



Phase 2 randomized study on chloroquine, hydroxychloroquine or ivermectin in hospitalized patients with severe manifestations of SARS-CoV-2 infection

Luis Enrique Bermejo Galan, Nayara Melo dos Santos, Mauro Shosuka Asato, Jucineide Vieira Araújo, Adriana de Lima Moreira, Aléxia Mahara Marques Araújo, Artur Diogenes Pinheiro Paiva, Diego Guilherme Santos Portella, Frank Silas Saldanha Marques, Gabriel Melo Alexandre Silva, Joana de Sousa Resende, Marycassielly Rodrigues Tizolim, Poliana Lucenados Santos, Steffi Ferreira Bittenbender, Stephanye Batista de Andrade, Roberto Carlos Cruz Carbonell, Juliana Gomes Da Rocha, Ruy Guilherme Silveira de Souza & Alex Jardim da Fonseca

To cite this article: Luis Enrique Bermejo Galan, Nayara Melo dos Santos, Mauro Shosuka Asato, Jucineide Vieira Araújo, Adriana de Lima Moreira, Aléxia Mahara Marques Araújo, Artur Diogenes Pinheiro Paiva, Diego Guilherme Santos Portella, Frank Silas Saldanha Marques, Gabriel Melo Alexandre Silva, Joana de Sousa Resende, Marycassielly Rodrigues Tizolim, Poliana Lucenados Santos, Steffi Ferreira Bittenbender, Stephanye Batista de Andrade, Roberto Carlos Cruz Carbonell, Juliana Gomes Da Rocha, Ruy Guilherme Silveira de Souza & Alex Jardim da Fonseca (2021): Phase 2 randomized study on chloroquine, hydroxychloroquine or ivermectin in hospitalized patients with severe manifestations of SARS-CoV-2 infection, Pathogens and Global Health, DOI: [10.1080/20477724.2021.1890887](https://doi.org/10.1080/20477724.2021.1890887)

To link to this article: <https://doi.org/10.1080/20477724.2021.1890887>



Published online: 08 Mar 2021.



Submit your article to this journal [↗](#)



Article views: 44



View related articles [↗](#)



View Crossmark data [↗](#)

Full Terms & Conditions of access and use can be found at

<https://www.tandfonline.com/action/journalInformation?journalCode=ypgh20>

ARTICLE



Phase 2 randomized study on chloroquine, hydroxychloroquine or ivermectin in hospitalized patients with severe manifestations of SARS-CoV-2 infection

Luis Enrique Bermejo Galan^{a,b}, Nayara Melo dos Santos^a, Mauro Shosuka Asato^a, Jucineide Vieira Araújo^{a,b}, Adriana de Lima Moreira^b, Aléxia Mahara Marques Araújo^b, Artur Diogenes Pinheiro Paiva^b, Diego Guilherme Santos Portella^b, Frank Silas Saldanha Marques^b, Gabriel Melo Alexandre Silva^b, Joana de Sousa Resende^b, Marycassielly Rodrigues Tizolim^b, Poliana Lucenados Santos^b, Steffi Ferreira Bittenbender^b, Stephanye Batista de Andrade^b, Roberto Carlos Cruz Carbonell^{a,b}, Juliana Gomes Da Rocha^c, Ruy Guilherme Silveira de Souza^b and Alex Jardim da Fonseca^{a,b,c}

^aDepartment of Infectious Diseases of Hospital Geral De Roraima. Universidade Federal De Roraima. Boa Vista Brazil; ^bPost Graduate Program in Health Science of Universidade Federal De Roraima, Health Science Center, Boa Vista, Brazil; ^cClinical Research Center of the Oncological Center of Roraima - Boa Vista - Brazil

ABSTRACT

Objective: Given the urgent need for strategies to minimize the damage caused by this pandemic, this study performed a randomized, double-blind phase 2 study to assess the safety of the effectiveness of chloroquine (CQ), hydroxychloroquine (HCQ) or ivermectin in severe forms of COVID-19, in addition to identifying predictors of mortality in this group of patients.

Methods: Phase 2, double-blind, randomized study to assess the safety and efficacy of enteral CQ, HCQ or ivermectin in patients hospitalized for SARS-CoV-2 infection, admitted to a Reference Hospital in Roraima (Brazil) in may 2020. Patients were randomized in a 1:1:1 ratio. The endpoints were need of supplemental O₂, invasive ventilation, admission in ICU and death. The study was approved by an independent IRB.

Results: 168 patients were randomized. The mean age was 53.4 years (± 15.6), most participants were male ($n = 95$; 58.2%). Therapy with corticosteroid, anticoagulant or antibiotics was a decision of the attending physicians, and there was no difference between the groups. The mortality was similar in three groups (22.2%; 21.3% and 23.0%) suggesting ineffectiveness of the drugs. No difference in the incidence of serious adverse events were observed. To be older than 60 years of age, obesity, diabetes, extensive pulmonary involvement and low SaO₂ at hospital admission due to independent risk factors for mortality.

Conclusion: Although CQ, HCQ or ivermectin revealed a favorable safety profile, the tested drugs do not reduce the need for supplemental oxygen, ICU admission, invasive ventilation or death, in patients hospitalized with a severe form of COVID-19.

KEYWORDS

coronavirus;
hydroxychloroquine;
chloroquine; ivermectin;
COVID-19

BACKGROUND

The current pandemic expansion of the COVID-19 calls for initiatives from the scientific community to accelerate the production of data that can bring solutions to the serious challenges that threaten public health globally. Appearing at the end of 2019 in the Hubei province of the People's Republic of China, it was declared a world emergency on 30 January 2020 [1]. One of the main challenges is its dynamic evolution, which moved the largest number of the case reported from Asia to Europe to the Americas in less than a month [2].

SARS-CoV-2 is a beta-coronavirus with a similar structure to SARS-CoV-1 and MERS-CoV, viruses responsible for epidemic outbreaks of acute respiratory syndrome cases since 2002 [3]. The rapid deterioration of lung function and the need for long-term respiratory support is the clinical feature related to the most harmful effect of this pandemic: the depletion of a community's health resources. One of the causes of

this fast clinical evolution is an exacerbated release of cytokines [4]. Given the absence of effective preventive strategies (such as vaccines), an urgent need for therapies to suppress this 'cytokine storm' emerged, to reduce its mortality rate and also to contribute to adequate and faster respiratory rehabilitation [5].

Among the therapeutic possibilities, hydroxychloroquine (HCQ), an agent widely used as a disease-modifying drug in rheumatoid arthritis, and chloroquine (CQ), an agent used in the treatment of malaria, have been suggested as possibilities due to their remarkable immuno-modulatory activities [6]. Recently, several authors have proposed the use of CQ and HCQ for the treatment of severe forms of COVID-19 due to their immuno-suppressive effects and low cost [7,8]. In light of this preliminary evidence, the Ministry of Health of Brazil published a nationwide guideline for severe

(hospitalized) forms of COVID-19, using CQ or HCQ as adjuvant therapy [9]. Additionally, in the search for short-term therapeutic solutions given the health services depletion, a natural path was to look for agents with in vitro viral inhibition action. Ivermectin had in its history several reports of in vitro inhibition of RNA viruses replication in addition to a favorable safety profile [10,11]. A clinical trial in the treatment of dengue demonstrated the safety of a 3-day treatment (400 µg/kg per day), although with modest results [11]. A recent publication by Caly et al. demonstrated the antiviral action of ivermectin in cell culture infected with SARS-CoV-2, strengthening the rationale to test ivermectin in COVID-19 cases [12].

The objective of this study is to assess the safety and efficacy of HCQ, CQ, or ivermectin in severe forms of COVID-19, through a randomized, double-blind phase 2 study in a Reference Hospital in Roraima, north of Brazil, in addition to identifying mortality predictors in this group of patients.

METHODS

Ethical issues

This study was conducted following the principles of the Declaration of Helsinki and the Good Clinical Practice guidelines of the International Conference on Harmonization. It was approved by an independent IRB/CONEP (CAAE 30605020.7.0000.5302/2020). The study was registered in REBEC, the Brazilian clinical trial database (RBR-8h7q82 – The effect of chloroquine, hydroxychloroquine or ivermectin in patients with severe manifestations of coronavirus) on 10 February 2020 (UTN code: 30605020.7.0000.5302). A virtual independent Data Safety and Monitoring Board (DSMB), with epidemiologists, cardiologists and experts in infectious diseases, was implemented to review the protocol and the preliminary results every 50 participants included to follow up the activities of the study. The study was financed with the institution's own resources. The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Study design

This study was a 1-institution, phase 2, double-blind, randomized clinical trial conducted from May 1 2020, to 16 July 2020. Patients were randomized in a 1:1:1 ratio using simple randomization to the treatment group with HCQ or CQ or ivermectin. No placebo was given to any group.

Population and setting

The Hospital Geral de Roraima is a regional reference for the treatment of COVID-19, located in Roraima, a state in the Brazilian Amazonian Region, and characterized by housing the largest proportion of the indigenous population in the country (15%) and by presenting 2 international borders. Both characteristics reveal important population groups of high vulnerability in Roraima. In particular, the border between Roraima and Venezuela has been, since 2018, the main immigration route for Venezuelan citizens to Brazil, due to the humanitarian crisis that the country is going through.

Sample and sampling

Considering the main objective of assessing the mortality among patients hospitalized for COVID-19, with a tolerable error of $\pm 5\%$, and estimated mortality of 20% in the hospitalized population infected with SARS-CoV-2, based on the Huang et al.'s report [13], and critical value for a 95% confidence level ($Z = 1.96$), a minimum sample size of 152 patients was achieved. Considering a loss of 10% of the sample, a final sample of 167 hospitalized patients was obtained. The sampling method was systematic.

Intervention, assessments and study endpoints

All patients (or their legal representatives, in case of clinical severity) who were hospitalized by COVID-19 were invited to participate in the study, consecutively, without patient selection. Inclusion criteria were as follows: (1) laboratory test confirming infection by SARS-CoV-2 (positive serologic test IgM or rt-PCR); (2) hospitalized with a clinical, epidemiological, and radiological picture compatible with COVID-19; (3) over 18 years old; (4) present a severe form of the disease characterized by one of the following clinical signs: dyspnea, tachypnea (>30 bpm), peripheral oxygen saturation $<93\%$ (pulse oximeter evaluation), $\text{PaO}_2/\text{FiO}_2$ ratio <300 , or infiltrate pulmonary $>50\%$ of the parenchyma seen on chest tomography or chest radiography. The exclusion criteria were as follows: (1) under 18 years old; (2) indigenous people; (3) patients not fluent in Portuguese; (4) unable to understand the objectives and methods of the study; (5) critically ill patients who are not accompanied by legal representatives; (6) those who reject participation in the study; (7) patients with cardiac arrhythmia that include prolongation of the QT interval; (8) previous use of any of the medications surveyed for more than 24 h.

Eligible participants were allocated at a 1:1:1 ratio to receive orally (or via a nasogastric tube in case of orotracheal intubation) either: (A) CQ difosfato

(450 mg, twice on day 0, and once daily from day 1 to day 4, total dose 2.7 g); or (B) HCQ sulfate (400 mg twice on day 0, and once daily from day 1 to day 4, total dose 2.4 g); or (C) ivermectin (14 mg once at day 0 + 1 placebo tablet at day 0, and once daily from day 1 to day 2, + 1 placebo tablet daily from day 3 to 4, total dose 42 mg). For participants with body weight under 55 kg, the ivermectin dose was adjusted to 10 mg each dose. Investigational products were produced blindly by an independent pharmacy. Placebo tablets were also produced by the same pharmacy to standardize treatment and blinding of the research team and participants (Figure 1).

An electronically generated randomization list was prepared by an independent statistician. This randomization list linked the participant in chronological order of inclusion to the numbered treatment bottle, blindly. A non-blinded pharmacist was responsible to assign the intervention. The bottles were numbered, and they contained an equal number of tablets, equally arranged in blister sheet with the daily intake schedule. The information about the investigational product was restricted to the non-blinded pharmacist, in an attempt to minimize observer bias. Unmasking was available to DSMB members in case of severe adverse events.

As per hospital protocol, every patient without contraindication received prophylactic doses of enoxaparin (1 mg/kg once a day). All patients meeting the criteria of acute respiratory distress syndrome used azithromycin (500 mg 1× for 5 days) and ceftriaxone (1 g 2× for 7 days). Oseltamivir (75 mg 2× for 5 days) was also prescribed when influenza infection was suspected. The use of corticosteroids was a decision of the assistant physician, as new scientific evidence on its effectiveness emerged during the course of the study.

Clinical parameters were measured daily by the research staff from day 0 to discharge or death. For discharged participants, vital status and clinical data were assessed, for safety and outcome assessment, until day 90 after randomization. Biochemistry and hematology exams were performed daily. All patients

underwent a chest CT scan at inclusion, and the repetition of this test was based on the recommendation of the assistant medical team. Electrocardiograms were performed weekly. The occurrence of cardiac arrhythmia was the monitored adverse event of greatest interest.

Following were the study endpoints: the need for supplemental oxygen, the need for invasive ventilation, the need for admission to the intensive care unit (ICU), mortality. Predictor variables, in addition to the intervention programmed in the study, were used for independent survival analysis. Outcome analysis was performed by intention to treat.

Data analysis

Descriptive statistical analysis was performed, including frequency of the distribution for categorical variables, and means (with standard deviation) for continuous variables, with distribution. The prevalence of outcome variables and their 95% confidence intervals (95% CI) were estimated based on the binomial distribution. To compare sample means, Student's *t*-test was used for variables with normal distribution and with homogeneity of sample variances. Otherwise, the Mann–Whitney test was used for this purpose. The χ -square test was used to compare differences in the proportions of categorical variables. The analysis of survival data was performed using the Cox Proportional-Hazards Model. Additionally, Kaplan–Meier curves were generated and log-rank tests were used when the predictor variable was categorical. The data were tabulated and analyzed using SPSS®. The level of significance considered was 5%.

RESULTS

A total of 434 patients were invited to participate. Of these, 266 (61.2%) were excluded. The main cause of exclusion was the previous use of any of the investigated medications before hospitalization. A total of 168 participants were randomized. The mean age was 53.4 years (\pm

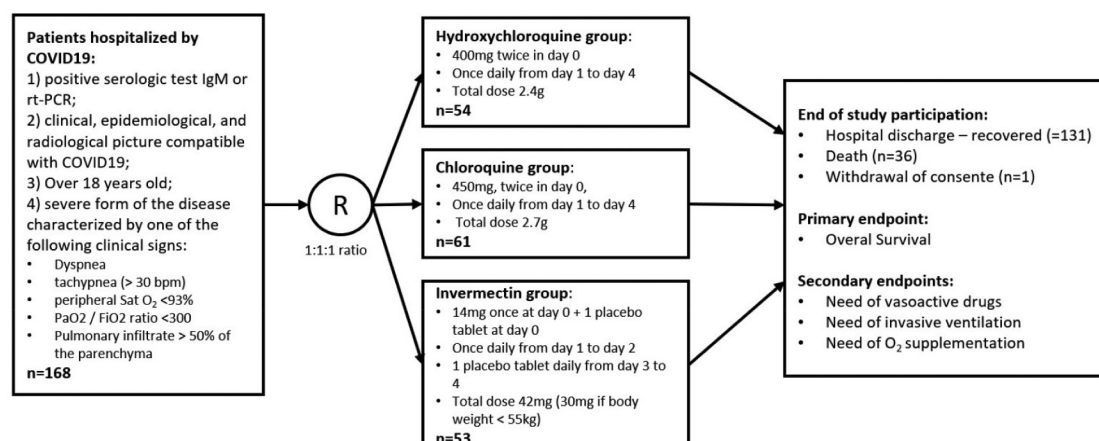


Figure 1. Study flowchart.

15.6), the majority of participants were male ($n = 95$; 58.2%), and Hispanic origin people (78.9%). The proportion of obese people ($\text{BMI} > 30 \text{ kg/m}^2$) was 37.5%. Near half of the sample denied the use of tobacco (46.9%), and only 9.7% of the participants were active smokers. Only 14.8% reported regular use of alcohol. The most common associated clinical conditions were: systemic arterial hypertension (43.4%), diabetes mellitus (28.1%), and previous chronic lung diseases (5.3%). The most common syndromic clinical presentation at hospital admission was a respiratory failure (76.5%), followed by pneumonic syndrome (42.5%). The mean value of oxygen saturation in arterial blood gas analysis at admission was 89.9% (± 5.4), without oxygen supplementation. The most common radiological findings on admission chest CT were ground-glass opacity (85.0%) and pulmonary consolidation (68.8%). And 38.1% of the patients had a magnitude of involvement from 25% to 50% of the lung parenchyma.

There was no significant difference between the proportions and means of the clinical and demographic variables between the treatment groups, except for a higher proportion of nonsmokers in the CQ group compared to the ivermectin group (56.6% vs 37.2%, respectively; $p = 0.03$), and a lower proportion of former smokers in the CQ group compared to the other two groups (28.3% vs 49.2%, respectively; $p = 0.02$). Table 1 describes and compares demographic and clinical variables between treatment groups.

Regarding in-hospital variables, we observed a high proportion of participants who completed the 5-day

test treatment (>90%), with no difference in proportion between the treatment groups. Approximately 90% of the participants in each group needed oxygen supplementation at some point during hospitalization, and there was no difference in the average number of days of supplemental oxygen need (7.8 vs 7.9 vs 8.1). Almost all participants in the three groups were treated with corticosteroids, most commonly, dexamethasone and methylprednisolone, and approximately one-third of the participants in each group were treated with anticoagulant (enoxaparin 1 mg/kg bid), with no statistically significant difference between them.

There was no significant difference between the secondary and primary endpoints between the three groups. The incidence of admission to the Intensive Care Unit was similar in the HCQ, CQ and ivermectin groups, 21.1% vs 22.4% vs 26.0%, respectively. The need for vasoactive drugs was also similar between the groups, varying between 20.6% and 28.0%, with no statistically significant difference, as well as the need for invasive ventilation. No adverse arrhythmia events were recorded in the three groups, as well as there was no difference in hematological or hepatic adverse events. Mortality was similar in the three groups, 22.2% for the HCQ group, 21.3% for the CQ group, and 23.0% for the ivermectin group, with no statistically significant difference. Table 2 and Figure 2 describe in detail the comparison of hospital outcomes between treatment groups.

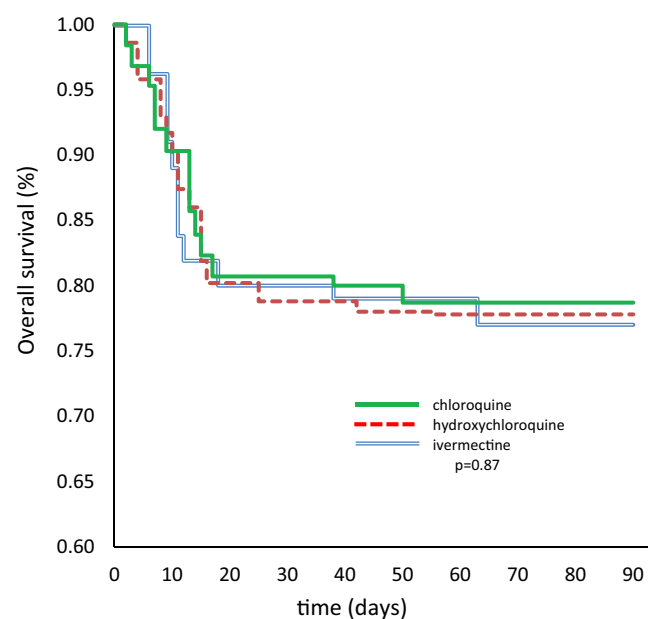
Table 1. Demographic and clinical data at hospital admission. Comparison between treatment groups.

Demographics and clinical data on admission	Total	HCQ group	CQ group	Ivermect. group	<i>p</i> Value
<i>n</i>	168	54	61	53	-
Age	53.4 (± 15.6)	54.8 (± 15.5)	51.9 (± 14.0)	53.2 (± 17.3)	ns
Male gender	95 (58.2%)	29 (56.8%)	35 (57.8%)	31 (60.7%)	ns
Hispanic origin race	130 (78.9%)	44 (33.8%)	48 (36.9%)	38 (29.3%)	ns
Obesity ($\text{BMI} > 30 \text{ kg/m}^2$)	63 (37.5%)	21 (38.8%)	20 (37.7%)	22 (36.0%)	ns
Smoking					
No, never	77 (46.9%)	24 (45.2%)	34 (56.6%)	19 (37.2%)	0.03
Former smoker	69 (42.0%)	26 (49.0%)	17 (28.3%)	26 (49.5%)	0.02
Yes (active smoker)	16 (9.7%)	3 (5.6%)	8 (13.3%)	5 (9.8%)	ns
Alcohol consumption					
Occasional	19 (11.3%)	5 (9.2%)	9 (14.7%)	5 (9.4%)	ns
Regular	25 (14.8%)	6 (11.1%)	5 (8.1%)	14 (26.4%)	ns
Associated conditions					
Systemic arterial hypertension	69 (43.4%)	24 (45.2%)	24 (42.1%)	21 (42.8%)	ns
Diabetes mellitus	45 (28.1%)	13 (24.5%)	18 (31.0%)	14 (28.5%)	ns
Chronic renal failure	4 (2.5%)	0	2 (3.4%)	2 (4.0%)	ns
Previous pulmonary disease (any)	9 (5.3%)	3 (5.5%)	4 (6.5%)	2 (3.7%)	ns
Cancer	5 (3.0%)	2 (3.7%)	1 (1.6%)	2 (4.0%)	ns
Clinical syndrome on admission (not mutually exclusive)					
Flu-like syndrome	60 (35.9%)	21 (38.9%)	23 (37.7%)	16 (26.6%)	ns
Pneumonic syndrome	71 (42.5%)	23 (42.6%)	27 (44.2%)	21 (40.4%)	ns
Respiratory insufficiency	127 (76.5%)	39 (72.2%)	44 (72.1%)	44 (84.4%)	ns
Septic shock	1 (0.6%)	0	0	1 (1.9%)	ns
O_2 saturation ($\text{FiO}_2 = 0.21$) blood gas analysis at admission	89.9 (± 5.4)	88.2 (± 5.5)	87.3 (± 7.2)	90.2 (± 5.8)	ns
Chest CT scan at admission					
Ground glass opacity	142 (85.0%)	42 (77.8%)	55 (90.1%)	45 (86.5%)	ns
Pulmonary consolidation	115 (68.8%)	35 (64.8%)	44 (72.1%)	36 (69.2%)	ns
Pleural effusion	14 (8.3%)	3 (5.5%)	7 (11.4%)	4 (7.7%)	ns
Atelectasis	5 (2.9%)	1 (1.8%)	3 (4.9%)	1 (1.9%)	ns
Magnitude of pulmonar parenchyma involvement					
up to 25%	54 (37.4%)	14 (32.5%)	23 (41.0%)	17 (37.7%)	ns
25– 50%	55 (38.1%)	16 (37.2%)	21 (37.5%)	18 (40.0%)	ns
More than 50%	35 (24.3%)	13 (30.2%)	12 (21.4%)	10 (22.2%)	ns

Table 2. Intrahospital variables, safety data, and clinical endpoints.

Intrahospital variables	Hydroxycyl.	Chloroqu.	Ivermect.	<i>p</i> Value
Completed investigational therapy	92.8%	90.5%	93.5%	ns
Oxygen supplementation (need)	90.2%	88.5%	88.4%	ns
Duration of oxygen need (days)	7.8 (±2.1)	7.9 (±2.3)	8.1 (±2.0)	ns
Corticosteroid therapy	100%	98%	97%	ns
Duration of corticosteroid	6.8 (±2.0)	7.2 (±1.8)	6.9 (±1.7)	ns
Anticoagulant therapy	32.9%	36.7%	30.4%	ns
Adverse events				
Arrhythmia (clinically significant)	0	0	0	-
Elevated liver Transaminases G1/G2	15.2%	13.4%	14.8%	ns
Elevated liver Transaminases G3/G4	10.2%	8.5%	11.2%	ns
Anemia (hemoglobin < 8 g/dL)	5.5%	5.3%	7.8%	ns
Leukopenia (<1500/mm ³)	5.5%	3.2%	2.2%	ns
UCI admission	21.1%	22.4%	28.0%	ns
Need of vasoactive drugs	21.1%	20.6%	26.0%	ns
Need for invasive ventilation	21.1%	20.6%	23.5%	ns
Death due to COVID complications	22.2%	21.3%	23.0%	ns

Hydroxycyl., hydroxychloroquine; Chloroqu., chloroquine; Ivermect., ivermectin.

**Figure 2.** Kaplan–Meier curve: overall survival of treatment groups.

The correlation between variables at hospital admission and mortality (Table 3) demonstrated that being over 60 years of age significantly increased mortality in relation to younger people (37.0% vs 15.0%, respectively; HR = 2.44; 95% CI = 1.40–4.30), and with those older than 70 years, the risk of death more than doubled (HR = 2.14; 95% CI = 1.15–3.99). Among the associated medical conditions, having diabetes mellitus proved to be an independent risk factor, with a mortality of 32.8%, and HR = 1.87 (95% CI = 1.02–2.59). Moderate to severe obesity (considering the cut-off point BMI > 33 kg/m²) was also associated with higher mortality compared to those without this condition (32.5% vs 18.5%, respectively, *p* = 0.04), almost doubling the risk of death (HR = 1.95; 95% CI = 1.07–3.09). Those with less than 7 days between onset of symptoms and hospitalization also presented a higher mortality (35.0% vs 19.5%; *p* = 0.04, HR = 2.28; 95% CI = 1.03–3.24). Low oxygen saturation at hospital admission was the main risk factor in this analysis. Using the

Sat O2 cutoff point <90% (arterial gasometry), patients with low oxygen saturation had 46.6% mortality, significantly higher than those with higher saturation (13.1%), with HR = 5.79 (95% CI = 2.63–12.7). More than 75% of pulmonary parenchyma involvement also proved to be an independent risk factor (HR = 1.92; 95% CI = 1.11–3.22). The only variable assigned as a protective factor was the presentation of diarrhea at hospital admission. The occurrence of this gastrointestinal form of COVID-19 was associated with lower mortality compared to those without this manifestation (11.1% vs 32.5%; *p* = 0.03), reducing mortality by half (HR = 0.47; 95% CI = 0.12–0.96).

DISCUSSION

To the best of our knowledge, this is the first study comparing the efficacy of CQ, HCQ and ivermectin and patients hospitalized for severe COVID-19. The design of this study had a particular ethical issue. On

Table 3. Survival analysis: correlation between variables at hospital admission and COVID-19-related mortality.

Independent variables	n	Mortality (%)	p vValue	Hazard ratio (95% CI)
CQ/Hydroxychloroquine groups	115	21.7	ns	0.94 (0.51–1.72)
Ivermectine group	52	23.0		1
Age >50 years	88	26.1	ns	1.44 (0.79–2.54)
Age >60 years	54	37.0	0.001	2.44 (1.40–4.30)
Age >70 years	19	42.1	0.02	2.14 (1.15–3.99)
Male gender	95	24.1	ns	1.37 (0.73–2.56)
Female gender	68	17.6		1
Associated conditions				
Diabetes	45	32.8	0.04	1.87 (1.02–2.59)
Systemic arterial Hypertension	69	27.5	ns	1.63 (0.77–3.44)
Smoking (current/former)	90	23.3	ns	1.12 (0.63–1.99)
Alcohol use	50	24.7	ns	1.42 (0.81–2.21)
Obesity				
BMI >30 kg/m ²	74	24.3	ns	1.19 (0.67–2.10)
BMI >33 kg/m ²	43	32.5	0.04	1.95 (1.07–3.09)
SatO ₂ <90% at hospital admission	45	46.6	<0.0001	5.79 (2.63–12.7)
No	122	13.1		1
Practice of physical activity	57	20.7	ns	0.92 (0.68–1.58)
No	99	22.2		1
Symptom onset to hospitalization (time)				
<7 days	50	35.0	0.04	2.28 (1.03–3.24)
7 days or more	125	19.5		1
Symptom before hospitalization				
Diarrhea	28	11.1	0.03	0.47 (0.12–0.96)
Dyspnea	24	20.8	ns	0.93 (0.31–2.63)
Fever	109	21.1	ns	0.87 (0.48–1.53)
Pulmonary involvement at admission CT scan				
≥25%	136	22.0	ns	0.97 (0.47–2.01)
≥50%	90	25.6	ns	1.46 (0.78–2.53)
≥75%	35	38.2	0.03	1.92 (1.11–3.22)

27 March 2020, the Brazilian Federal Government established a nationwide treatment guideline for the use of HCQ and CQ in patients with severe forms of COVID-19 [9], even without definitive scientific evidence on its efficacy and safety. Therefore, we were unable to institute a placebo arm. This compelled the authors to add an arm with ivermectin, a medication with a mechanism of action totally different from CQ and derivatives, to strengthen the comparison of efficacy and safety.

The rationale to test those drugs is based on clinical and preclinical studies. Shukla et al. demonstrated in vitro antiviral activity of CQ against SARS-CoV by changing the pH of cell membrane surfaces and consequent inhibition of terminal glycosylation of the angiotensin-converting enzyme receptor 2, impairing the virus fusion with human cells [14]. Additionally, HCQ and CQ reduce the secretion of proinflammatory cytokines, with emphasis on IL-1, TNF α , and IFN γ , by alveolar macrophages, with a potential reduction in the cytokine storm [15]. But clinical studies on the role of CQ and derivatives in patients with a severe form of COVID-19 are scarce and conflicting. In a randomized clinical trial with 504 hospitalized patients, Cavalcanti et al. found no clinical benefit in the use of 400 mg HCQ, associated or not with azithromycin [16]. In that study, a significant increase in liver enzyme levels was reported in these patients, in addition to the prolongation of the QTc interval, with no clinical implications, according to the authors. Something similar has

been reported by a case-control study that compared individuals infected with COVID-19 treated with HCQ and azithromycin [17,18]. In a retrospective observational study by Geleris et al., the use of HCQ did not reduce the need for invasive ventilation or mortality [18]. In another randomized, unblinded clinical trial for mild to moderate forms of COVID-19, 150 hospitalized patients were analyzed, of which 75 underwent conventional treatment (best supportive care), and the remaining patients received CQ in addition to supportive treatment. The use of QC also did not increase survival or the likelihood of viral clearance, raising issues about its possible in vivo antiviral activity [19]. On the other hand, a controlled and randomized clinical trial conducted with 63 patients in Wuhan, China, reported a shorter mean time to clinical recovery in the experimental group (400 mg of HCQ for 5 days) compared to the control group. Although without a difference in mortality, the HCQ treatment group had a significantly shorter time to reach afebrile status and had significantly less time to relieve coughing [20].

Although ivermectin has been approved as a safe antiparasitic drug for decades, some recent studies have revealed that ivermectin has antiviral action against RNA viruses, like the Zika virus [21], influenza A [22], Chikungunya virus [23] and HIV-1 [24]. This is due to the inhibition of nuclear transport mediated by α/β importin, which in turn blocks nuclear trafficking in viral proteins (HIV-1, SV40), which is necessary for replication

[23]. Although SARS-CoV-2 is believed to replicate in cytosol, most RNA viruses depend on $\alpha/\beta 1$ importin during the replication process. Consequently, ivermectin was presented as a therapeutic possibility in the face of the COVID-19 pandemic [25]. Caly et al. documented the antiviral activity of ivermectin against SARS-CoV-2 in vitro [12]. Based on this evidence, some centers have started treatment with ivermectin at a dose of 150 $\mu\text{g/kg}$ for patients infected with SARS-CoV-2, and some authors have reported their possible benefits [26].

Although we found a favorable safety profile for the three drugs, in terms of cardiac, hematologic and hepatic toxicity, our study showed no benefit in terms of reducing the need for supplemental oxygen, mechanical ventilation, or mortality for the treated groups with HCQ or CQ in relation to ivermectin in hospitalized patients. Mortality was very similar in the three arms (ranging from 21.3% to 23.0%), suggesting that the 3 drugs are equally ineffective in this setting. The mortality rates of the three groups are very similar to the historical reports of other studies that used placebo in hospitalized patients, strengthening the hypothesis of ineffectiveness.

Regarding the analysis of predictive factors for mortality, our study showed worse progression in obese patients ($\text{BMI} > 33 \text{ kg/m}^2$). Marked deregulation of lymphoid responses and exacerbation of pro-inflammatory cytokines has been described in obese patients, especially IL-6 and TNF [27,28]. IL-6 amplifies and supports the activation of various cytokine pathways for many days after the initial immune injury [29]. Thus, our data corroborate that obesity is an independent risk factor, doubling mortality, possibly because it contributes to the cytokine storm. Age was also a major predictor associated with fatality. The risk associated with increasing age has been a consistent finding among published works [4], but different from these reports, the present findings point to an earlier crucial point. While in different countries like Korea, China and Italy, the highest acceleration in fatality rates happened in patients over 70 years [30,31], in our data the most significant increase in fatality rates happened in patients over 60 years. Further studies should be directed to the role of local conditions in the lowering of the age range. Male gender has been related to a worse outcome [32,33], and although our data showed a trend to higher mortality, the difference was not statistically significant.

Low oxygen saturation ($\text{SatO}_2 < 90\%$) at admission was also a risk factor for death. This has also been observed in other studies, with small variations in the cutoff point [34,35]. In New York, SatO_2 less than 92% was associated with an increased risk of hospital lethality [36]. In our study, patients who were hospitalized less than 7 days after the onset of symptoms also had a worse prognosis. We did not find similar data in the literature. On the contrary, a retrospective study in

Wuhan, China, that evaluated hospitalized patients reported higher mortality in the group that was admitted after the tenth day of symptoms [37]. Differences in patient severity between the Chinese study and ours may explain this discrepancy. The earlier hospitalization may be related to a more serious evolution of the disease, possibly due to a higher viral load and systemic inflammation. Studies have already correlated higher viral load and clinical severity, showing that an increase in viral load precedes clinical deterioration, and a reduction in symptom improvement [38].

The presentation of diarrhea before hospitalization was a protective factor in our data. We did not find similar data in the literature. Kumar et al. did not observe a statistically significant difference in mortality between patients with a gastrointestinal manifestation of COVID-19 in relation to those with purely respiratory manifestation [39]. In a meta-analysis, the proportion of patients with gastrointestinal symptoms was higher in the group of critically ill patients when compared to the non-severe group; however, mortality was similar between patients with or without diarrhea [40]. Unlike our study, this meta-analysis assessed the effect of diarrhea during hospitalization, and not as a prognostic factor at hospital admission.

We conclude that the use of CQ, HCQ or ivermectin is not related to a reduction in the need for supplemental oxygen, admission to ICU, invasive ventilation or death, in hospitalized patients with a severe form of COVID-19. In the doses used, we observed a favorable safety profile of the tested drugs, especially regarding serious adverse cardiac, hematologic or hepatic events. Age over 60 years, obesity, diabetes, extensive pulmonary involvement and low oxygen saturation at hospital admission were independent risk factors for mortality in this cohort.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Allex Jardim da Fonseca  <http://orcid.org/0000-0002-1715-5469>

References

- [1] Velavan TP, Meyer CG. The COVID-19 epidemic. *Trop Med Int Health*. 2020;25(3):278–280.
- [2] Wu D, Wu T, Liu Q, et al. The SARS-CoV-2 outbreak: what we know. *Int J Infect Dis*. 2020;94:44–48.
- [3] Petrosillo N, Viceconte G, Ergonul O, et al. SARS and MERS: are they closely related? *Clin Microbiol Infect*. 2020;26(6):729–734.
- [4] Baj J, Karakula-Juchnowicz H, Teresiński G, et al. COVID-19: specific and non-specific clinical manifestations and

- symptoms: the current state of knowledge. *J Clin Med*. 2020;9(6):6.
- [5] Ye Q, Wang B, Mao J. The pathogenesis and treatment of the 'cytokine storm' in COVID-19. *J Infect*. 2020;80(6):607–613.
 - [6] Ruiz-Irastorza G, Ramos-Casals M, Brito-Zeron P, et al. Clinical efficacy and side effects of antimalarials in systemic lupus erythematosus: a systematic review. *Ann Rheum Dis*. 2010;69(1):20–28.
 - [7] Yao X, Ye F, Zhang M, et al. In vitro antiviral activity and projection of optimized dosing design of hydroxychloroquine for the treatment of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). *Clin Infect Dis*. 2020;71(15):732–739.
 - [8] Gao J, Tian Z, Breakthrough: YX. Chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. *Biosci Trends*. 2020;14(1):72–73.
 - [9] Brazil. Ministry of Health. NOTA INFORMATIVA Nº 5/2020-DAF/SCTIE/MS. Uso da Cloroquina como terapia adjuvante no tratamento de formas graves do COVID-19. Brasília. 2020.
 - [10] Lee YJ, Lee C. Ivermectin inhibits porcine reproductive and respiratory syndrome virus in cultured porcine alveolar macrophages. *Arch Virol*. 2016;161(2):257–268.
 - [11] Xu TL, Han Y, Liu W, et al. Antivirus effectiveness of ivermectin on dengue virus type 2 in *Aedes albopictus*. *PLoS Negl Trop Dis*. 2018;12(11):e0006934.
 - [12] Caly L, Druce JD, Catton MG, et al. The FDA-approved drug ivermectin inhibits the replication of SARS-CoV-2 in vitro. *Antiviral Res*. 2020;178:104787.
 - [13] Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020;395(10223):497–506.
 - [14] Shukla AM, Archibald LK, Wagle Shukla A, et al. Chloroquine and hydroxychloroquine in the context of COVID-19. *Drugs Context*. 2020;9:2020-4-5.doi: 10.7573/dic.2020-4-5.eCollection 2020.
 - [15] Zhao M. Cytokine storm and immunomodulatory therapy in COVID-19: role of chloroquine and anti-IL-6 monoclonal antibodies. *Int J Antimicrob Agents*. 2020;55(6):105982.
 - [16] Cavalcanti AB, Zampieri FG, Rosa RG, et al. Hydroxychloroquine with or without azithromycin in mild-to-moderate Covid-19. *N Engl J Med*. 2020. 10.1056/NEJMoa2019014.
 - [17] Cipriani A, Zorzi A, Ceccato D, et al. Arrhythmic profile and 24-hour QT interval variability in COVID-19 patients treated with hydroxychloroquine and azithromycin. *Int J Cardiol*. 2020;316:280–284.
 - [18] Geleris J, Sun Y, Platt J, et al. Observational study of hydroxychloroquine in hospitalized patients with Covid-19. *N Engl J Med*. 2020;382(25):2411–2418.
 - [19] Tang W, Cao Z, Han M, et al. Hydroxychloroquine in patients with mainly mild to moderate coronavirus disease 2019: open label, randomised controlled trial. *BMJ*. 2020;369:m1849.
 - [20] Chen J, Liu D, Liu L, et al. [A pilot study of hydroxychloroquine in treatment of patients with moderate COVID-19]. *Zhejiang Da Xue Xue Bao Yi Xue Ban*. 2020;49(2):215–219.
 - [21] Barrows NJ, Campos RK, Powell ST, et al. A Screen of FDA-approved drugs for inhibitors of Zika virus infection. *Cell Host Microbe*. 2016;20(2):259–270.
 - [22] Götz V, Magar L, Dornfeld D, et al. Influenza A viruses escape from MxA restriction at the expense of efficient nuclear vRNP import. *Sci Rep*. 2016;6(1):23138.
 - [23] Yang SNY, Atkinson SC, Wang C, et al. The broad spectrum antiviral ivermectin targets the host nuclear transport importin $\alpha/\beta 1$ heterodimer. *Antiviral Res*. 2020;177:104760.
 - [24] Wagstaff KM, Sivakumaran H, Heaton SM, et al. Ivermectin is a specific inhibitor of importin α/β -mediated nuclear import able to inhibit replication of HIV-1 and dengue virus. *Biochem J*. 2012;443(3):851–856.
 - [25] Sharun K, Dhama K, Patel SK, et al. Ivermectin, a new candidate therapeutic against SARS-CoV-2/COVID-19. *Ann Clin Microbiol Antimicrob*. 2020;19(1):23.
 - [26] Heidary F, Gharebaghi R. Ivermectin: a systematic review from antiviral effects to COVID-19 complementary regimen. *J Antibiot (Tokyo)*. 2020;73(9):593–602.
 - [27] Mraz M, Haluzik M. The role of adipose tissue immune cells in obesity and low-grade inflammation. *J Endocrinol*. 2014;222(3):R113–27.
 - [28] Kern L, Mittenbühler MJ, Vesting AJ, et al. Obesity-induced TNF α and IL-6 signaling: the missing link between obesity and inflammation-driven liver and colorectal cancers. *Cancers (Basel)*. 2018;11:1.
 - [29] Yiu HH, Graham AL, Stengel RF. Dynamics of a cytokine storm. *PLoS One*. 2012;7(10):e45027.
 - [30] Report on the Epidemiological features of coronavirus disease 2019 (COVID-19) outbreak in the Republic of Korea from January 19 to March 2, 2020. *J Korean Med Sci*. 2020;35(10):e112.
 - [31] Porcheddu R, Serra C, Kelvin D, et al. Similarity in case fatality rates (CFR) of COVID-19/SARS-COV-2 in Italy and China. *J Infect Dev Ctries*. 2020;14(2):125–128.
 - [32] Yang X, Yu Y, Xu J, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med*. 2020;8(5):475–481.
 - [33] Guan WJ, Ni ZY, Hu Y, et al. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med*. 2020;382(18):1708–1720.
 - [34] Yadaw AS, Li YC, Bose S, et al. Clinical predictors of COVID-19 mortality. *medRxiv*. 2020. doi:10.1016/S2589-7500(20)30217-X
 - [35] Du RH, Liang LR, Yang CQ, et al. Predictors of mortality for patients with COVID-19 pneumonia caused by SARS-CoV-2: a prospective cohort study. *Eur Respir J*. 2020;55(5):5.
 - [36] Mikami T, Miyashita H, Yamada T, et al. Risk factors for mortality in patients with COVID-19 in New York City. *J Gen Intern Med*. 2021 Jan;36(1):17–26. doi: 10.1007/s11606-020-05983-z.
 - [37] Deng Y, Liu W, Liu K, et al. Clinical characteristics of fatal and recovered cases of coronavirus disease 2019 in Wuhan, China: a retrospective study. *Chin Med J (Engl)*. 2020;133(11):1261–1267.
 - [38] Pujadas E, Chaudhry F, McBride R, et al. SARS-CoV-2 viral load predicts COVID-19 mortality. *Lancet Respir Med*. 2020;8(9):e70.
 - [39] Suresh Kumar VC, Mukherjee S, Harne PS, et al. Novelty in the gut: a systematic review and meta-analysis of the gastrointestinal manifestations of COVID-19. *BMJ Open Gastroenterol*. 2020;7(1):1.
 - [40] Mao R, Qiu Y, He JS, et al. Manifestations and prognosis of gastrointestinal and liver involvement in patients with COVID-19: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol*. 2020;5(7):667–678.