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Randomized Clinical Trial to Compare the Efficacy of Ivermectin Vs. Placebo to Negativize Nasopharyngeal PCR in Patients with Early Covid-19 in Peru (Saint-Peru)

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Abstract

Ivermectin was a popular drug used for COVID-19 in Latin America, although there was no clinical evidence about its efficacy. We conducted a randomized, placebo-controlled trial to evaluate the effect of ivermectin on the detection of SARS-CoV-2 RNA by PCR in non-severe COVID-19 patients in Peru.

Patients with 96 hours of COVID-19 related symptoms were preselected at the emergency services of two national hospitals. Those eligible were randomized to one dose of 300 mcg/kg ivermectin or placebo daily for 3 days and were followed for 21 days. The primary outcome was the proportion of patients with a positive SARS-CoV-2 PCR from a nasopharyngeal swab at day 7. 22/24 in the placebo and 16/16 in the ivermectin arm (p=0.236) were PCR positive at day 7, and participants in the placebo arm had cycle threshold (Ct) values significantly higher compared to the ivermectin arm (p=0.028 and 0.014 for genes N1 and N2 respectively). There was no difference between arms in disease progression, adverse events, IgG seroconversion rate or median IgG titers at day 21.

We found no difference in the PCR positivity between those treated with ivermectin or placebo suggesting no benefit from the use of ivermectin among patients with non-severe COVID-19.

Keywords: COVID-19; Ivermectin; Randomized clinical trial

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Introduction

The COVID-19 pandemic has already exceeded 183 million cases and 4 million deaths worldwide. And yet, there is no current specific and effective antiviral treatment available for this disease.[1]

Ivermectin is an affordable and widely used antiparasitic drug with an excellent safety profile and known properties against single-strain RNA viruses.[2,3] It was proposed as a candidate repurposed drug against COVID-19 due to initial studies showing its potential to inhibit *in vitro* replication of SARS-CoV-2.[4] The scientific community debated whether these results warranted further studies since the *in vitro* micromolar concentrations used were much higher than the achieved with routine antiparasitic doses.[5-7] Even when evidence from animal models showed that ivermectin could potentially reach concentrations three-fold higher in lung tissue than in plasma, those levels were still below those found effective *in vitro*.[8,9]

Many ecological, observational, and a few experimental studies have investigated the use of ivermectin in blocking viral transmission and disease progression in COVID-19 patients. Clinical outcomes of ivermectin in COVID-19 have also been studied.[10] Yet, there is still no conclusive evidence to appropriately inform policy decisions.

In Latin America, ivermectin for COVID-19 began to be used early in the pandemic, based on the *in vitro* study and the results of an observational study (pre-print) reporting a strong positive association between ivermectin and survival in patients with COVID-19 in April 2020. That study used a database owned by Surgisphere, now-discredited company, and was eventually withdrawn. However, it started to be cited in white papers and was used by governments in countries like Bolivia and Peru to release clinical guidelines promoting widely ivermectin use for COVID-19.[11-13]

In order to collect information that could guide better policy decisions, we implemented a randomized control trial with the primary objective of evaluating the effect of ivermectin on the detection of SARS-CoV-2 RNA by PCR when administered on the first days of disease to non-severe COVID-19 patients in Peru. The secondary objectives were to evaluate the viral load, clinical improvement, and seroconversion, safety of proposed dose and magnitude of the immune response.

Methods

Study design

This was a triple-blinded, placebo-controlled, parallel-arm, randomized clinical trial that compared ivermectin *vs.* placebo in patients with non-severe COVID-19. The study was conducted in two national hospitals in Lima- Peru from August 2020 to April 2021. The clinical team, the statistician, and the participants were all blinded as to arm allocation. The study pharmacist was the only person unblinded. The protocol was approved by the Peruvian National COVID-19 Ethics Committee and is published.[14] All participants in the trial provided two consents: a preselection consent, and a consent for participation in the trial. This study is registered at ClinicalTrials. gov: NCT04635943.

Participants

Potential participants were identified at the emergency services of the study sites. The pre-selection criteria were: COVID-19 symptomatology lasting no more than 96 hours, age 18 or older, and no use of ivermectin in the prior month, no known history of ivermectin allergy, and no current use of CYP 3A4 or P-gp inhibitor drugs or critical CYP3A4 substrate drugs. The exclusion criteria were having clinical signs of pneumonia (oxygen saturation <

95% or crackles at lung examination), and/or a positive pregnancy test and/or a positive SARS- CoV-2 rapid serological test (STANDARD Q COVID-19 IgM/IgG, SD Biosensor, Gyeonggi-do, South Korea) at baseline. PCR test for SARS-CoV-2 was taken on participants on the day of recruitment to confirm the diagnosis. Participants were randomized before having the results of the PCR to assure an early start of treatment. Starting in February 2021 a positive antigen test result for SARS-CoV-2 was added as an inclusion criterion.

Randomization and concealment

Participants were randomized 1:1 to receive one dose of 300 mcg/kg ivermectin or placebo (solution) daily for three consecutive days. The statistician generated a list of correlative numbers, in randomized blocks of size 4, with the assignment to the treatment groups (a and b). This list was handed directly to the pharmacist. The randomization list was kept in an encrypted file accessible only to the trial statistician and the pharmacist. Independently, the principal investigator randomly assigned the intervention (ivermectin) to one of the two groups (a or b) by tossing a coin but never had access to the list of randomized numbers. She informed the pharmacist of the result of this process. The pharmacist prepared and labeled the treatment vials according to the randomization list prepared by the statistician and the treatment assignment given by the principal investigator. The vials with placebo were visibly identical to the ones with the active drug.

Procedures

Patients seen at the emergency of the participating hospitals, with a history of less than 4 days of symptoms compatible with COVID-19 and no use of ivermectin were referred to the trial team to assess them for eligibility. If they fulfilled pre-selection criteria, they were invited to participate and signed a pre-selection written informed consent. Subsequently, study investigators measured oxygen saturation, examine their lungs for crackles, took a SARS-CoV-2 rapid serological test, a pregnancy test for women in childbearing age, and a SARS-CoV-2 antigen test. If no exclusion criteria were met, participants were invited to sign a written consent to participate in the clinical trial. A nasopharyngeal swab was taken to confirm the presence of SARS-CoV-2 RNA by PCR. We collected data on demographics, comorbidities, baseline symptoms, vital functions, physical examination, and concomitant medication. Additionally, blood and stool samples were collected at baseline. Finally, the participant was given the first dose of the investigational product and was monitored for 30 minutes for any adverse events. This was considered day 1 of the study. Two additional vials of the investigational product with the instructions of the number of drops to take according to their weight were given to the patient for self-administration on day 2 and 3.

Participants were followed for a total of 21 days. Daily follow-up calls were carried out from day 2 to day 21 of the study. During the calls, study staff informed participants about the results of their baseline PCR test for SARS-CoV-2. If it was negative, participants were asked to stop taking the investigational product and informed that they were excluded from the study. This was done due to the 24-48 hours waiting time for PCR results in Lima which precluded inclusion upon results. Data on symptoms, adverse events and concomitant medication was collected by telephone. Additionally, study physicians conducted home or hospital (in case participants were hospitalized) visits at days 4, 7, 14 and 21. During these visits, study physicians collected data on symptoms, vital functions, and physical examination. A SARS-CoV-2 rapid serological test and a blood sample were taken at days 7, 14 and 21. An additional stool sample was collected on day 14. A nasopharyngeal swab for SARS-CoV-2 PCR was repeated at all visits. All samples for PCR testing (including baseline), were processed for genes N1 and N2 of SARS-CoV-2 (2019-nCoV TaqMan RT-PCR, Norgen Biotek, Canada). Unfortunately, in Peru viral cultures

for SARS-CoV-2 were not feasible at the time of the study, so we used the cycle threshold (Ct) values as proxy to viral load. IgG antibodies to the full-length SARS-CoV-2 spike protein (S crg), its sub-region (S2), RBD that lies within the S1 region (RBD kr), the nucleocapsid protein full length protein (N-FL) and its specific C-terminal region (N-C term) were measured on samples from all patients on day 21 by Luminex based on a previously described protocol.[15]

Stools (baseline and day 14) were examined for the presence of parasites by microscopy, both directly and after formalin ether concentration and sedimentation.[16]

Outcomes

The primary outcome for the study was the proportion of patients with a positive SARS-CoV-2 PCR from a nasopharyngeal swab at day 7. Secondary outcomes were 1). The proportion of patients with fever and cough at days 4, 7, 14, and 21 as well as the proportion of patients progressing to severe disease or death during the trial; 2). The proportion of drug-related adverse events during the trial; 3). Ct values on days 4, 7, 14, and 21; 4). The proportion of patients with a positive IgG with the rapid serology test at day 21; 5). The change in proportion of intestinal helminths between baseline and day 14; 6). The median IgG levels at day 21.

Sample size and Statistical analysis

The sample size was calculated to compare two proportions. We estimated a minimum of an effective sample of 22 (11 per arm) could provide 80% power at the 5% level of significance using Fisher's one-sided test to determine a difference of at least 45% (100% *vs.* 55%) in the proportion of participants with a positive PCR result on day 7 post-treatment. The estimation of 100% PCR positivity on day 7 is based on the study by Chaccour in Navarra.[17] The effective sample size (16 in the intervention and 24 in the control arm) provides a power of 81% to detect differences of 39% (61% in intervention, 100% in the control group). For the statistical analysis and generation of graphs Stata Statistical Software Version 17.0 was used. Descriptive analyses used frequencies and percentages for qualitative variables, and medians and interquartile ranges for quantitative variables. Baseline data was compared between study arms using Fisher's exact test for qualitative variables and Mann-Whitney U test for quantitative variables. The proportion of participants with positive PCR tests and the proportion of participants with positive rapid serological test were calculated at each visit. Proportions were compared between study arms using Fisher's exact test and presented as a Relative Risk (RR) with their corresponding 95% Confidence Interval (CI). To evaluate the potential effect of lost to follow-up, sensitivity analysis assuming the worst-best and best-worst possible scenarios for missing data were performed. For the analysis of the symptoms reported by patients at the visits, the proportion of patients with each symptom was calculated and compared between study arms using Fisher's exact test. For each symptom and patient, the proportion of effective daily phone calls with report of that symptom was calculated. Then, the mean of the proportions of days with each symptom were compared between study arms using a student's t test. Adverse events and concomitant medication during the trial were also reported by patients in the daily follow up calls. The proportion of patients with at least one adverse event or administration of a medication on one occasion was calculated. Proportions were compared between study arms using Fisher's exact test. Significance was set at 0.05. The Ct for genes N1 and N2 are right censored for PCR negative samples, so they were analyzed as a time to event variable in survival analysis. PCR negative samples were considered as censored at time 40. Log rank test was used to test for differences in survival associated with treatment arm.

Results

Patient characteristics

Of 514 participants assessed, 342 did not meet eligibility criteria, 9 declined to participate and 163 were preselected. Of this 163, 26 were excluded, 1 withdrew from the study and 136 signed the written consent and were randomized and received either ivermectin or placebo.

Of the 67 randomized in the placebo arm, 41 were excluded due to negative PCR test for SARS-CoV-2, one was lost to follow up before day 4 and one withdrew from the study. Of the 69 randomized in the ivermectin arm, 49 were excluded because their PCR test for SARS-CoV-2 was negative, 3 withdrew from the study before the day 4, and 1 was lost to follow up before day 7. Finally, 24 and 16 were included in primary analysis (day 7) in the placebo and intervention arm, respectively. One participant from the ivermectin group did not complete visit from day 21 (Figure 1).

The characteristics of the population at baseline by arm are presented in Table 1. The median age was 35.5 and 34 years old for the placebo and ivermectin arms, respectively. There was a higher proportion of females in the placebo arm (66.7% *vs.* 50%), however this difference was not statistically significant. The median Body Mass Index (BMI) was over 25 for both groups. Very few (4 in the placebo and 3 in the ivermectin arm) reported an underlying condition. There were no significant differences between groups for symptoms or vital signs on presentation. The median time from symptoms to randomization was 72 hours for both groups.

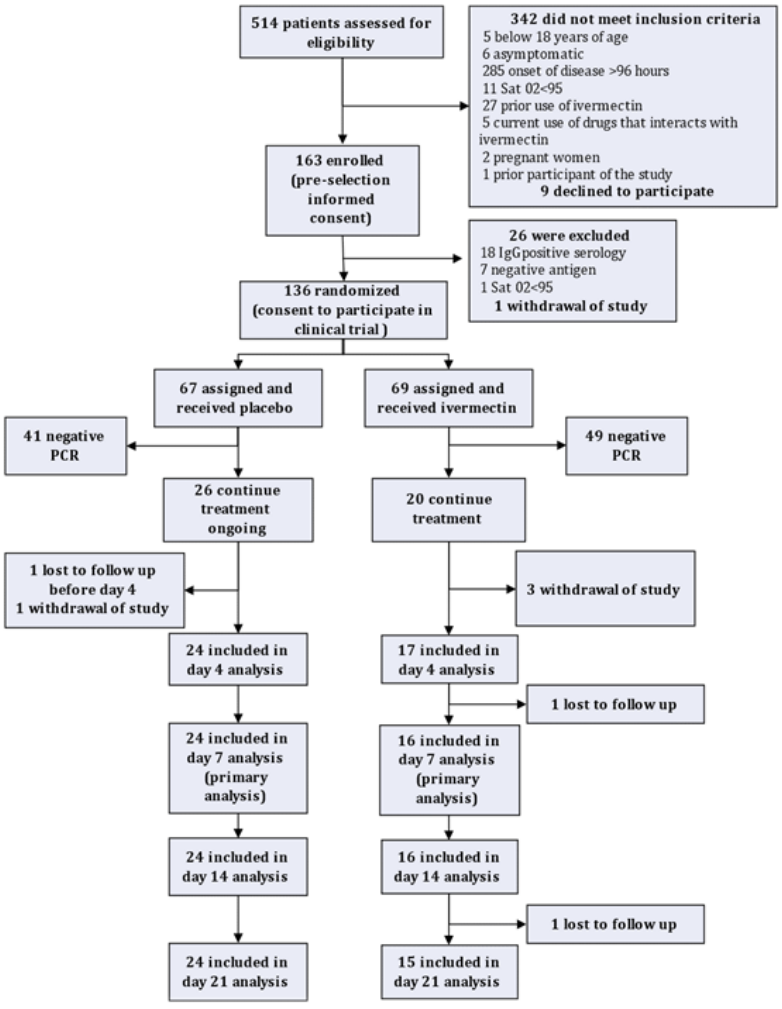


Figure 1: Enrollment and patient flow.

Table 1. Demographic and Clinical Characteristics of the Primary Analysis Population at Baseline by arm

	Placebo (N =24)	Ivermectin (N=16)
Age, median (IQR)	35.5(25.5-46.5)	34 (30-45.5)
Gender, n (%)		
Male	8 (33.3)	8 (50.0)
Female	16 (66.7)	8 (50.0)
BMI, median (IQR)	26.9 (22.7-31.7)	28.1 (22.7-30)
Coexisting conditions		
Hypertension, n (%)	1 (4.2)	0 (0.0)
Diabetes, n (%)	0 (0.0)	1 (6.3)
Asthma, n (%)	2 (8.3)	2 (12.5)
Hepatic disease, n (%)	1 (4.2)	0 (0.0)
Symptoms		
Fever n (%)	16 (66.7)	10 (58.8)
Cough n (%)	15 (62.5)	14 (82.4)
Malaise n (%)	21 (87.5)	15 (88.2)
Headache n (%)	23 (95.8)	16 (94.1)
Odynophagia n (%)	16 (66.7)	13 (76.5)
Diarrhea n (%)	4 (16.7)	5 (29.4)
Anosmia n (%)	5 (20.8)	3 (17.6)
Median time (IQR) from symptoms to randomization (in hours)	72 (70-96)	72 (72-93)
Vital signs		
Respiratory rate, median (IQR), bpm	18 (16.5-18)	17.5 (16-18)
Temperature, median (IQR), °C	36.8 (36.6-37)	36.6 (36.5-37.1)
Oxygen saturation, median (IQR), %	99 (98-99)	98.5 (98-99)

Primary outcome, PCR results and cycle threshold (Ct) analysis

There was no significant difference in the proportion of patients with a positive PCR at day 7 post treatment, 22/24 (91.7%) in the placebo group and 16/16 (100%) in the ivermectin arm had a positive PCR (RR 0.92, 95% CI: 0.81-1.03, p=0.236).

Additionally, the PCR positivity on follow up at day 4, 14 or 21 was not significantly different between groups (Table 2). Ct values were similar at baseline (day 1) between treatment arms (p=0.779 and 0.856 for genes N1 and N2 respectively). Ct values were significantly higher for the placebo arm at day 7 (p=0.028 and 0.014 for genes N1 and N2 respectively (Figure 2) (Table 3).

Table 2. Patients with a positive SARS-CoV-2 PCR from a nasopharyngeal swab by arm and day of follow-up. The primary end point is calculated based on day 7 follow up.

	Placebo (N=24)	Ivermectin (N=17)	Effect estimate PR[a] (95% CI)	p-value
Positive PCR at day 4, n/N (%)	22/24 (91.7)	17/17 (100)	1.09 (0.97-1.23)	0.502
Positive PCR at day 7, n/N (%)	22/24 (91.7)	16/16[b] (100)	1.09 (0.97-1.23)	0.508
Positive PCR at day 14, n/N (%)	11/24 (45.8)	8/16 (50)	1.09 (0.57-2.10)	1.000
Positive PCR at day 21, n/N (%)	7/24 (29.2)	6/14[c] (42.9)	1.47 (0.62-3.50)	0.486

a PR: prevalence ratio

b 1 participant was lost after day 4.

c 1 participant didn't complete day 21 visit and in 1 case the result of the PCR was inconclusive, so it was not considered in the analysis.

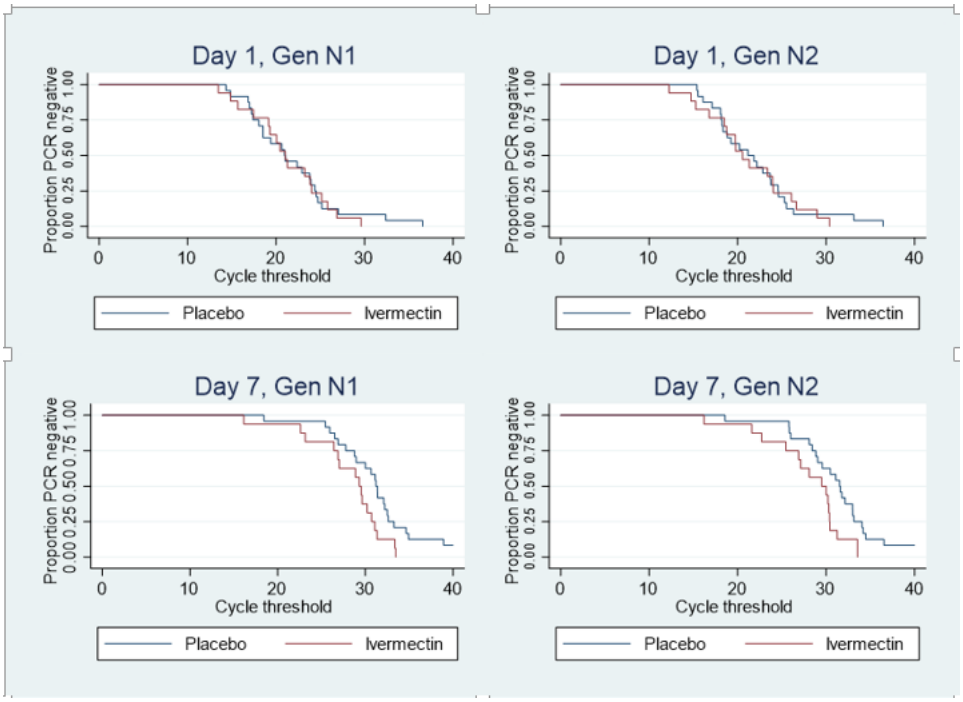


Figure 2: Kaplan-Meier survival of Cycle threshold (Ct) for genes N1 and N2 for samples collected at day 1 and 7. Ct was used as time to event. PCR negative samples were considered censored at time 40.

Table 3: CT values by study arm and day of sample collection

	Gene N1			Gene N2		
	Ivermectin	**Placebo**	**p***	**Ivermectin**	**Placebo**	**p***
	Cycle threshold, median (min – max)			**Cycle threshold, median (min – max)**		
Day 1	21.0 (13.5 – 29.6)	21.0 (14.3 – 36.6)	0.779	20.6 (12.3 – 30.4)	21.5 (15.4 – 36.5)	0.856
Day 4	24.5 (16.1 – 31.3)	27.0 (17.7 – ≥40)	0.059	24.9 (16.2 – 32.2)	27.3 (17.2 – ≥40)	0.072
Day 7	29.4 (16.1 – 33.5)	31.3 (18.4 – ≥40)	0.028	29.8 (16.2 – 33.6)	31.6 (18.6 – ≥40)	0.014
Day 14	38.3 (27.1 – ≥40)	≥40 (28.0 – ≥40)	0.714	38.5 (27.4 – ≥40)	≥40 (28.5 – ≥40)	0.682
Day 21	≥40 (28.4 – ≥40)	≥40 (33.2 – ≥40)	0.325	≥40 (30.1 – ≥40)	≥40 (32.3 – ≥40)	0.354

*Log-rank test considering Ct as time to event and censoring at time 40 for PCR negative samples.

Sensitivity analysis for the main outcome

In the worst-best scenario, the four participants lost to follow up assigned to ivermectin would remain PCR positive by day 7, and the 2 lost to follow up in the placebo group would become PCR negative. This would still result in non-significant differences between the two groups (p=0.121). Likewise, in the best-worst scenario, the four participants assigned to ivermectin lost to follow up would become PCR negative at day 7, and the 2 lost to follow up in the placebo group would remain PCR positive. This again would result in non-significant differences between the two groups (p=0.380). The sensitivity analysis indicates that those lost to follow up could not significantly change the results of this trial.

Symptoms

There were no statistically significant differences between arms on the proportion of patients with symptoms at any of the visits, except for odynophagia at day 7, that was higher in the placebo arm (p=0.027) (Table 4).

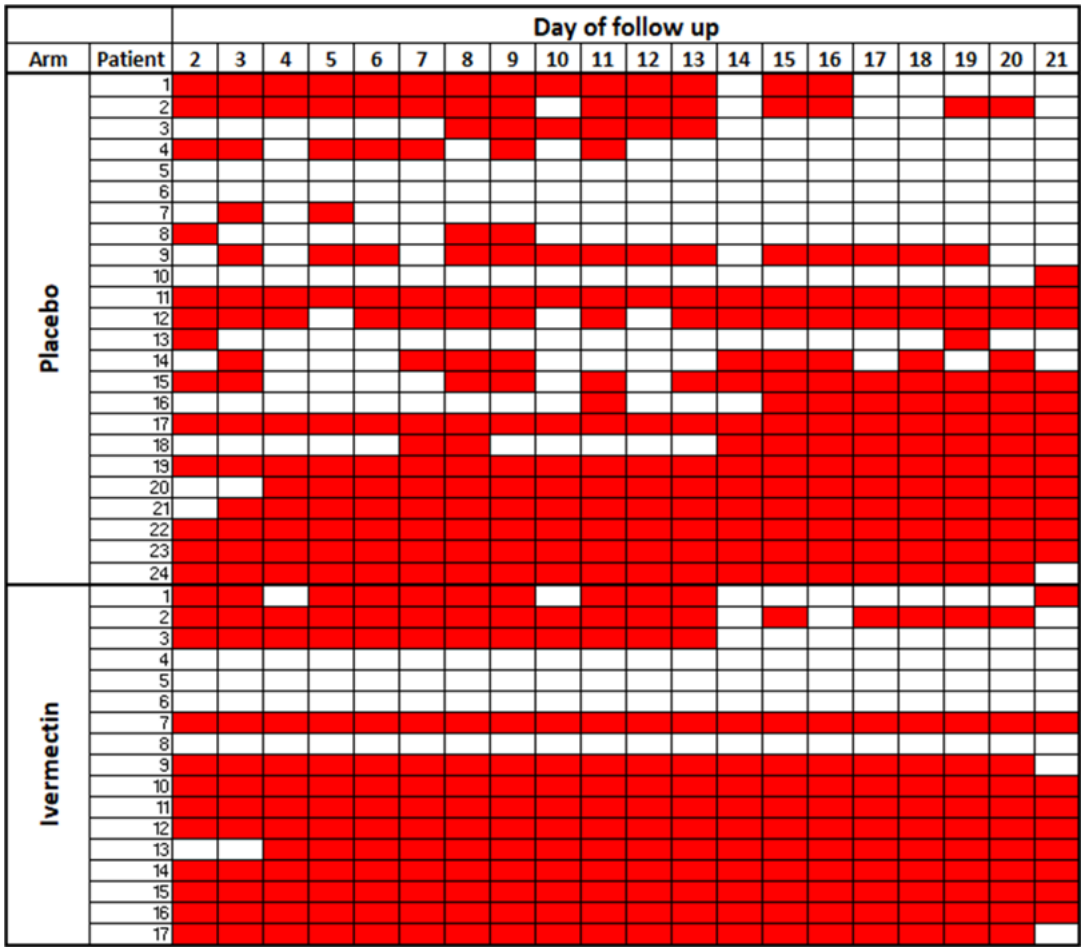
Table 4: Symptoms by arm and day of follow up

Symptoms	Day 4			Day 7			Day 14			Day 21		
	Placebo (N=24)	Ivermectin (N=17)	p-value	Placebo (N=24)	Ivermectin (N=16) [a]	p-value	Placebo (N=24)	Ivermectin (N=16) [a]	p-value	Placebo (N=24)	Ivermectin (N=15) [b]	p-value
Fever, n (%)	8 (33.3)	3 (17.7)	0.309	0 (0.0)	3 (18.8)	0.057	0 (0.0)	0 (0.0)	-	1 (4.2)	0 (0.0)	1.000
Cough, n (%)	18 (75)	15 (88.2)	0.433	14 (58.3)	8 (50)	0.748	6 (25)	6 (37.5)	0.490	4 (16.7)	3 (20)	1.000
General malaise, n (%)	16 (66.7)	12 (70.6)	1.000	15 (62.5)	9 (56.3)	0.750	3 (12.5)	2 (12.5)	1.000	2 (8.3)	2 (13.3)	0.631
Headache, n (%)	14 (58.3)	8 (47.1)	0.537	12 (50)	5 (31.3)	0.332	6 (25)	4 (25)	1.000	3 (12.5)	3 (20)	0.658
Odynophagia, n (%)	17 (70.8)	10 (58.8)	0.512	15 (62.5)	4 (25)	**0.027**	4 (16.7)	2 (12.5)	1.000	4 (16.7)	1 (6.7)	0.631
Diarrhea, No. (%)	5 (20.8)	2 (11.8)	0.679	3 (12.5)	5 (31.3)	0.229	5 (20.8)	2 (12.5)	0.681	3 (12.5)	1 (6.7)	1.000
Anosmia, No. (%)	9 (37.5)	9 (52.9)	0.358	13 (54.2)	6 (37.5)	0.349	11 (45.8)	7 (43.8)	1.000	8 (33.3)	4 (26.7)	0.734

[a] 1 participant was lost after day 4.
[b] 1 participant didn't complete day 21 visit

Symptoms were also collected during daily follow-up calls. Of the 820 daily phone calls planned for the 41 participants included in the analysis, 514 (62.7%) were completed, with 6 participants who didn't answer any of the calls, and 12 answering all 20 scheduled calls. There were 278 calls answered in the placebo and 236 in the ivermectin arm (Figure 3).

Figure 3: Phone calls to patients to collect information on symptoms



Patients were called daily starting when PCR result was available (which varied between 24 hours to 72 hours), however several times they didn't answer, or preferred not to talk because they were sick or were outside their home. Each row represents a study participant. We consider a call completed when the information about symptoms was collected. Highlighted in red are those days where the call was completed.

The median number of completed calls per participant in the ivermectin arm [19] was not significantly higher than the median for participants in the placebo arm,[14] (Kruskall-Wallis p: 0.284). There were no statistically significant differences between arms for any of the symptoms collected through phone calls (Table 5).

Table 5: Proportion of days with symptoms by arm, from phone calls

Symptom	A (n=22)[a]	B (n=15)[a]	p-value
Mean proportion of days with fever	5.5%	13.1%	0.229
Mean proportion of days with cough	37.8%	46.6%	0.450
Mean proportion of days with general malaise	30.4%	29.7%	0.945
Mean proportion of days with headache	27.9%	33.1%	0.549
Mean proportion of days with odynophagia	21.1%	28.9%	0.397
Mean proportion of days with diarrhea	7.0%	11.4%	0.518
Mean proportion of days with anosmia	50.8%	60.8%	0.449

[a] Number of patients with at least one follow-up phone call. For each participant, the proportion of days with each symptom was calculated. The table presents the means of these values by treatment arm.

Five participants were hospitalized during their follow up, 4 in the control arm and one in the ivermectin arm (p= 0.625). No patient from either group progressed to severe disease or died during the trial.

Serology

Almost all patients in the study had seroconverted by day 21. 23/24 (95.8%) patients had a positive rapid serological test by day 21 in the placebo arm and 15/15 (100%) in the ivermectin arm. (RR 0.96, 95% CI: 0.88-1.04, p=0.423). The proportion of patients with a positive rapid serological test at various stages of the trial is presented in Table 6.

Table 6: Patients with a positive rapid serological test by study arm and day of sample collection

	Placebo (N =24)	**Ivermectin (N =16)**	**Effect estimate RR (95% CI)**	**p-value**
Patients with positive rapid serological test at day 7, n/N (%)	7/24 (29.2)	6/15[a] (40)	0.73 (0.30-1.76)	0.485
Patients with a positive rapid serological test at day 14, n/N (%)	20/24 (83.3)	16/16 (100)	0.83 (0.70-0.10)	0.085
Patients with a positive rapid serological test at day 21, n/N (%)	23/24 (95.8)	15/15 (100)	0.96 (0.88-1.04)	0.423
[a]1 of the 16 patients in the ivermectin arm who was visited at day 7, did not have a rapid serological test.				

The median IgG titers (measured as the C-terminal region median fluorescent intensity- MFI)) for both arms at day 21 were similar (48 734.1 MFI for the placebo *vs.* 57365.1 MFI for ivermectin p=0.34) (Figure 4).

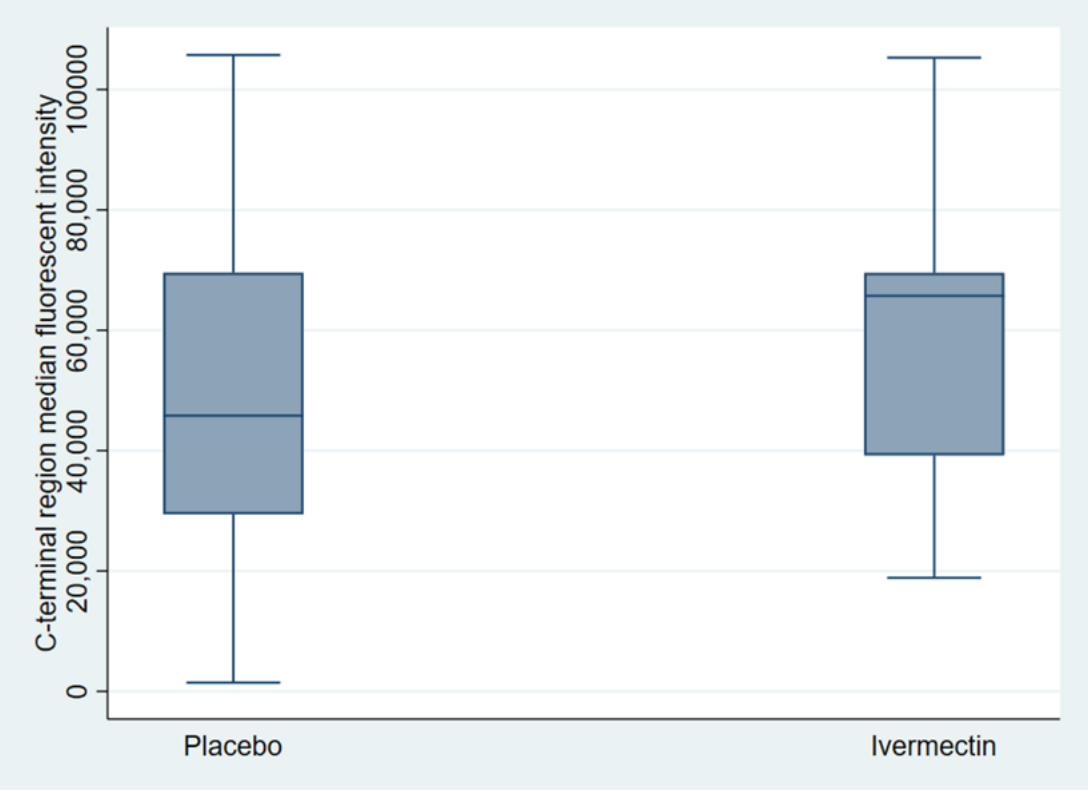


Figure 4: Comparison median IgG antibody levels (measured using the C-terminal region median fluorescent intensity) between Placebo and Ivermectin group at 21 days.

Intestinal parasites

13/24 participants from the placebo group and 5/17 from the ivermectin group provided a stool sample within three days of enrollment, and a second sample between day 12 and 20. No helminths were found on the baseline samples. Parasites such as Blastocystis hominis, Entamoeba coli, Chilomastix meslini and Endolimax nana were

founded in stool samples of patients in both arms. Only one patient had a nematode (Enterobius vermicularis) detected at day 14 in the ivermectin arm.

Safety

A list of 13 ivermectin-related adverse events were solicited daily by phone for 3 days after enrollment. 18/24 (75%) of the placebo participants and 13/17 (76.5%) of the ivermectin arm had at least one follow up call during those first days. Of these, 66.7% in the placebo and 69.2% in the ivermectin arm reported one or more solicited adverse event. There were no statistically significant differences between placebo and control arms for any of the adverse events. The most common adverse events were dizziness (33.3% *vs.* 46.2%), somnolence (38.9% *vs.* 38.5%), confusion (16.7% *vs.* 30.8%), for placebo and ivermectin respectively. Other adverse events were tremor (11.1% *vs.* 15.4%), skin rash (0% *vs.* 15.4%), vertigo (11.1% *vs.* 0%) and pruritus (0% *vs.* 7.7%) (Table 7).

Table 7: Solicited adverse events by arm

Solicited adverse event[a]	Placebo (N=18)[b]	Ivermectin (N=13)[b]	p-value
Dizziness, n (%)	6 (33.3)	6 (46.2)	0.710
Tremor, n (%)	2 (11.1)	2 (15.4)	1.000
Somnolence, n (%)	7 (38.9)	5 (38.5)	1.000
Skin rash, n (%)	0 (0.0)	2 (15.4)	0.168
Confusion, n (%)	3 (16.7)	4 (30.8)	0.413
Vertigo, n (%)	2 (11.1)	0 (0.0)	0.497
Pruritus, n (%)	0 (0.0)	1 (7.7)	0.419
One or more adverse events, n (%)	12 (66.7)	9 (69.2)	1.000
[a]A list of 13 drug-related adverse events were solicited by phone at each follow-up call during the first 3 days of follow up. No participant reported visual abnormalities (e.g blurred vision, peripheral vision loss, difficulty focusing on objects or reading, abnormal forms or colors, blind spots or floating spots). [b]Number of patients that have at least one follow-up call in the first 4 days of study.			

Concomitant medications

The most common used medication participants reported during the trial was paracetamol in both arms (63.6% in placebo arm and 92.3% in ivermectin arm), followed by NSAIDs (27.3% *vs.* 38.5%), and glucocorticoids (18.2% *vs.* 23.1%) and non-macrolide antibiotics (22.7% *vs.* 15.4%). There were not statistically significant differences between groups for any medication (Table 8).

Table 8: Reported concomitant medications during the trial, by arm

Medication	Placebo (N=22)[a]	Ivermectin (N=13)[a]	p-value
Paracetamol n (%)	14 (63.6)	12 (92.3)	0.109
NSAIDs n (%)	6 (27.3)	5 (38.5)	0.708
Glucocorticoids n (%)	4 (18.2)	3 (23.1)	1.000
Non-macrolides antibiotics n (%)	5 (22.7)	2 (15.4)	0.689
Macrolides antibiotics n (%)	2 (9.1)	2 (15.4)	0.618
Enoxaparin n (%)	4 (18.2)	1 (7.7)	0.630
[a] Number of patients that have at least one follow-up call in the 21 days of study.			

Discussion

In this triple-blinded randomized trial of patients with early non severe-COVID-19, we found that patients in the placebo arm had Cycle threshold (Ct) values significant higher at day 7 compared to those from the ivermectin arm reflecting lower viral loads; and that there were no statistically significant differences between arms on the immunological response, adverse events or the proportion of patients with symptoms at any of the visits, except for odynophagia at day 7, that was higher in the placebo arm.

This study was designed in early 2020 when, due to its preliminary antiviral properties, good safety profile, low cost, and wide availability, ivermectin received large attention in Latin America. Unfortunately, Peru was one of the earliest adopters of Ivermectin for COVID-19, although there was not enough evidence for its use. It was included in the National Treatment Guidelines from the Ministry of Health of Peru on May 2020 for mild, moderate, and severe cases,[18] resulting in physicians prescribing ivermectin widely. The government created "treatment packages" including this drug and distributed them especially in peripheral districts. Since it was freely available at pharmacies, the stock of human ivermectin were quickly consumed. So eventually veterinarian formulations of the drug started to be used at scale with previously unreported side effects related to injectables.[19] Later, in October 2020, the Ministry of Health removed the drug from the guidelines for the management of hospitalized patients with COVID-19, but kept the use in ambulatory cases, until March 2021. Nevertheless, it remained a popular medication use over the counter for "prevention" and for treatment of COVID-19. A survey in February 2021, in 1219 individuals from urban and rural Peru, showed that 32% of the respondents have or were taking ivermectin at the time of the interview, 26% as "prevention".[20] Due to all these contextual issues, it was challenging to recruit patients who have not taken ivermectin in the prior month.

Observational studies related to ivermectin use for COVID-19 in Peru have shown mixed results. One preprint suggested a link between ivermectin use with a decline in mortality in some Peruvian states using a misleading ecological approach,[21] while a retrospective cohort study in COVID-19 hospitalized patients found no association of ivermectin with all-cause mortality, death or oxygen requirement.[22]

Ivermectin, as an early intervention in COVID-19, has been evaluated for a potential effect to reduce viral load faster and influence disease transmission. Our study did not show an effect of ivermectin on the PCR positivity at day 7. Moreover, even at day 21, the proportion of patients PCR positive were higher in the ivermectin arm compared with the placebo, this difference however, was not statistically significant. Additionally, since we couldn't determine viral loads, we used the Cycle thresholds (Ct) values as proxy and we found similar baseline values for both arms, but on day 7 the Ct values were significant higher for the placebo, reflecting a lower viral load compared to the ivermectin arm. These results differ from three published studies from Bangladesh, and two preprints, one from Pakistan and one from Egypt. The first publication is a retrospective study in which the median PCR positivity was 4 days for patients in ivermectin, and 15 for those in the control group.[23] The second study from Bangladesh is a blinded, placebo-controlled trial in patients with mild-to-moderate COVID- 19 symptoms, which were randomized to ivermectin plus doxycycline vs. placebo, both associated with the standard of care. This study found that the proportion of patients who remained PCR positive on day 14 was significantly lower in the treatment than in the placebo group.[24] The third study from Bangladesh was a randomized, double-blind trial with three arms: ivermectin alone, ivermectin plus doxycycline, and placebo. They reported that the mean number of days to achieve RT-PCR negativity was significantly smaller in the ivermectin arm compared to the placebo group (9.7 days *vs.* 12.7 days; $p = 0.02$).[25] The study from Pakistan was an open label trial.[26] The study from Egypt was a preprint that was withdrawal from the platform due to allegations of flaws in the data and ethical concerns.[27]

On the other hand, our findings aligned with several clinical trials with similar primary outcomes: one from Spain, two from India and two studies from Argentina, all reporting no significant differences in the positivity of PCR or viral loads between placebo or ivermectin groups at 5 or 7 days of follow up.[17,28-31]

A recent systematic review of Randomized Control Trials (RCT) on the use of ivermectin for COVID-19, identified ten RCTs eligible for their metanalysis. They found heterogeneity in the total doses used and in the duration of treatment, as well as in the follow up. All were patients with mild or mild to moderate disease, but only 2 RCTs included non-hospitalized patients. Of note, the metanalysis showed that compared with placebo or standard of care, the use of ivermectin did not reduce mortality, or length of stay in hospital or viral clearance.[32] While there was good compliance with the study visits, there was sub-optimal compliance with the daily follow up calls. Several recruited participants were not at home most of time during their disease and avoided answering the phone since they were working, although they knew about their positive PCR and were counseled on how the virus is transmitted and the importance of staying home, in quarantine, to avoid their family and others to get infected. We found no differences between arms in the resolution of symptoms at the day 7 clinical visit, except for odynophagia in the placebo arm. Moreover, there were no differences between arms in the daily collection of symptoms through the phone calls during the 21-day follow up. Most trials around the world, including one from Colombia,[33] have found no significant differences in mean recovery time, median resolution of symptoms, or symptomatic status at day 6 or 7, which is consistent with our findings.[10,25,28-30,34] However, one randomized clinical trial in Spain found that patients in the ivermectin group recovered earlier from hyposmia/anosmia and cough.[17]

Our study was not designed to evaluate hospitalization or mortality as outcomes. There were only 5 participants hospitalized, none progressed to severe disease or died and there was no difference between groups. Since most of the studies on ivermectin have included participants with mild-to-moderate COVID-19, the number of mortality events are small. A PAHO meta-analysis of patients receiving ivermectin, based on four RCT considered low risk of bias, found no statistically significant reduction of mortality in comparison with controls. Another issue highlighted in the report was that several studies had to be excluded due to methodological limitations.[35]

We didn't find any significant differences in the seroconversion rate or median IgG titers at day 21 between placebo or ivermectin, although the median levels were slightly higher for the ivermectin group. It is interesting that Chaccour et al. report the opposite, a tendency to lower IgG levels in the ivermectin group, though this difference was not statistically significant, and the method used to measure serology was not the same as ours.[17]

In our study we included the evaluation of stools for parasites since there is some evidence that helminths could have an immunomodulatory effect on their host in general.[36] If that is the case the use of ivermectin, due to the antiparasitic effect, could result in the reduction of its immunomodulatory effect (if there is one), and in theory, it could cause a more torpid course of the disease.[37] Yet, we were not able to test the hypothesis, since it was challenging to collect the stool samples and we did not find helminths in the baseline studies.

When assessing ivermectin safety, no serious adverse events were observed and although the percent of elicited symptoms like dizziness, somnolence, confusion, and skin rash were more common in the ivermectin group, there were no statistically significant differences compared to placebo. Many observed events could have been related to the course of the disease without being able to differentiate whether they were attributed to the study drug.

Our study has several strengths: it is the first randomized clinical trial of the use of ivermectin in Peru, was triple blinded, and included objective outcome measures. However, we faced several hassles during its implementation: the lack of local funding, the lack of COVID-19 diagnostic tests (PCR), supplies and personal protection equipment due to the disruptions in the supply chain which delayed the initiation of the study and the impossibility to perform viral loads. Another issue was that PCR was not easily available for diagnosis in the country, so we

recruited patients with symptoms compatible with COVID-19 who were tested and randomized before having the results of the PCR, to assure an early start of treatment. This resulted in low efficiency since many patients were excluded from the study when their PCR test results came back negative. For this reason, in February 2021, the protocol was amended to add a positive antigen test result for SARS-CoV-2 as a preselection criterion at recruitment. The strong agreement between the performance of the antigen test and the PCR test,[38] reduced the proportion of randomized participants that were later excluded because of a negative PCR test at baseline. Unfortunately, the antigen test for SARS-CoV-2 was not available in the country when the protocol was first designed. Probably the most important limitation of the study is that we faced slow recruitment of participants since most patients seen at the hospital have already taken ivermectin before assessment or they came with several days of disease when they were severely sick and were not eligible for the trial. Nevertheless, through this local randomized trial, although small, we contributed to the country policy change and eventually ivermectin was not anymore recommended for use by the ministry of health.

Other large studies on ivermectin are still under way like PRINCIPLE, a UK study aimed to recruit 5000 volunteers to evaluate if ivermectin can speed up recovery, reduce the severity of symptoms and prevent the need for hospital admission.[39] The other large ongoing trial is ANTICOV which is testing the efficacy and safety of ivermectin aiming to recruit 3000 participants with mild to moderate COVID-19 in Africa.[40] But to date the World Health Organization (WHO) do not recommend Ivermectin for treatment of COVID-19 outside randomized controlled trials.[41]

As a conclusion, our study found no difference in PCR positivity at day 7 in patients receiving placebo *vs.* ivermectin and even more, the placebo arm patients had lower viral loads at day 7 compared with those receiving ivermectin. Our results are not definitive but suggest no benefit of the use of ivermectin for the treatment of COVID-19, adding information to the existing data available.

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Author Contributions

Conceptualization, Patricia Garcia, Carlos Chaccour and Cesar Carcamo; Data curation, Cesar Carcamo; Formal analysis, Patricia Garcia, Flavia Moran and Cesar Carcamo; Funding acquisition, Patricia Garcia; Investigation, César Ugarte-Gil, Patricia Leon , Jesus Chacaltana, German Malaga, Hansel Mundaca, Carlota Dobaño and Gemma Moncunill; Methodology, Patricia Garcia; Supervision, Patricia Garcia; Writing – original draft, Patricia

Garcia and Flavia Moran; Writing – review & editing, César Ugarte-Gil, Patricia Leon , Jesus Chacaltana, German Malaga, Hansel Mundaca, Carlota Dobaño, Gemma Moncunill, Carlos Chaccour and Cesar Carcamo.

Institutional Review Board Statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and the protocol was approved by the Peruvian National COVID-19 Ethics Committee and it is published. The study is registered at ClinicalTrials. gov: NCT04635943

Informed Consent Statement

All participants in the trial provided two consents: a preselection consent, and a consent for participation in the trial.

Conflicts of Interest

The authors declare no conflict of interest. The sponsors had no role in the design, execution, interpretation, or writing of the study.

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