study	RR (95% CI)	Weight
Abadan	1.82 (1.25, 2.64)	24.01
Sari	1.14 (0.96, 1.35)	43.49
Tehran	1.32 (1.00, 1.73)	32.50
Overall (I-squared = 61.6%)	1.34 (1.05, 1.71)	100.00

.5 1 3

Favours SOC   Favours SOF/DCV

(b) All-cause mortality

Study	RR (95% CI)	Weight
Abadan	0.17 (0.04, 0.73)	41.64
Sari	0.14 (0.01, 2.62)	10.30
Tehran	0.60 (0.16, 2.31)	48.06
Overall (I-squared = 0.0%)	0.31 (0.12, 0.78)	100.00

.1 1 6

Favours SOF/DCV   Favours SOC

(c) Cumulative incidence of recovery by treatment group

Subhazard ratio 2.03 (95% CI 1.33 – 3.08)

Cumulative incidence

Days

—— SOF/DCV   ······ Standard of care

Figure 1. Primary outcomes in the ITT population. (a and b) Forest plots for the relative risk of clinical recovery within 14 days and all-cause mortality, respectively. (c) Cumulative incidence of clinical recovery by treatment group and the SHR. All analyses were conducted including the three trials in the ITT population. DCV, daclatasvir; SOF, sofosbuvir. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

($P = 0.019$). The cause-specific HR for recovery was 1.63 (95% CI = 1.10–2.43) ($P = 0.016$). The difference in all-cause mortality was no longer significant [RR = 0.47 (95% CI = 0.14–1.58), $P = 0.221$]. In the second sensitivity analysis, one trial excluded four randomized individuals from the ITT population for not meeting inclusion criteria (two per arm). Imputing these as failures, the RR for clinical recovery was 1.34 (95% CI = 1.04–1.72) ($P = 0.023$) and for all-cause mortality was 0.35 (95% CI = 0.12–1.05) ($P = 0.060$). In the final sensitivity analysis, using the IPW estimator, the effect of sofosbuvir/daclatasvir on clinical recovery remained significant [average treatment effect (ATE) = 22.1% (95% CI = 0.9%–43.3%), $P = 0.041$]; however, the effect on all-cause mortality was not significant [ATE = −12.0% (95% CI = −25.3%–1.3%), $P = 0.078$].

Discussion

Our meta-analysis showed significant differences in clinical recovery and all-cause mortality in favour of sofosbuvir/daclatasvir regimens over the three trials included. The effect of sofosbuvir/daclatasvir on clinical recovery was sustained in sensitivity analyses.

This meta-analysis has several key limitations. Primarily, the number of studies and the overall population size are small. Caution should be taken in interpreting the results, which are subject to bias and potentially influential sampling error. Further, the meta-analysis can only be as reliable as the effect captured in the primary studies and cannot overcome problems inherent in the design and execution of individual trials. We included a non-randomized study and as such treatment effects may be confounded. The included studies were not fully blinded and therefore investigator bias is a possibility; however, managing a placebo-controlled clinical trial during a fast-moving pandemic remains challenging. Furthermore, the comparator arms across the included studies varied, reflecting the change in national guidelines in Iran during the course of the trials. Primary outcomes over studies were not uniform and no viral outcomes were measured. Viral load is an important parameter to demonstrate an effective antiviral therapy. Future studies should include viral load kinetics as a measure of clinical progress.

Ribavirin was administered in the treatment arm in one study.[15] Ribavirin has predicted binding affinity for SARS-CoV-2[10] and therefore it is difficult to determine whether sofosbuvir/daclatasvir or ribavirin has a superior clinical benefit. Furthermore, a combination of antivirals may act synergistically against COVID-19. Hung et al.[18] demonstrated that a triple combination of lopinavir/ritonavir, IFN-β-1b and ribavirin had a shorter time to negative nasopharyngeal swab than lopinavir/ritonavir alone.

In a sensitivity analysis excluding non-randomized trials and in methods adjusting for non-random treatment assignment, all-cause mortality was no longer significant. In the excluded non-randomized study, participants were allocated to either a sofosbuvir/daclatasvir or ribavirin arm.[17] A retrospective cohort study, investigating ribavirin for the treatment of SARS, demonstrated that ribavirin is associated with greater adverse events.[19] These adverse events may have contributed to the increased mortality measured in the ribavirin arm of this particular study.

More detailed in vitro studies are needed to evaluate whether sofosbuvir or daclatasvir is the most active agent in the sofosbuvir/daclatasvir combination or if there is synergistic activity. Use of sofosbuvir/daclatasvir in combination with other antivirals, such as favipiravir or atazanavir/ritonavir, might further improve efficacy. There are guidelines from the US FDA on optimal trial design.[20] The recent approval of remdesivir to treat COVID-19 was based on several randomized trials in over 2000 patients. Similar sample sizes may be needed to establish the evidence to support worldwide approval of sofosbuvir/daclatasvir. If the results from this meta-analysis were confirmed in larger randomized trials, sofosbuvir/daclatasvir would be an important treatment option. The oral dosing, established safety profile, low costs of production and large-scale manufacture of sofosbuvir/daclatasvir could allow rapid expansion to worldwide use in the treatment of COVID-19.

Transparency declarations

All authors were involved in the original analysis of the clinical trials.

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