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Statistical review of *Efficacy and safety of lopinavir/ritonavir or arbidol in adult patients with mild/moderate COVID-19: an exploratory randomized controlled trial*

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The following review has been prepared in collaboration with members of the MRC-NIHR Trials Methodology Research Partnership [1]. The reviewers named above, and other, unnamed discussants of the paper, are all qualified statisticians with experience in clinical trials. Our objective is to provide a rapid review of publications, preprints and protocols from clinical trials of COVID-19 treatments, independent of journal specific review processes. We aim to provide timely, constructive, focused, clear advice aimed at improving both the research outputs under review, as well as future studies. Given our collective expertise (clinical trial statistics) our reviews focus on the designs of the trials and other statistical content (methods, presentation and accuracy of results, inferences). This review reflects the expert opinions of the named authors, and does not imply endorsement by the MRC-NIHR Trials Methodology Research Partnership, its wider membership, or any other organization.

Here we review *Efficacy and safety of lopinavir/ritonavir or arbidol in adult patients with mild/moderate COVID-19: an exploratory randomized controlled trial, by Li et al* [2] . A pre-proof version of the accepted, peer-reviewed manuscript was published by Med (Cell Press) https://marlin-prod.literatumonline.com/pb-assets/products/coronavirus/MEDJ1.pdf, following posting of a preprint on medRxiv on April 15th, 2020: https://www.medrxiv.org/content/10.1101/2020.03.19.20038984v2.

Positively, this trial randomly allocated participants to study groups, and ensured that the allocation was concealed at recruitment by using a web-based system. However, the sample size was small, as pandemic control measures limited the number of eligible patients. As a result, the ability of the study to detect meaningful differences between treatment regimens was limited, and the reader should understand that considerable uncertainty remains about the effects of these treatments compared to standard of care, particularly if basing decisions on this study alone.

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Study Summary

The paper reports a three-arm parallel randomised controlled trial planned in 125 patients with mild/moderate COVID-19. The study was conducted in a single centre in China between February 1st and March 28th, 2020. Patients were randomised in a 2:2:1 allocation ratio to receive 200mg lopinavir and 50mg ritonavir, orally administered, twice daily, for 7-14 days; 100mg arbidol, orally administered, three times daily for 7-14 days; or to standard-of-care. The primary outcome was the time it took for patients to experience positive-to-negative conversion of SARS-CoV-2 nucleic acid from the initiation of treatment to day 21. Secondary outcomes included proportion of negative conversions by day 14, resolution of fever, cough alleviation, improvement of chest CT at days 7 and 14, and deterioration of clinical status from mild/ moderate to severe/ critical during the study period.

The study enrolled 86 participants before stopping recruitment (n = 34, 35, 17), citing the limited number of new cases. By the end of the study period, all patients were negative for SARS-CoV-2. Further, there were no substantial treatment differences in the proportion of patients with a negative test at day 7 (35% lopinavir/ritonavir, 37% arbidol, 41% standard of care; p = 0.97) or day 14 (85, 91, 77; p = 0.35); nor any meaningful differences in the mean time for negative conversion during the study period (9 days [SD 5], 9.1 [4.4], 9.3 [5.2]; p = 0.98). Though a higher proportion of patients in the lopinavir/ritonavir arm progressed to severe/critical status (8/34 vs 3/34 and 2/17), and experienced more adverse events (12/34 vs 5/35 and 0/17), the data were not inconsistent with a null hypothesis of no difference between groups.

The authors concluded that neither the lopinavir/ritonavir nor the arbidol protocols offered any benefit compared to standard of care, while noting the observed increase in adverse events in the active treatment arms.

We sincerely thank the authors for their contribution to our collective understanding of COVID-19, and for their commitment to the timely dissemination of research results.

Major comments

Absence of statistical significance should not be interpreted as “no difference” between the study arms.

Throughout, the authors interpret absence of statistical significance as indicating that there was no benefit of the active treatments compared to standard of care. This may not be warranted given the small sample size in the study, and the data are likely to be compatible with other scenarios (for example, in which either or both of the active treatment regimens are more or less effective than standard care) [3]. This point is difficult for the reader to assess because the authors do not present measures of precision, such as confidence intervals.

Recommendations:

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For future studies

- Future trialists are encouraged to include measures of uncertainty for the study results, such as confidence intervals, to aid interpretation. Using a non-significant result to conclude lack of a notable treatment effect may be warranted when a study is well-powered to detect clinically relevant benefits, but this does not apply to the present study.

For the reader

- The results should not be interpreted as showing that the tested treatment regimens are equivalent. It remains possible that either, or both, of the tested treatment regimens are superior (or inferior) to standard of care.

There were inconsistencies in how study outcomes were described in the paper and the trial registration.

The trial registration did not specify the primary outcome's time point of measurement, instead listing time points of +2, 4, 7, 10, 14 and 21 days; while the paper refers to +21 days as the time of primary outcome assessment, and reports additional results for 14 and 7 days as secondary outcomes. The timings of other outcomes (e.g. chest imaging) were also inconsistently described across the registry and the paper. The registration listed a number of secondary outcomes such as respiratory rate, oxygen saturation, and blood pressure, but in the paper these were combined into a single measure, *deterioration of clinical status*, which was not mentioned in the registry. One outcome reported in the paper (cough alleviation) did not appear on the trial registry. It is important that study outcome measures are properly described in advance, to limit the scope for selective reporting and for post-hoc outcome definition, which may undermine the integrity of statistical inferences and the trustworthiness of results.

Recommendations:

For future studies

- Future studies should prospectively register the study outcomes with sufficient detail, including their timings, to prevent flexibility in their selection and reporting.
- Where there is a valid reason to change the study outcome post-registration, there needs to be a clear process for independent oversight of those changes and transparent reporting of the rationale behind them.

For the reader

- It is important that the analyses performed and reported in a trial, including the choice of outcomes, were not selected once the data were known. Without clear and complete prospective registration of study outcomes, it is impossible to distinguish prespecified from data-driven choices.

Methods of statistical analysis appear to be suboptimal

The authors have reported the time to positive-to-negative conversion of SARS-CoV-2 nucleic acid. Neither this, nor other statistical analyses included in the paper, appear to have been

prospectively registered, although a study protocol was not available for review. Their analysis of the primary outcome appears to have been a one-way ANOVA of days to negative conversion, resulting in an estimate of the mean number of days in each group. A time-to-event analysis, for example using Cox regression, would have been more appropriate here, and would have permitted for the adjustment of (prospectively declared) prognostic variables in order to increase precision [4]. It may have been possible to perform adjusted analyses for other outcomes in the study, although low numbers of events may have proven prohibitive.

Recommendations:
For future studies

- Authors should prospectively declare their statistical analysis plans, to reduce flexibility once the data are known. Authors should generally analyse time to event data using methods developed for this purpose. Authors should consider adjusting for prognostic variables, as this may increase power/precision.

Lack of blinding may have influenced study results.

The authors correctly ensured prospective allocation concealment by using a web-based randomisation system. However, while they noted that that allocation was not revealed to physicians and radiologists reviewing data and radiological images, they also said that the recruiting clinicians and research staff were unblinded. Further, although the authors stated that participants were blinded, it is unclear whether and how this could be maintained, since it does not appear that dummy treatments were used (though we acknowledge that the production and administration of dummy medications to maintain blinding would have been challenging given the circumstances).

Recommendations:
For future studies

- Where possible, trialists should attempt to blind participants and relevant personnel to treatment allocation.

For the reader

- Lack of blinding can lead to differences in the way both participants and clinicians behave, compared to what would happen outside of the context of a clinical trial. Lack of blinding can also influence the way in which some subjective outcome measures are assessed.

The rationale for using an unequal allocation ratio was unclear.

Twice as many participants were allocated to each of the active treatment arms compared to the standard of care arm. This results in reduced power for comparisons of each active treatment against the control arm. Dividing a given number of participants equally between treatment groups will yield the greatest efficiency (i.e. result in more precise estimates, or greater power to detect a given effect). There can be other reasons to use an unequal allocation ratio (for example, to make the study more attractive for potential participants, thereby boosting recruitment). However, no explanation was given.

Recommendations:
For future studies

- Randomly allocating participants across arms in equal numbers will provide the greatest power, and should generally be done unless there are compelling reasons to do otherwise (which should be justified in the trial reporting).

Minor comments

- Authors refer to ‘rate’ of positive-to-negative conversion of SARS-CoV-2 nucleic acid at days 7, 14, 21, but actually mean ‘proportion experiencing the event’. The term ‘rate’ really means the frequency of something happening per unit of another measure, usually time (although we acknowledge that its use to mean ‘proportion’ has become ubiquitous in some fields). Readers may misunderstand the intent.

- Results were typically presented as within-group estimates (e.g. the mean time to negative conversion in the standard-of-care arm), whereas it is better to report estimates of the differences between groups (e.g. the difference in mean time to negative conversion comparing the arbidol arm to standard-of-care), since this is more aligned with the purpose of the trial as a comparative tool.

Open Data

No

Open Analysis Code

No

Pre-registered study design

Registered several days after recruitment opened (NCT04252885).

PubPeer

There may be comments on the PubPeer page for the published version of this paper.
https://pubpeer.com/publications/4082F2BDA9963A7D97A2C352A68AC0

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CONSORT CHECKLIST

To support the review, we completed the CONSORT checklist [5] below. Material taken directly from the paper (or trial registry) is in *italics.* Our additional comments are in **bold**.

Title and abstract

1a Identification as a randomised trial in the title

Efficacy and safety of lopinavir/ritonavir or arbidol in adult patients with mild/moderate COVID-19: an exploratory randomized controlled trial

1b Structured summary of trial design, methods, results, and conclusions.

Title: Identification of the study as randomised	Yes
Authors: Contact details for the corresponding author	Yes
Trial design: Description of the trial design (eg, parallel, cluster, non-inferiority)	No
Methods	
Participants: Eligibility criteria for participants and the settings where the data were collected	Yes
Interventions: Interventions intended for each group	Yes
Objective: Specific objective or hypothesis	Yes
Outcome: Clearly defined primary outcome for this report	No
Randomisation: How participants were allocated to interventions	No
Blinding (masking): Whether or not participants, care givers, and those assessing the outcomes were blinded to group assignment	Yes
Results	
Numbers randomised: Number of participants randomised to each group	Yes
Recruitment: Trial status	Yes
Numbers analysed: Number of participants analysed in each group	Yes
Outcome: For the primary outcome, a result for each group and the estimated effect size and its precision	No
Harms: Important adverse events or side-effects	Yes
Conclusions: General interpretation of the results	Yes
Trial registration: Registration number and name of trial register	Yes
Funding: Source of funding	Yes

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Introduction

Background and objectives

2a Scientific background and explanation of rationale

See Introduction

2b Specific objectives or hypotheses

"...aiming to provide a preliminary evaluation of the efficacy and safety of monotherapy with LPV/r or arbidol in the treatment of patients with mild/moderate COVID-19" **[introduction]**

Methods

Trial design

3a Description of trial design (such as parallel, factorial) including allocation ratio

"an exploratory randomized (2:2:1) controlled trial" **[abstract]**
"who were randomly assigned (2:2:1) into 3 groups as follows:" **[study design and participants]**

3b Important changes to methods after trial commencement (such as eligibility criteria), with reasons

"we initially estimated that a maximum of 125 patients could meet the inclusion criteria, however, only 86 were ultimately recruited because few new cases developed in Guangzhou with the epidemic under control." **[quantification and statistical analysis]**

Participants

4a Eligibility criteria for participants

"1) age between 18 and 80
years; 2) SARS-CoV-2 infection confirmed by real-time PCR (RT-PCR) from pharyngeal swab; 3) mild clinical status, defined as having mild clinical symptoms but no signs of pneumonia on imaging or moderate clinical status, defined as having fever, respiratory symptoms and pneumonia on imaging;5 4) the following lab findings: creatinine ≤110μmol/L, creatinine clearance rate (eGFR) ≥60 ml/min/1.73m2, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤5 × ULN, and total bilirubin (TBIL) ≤2 × ULN; 5) willingness to participate in the study and provide informed consent. Patients were excluded based on the following criteria: 1) known or suspected to be

allergic to LPV/r or arbidol; 2) having severe nausea, vomiting, diarrhea or other complaints affecting oral intake or absorption in the digestive tract; 3) taking other drugs that may interact with LPV/r or arbidol; 4) having serious underlying diseases, including but not limited to heart, lung, or kidney disease, liver malfunction, or mental illnesses affecting treatment compliance; 5) complications with pancreatitis or hemophilia prior to the trial; 6) Pregnant or lactating women; 7) suspected or confirmed history of alcohol or substance use disorder; 8) participation in other drug trials within the past month; 9) deemed otherwise unsuitable for the study by researchers." **[study design and participants]**

4b Settings and locations where the data were collected

"Guangzhou Eighth People's Hospital is a designated hospital for the treatment of COVID-19 patients and over 80% of the patients confirmed with COVID-19 in Guangzhou were hospitalized at this facility" **[introduction]**

Interventions

5 The interventions for each group with sufficient details to allow replication, including how and when they were actually administered

"group A (LPV/r group), 34 patients were administered lopinavir (200mg) boosted by ritonavir (50mg) (orally administed, twice daily, 500 mg, each time for 7-14 days). In group B (arbidol group), 35 patients were given arbidol (100mg) (orally administed, 200mg three times daily for 7-14 days). In group C (control group), 17 patients were not given any antiviral therapy. All three groups were followed for up to 21 days. All three groups were treated with supportive care and effective oxygen therapy if in need.5 Antiviral treatment was discontinued for patients who 1) had been treated for more than 7 days and tested negative for SARS-CoV-2 nucleic acid in two consecutive tests separated by more than 24 hours, or 2) were discharged from hospital, or 3) had intolerable side effects." **[study design and participants]**

Outcomes

6a Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed

"The rate of virus inhibition [Time Frame: Day 0, 2, 4, 7, 10, 14 and 21]
Novel coronaviral nucleic acid is measured in nose / throat swab at each time point" **[registry]**

"The primary outcome was the rate of positive-to-negative conversion of SARS-CoV-2 nucleic acid from the initiation of treatment to day 21" **[outcomes]**

6b Any changes to trial outcomes after the trial commenced, with reasons

No reasons provided

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Sample size

7a How sample size was determined

“Based on theestimated number of patients admitted to the hospital at that time, we initially estimated that a maximum of 125 patients could meet the inclusion criteria, however, only 86 were ultimately recruited because few new cases developed in Guangzhou with the epidemic under control.” **[quantification and statistical analysis]**

7b When applicable, explanation of any interim analyses and stopping guidelines

N/A

Randomisation

Sequence generation

8a Method used to generate the random allocation sequence

“The randomization numbers were computer-generated.” **[randomization and masking]**

8b Type of randomisation; details of any restriction (such as blocking and block size)

None provided
Allocation concealment mechanism

9 Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned

Implementation

10 Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions

“Allocation concealment was achieved using a centralized web-based randomization system in which the participant identifier (hospitalization number) was entered before the allocation was revealed. The randomization numbers were used in case report form (CRF) pages.”
[randomization and masking]

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Blinding

11a If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how

"The study was blinded to participants and those physicians and radiologists who reviewed the data and radiological images but open-label to clinicians who recruited patients and research staff." **[randomization and masking]**

11b If relevant, description of the similarity of interventions

None

Statistical methods

12a Statistical methods used to compare groups for primary and secondary outcomes

"Means for continuous variables were compared using one-way ANOVA when the data were normally distributed; otherwise, the Mann-Whitney test was used. Proportions for categorical variables were compared using the $\chi 2$ test or Fisher's exact tests. A two-sided α of less than 0.05 was considered statistically significant." **[quantification and statistical analysis]**

12b Methods for additional analyses, such as subgroup analyses and adjusted analyses

N/A

Results

Participant flow (a diagram is strongly recommended)

13a For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome

Figure 1

13b For each group, losses and exclusions after randomisation, together with reasons

Figure 1

Recruitment

14a Dates defining the periods of recruitment and follow-up

"From Feb 1 to March 28, 2020, 105 patients with COVID-19 were screened for this study, among whom 86 patients (mean age of 49.4 years [SD 14.7, range 19-79]) including 40 men and 46 women were successful enrolled...All patients were followed up for 21 days." **[baseline characteristics of patients]**

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14b Why the trial ended or was stopped

“we initially estimated that a maximum of 125 patients could meet the inclusion criteria, however, only 86 were ultimately recruited because few new cases developed in Guangzhou with the epidemic under control.” **[quantification and statistical analysis]**

Baseline data

15 A table showing baseline demographic and clinical characteristics for each group

Table 1

Numbers analysed

16 For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups

Table 2

Outcomes and estimation

17a For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)

Table 2 (no effect size or precision)

17b For binary outcomes, presentation of both absolute and relative effect sizes is recommended

None provided

Ancillary analyses

18 Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory

None

Harms

19 All important harms or unintended effects in each group (for specific guidance see CONSORT for harms42)

“During the follow-up period, 12 (35.3%) patients in the LPV/r group experienced adverse events including diarrhea (9/34, 26.5%), loss of appetite (5/34, 14.7%) and elevation of ALT

over 2.5-fold above the normal limit (1/21, 4.8%). In addition, 5 (14.3%) patients in the arbidol group experienced adverse events including diarrhea (3/35, 8.6%) and nausea (2/34, 5.9%). No apparent adverse events occurred in the control group. Notably, one serious adverse event occurred in a 79-year-old man with underlying diseases including diabetes and hypertension in the LPV/r group, characterized by severe diarrhea on day 3. The patient withdrew from this study and tested positive for SARS-CoV-2 nucleic acid lasting over 14 days of the follow-up period. This patient progressed to critical condition and received extracorporeal membrane oxygenation (ECMO). Fortunately, he recovered and stopped needing ECMO by the observation endpoint of this study." **[safety outcomes]**

Discussion

Limitations

20 Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses

"Limitations of study Our study is not without its limitations. First, we recognize that our sample size was small. Second, the study did not enroll severely or critically ill patients, or patients at increased risk of poor outcome with many comorbidities and was conducted in only one center. Third, the study was not completely blinded, possibly influencing the outcome to some extent. We will continue to follow these patients to evaluate their long-term prognosis" **[Limitations of study]**

Generalisability

21 Generalisability (external validity, applicability) of the trial findings

"the study did not enroll severely or critically ill patients, or patients at increased risk of poor outcome with many comorbidities and was conducted in only one center. " **[limitations of study]**

Interpretation

22 Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence

"In conclusion, our study found that LPV/r or arbidol monotherapy presents little benefit for improving the clinical outcome of hospitalized patients with mild/moderate COVID-19 beyond symptomatic and supportive care, causing instead more adverse events. Further work is needed to confirm these results." **[conclusion]**

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Other information

Registration

23 Registration number and name of trial registry

"registered on ClinicalTrials.gov (NCT04252885" **[experimental model and subject details]**)

Protocol

24 Where the full trial protocol can be accessed, if available

N/A

Funding

25 Sources of funding and other support (such as supply of drugs), role of funders

"This study was supported by project 2018ZX10302103-002, 2017ZX10202102-003-004 and Infectious Disease Specialty of Guangzhou High-level Clinical Key Specialty (2019-2021)."
[abstract]