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Post-exposure Lopinavir-Ritonavir Prophylaxis versus Surveillance for Individuals Exposed to SARS-CoV-2: The COPEP Pragmatic Open-Label, Cluster Randomized Trial

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ABSTRACT

Background: Since the beginning of the COVID-19 pandemic, no direct antiviral treatment is effective as post-exposure prophylaxis (PEP). Lopinavir/ritonavir (LPV/r) was repurposed as a potential PEP agent against COVID-19.

Methods: We conducted a pragmatic open-label, parallel, cluster-randomised superiority trial in four sites in Switzerland and Brazil between March 2020 to March 2021. Clusters were randomised to receive LPV/r PEP (400/100 mg) twice daily for 5 days or no PEP (surveillance). Exposure to SARS-CoV-2 was defined as a close contact of >15 minutes in <2 metres distance or having shared a closed space for ≥2 hours with a person with confirmed SARS-CoV-2 infection. The primary outcome is the occurrence of COVID-19 defined by a SARS-CoV-2 infection (positive oropharyngeal SARS-CoV-2 PCR and/or a seroconversion) and ≥1 compatible symptom within 21 days post-enrolment. ClinicalTrials.gov (Identifier: NCT04364022); Swiss National Clinical Trial Portal: SNCTP 000003732.

Findings: Of 318 participants, 157 (49.4%) were women; median age was 39 (interquartile range, 28-50) years. A total of 209 (179 clusters) participants were randomised to LPV/r PEP and 109 (95 clusters) to surveillance. Baseline characteristics were similar, with the exception of baseline SARS-CoV-2 PCR positivity,

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which was 3-fold more frequent in the LPV/r arm (34/209 [16.3%] vs 6/109 [5.5%], respectively). During 21-day follow-up, 48/318 (15.1%) participants developed COVID-19: 35/209 (16.7%) in the LPV/r group and 13/109 (11.9%) in the surveillance group (unadjusted hazard ratio 1.44; 95% CI, 0.76-2.73). In the primary endpoint analysis, which was adjuted for baseline imbalance, the hazard ratio for developing COVID-19 in the LPV/r group vs surveillance was 0.60 (95% CI, 0.29-1.26; p =0.18).

Interpretation: The role of LPV/r as PEP for COVID-19 remains unanswered. Although LPV/r over 5 days did not significantly reduce the incidence of COVID-19 in exposed individuals, we observed a change in the directionality of the effect in favour of LPV/r after adjusting for baseline imbalance. LPV/r for this indication merits further testing against SARS-CoV-2 in clinical trials.

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Research in context

Evidence before the study

Given the delay in the roll-out of effective vaccines in many regions worldwide, there is an urgent need to find a simple and effective preventive treatment that could be widely implemented in the early stage of SARS-CoV-2 infection. Preclinical data suggest that lopinavir/ritonavir (LPV/r) has a direct antiviral action against SARS-CoV-2. To our knowledge, there is no published trial assessing the role of oral LPV/r-based post-exposure prophylaxis (PEP) to prevent COVID-19 in persons exposed to SARS-CoV2.

Added value of this study

In this trial, LPV/r over 5 days did not significantly reduce incidence of COVID-19 in exposed individuals. An imbalance in baseline SARS-CoV-2 PCR positivity resulted in a higher COVID-19 incidence in the LPV/r arm (35/209 (16.7%) vs 13/109 (11.9%)). However, in the pre-planned primary end point analysis, the adjusted hazard ratio for developing COVID-19 in the LPV/r group vs surveillance was 0.60 (95% CI, 0.29-1.26; p =0.18).

Implications of all the available evidence

LPV/r efficacy in PEP remains unproven, but our study suggests a possible role for LPV/r for this indication that needs to be confirmed or disproved in future trials.

1. Introduction

The SARS-CoV-2 pandemic continues to exert intense pressure on societies and health systems worldwide [1]. SARS-CoV-2 is more transmissible in households than other coronaviruses, with a high infectivity during the incubation period [2]. Although mass vaccination will reduce secondary attack rates, estimated to be 16.6% for household transmission in a meta-analysis [3], and the occurrence of severe COVID-19 cases, vaccine hesitancy [4,5], delays in vaccine roll-out, waning immunity post-vaccination [6], non-response to vaccine, and emerging virus variants of concern [7,8] are reasons for SARS-CoV-2 to maintain its outbreak potential in the years to come. In addition to vaccination, contact tracing, mass testing, isolation and quarantine, an affordable, easy-to-use and safe post-exposure prophylaxis (PEP) would be an effective tool to contain future outbreaks in vulnerable populations [9].

A range of PEP candidates against COVID-19 are currently under evaluation in clinical trials. Recent data on the use of combinations of the monoclonal antibodies casirivimab/indevimab etesevimab and bamlanivimab are encouraging [10–12]. However, there are major constraints to their wide use, including costs, manufacturing capacities, and the need for in-hospital administration. Several drugs have been repurposed as potential prophylactic agents against COVID-19, including hydroxychloroquine [13,14], tenofovir disoproxil fumarate [14], lopinavir/ritonavir (LPV/r), favipiravir [15], and ivermectin [16,17]. Recent systematic reviews and network meta-analysis found little or no effect of hydroxychloroquine on reducing mortality, hospital admission or the incidence of SARS-CoV-2 infection [18,19]. LPV/r, a protease inhibitor used in antiretroviral therapy for HIV infection, was repurposed as a PEP agent after studies demonstrated in vitro action against SARS-CoV-2 [20] following reports of promising clinical data for Middle East Respiratory Syndrome coronavirus infection [21]. In four published randomised clinical trials on treatment for hospitalised patients with severe COVID-19, LPV/r did not show any effect on clinical endpoints [22–25], but no randomised clinical trial assessing LPV/r as PEP against COVID-19 has been published to date.

The purpose of this multicentre, pragmatic open-label, cluster randomised trial was to evaluate a 5-day course of LPV/r PEP among asymptomatic individuals with documented exposure to SARS-CoV-2 in Brazil and Switzerland. We aimed to test a community-based strategy by enrolling asymptomatic contacts without waiting for the SARS-CoV-2 PCR test result in contacts for PEP to be initiated.

2. Methods

2.1. Study design and trial oversight

The COPEP (COronavirus Post-Exposure Prophylaxis) Trial is a multicenter, pragmatic open-label, two-arm, parallel group, cluster randomised, investigator-initiated, superiority clinical trial to investigate the efficacy of LPV/r PEP in asymptomatic individuals exposed to SARS-CoV-2 vs no PEP (symptom monitoring alone). During the course of the trial, the protocol underwent several critical amendments. Initially, the protocol was designed as a three-arm parallel group (1:1:1) trial with a hydroxychloroquine 800 mg single-dose as the third arm. This arm was stopped seven weeks after the start of the trial on June 18, 2020, upon request of the Swiss regulatory agency (Swissmedic [Swiss Agency for Therapeutic Products]). At that time, the evidence for the clinical efficacy of hydroxychloroquine in the treatment and prevention of COVID-19 had not been established. Furthermore, in a controversial study published in The Lancet, [26] hydroxychloroquine was associated, either alone or in combination with other molecules, with an increase in adverse effects, particularly cardiac. The benefit/risk ratio was considered to be unfavourable and the prescription of hydroxychloroquine was no longer recommended for the treatment or prophylaxis of COVID-19. Only four participants had been assigned to the hydroxychloroquine arm. The remaining two arms were maintained with 2:1 allocation in favour of LPV/r. Originally conceived as a two-centre trial (Geneva

and Basel), the protocol was amended on June 15, 2020, to include a third Swiss site (Lugano) and a Brazilian site (Rio de Janeiro). On February 4, 2021, as a further amendment, the definition of SARS-CoV-2 infection and COVID-19 was broadened to include not only PCRs, but also rapid antigen tests, and vaccination against SARS-CoV-2 was introduced as an exclusion criterion.

The protocol and amendments were approved by Swissmedic and the local ethics committees in Switzerland and Brazil. An independent medical monitor, data safety monitoring board, and the COPEP trial steering committee provided an oversight of safety and efficacy endpoints. The initial/amended trial protocols and the statistical analysis plan are listed in Supplements 1-3. ClinicalTrials.gov (Identifier: NCT04364022); Swiss National Clinical Trial Portal: SNCTP 000003732.

2.2. Participants

In Switzerland, participants were recruited at Geneva and Basel University Hospitals and the Lugano Regional Hospital. In Brazil, participants were recruited at the Evandro Chagas National Institute of Infectious Diseases in Rio de Janeiro. All individuals aged $\geq$16 years who had a documented close contact with a person with a confirmed SARS-CoV-2 infection (index case), defined as having spent $>$15 minutes in $<$2 metres distance or having shared closed space for $\geq$2 hours, were eligible for inclusion. Index cases had to be diagnosed by either a PCR oro- or nasopharyngeal swab test, or a validated rapid antigen test at an authorized SARS-CoV-2 test centre. Participants were only eligible for inclusion if contact occurred no more than 48 hours before onset of symptoms in the index case and within 7 days of enrolment, but no more than 72 hours after diagnosis of the index case. Participants provided written informed consent before study entry.

Exclusion criteria included symptoms compatible with COVID-19 (tympanic body temperature $>$38.0°, cough, dyspnea, new anosmia or ageusia, sore throat, myalgia, fatigue), known previous SARS-CoV-2 infection, previous vaccination against SARS-CoV-2, or contraindications to LPV/r (impaired liver function, hypersensitivity to LPV/r, drug-drug interactions with the participant's usual medication, persons already taking protease inhibitors) (Supplement 1). Baseline SARS-CoV-2 PCR or antigen positivity were not an exclusion criterion because participants were enrolled as soon as possible without waiting for the PCR result. This approach was chosen to test an easy-to-apply and pragmatic strategy that – if proven effective - would allow to rapidly provide PEP to all asymptomatic contacts of an index case.

2.3. Interventions and randomisation

Individuals who lived in the same household formed a cluster. The rationale for randomisation by household was the risk of cross-contamination and the difficulty in assigning household members to different trial arms. Clusters were randomised in a 1:2 allocation to the control and intervention groups, respectively. Randomisation was done in variable block sizes (6 or 9) in random sequence stratified by site. A statistician not involved in patient recruitment generated the randomisation list for each site and the allocation was placed into sequentially-numbered, opaque sealed envelopes. During enrolment, site investigators followed the numbering sequence of the envelopes.

Participants assigned to the intervention group received 20 prepacked tablets of LPV/r 200/50 mg (WHO prequalified generic formulation; Alletra® , Mylan, India) with the instruction to take two tablets twice daily for 5 days. The first dose was taken under the direct supervision of the study physician. Participants assigned to the surveillance arm received no medication. This was an open-label study. The study doctors and nurses who conducted the visits were aware of the group assigned to each participant, unlike the principal investigator and the trial statistician who were not aware of allocation until the database was sealed, the statistical analysis plan signed, and the primary endpoint analyzed.

2.4. Study procedures

The recruitment procedure varied by study site. In Switzerland, the three regional public health authorities were mandated to contact all SARS-CoV-2-positive individuals and perform detailed contact tracing to inform them of quarantine procedures. As part of the study, the contact information of consenting close contacts was forwarded by the regional public health authorities to the study team who then conducted telephone interviews with these persons, proposed the study, and conducted the pre-screening process. Eligible individuals were then invited to the study site for further eligibility assessment and enrolment. In Brazil, the study team recruited family members of hospitalised patients recently diagnosed with COVID-19 and healthcare workers exposed to SARS-CoV-2 as a result of their professional or private activities.

At enrolment, participants were interviewed and underwent a physical examination. An oropharyngeal swab for a SARS-CoV-2 PCR and a venous blood draw for SARS-CoV-2 serology were also taken. Participants were asked to complete a daily online COVID-19 symptom questionnaire during the 21-day follow-up period, which generated alerts to site investigators if individuals reported a COVID-19-associated symptom and if participants did not complete the questionnaire for two consecutive days. If the alert was related to a COVID-19-related symptom, a member of the study team performed a medical evaluation by telephone and invited the participant for a clinical examination and oropharyngeal swab for a SARS-CoV-2 PCR. Online questionnaires were available in English, French, German, Italian, Portuguese and Spanish.

On day 21, all participants presented for an on-site clinical assessment, which included an oropharyngeal swab for SARS-CoV-2 PCR and a venous blood draw for serology. Any participant with a positive SARS-CoV-2 PCR result during follow-up or at day 21 received care according to current treatment protocols at all sites, as well as a follow-up visit 14 days after the positive test result to assess disease severity on an 8-point ordinal scale ranging from 0 (no COVID-19) to 7 (death). Additionally, participants in Geneva and Basel were asked to provide a dried blood spot via capillary puncture on day 5 to assess the LPV/r level in blood. In Lugano, only participants in the intervention arm were asked to provide dried blood spot samples; no dried blood spots were requested from participants at the Brazil site.

2.5. Study endpoints

The primary endpoint was the 21-day incidence of COVID-19. The analysis included all individuals enrolled (intention-to-treat [ITT] analysis). We defined COVID-19 as evidence of SARS-CoV-2 infection and $\geq$1 compatible symptom between days 1 and 21. SARS-CoV-2 infection was defined as a positive oropharyngeal SARS-CoV-2 PCR and/or a seroconversion of IgG only, or IgG and IgA for SARS-CoV-2 at day 21 if baseline serology was negative. SARS-CoV-2 PCRs were conducted on Cobas 6800 (Roche Diagnostics, Rotkreuz, Switzerland) for Swiss site participants, and Applied Biosystems 7500 Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA) in Rio de Janeiro. SARS-CoV-2 serologies were performed on Roche Elecsys® Anti-SARS-CoV-2 S (Roche Diagnostics) and IgG S-rIFA for blood samples in Switzerland, and on Abbott Architect SARS-CoV-2 IgG (Abbott Diagnostics, Lake Forest, IL, USA) in Brazil.

Secondary endpoints were: 1) 21-day incidence of COVID-19 only in participants with a negative SARS-CoV-2 PCR and/or negative serology at baseline (modified (m) ITT); 2) 21-day incidence of new SARS-CoV-2 infection (irrespective if symptomatic or not) in participants with a negative SARS-CoV-2 PCR and/or negative serology at baseline (mITT); 3) severity of COVID-19 according to an 8-point

ordinal scale in those with COVID-19 (ITT analysis); and 4) incidence of serious adverse events.

Further pre-specified endpoints were acceptability of LPV/r prophylaxis for COVID-19 assessed at day 21, self-reported adherence to LPV/r, and drug levels of LPV/r on day 5 after enrolment. A participant was defined adherent to treatment if he/she took at least 80% of the tablets prescribed. We explored the occurrence of COVID-19 in a per-protocol analysis including only those with at least an 80% pill intake in the LPV/r arm vs all participants in the control arm.

2.6. Statistical analyses

For the primary endpoint, we assumed that 20% of close contacts would develop COVID-19 without treatment. A sample size of 200 participants for the LPV/r group and 100 for the standard of care, or 300 in total, was needed to detect an absolute risk reduction from 20% to 8%, with 80% power, an alpha of 5%, and accounting for a design effect of 1.1 (based on an average cluster size of 3 and an intra-class correlation coefficient of 0.05).

The primary analysis used a complementary log-log regression model for the occurrence of COVID-19. We used a mixed model, with a random intercept for each cluster (randomisation unit). The main fixed predictor was the treatment as randomised. Additionally, the model was adjusted for risk factors for COVID-19 via an adjustment of the baseline imbalance score obtained from a logistic regression model with randomization to PEP as the dependent variable and four independent variables: age (categorized in 10-year age groups); number of risk factors for severe COVID-19 (hypertension, diabetes, heart disease, liver disease, cardiovascular disease, and/or the presence of conditions or therapies that weaken the immune system); a positive serology for SARS-CoV-2; and a positive PCR test for SARS-CoV-2 at baseline. The regression coefficient associated with

Figure 1. Flow Diagram of Participants in the COPEP TrialAbbreviations: HCQ - hydroxychloroquine; LPV/r - lopinavir-ritonavir; ITT - intention to treat; PP - per-protocol; PCR - polymerase chain reaction; DBS - dried blood spot

treatment is the logarithm of a relative hazard. As post-hoc exploratory analyses, we ran the same model once with adding the recruitment site and once with adding body mass index and gender as covariates.

The same model was used for the secondary analyses of COVID-19 and SARS-CoV-2 infection in the mITT population. Due to sparse data, the ordinal 8-level variable measuring the highest severity of COVID-19 was reduced to 3 levels: no COVID-19, COVID-19 without any limitations, COVID-19 of greater severity. This variable was analyzed using ordinal logistic regression, an adjustment of baseline imbalance, and yielded odds ratios for greater severity. The occurrence of adverse events was reported as proportions compared by chi-square tests. Regarding analysis of the dried blood spot test, the proportion of participants who developed COVID-19 was compared between the surveillance arm and the LPV/r arm with quartiles of lopinavir concentrations.

Role of the funding source

The funding source had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

3. Results

3.1. Participants

Between April 23, 2020, and February 26, 2021, 326 participants were enrolled (207 in Geneva; 10 in Basel; 27 in Lugano; 82 in Rio de Janeiro). Eight participants were excluded retrospectively (4 were randomised to the hydroxychloroquine arm before the first protocol amendment removing this arm; 1 excluded due to ineligibility; 3 discontinued the study). Of the remaining 318 participants (in 274 clusters) who had available outcome data, 209 (in 179 clusters) were assigned to LPV/r and 109 (in 95 clusters) to the surveillance arm (Fig. 1).

Exposure occurred most frequently in households (61.0%), followed by leisure activities (19.2%) and the workplace (11.3%). Type of exposure was similar across sites with the only difference of more workplace (17.1%) and less leisure exposures (4.9%) at the Brazilian site compared to Swiss sites (Table S1). Median age was 39 (interquartile range, 28-50) years, with a slightly higher proportion of participants >65 years in the LPV/r compared to the surveillance arm (3.3% vs 0.9%, respectively); 157 (49.4%) were female with a higher proportion in the LPV/r arm (52.2% vs 44%, p=0.13). Most (263/318 [82.7%]) participants had no known risk factor for severe COVID-19. Baseline SARS-CoV-2 PCR was positive in 40/318 (12.6%) participants, with a significant imbalance towards the LPV/r arm (34/209 [16.3%] vs 6/109 [5.5%], respectively; p=0.007) (Table 1). Baseline PCR positivity tended to be higher in household contacts (14.4%) than in participants reporting exposure during leisure activities (9.8%) or at the workplace (5.6%), but this difference was not significant (Tables S2, S3). The average days elapsed between exposure to the index case and enrolment in the trial was shorter in the LPV/r arm (2.75 vs 3.63 days, respectively) (Tables S4, S5).

Table 1
Baseline characteristics of COPEP participants randomized to lopinavir-ritonavir or surveillance following exposure to SARS-CoV-2

	Lopinavir-ritonavir	Surveillance
N	209	109
Women, N (%)	109 (52.2)	48 (44.0)
Age, mean (SD), y	40.5 (13.7)	38.2 (14.0)
Body mass index, mean (SD), kg/m^2	26.6 (6.0)	25.9 (5.3)
Smoking (%)		
Current	55 (26.3)	27 (24.8)
Past	33 (15.8)	13 (11.9)
Never	121 (57.9)	69 (63.3)
Place of exposure to SARS-CoV-2 (%)	135 (64.6)	59 (54.1)
Household	32 (15.3)	29 (26.6)
Leisure activity	24 (11.5)	12 (11.0)
Workplace	2 (1.0)	0 (0.0)
Healthcare	16 (7.7)	9 (8.3)
Other		
Risk factors for severe COVID-19 (%)	7 (3.3)	1 (0.9)
Age ≥65 years	0 (0.0)	2 (1.8)
Cancer	7 (3.3)	1 (0.9)
Cardiovascular disease	9 (4.3)	8 (7.3)
Respiratory disease	1 (0.5)	0 (0.0)
Immunosuppression	6 (2.9)	4 (3.7)
Diabetes mellitus	18 (8.6)	8 (7.3)
High blood pressure		
Number of risk factors for COVID-19 (%)		
0	173 (82.8)	90 (82.6)
1	28 (13.4)	14 (12.8)
2	4 (1.9)	5 (4.6)
3	4 (1.9)	0 (0.0)
Positive PCR for SARS-CoV-2 (%)	34 (16.3)	6 (5.5)
Positive serology for SARS-CoV-2 (%)	20 (9.6)	13 (11.9)
Cluster size, N (%)		
1	153 (73.2)	85 (78.0)
2	22 clusters, 44 (21.1)	8 clusters, 16 (14.7)
3	4 clusters, 12 (5.7)	1 cluster, 3 (2.8)
5		1 cluster, 5 (4.6)

3.2. Efficacy endpoint analyses

During the 21-day follow-up, 48/318 (15.1%) participants developed COVID-19, 35/209 (16.7%) in the LPV/r and 13/109 (11.9%) in the surveillance group (Tables S6, S7, S8). Overall COVID-19 incidence depended strongly on the presence or absence of SARS-CoV-2 in the baseline PCR test. Among baseline PCR-positive participants, most developed COVID-19 (27/34 in the LPV/r [79.4%]) vs 6/6 in the surveillance arm [100%]). Among baseline PCR-negative participants, the risk of COVID-19 was lower in the LPV/r arm compared to the surveillance arm (4.6% [8/175] vs 6.8% [7/103], respectively). Due to the higher SARS-CoV-2 PCR positivity in the LPV/r arm at baseline, the LPV/r effect was detrimental in the overall sample, but tended towards protection for both subgroups, ie, individuals who were SARS-COV-2 PCR-positive and -negative at baseline (Table 2). In an unadjusted analysis, the hazard ratio (HR) for COVID-19 in the LPV/r vs surveillance was 1.44 (95% CI 0.76-2.73). In the pre-specified primary endpoint analysis in a mixed model adjusted for baseline imbalance, the HR for developing COVID-19 in the LPV/r vs surveillance arm 0.60 (95% CI, 0.29−1.26; p=0.18) (Table 2). Results were similar in two post hoc exploratory analyses (Table S9). There was no significant difference in the occurrence of COVID-19 across the four study sites. Similarly, there was no significant difference in the average number of days between exposure and enrolment between participants who developed COVID-19 and those who did not (Table S10).

Secondary endpoints are displayed in Table 2. In the mITT including only participants with a negative SARS-CoV-2 PCR at baseline, 8/175 (4.6%) and 7/103 (6.8%) developed COVID-19 in the LPV/r and surveillance arm, respectively (HR, 0.58; 95% CI 0.16-2.07; p=0.40). Results were similar for participants with a negative serology (HR, 0.61; 95% CI 0.30-1.26; p=0.18) or a negative serology and PCR at baseline (HR, 0.59; 95% CI 0.17-2.02; p=0.40). Occurrence of SARS-CoV-2 infection (with or without COVID-19) in participants with negative baseline serology and PCR at baseline did not differ between LPV/r and surveillance (1.03; 95% CI 0.37- 2.91, p=0.95) (Table 2).

Of the 48 participants who developed COVID-19, 33 (69%) had no limitation on daily activity (level 1), 13 (27%) had limitations (level

Table 2
Efficacy outcomes

	Lopinavir-ritonavir (%)	Surveillance (%)	Adjusted hazard ratio (95% CI) *	Adjusted p-value*
21-day incidence of COVID-19 (primary outcome, intention to treat)	35/209 (16.7)	13/109 (11.9)	0.60 (0.29-1.26)	0.18
21-day incidence of COVID-19 among participants with negative SARS-CoV-2 serology and negative PCR at baseline (modified intention to treat)	8/159 (5.0)	7/90 (7.8)	0.59 (0.17-2.02)	0.40
21-day incidence of COVID-19 among participants with negative PCR at baseline (modified intention to treat)	8/175 (4.6)	7/103 (6.8)	0.58 (0.16-2.07)	0.40
21-day incidence of COVID-19 among participants with negative serology at baseline (modified intention to treat)	33/189 (17.5)	13/96 (13.5)	0.61 (0.30-1.26)	0.18
21-day incidence of SARS-CoV-2 infection among participants with negative SARS-CoV-2 serology and negative PCR at baseline (modified intention to treat)	12/158 (7.6)	7/90 (7.8)	1.03 (0.37- 2.91)	0.95
21-day incidence of SARS-CoV-2 infection among participants with negative PCR at baseline (modified intention to treat)	12/174 (6.9)	7/103 (6.8)	1.02 (0.34- 3.07)	0.97
21-day incidence of SARS-CoV-2 infection among participants with negative SARS-CoV-2 serology (modified intention to treat)	38/188 (20.2)	13/96 (13.5)	0.87 (0.45-1.70)	0.68
21-day incidence of COVID-19 among participants whose adherence was ≥80% in the PEP arm, vs. all in surveillance arm (per protocol)	31/182 (17.0)	13/109 (11.9)	0.63 (0.30-1.32)	0.22
21-day incidence of COVID-19 by lopinavir concentration in the post-exposure prophyaxis arm among participants with a dried blood spot test (exploratory analysis):		12/72 (16.7)	1 (reference)	
Quartile 1: 0-2299 ng/ μL	5/36 (13.9)		0.56 (0.19-1.62)	0.28
Quartile 2: 2300-4399 ng/ μL	8/36 (22.2)		0.75 (0.29-1.92)	0.55
Quartile 3: 4400-6099 ng/ μL	6/36 (16.7)		0.27 (0.09-0.87)	0.028
Quartile 4: 6100 ng/ μL and more	7/37 (18.9)		0.61 (0.25-1.50)	0.39
Degree of severity of COVID-19 (3 levels) (intention to treat)			Adjusted ordinal odds ratio (95% CI) *	
0: no COVID-19	174 (83.3)	96 (88.1)	0.73 (0.33-1.63)	0.44
1: no limitation	21 (10.0)	12 (11.0)		
2-4	14 (6.7)	1 (0.9)		

*Mixed effects model adjusted for baseline imbalance

2), and two participants (4%) were hospitalised (one required supplemental oxygen [level 4] and one did not [level 3]). No participant required further medical assistance (Table 2).

3.3. Exploratory analyses

At the end of the study, 182/209 (87.1%) in the LPV/r arm judged an intake of LPV/r for 5 days as acceptable for COVID-19 prevention, 13 found it unacceptable (6.2%), and 14 (6.7%) had no opinion. In the LPV/r group, 182 (87.1%) participants self-reported an adherence of 80% or higher. Most cited reasons for non-adherence were gastrointestinal side-effects (57.7%), dosage error (23.1%) and pill size (3.8%). When considering only the subgroup with at least 80% adherence to LPV/r vs surveillance, 31/182 vs 13/109 developed COVID-19 (HR, 0.63; 95% CI 0.30-1.32). Overall, 145/209 (69.4%) and 72/109 (66.1%) had a dried blood spot test result in the LPV/r and surveillance arm, respectively (Tables S11, S12, S13). There was no clear correlation between quartile of LPV concentration and the hazard to develop COVID-19 (Table 2).

3.4. Adverse events

Adverse events were reported by 175/207 (84.5%) in the LPV/r and 33/107 (30.8%) in the surveillance arm (missing data in four participants). Two serious adverse events occurred, both LPV/r unrelated (Table 3).

4. Discussion

Our trial was inconclusive and produced only weak evidence in favour of LPV/r as prophylactic treatment for persons in close contact with SARS-CoV-2. The point estimate (HR, 0.60) was consistent with a clinically meaningful effect, but the confidence interval was wide (range 0.29-1.26) and the result was not statistically significant. This suggests that LPV/r may merits further consideration as a PEP candidate but additional trials are needed to strengthen the evidence for or against this prophylactic option.

We initiated this trial at the very beginning of the sanitary crisis in April 2020. The pragmatic approach and the multi-site enrolment in two countries heavily affected by the COVID-19 pandemic were challenging. Despite this, we succeeded in completing the recruitment in due time with little or no lost to follow-up, and with an excellent adherence to the study intervention.

In interpreting the results, we faced two methodological issues. First, the sample size was too small to reliably detect an HR of 0.60. In planning the trial, we aimed to detect a reduction in the risk of COVID-19 from 20% to 8%, i.e., an HR of 0.37; in retrospect, this was too optimistic. Second, the distribution of the SARS-CoV-2 positivity among the two arms was uneven at study entry, despite standard randomization procedures followed at all sites. The most likely explanation is chance. Indeed, randomization can on occasion produce large differences between trial arms. Unfortunately, in our trial, this

Table 3
Adverse events reported by COPEP participants by treatment arm

	Lopinavir-ritonavir (%)	Surveillance (%)	P (exact)
N	207	107	
Any	175 (84.5)	33 (30.8)	<0.001
Nausea	70 (33.8)	4 (3.7)	<0.001
Vomiting	10 (4.8)	2 (1.9)	0.23
Diarrhea	115 (55.6)	7 (6.5)	<0.001
Abdominal pain	67 (32.4)	5 (4.7)	<0.001
Lack of appetite	47 (22.7)	4 (3.7)	<0.001
Itching	20 (9.7)	1 (0.9)	0.003
Bloating	61 (29.5)	2 (1.9)	<0.001
Other	86 (41.5)	25 (23.5)	0.002

large difference affected the strongest predictor of any infectious disease – the presence of the infectious agent in the target organ of the host. However, through our past experience in conducting such multi-site trials, we were aware of the necessity to adjust for baseline SARS-CoV-2 positivity (and other potential confounders) due to expected imbalances and this was planned in the primary analysis. This well illustrates the utility of pre-specifying an adjusted analysis of the main outcome, especially in smaller trials.

The rate of COVID-19 observed in participants who were SARS-CoV-2 -positive or -negative at baseline aligns with previously published studies. In prevention trials, such as large phase III randomized studies testing the prophylactic effect of monoclonal antibodies [27,28], the treatment effect was analysed separately for participants with a negative baseline PCR (so-called "prevention population") and those with a positive baseline SARS-CoV-2 PCR ("preemptive treatment" population), thus accounting for the major differences in these two groups. Interestingly, among those with a positive SARS-CoV-2 PCR during the phase III trial evaluating the efficacy of casirivimab with imdevimab in prevention, placebo recipients had a 100-fold greater peak viral load and SARS-CoV-2 became undetectable within a week in the active arm, but persisted in 40% of placebo recipients at 3-4 weeks [28]. When administered early in the course of a mild symptomatic disease, other oral antivirals, such as molnupiravir, also showed their ability to decrease virus infectivity at day 5 compared with placebo in a small phase II trial [29]. The target dose of LPV/r in COVID-19 remains unknown, but it has been suggested that the low concentration in the lungs partly explained its lack of efficacy in treatment [30].

Our study has several limitations. First, the sample size was too small, similar to the number of events, as the observed incidence of COVID-19 in the surveillance arm was less than expected (11.9% versus 20%, respectively). The addition of data from ongoing clinical trials [31] assessing LPV/r given as a chemoprophylactic agent will offer more power to assess LPV/r efficacy for this indication. Second, LPV/r has well documented, short-term adverse effects and a considerable number of drug-drug interactions, thus limiting its wide use, especially in high-risk groups with co-medications. This restricted our ability to recruit the most vulnerable populations and may also reduce its attractiveness as a future PEP if proven efficacious. Third, the uneven distribution of PCR positivity may have played a role and a more balanced distribution might have increased the power of the trial. Finally, recruitment was largely driven by intrafamilial transmission and we were unable to assess the ability of LPV/r to produce a beneficial effect to protect healthcare workers.

In COVID-19, vaccines are indeed the most powerful preventive strategy so far. However, we believe that the use of multifaceted interventions, including repurposed antivirals or antiviral-based antibodies, could add a tailored approach by helping not only to prevent infection, but also to attenuate disease severity and lower its transmission to contacts [32]. Importantly, the requirements for an effective antiviral drug to be given prophylactically or early after diagnosis will be high and cost, convenience and tolerability will be critical issues to assess before recommending such interventions on a large scale.

In summary, LPV/r 400/100mg used as PEP for asymptomatic individuals recently exposed to SARS-CoV-2 provided no definite conclusions with regards to its effectiveness. The point estimate of the effect after adjustment for baseline imbalances showed a statistically non-significant trend towards protection in the LPV/r treated group in some but not all mITT analyses.

Author contributions

AC and TP had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Concept and design: NDL, AC, MS
Drafting the manuscript: NDL, AC, IP
Critical revision of the manuscript for important intellectual content: All authors
Statistical analysis: TP
Obtained funding: AC, NDL
Administrative, technical, or material support: DOA, BM, LAD, JS, FJ, HS, DL, EB, SWC, BG, MS
Overall study responsibility: AC

Data sharing statement

Anonymized data set with core-data from the trial will be made available on Zenodo upon publication of the manuscript. The full dataset will be available upon reasonable request to the corresponding author.

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Declaration of Competing Interest

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.eclinm.2021.101188.

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