



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



Hydroxychloroquine / azithromycin in COVID-19: The association between time to treatment and case fatality rate

Roberto Alfonso Accinelli^{a,b,c,*}, Grisel Jesús Ynga-Meléndez^d, Juan Alonso León-Abarca^a, Lidia Marianella López^a, Juan Carlos Madrid-Cisneros^d, Juan Diego Mendoza-Saldaña^b

^a Instituto de Investigaciones de la Altura. Universidad Peruana Cayetano Heredia, Lima, Peru

^b Facultad de Medicina Alberto Hurtado, Universidad Peruana Cayetano Heredia, Lima, Peru

^c Hospital Cayetano Heredia, Peru

^d Centro Materno-Infantil Tahuantinsuyo Bajo, Lima, Peru

ARTICLE INFO

Keywords:

Hydroxychloroquine
Azithromycin
SARS-CoV-2
COVID-19
Mortality
Time-to-Treatment

ABSTRACT

Background: Currently, there is no formally accepted pharmacological treatment for COVID-19.

Materials and methods: We included COVID-19 outpatients of a Peruvian primary care center from Lima, Peru, who were treated between April 30 - September 30, 2020, with hydroxychloroquine and azithromycin. Logistic regression was applied to determine factors associated with case-fatality rate.

Results: A total of 1265 COVID-19 patients with an average age of 44.5 years were studied. Women represented 50.1% of patients, with an overall 5.9 symptom days, SpO₂ 97%, temperature of 37.3 °C, 41% with at least one comorbidity and 96.1% one symptom or sign. No patient treated within the first 72 h of illness died. The factors associated with higher case fatality rate were age (OR = 1.06; 95% CI 1.01–1.11, p = 0.021), SpO₂ (OR = 0.87; 95% CI 0.79–0.96, p = 0.005) and treatment onset (OR = 1.16; 95% CI 1.06–1.27, p = 0.002), being the latter the only associated in the multivariate analysis (OR = 1.18; 95% CI 1.05–1.32, p = 0.005). 0.6% of our patients died.

Conclusions: The case fatality rate in COVID-19 outpatients treated with hydroxychloroquine/azithromycin was associated with the number of days of illness on which treatment was started.

1. Introduction

The rapid spread of the virus referred to as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has led to a devastating worldwide pandemic. Despite the astonishingly rapid development of effective vaccines, most countries continue to suffer from the tragic consequences of the coronavirus disease 2019 (COVID-19). There is still a need for drugs that effectively control the disease. Unfortunately, COVID-19 has proven elusive and non-responsive to most treatment options as indicated by several clinical trials that failed to demonstrate significant reduction in morbidity and mortality of COVID-19 patients [1,2]. Perhaps most disheartening is the fact that drugs proven to possess strong anti-infectious and anti-inflammatory properties and that have

been successfully employed in other viral diseases failed to show statistical improvement in several clinical trials in COVID-19 patients. Two concrete examples are Chloroquine (CQ) and its metabolite Hydroxychloroquine (HCQ). Successfully used to prevent and treat malaria and amebiasis for many years [3], these drugs yielded conflicting results in various clinical trials [4]. Furthermore, its usage or treatment interruption could be confounded by the known side effects of CQ and HCQ which include mild gastrointestinal and more serious cardiovascular and neurological effects. This is a particularly important consideration when treating patients at risk of developing severe forms of COVID-19 [4,5].

However, notwithstanding the known limitations and well-justified reservation for the use of these drugs, there is one aspect that requires further investigation: the fact that in viral infections such as influenza,

Abbreviations: Hydroxychloroquine, (HCQ); Azithromycin, (AZIT); Severe acute respiratory syndrome coronavirus 2, (SARS-CoV-2); Coronavirus disease 2019, (COVID-19); Angiotensin converting enzyme 2, (ACE2); Selectivity Index, (SI); Mean Maximum Inhibitory Concentration, (MIC50%); Mean Cytotoxic Concentration, (CC50%); Mean Effective Concentration, (EC50); Maternal-Infant Center, (MIC).

* Corresponding author. Instituto de Investigaciones de la Altura. Facultad de Medicina Alberto Hurtado. Universidad Peruana Cayetano Heredia, Av. Honorio Delgado 430, San Martín de Porres 15102, Lima, Peru.

E-mail address: roberto.accinelli@upch.pe (R.A. Accinelli).

<https://doi.org/10.1016/j.tmaid.2021.102163>

Received 26 April 2021; Received in revised form 27 August 2021; Accepted 9 September 2021

Available online 14 September 2021

1477-8939/© 2021 Published by Elsevier Ltd.

there is a relationship between early antiviral therapy and survival. It is the rapid elimination of pathogens and the early reduction in the viral load that seems to be decisive to avoid irreversible injury due to progression of the disease [6]. This is particularly relevant for the use of CQ and HCQ in the context of COVID-19 [7], and much can be learned from countries that have considerable clinical experience with the use of these drugs in the treatment of malaria and other infectious diseases. Indeed, the healing properties of the bark of the tree *Cinchona officinalis*, the source of the natural quinine, was first discovered by the Incas, and clinically applied to cure malaria as early as the 1600's, making the *Cinchona* tree the national tree of Peru.

Thus, when the first case of COVID-19 was diagnosed in Peru on March 6th, 2020, clinicians experienced with the use of HCQ in the treatment of other diseases employed this drug to combat COVID-19. They based its use on their clinical experience and the knowledge that HCQ significantly decreases viral load in particular when associated with azithromycin (AZIT). To these clinicians, the long-term use of low doses of AZIT was known to reduce exacerbations of poorly controlled asthma, which has been attributed to the suppressive effect of AZIT on the inflammatory TNF pathways [8]. The use of this treatment regimen was further encouraged by early reports that HCQ not only decreased significantly the viral load, but when associated with AZIT, was also able to control the infection in COVID-19 patients [9].

Chloroquine, the derivative of the natural quinine, is a 9-aminoquinoline that has first been described in 1934, and used to combat various viruses since 1960 [10]. In 1978 it was demonstrated that CQ is an acidotropic dibasic agent that increases the pH of lysosomes [11], and alters cellular metabolism [12]. This pH effect in lysosomes and other cytoplasmic organelles contributes to the suppression of viral replication. Together with its anti-inflammatory action as a suppressor of TNF alpha and Interleukin 6, this drug seems to be an ideal candidate to treat patients infected with SARS-CoV-2 [13]. In 2003 it was discovered that the S1 domain of the SARS-CoV protein binds to angiotensin-converting enzyme 2 (ACE2) for cell entry which opened the way for further in-depth mechanistic *in vitro* studies [14]. One of these studies demonstrated that CQ is an effective inhibitor of replication of the coronavirus SARS-CoV. These cell culture experiments demonstrated that the IC50 for the antiviral activity of CQ was significantly lower than its cytostatic activity, which was reflected in a high selectivity index of 30. Specifically, these studies indicated that the maximal concentration of its antiviral action (8.8 μM) was much lower, than the concentration required for its mean cytotoxic effect (261.3 μM). Clearly, as is the case for all *in vitro* assays, there are numerous caveats associated with the translation of such preclinical findings into the clinic. Nevertheless, such experiments are encouraging, since concentrations (CC50%) of 261.3 μM are much higher than those achieved in the blood at the therapeutic level of MIC50%, suggesting that CQ could potentially be an effective clinical agent against SARS-CoV [15]. Moreover, as demonstrated in another study cells previously treated with CQ are refractory to SARS-CoV infection, and when cells are already infected, CQ can prevent viral replication [16].

These encouraging results laid a solid scientific foundation for the use of CQ in the treatment of SARS-CoV-2. Indeed, various studies confirmed that the known antiviral properties of CQ also potentially blocked SARS-CoV-2 infection at low concentration, with mild cytotoxicity and a high selectivity index (mean effective concentration (EC50%) = 1.13 μM ; CC50 > 100 μM , SI > 88.50) [17]. Moreover additional mechanisms have been identified. One study suggests that binding of HCQ/CQ to sialic acids and ECA-2 receptor gangliosides prevents viral S protein from entering the cell [18], and a cell culture assay confirmed that HCQ/CQ blocks the transport of SARS-CoV-2 from early to late endosomes [19]. Another study tested 1520 compounds that are in clinical use and identified fifteen products effective against SARS-CoV-2. Among these products HCQ and AZIT showed the highest antiviral activities (CE50% = 4.17 μM and 2.12 μM , respectively) and highest SIs [20]. In both plasma and lung, CQ/HCQ have mean/median

Cmax concentrations above the EC50, and both plus AZIT would reach lung concentrations 10 times higher than the EC50 [21]. Structurally, AZIT resembles the GM1 ganglioside of the ECA2 receptor, so it binds to the tip of the spike protein, while the CQ/HCQ molecules bind to the virus binding sites of sialic acids and ECA-2 gangliosides, generating a synergistic antiviral mechanism [22]. These *in vitro* studies suggest that the HCQ-AZIT combination has a synergistic effect on SARS-CoV-2 at concentrations that are compatible with those obtained in the human lung [23]. Furthermore, by binding to Sigma 1 and Sigma 2 receptors, HCQ effectively reduces the infectivity of SARS-CoV-2 [24].

The scientific data obtained for the Coronavirus as early as 2006 and confirmed for SARS-CoV-2 at the beginning of the pandemic, together with our extensive clinical experience in the use of CQ in treating malaria and other infectious diseases [3] provided a strong rationale for the therapeutic use of CQ in patients infected during the new coronavirus epidemic COVID-19. It also provided an impetus to test QC immediately in clinical trials [25].

In Peru, the Ministry of Health decided to use the HCQ/CQ combination with AZIT [26]. In the absence of clinical trial results during the early phase of the pandemic, physicians were instructed to apply their extensive clinical experience with the use of this drug combination in the context of the emerging understanding of the pathophysiology of SARS CoV-2 infection in order to determine the impact of early outpatient treatment on hospitalization and mortality [27]. In this report we present the results in 1265 patients treated on an outpatient basis at the Centro Materno-Infantil (CMI) de Tahuantinsuyo Bajo, a I-4-level health center in the city of Lima.

2. Material and methods

The present study analyzed anonymized data from the database of COVID-19 patients attended at the CMI Tahuantinsuyo Bajo, a primary care facility in the city of Lima, between April 30 and September 30, 2020.

Patients arrived at a dedicated triage site for patients with suspected COVID-19 infection. There, vital signs were taken, including SpO₂, and the attending physicians took the patient history and performed a clinical examination to determine whether they met COVID-19 patient clinical criteria according to the guidelines of the Peruvian Ministry of Health. All patients were registered in the respective epidemiological data file and the information included vital signs, comorbidities, symptoms and treatment onset, consisting of 200 mg HCQ every 8 h for 7–10 days in combination with 500 mg AZIT on the first day, followed by 250 mg for 4 days. Data on days from symptom onset to treatment was collected as well. The patients were followed up with daily telephone controls and if any symptoms of deterioration or side effects appeared, they were summoned to the clinical facility. Follow-up was carried out not only with the patients but also with their contacts, with the aim of providing treatment as soon as the first symptoms appeared. Every day the epidemiology team recorded and shared patient's information to the physician coordinating the COVID-19 registry. The information was transcribed into an Excel spreadsheet and the cases were followed up after discharge until they were sure of their condition. If the information could not be obtained by telephone, a home visit was done by the rapid a response team also established under Peruvian COVID-19 guidelines.

The treatment started as soon as the attending physician determined that the patient exhibited symptoms that met the COVID-19 patient clinical criteria according to the guidelines of the Peruvian Ministry of Health. Some of these patients arrived at the hospital with a positive test, but most did not. Those that were not tested before arrival were asked to take the test. This test was not readily available at the center, albeit it continues to be offered at no cost at some government testing sites. Given that during the study period it could take almost a week to process and register the result of the NAAT test, and because tests tend to be less accurate within three days of exposure, the treatment regimen was

started irrespective of any result if a patient met all the clinical criteria for COVID-19. Statistical analysis was performed with the Stata 14 statistical package (Stata Corporation, College Station, Texas, USA). Categorical variables were presented as frequencies and percentages and their respective 95% confidence intervals (95%CI), continuous variables as means or medians along standard deviations (SD) or interquartile ranges (IQR). To determine the risk factors associated with death, a logistic regression analysis was performed, odds ratios were presented with their respective 95%CI and a p value of less than 0.05 was considered statistically significant.

This study was approved by the Institutional Human Ethics Committee of the Universidad Peruana Cayetano Heredia (approval code: 203939). This study did not require individual consent from the participants because it analyzed de-identified data from an already existing database. Cayetano Heredia University’s researchers analyzed the information that was previously registered and systematized by the team of physicians in charge of primary care at the health center.

3. Results

A total of 1265 clinically diagnosed COVID-19 patients were studied with an average age of 44.5 years, 50.1% being women, with a time of symptom onset to treatment of 5.9 days, SpO2 of 97%, temperature of 37.3 °C, with 41% with at least one comorbidity and 96.1% with at least one symptom or sign (Table 1). The most common comorbidities were obesity (17.3%), hypertension (8.3%), chronic respiratory disease (7.2%) and diabetes (6.1%) (Table 2). The most common symptoms were cough (85.1%), malaise (81.7%), sore throat (76.7%), sensation of thermal rise (54.2%) and dyspnea (33.8%) (Table 3).

At follow-up, there were 7 deaths in total, all men with a mean age of 57.7 years, SatO2 96%. Four of the deceased patients carried one known comorbidity (hypertension, obesity, diabetes and chronic respiratory disease), three had no comorbidity. The one aged 29 years old had

Table 1
Characteristics of COVID-19 patients treated with hydroxychloroquine and azithromycin at CMI Tahuantinsuyo Bajo.

Variables	All patients (n = 1265)			Dead (n = 7)	
	n	Mean	SD	Mean	SD
Age (years)	1265	44.5	14.8	57.7	20.6
SpO2 (%)*	1057	97	2	96	4
Temperature (°C)	1108	37.3	0.8	37.4	0.7
Time from symptom onset to treatment (days)	1202	5.9	4	10.3	9.5
Symptoms (number)*	1265	5	3	6	4
Comorbidities (number)*	1265	0	1	1	1
Height (meters)	420	1.62	0.09	1.64	0.1
Weight (kg)	418	72.9	13	73	25.7
BMI (kg/m2)	418	27.8	4.2	26.8	7.5
Characteristics	n	%	CI 95%	n	%
0–20 years	39/1265	3.1	2.3–4.2%	0	0
20–40 years	439/1265	34.7	32.1–37.4%	2	28.6
40–60 years	590/1265	46.6	43.9–49.4%	2	28.6
60–80 years	177/1265	14	12.2–16.0%	1	14.3
80–100 years	20/1265	1.6	1.0–2.4%	2	28.6
Sex (female)	634/1265	50.1	47.4–52.9%	0	0
At least one comorbidity	470/1265	37.2	34.5–39.9%	4	57.1
SpO2 <92%	32/1265	2.5	1.8–3.6%	1	14.3
Tested for SARS-CoV-2 (any test)	134/1265	10.6	9.2–12.7%	5	3.6
Treatment discontinuation	10/1265	0.8	0.4–1.5%	1	10%

Table 2
Comorbidities in COVID-19 patients who were treated with hydroxychloroquine and azithromycin.

Comorbidities	All patients (n = 1265)			Dead (n = 7)	
	n	%	IC 95%	n	%
Obesity	219	17.3	15.3 19.5	1	14.3
Hypertension	105	8.3	6.9 10	1	14.3
Respiratory disease	91	7.2	5.9 8.8	1	14.3
Diabetes	77	6.1	4.9 7.5	1	14.3
Endocrinological disease	18	1.4	0.9 2.2	0	0
Cardiovascular disease	13	1	0.6 1.8	0	0
Gastrointestinal disease	12	0.9	0.5 1.7	0	0
Neurological disease	10	0.8	0.4 1.5	0	0
Rheumatologic disease	7	0.6	0.3 1.2	0	0
Immunosuppression	7	0.6	0.3 1.2	0	0
Pregnant women	6	0.5	0.2 1.1	0	0
Psychiatric disease	5	0.4	0.2 0.9	0	0
Surgical pathology	4	0.3	0.1 0.8	0	0
Hematologic disease	3	0.2	0.1 0.7	0	0
Renal disease	2	0.2	0 0.6	0	0
Neoplastic disease	2	0.2	0 0.6	0	0

Table 3
Symptoms presented by COVID-19 patients who were treated with hydroxychloroquine and azithromycin.

Symptoms	All patients (HCQ + AZIT) (n = 1265)			Dead (n = 7)	
	n	%	CI 95%	n	%
Cough	1076	85.1	83 86.9	7	100
General discomfort	1034	81.7	79.5 83.8	7	100
Sore throat	970	76.7	74.3 78.9	6	85.7
Fever	685	54.2	51.4 56.9	3	42.9
Dyspnea	427	33.8	31.2 36.4	5	71.4
Nasal congestion	397	31.4	28.9 34	3	42.9
Headache	360	28.5	26 31	2	28.6
Chills	259	20.5	18.3 22.8	0	0
Muscle pain	207	16.4	14.4 18.5	2	28.6
Joint pain	223	17.6	15.6 19.8	1	14.3
Chest pain	141	11.2	9.5 13	1	14.3
Diarrhea	103	8.1	6.8 9.8	1	14.3
Nausea	82	6.5	5.2 8	0	0
Anosmia	65	5.1	4 6.5	1	14.3
Ageusia	49	3.9	2.9 5.1	1	14.3
Vomiting	39	3.1	2.3 4.2	0	0
Back pain	26	2.1	1.4 3	0	0
Unspecified pain	21	1.7	1.1 2.5	1	14.3
Abdominal pain	11	0.9	0.5 1.6	0	0
Irritability	8	0.6	0.3 1.3	0	0
Eye redness	4	0.3	0.1 0.8	0	0
Decreased appetite	3	0.2	0.1 0.7	0	0
Dizziness	1	0.1	0 0.6	0	0
Skin rash	1	0.1	0 0.6	0	0

obesity and a 98% SpO2 at first encounter, while the patient age 39 years old had no known comorbidities and a 98% SpO2. The most common symptoms were cough (100%), malaise (100%), sore throat (85.7%), dyspnea (71.4%), nasal congestion (42.9%) and febrile sensation (42.9%) (Table 3). Logistic regression showed that those factors associated with higher mortality were age (OR 1.06; 95% CI 1.01–1.11, p = 0.021), SpO2 (OR 0.87; 95% CI 0.79–0.96, p = 0.005) and number of days until the start of treatment (OR 1.16; 95% CI 1.06–1.27, p = 0.002). However, in a multivariate analysis the time of illness elapsed before receiving treatment was the only factor associated with higher mortality (OR 1.18; 95%CI 1.05–1.32, p = 0.005) (Table 4).

The case-fatality rate of this cohort of patients treated with HCQ-AZIT was 0.6. No female patient died and the mortality among males was 1.12%. (Table 5).

Remarkably, none of those treated in the first 72 h of illness onset died. Deaths occurred on days four, when two died, and on days six, seven, eight, twelve and thirty-one after onset. All but nine patients

Table 4

Odds ratio for death in patients with suspected COVID-19 who were treated with hydroxychloroquine + azithromycin. N = 904.

Variables	Univariate			Multivariate				
	OR	p	95% CI	OR	p	95% CI		
Age	1.06	0.021	1.01	1.11	1.06	0.087	0.99	1.13
Sex (female)	0.07	0.062	0	1.15	0.11	0.131	0.01	1.93
Risk factors (at least one)	2.18	0.275	0.54	8.87	1.16	0.855	0.24	5.54
SpO2 (first measurement)	0.87	0.005	0.79	0.96	0.93	0.423	0.78	1.11
Time from symptom onset to treatment (days)	1.16	0.002	1.06	1.27	1.18	0.005	1.05	1.32
Temperature (°C)	1.19	0.703	0.49	2.89	1.49	0.471	0.51	4.38

Table 5

Comparison of mortality between Peruvian COVID-19 patients and those treated at the Tahuantinsuyo Bajo CMI with hydroxychloroquine + azithromycin.

Age (women)	Deaths (Tahuantinsuyo)	Cases	Deaths (%)	95% IC	
0–19	0	24	0.00%	0	0
20–39	0	239	0.00%	0	0
40–59	0	277	0.00%	0	0
60–79	0	86	0.00%	0	0
80+	0	8	0.00%	0	0
Total	0	634	0.00%	0	0
(Women)					
Age (men)	Deaths (Tahuantinsuyo)	Cases	Deaths (%)	95% IC	
0–19	0	15	0.00%	–	–
20–39	2	198	1.00%	0.25%	3.93%
40–59	2	311	0.64%	0.16%	2.53%
60–79	1	90	1.10%	0.15%	7.49%
80+	2	10	16.67%	3.92%	49.48%
Total (Men)	7	624	1.12%	0.30%	1.95%
Total (all patients)	7	1265	0.60%	0.30%	1.20%

Table 6

Distribution according to days of symptom onset to treatment of COVID-19 patients and deaths of the CMI Tahuantinsuyo Bajo who were treated with hydroxychloroquine + azithromycin.

Time of illness	Deaths per day	Cases per day	%
1	0	83	0
2	0	124	0
3	0	153	0
4	2	157	1.27
5	0	143	0
6	1	106	0.94
7	1	139	0.72
8	1	81	1.23
9	0	35	0
10	0	39	0
11	0	44	0
12	1	29	3.45
13	0	17	0
14	0	8	0
15	0	10	0
16	0	12	0
17	0	2	0
18	0	1	0
19	0	4	0
20	0	6	0
21	0	0	0
22	0	1	0
23	0	1	0
24	0	0	0
25	0	0	0
26	0	2	0
27	0	1	0
28	0	1	0
29	0	0	0
30	0	2	0
31	1	1	100

(0.72%) reported having sought care within the first 20 days of symptom onset (Table 6). The proportion of patients seen within the first 72 h was different than the proportion of patients that arrived with longer duration of illness [28.06% (95% CI:25.65–30.61%) vs 71.94%, (95% CI: 69.39–74.35%), $p < 0.0001$] (Fig. 1). The percentage of case fatality increased progressively with the number of days after treatment initiation ($p = 0.0039$), reaching 0.89% (95% CI: 0.1–6.12%) among those who received treatment from day 10–12 of illness (Fig. 2). Importantly, no patients had cardiovascular side effects or had to be hospitalized for any effect attributable to the use of HCQ/AZT, but there were 0.79% (10/1265) who had their scheme suspended due to side effects, being nausea the most frequent present in 5 patients (Table 1).

4. Discussion

Of the 1265 COVID-19 patients treated at Tahuantinsuyo Bajo with HCQ-AZIT, 0.6% died (Table 1). This outcome is consistent with the first European study that used the same treatment regimen for 1061 patients. The case-fatality rate in this healthcare center was 0.75% [28]. The mortality rate among the 1265 patients treated with this specific regimen was six times lower than the national average [29]. As of September 1st, 2020, the updated case counts show that 74687 out of 694314 COVID-19 Peruvian patients died (case-fatality rate of 10.8%), being 13.2% (49121/372685) for men and 7.9% (25566/321629) for women [30]. Unfortunately, as of August 25th, Peru has one of the highest CFR of COVID-19 related deaths and ranks on seventh position with 6086 deaths per million inhabitants.

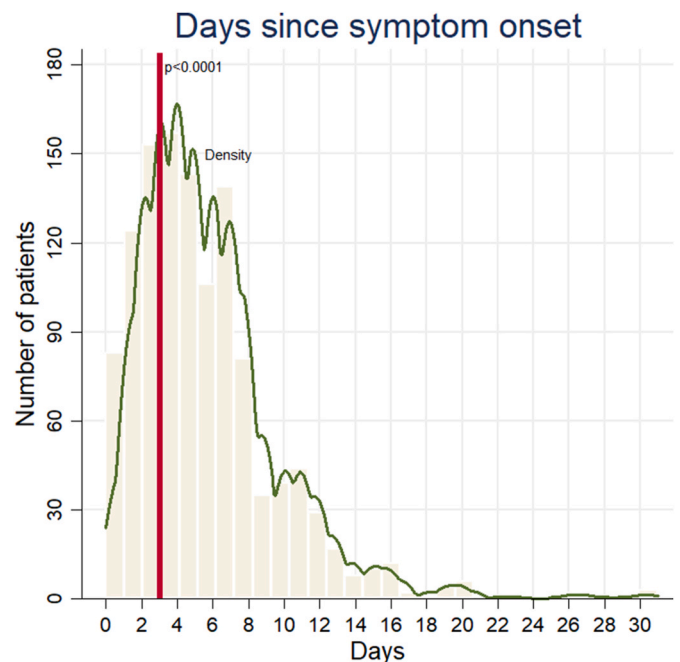


Fig. 1. Presentation by symptom onset to treatment of COVID-19 patients at Tahuantinsuyo Bajo.

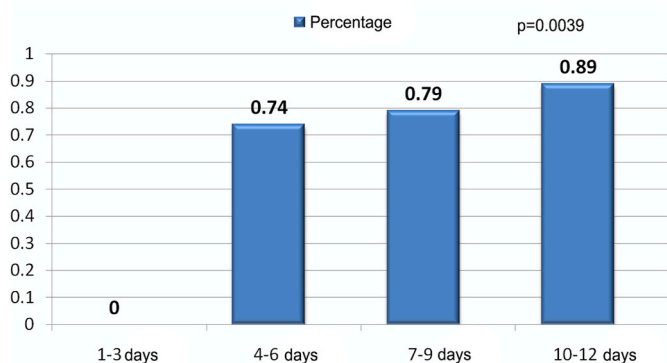


Fig. 2. Deaths by days from symptom onset to treatment ($p = 0.0039$).

4.1. Time of symptom onset to treatment before HCQ/AZIT

The first clinical manifestations at the onset of COVID-19 are thought to be attributable to viral replication, and it is generally assumed that the disease then moves into the inflammatory phase if the immune system fails to mount an adequate response. Thus, in patients who present late after the onset of the infection, the use of antiviral agents alone will not suffice to stop the ensuing cytokine storm, lung destruction and respiratory distress [31]. This could explain why HCQ has a better clinical efficiency when given earlier in the disease [32]. In the present study, treatment with HCQ-AZIT began on average 5.9 days after symptom onset, and there was no mortality among patients that received this drug combination during the first three days of symptom onset (Fig. 2).

This is consistent with the known relationship between early antiviral therapy and survival in influenza [6]. In a study of 657 influenza A/H1N1-2009 patients, those who received oseltamivir as an antiviral therapy within 48 h of symptom onset had lower ICU mortality and consumption of ICU resources and showed protection (OR 0.44, $p = 0.02$) [33], while in another research study those receiving treatment later had greater morbidity and mortality (OR 2.20, 95% CI: 1.47–3.57) [34]. While it has been observed that the interval for treatment onset was 2 days in community cases, 4 for those hospitalized and 6 in those admitted to the ICU ($p < 0.001$) [35]. Our study is most consistent with a study in which the use of HCQ before the fifth day of diagnosis was the only protective factor for prolonging viral shedding in patients with COVID-19 (OR = 0.111, $p = 0.001$) [7]. This conclusion is also consistent with the observation that HCQ had a protective effect in 67 patients who took this drug before hospitalization ($p < 0.001$), while there was no apparent protection among the 558 patients that received these drugs when they were already hospitalized [36].

Our results also corroborate other studies in which COVID-19 patients were treated from the first days of the disease. Among 141 HCQ-AZIT patients that were treated on average four days after symptoms onset only four (2.8%) were hospitalized, which was significantly less ($p < 0.001$) compared to the 58 hospitalized patients out of 377 (15.4%) (odds ratio 0.16, 95% CI 0.06–0.5) that were untreated [37]. Moreover, among 100 nursing home residents there was less mortality among those who received HCQ-AZIT on the first day of symptom onset (OR = 22.6; $p = 0.004$) [38]. In a cohort of 46 patients in Wuhan that had SatO₂ >93%, those treated with CQ and HCQ had a shorter clinical recovery time and viral RNA negativity [39]. In 57 patients with early treatment onset, 41% at the first day of symptoms, matched for age, sex and BMI in three groups, those who received HCQ-AZIT ($p = 0.0002$) or AZIT ($p = 0.0149$) recovered faster than those who did not use these drugs [40].

The early use of HCQ, within 5 days of diagnosis, was a protective factor associated with disease aggravation (95% CI: 0.040–0.575, $p = 0.006$). Clinical improvement by 20 days was significantly different between patients with HCQ used early and those with HCQ not used (p

= 0.016, 95% CI: 1.052–1.647). The median time to clinical improvement was 6 days in the HCQ used early group, compared with 9 days in the without HCQ used group and 8 days in the with HCQ not used early group ($p < 0.001$). HCQ used early was associated with earlier PCR conversion in both throat swabs (HR = 1.558, $p = 0.001$) and stool swabs (HR = 1.400, $p = 0.028$) [41].

In a cohort of 28,759 Iranian patients with COVID-19, 7295 (25.37%) with mild symptoms consented to receive and use HCQ within the first 3–7 days of diagnosis. HCQ reduced the odds of hospitalization by 38%, because it was required in 7.17% and 11.1% of patients who received and did not receive HCQ, respectively. A total of 314 patients died of COVID-19 complications, 27 (0.37%) and 287 (1.34%) in those who receive and did not receive HCQ, respectively, indicating a 73% mortality risk reduction on logistic regression model. The effect of HCQ on the outcome measures was maintained after adjusting for confounding factors and comorbidities. This effect remained significant whether patients were diagnosed based on positive RT-PCR or otherwise [42].

Among the 1067 outpatients with 5 days of COVID-19 in the propensity matched cohort, three hundred and five (31.4%) patients with no outpatient exposure to HCQ were hospitalized and 21 (21.6%) of patients with exposure to HCQ were hospitalized ($p = 0.045$), and 47 (4%) patients with no outpatient exposure died compared to 2 (2%) patients with outpatient exposure to HCQ [43]. In a group of Brazilian COVID-19 patients with an average delay from the start of symptoms to ER visit of 4.6 days the use of HCQ had a significant protective effect of 55% (OR 95% 0.45 (0.25–0.80), $p = 0.0065$) for hospitalizations [44].

In Saudi Arabia there were 5541 study participants, almost 33% ($n = 1817$) received HCQ in addition to SC while 67.2% ($n = 3724$) received the SC only, with significant fewer hospital admissions in the HCQ group compared to the SC (171 (9.36%) vs. 617 (16.6%), $p < 0.001$). This corresponded to a relative risk reduction in hospital admission of 43%. The rate of ICU admissions and mortality rate were also lower in the HCQ compared to the SC (0.77 vs. 1.5 ($p = 0.022$), and 0.39 vs. 1.45 ($p < 0.001$), respectively) [45].

So, all the studies with HCQ since the first week of symptoms in COVID-19 patients, including this, demonstrate protection for hospitalization and/or CFR. And with other drugs also used in COVID-19 for second indication, because their action over SARS-CoV-2 with excellent selectivity index, as ivermectin [46], colchicine [47], fluvoxamine [48], they have nice results when patients received them since the first symptoms days.

4.2. Sex

Although 50.1% of our patients were female, none of them died (Table 5). The sexual dimorphism in the evolution of COVID-19 may be hormonally based. Women produce higher levels of estrogens which is known to cause a more potent innate, cellular and humoral response, which is associated with a greater number of regulatory T cells and immunoglobulins [49]. Progesterone has higher levels in women and it is a Sigma R1/R2 active drug with antiviral action to SARS-CoV-2 [25]. Moreover, the immune cells of females exhibit a 10-fold higher expression of TLR [50]. Another factor could be the presence of two X chromosomes which confers a stronger innate and adaptive immune response to viral infections in women [51]. By contrast, males could have a higher susceptibility to SARS-CoV-2 as they express more RCT2 [52], which activity increases after ovariectomy and is reduced after orchiectomy [53], human bronchial epithelial cells treated with 17 β -estradiol express lower levels of RCT2 mRNA [54], and the serine protease gene TMPRSS2, required for virus entry [55], increases after exposure to androgens [56].

4.3. Age

With HCQ/AZIT treatment, mortality in male patients did not exceed

1% in the group younger than 80 years of age, while 16.67% in those older than 80 years (Table 5). Multiple factors contribute to the well-established age difference. In the elderly there is mild chronic inflammation, in which ACE2 expression, as well as autophagy and mitophagy are altered. There is also excess production of reactive oxygen species and senescent adipocyte activity, immunosenescence, as well as vitamin D deficiency. These and many other known factors will compromise the inflammatory response associated with cytokine storm in patients with severe COVID-19 resulting in an increased mortality among the elderly [57].

4.4. Comorbidities

The four most prevalent comorbidities of COVID-19 are hypertension, diabetes, cardiovascular and respiratory diseases, all of which are closely associated with obesity which is a major factor in the severity of morbidity and mortality of COVID-19 [58]. In the present cohort, 17.3% were obese (Table 2). This may affect ACE2 receptor expression in adipocytes and virus entry [59]. Moreover, in diabetic patients there is chronic inflammation, and in the elderly, immunological senescence aggravates the evolution of the disease, increasing the vulnerability in patients that do not control their glycemia, which may in part explain why diabetes is the comorbidity with the highest case fatality number [60]. Although the presence of comorbidities confers a much higher mortality risk in COVID-19 [61], their presence in our patients treated with HCQ/AZT was not associated with the case-fatality rate (Table 4).

A limitation of the present investigation is the underreporting of information, mainly of comorbidities and symptoms. In order to prevent an increased mortality among healthcare workers, forty percent of healthcare workers in Peru were sent home from the beginning of the pandemic, either because they were elderly with/without risk factors or because they had medical comorbidities. In addition, many of the healthcare workers that continued became ill in the meantime, which could have affected the completeness of the information.

Another limitation of the present investigation is that the diagnosis was primarily based on clinical guidelines [25]. In China, suspected COVID-19 cases are considered on the basis of epidemiological and clinical manifestations [62]. In Perú, the definition of a “suspected COVID-19 case” encompass a group of symptoms classically associated with COVID-19 according to national guidelines, while the definition of a “probable case” includes additional epidemiological or imaging criteria. Specific tests to detect the presence of the SARS-CoV-2 antigens or RT-PCR assays are only for diagnostic confirmation [63]. Of note, most healthcare centers faced difficulties securing enough tests for their assigned population. Of the 1265 patients evaluated in this study, only 134 (10.6%) had a test performed that was positive in 38 cases (28.4%), and both groups shared similar characteristics.

This limitation is typical for many countries including Peru, because the pandemic quickly overwhelmed the local health care systems. Global COVID-19 studies show that the median time from the first symptom onset to hospital admission is 7 days, 5–8 days for dyspnea, 8–9 days for acute respiratory distress syndrome, 10 to 5 days for mechanical ventilation and admission to hospital, and 5 days for mechanical ventilation and admission to the intensive care unit [64]. Any delays in the initiation of treatment would increase the frequency of hospitalization and worsen the outcomes in a healthcare system that experiences severe shortages in all aspects of clinical care. Moreover, during the first week of illness the positivity of RT-PCR is not more than 71% [65] and that of the combined IgM/IgG test is even lower at 39.3% [66]. Thus, relying on the positivity of any one of these tests in order to make the diagnosis of COVID-19 is inadequate because the diagnosis is delayed or never made. Thus, the Peruvian government decided to address this limitation by regulating that the diagnosis should be done on a clinical suspicion basis and the tests were only to confirm the patient as a COVID-19 case [26]. Indeed, as shown in the present study the reliance on the clinical diagnosis was an important prerequisite to ensure not

only the early treatment onset but also an increased therapeutic success.

The case-fatality rate with this treatment regimen was 0.6%, which was significantly lower than the national average. There is a large discrepancy between the case-fatality rate reported in this study and the rest of the country. Peruvian government put in its guidelines HCQ-AZT as one of the treatments for these patients [67]. At the same time, the World Health Organization stated no recommendation in favor of any specific treatment at inpatient settings to date, along Peruvian medical societies [68], physicians [69] and mass media [70]. This could have led to the underusage of the national COVID-19 guidelines which suggested diverse early treatment schemes. On another hand, the primary-care physicians at the Tahuantinsuyo Bajo Maternal and Child Center continued to treat patients with COVID-19 with HCQ-AZT, being the first primary care center in Lima that treated them early including patients outside its area of influence. This situation could explain the difference in the results between Tahuantinsuyo Bajo and the rest of the Peruvian territory.

In conclusion, our study showed that case-fatality rate in COVID-19 patients treated on an outpatient basis with HCQ/AZIT was associated with the number of days of illness when treatment was initiated.

CRedit authorship contribution statement

Roberto Alfonso Accinelli: Conceptualization, Resources, Data curation, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Grisel Jesús Ynga-Meléndez:** Investigation, Resources, Data curation, Writing – original draft, Funding acquisition. **Juan Alonso León-Abarca:** Methodology, Software, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Lidia Marianella López:** Methodology, Software, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Juan Carlos Madrid-Cisneros:** Investigation, Writing – original draft, Visualization. **Juan Diego Mendoza-Saldaña:** Investigation, Writing – original draft, Visualization.

Acknowledgments

The authors gratefully acknowledge the constructive suggestions given by Professor Nino Ramirez (Director, Center for Integrative Brain Research, Seattle Children’s Research Institute) on the final version of the present manuscript. We’d also like to acknowledge the members of the multidisciplinary team of the Centro Materno Infantil Tahuantinsuyo Bajo Lic. Rosa Chinchay Matta, Dr. Jose Luis Pando Herмосilla, Dr. Jose Roberto Espinoza Acosta, Dr. Veronica Cecilia Campos Aparcana, Dr. Cesar Vidal Osco Tamayo, and Dr. Cesar Vidal Osco Tamayo. Cesar Vidal Osco Tamayo, Dr. Henry Fabio Carrion Flores, Dr. Jean Huerta Jara, Lic. Victoria Sallo Accostupa, Lic. Ines Matta Bejarano, Lic. Maribel Reaño Rodriguez, Lic. Marisa Rios Jara, Tec. Jackie Yvonne Greenwilch Centeno, Tec. Irene Alejo Mendoza De Quispe, Tec. Hayde Mañuico Mallma, Tec. Liliana Janeth Hernandez Miñope and the students of the Faculty of Medicine of the Universidad Peruana Cayetano Heredia who confirmed the final condition of each patient Seungseo Choi, Niels Víctor Pacheco Barrios, José Enrique Vitón Rubio, Mayte Bryce Alberti, Arianna Portmann Baracco, Carlos Ruiz Sánchez and María Teresa Peña Gallardo.

References

- [1] Apaydın ÇB, Çınar G, Cihan-Üstündağ G. Small-molecule antiviral agents in ongoing clinical trials for COVID-19. *Curr Drug Targets* 2021 Feb 14. <https://doi.org/10.2174/1389450122666210215112150>. In press.
- [2] Ebina-Shibuya R, Namkoong H, Horita N, Kato H, Hara Y, Kobayashi N, Kaneko T. Hydroxychloroquine and chloroquine for treatment of coronavirus disease 19 (COVID-19): a systematic review and meta-analysis of randomized and non-randomized controlled trials. *J Thorac Dis* 2021 Jan;13(1):202–12.
- [3] Pastick KA, Okafor EC, Wang F, Lofgren SM, Skipper CP, Nicol MR, Pullen MF, Rajasingham R, McDonald EG, Lee TC, Schwartz IS. Review: hydroxychloroquine

- and chloroquine for treatment of SARS-CoV-2 (COVID-19). *Open Forum Infect Dis* 2020 Apr 1;7(4).
- [4] Younis NK, Zareef RO, Al Hassan SN, Bitar F, Eid AH, Arabi M. Hydroxychloroquine in COVID-19 patients: pros and cons. *Front Pharmacol* 2020; 11:597985.
- [5] Oscanoa TJ, Romero-Ortuno R, Carvajal A, Savarino A. A pharmacological perspective of chloroquine in SARS-CoV-2 infection: an old drug for the fight against a new coronavirus? *Int J Antimicrob Agents* 2020 Sep;56(3):106078.
- [6] Kumar A. Early versus late oseltamivir treatment in severely ill patients with 2009 pandemic influenza A (H1N1): speed is life. *J Antimicrob Chemother* 2011 May 1; 66(5):959–63.
- [7] Hong KS, Jang JG, Hur J, Lee JH, Kim HN, Lee W, Ahn JH. Early hydroxychloroquine administration for rapid severe acute respiratory syndrome coronavirus 2 eradication. *Infect Chemother* 2020 Sep;52(3):396.
- [8] Niessen NM, Gibson PG, Baines KJ, Barker D, Yang IA, Upham JW, et al. Sputum TNF markers are increased in neutrophilic and severe asthma and are reduced by azithromycin treatment. *Allergy* 2021 Mar 2;76(7):2090–101. <https://doi.org/10.1111/all.14768>. In this issue.
- [9] Gautret P, Lagier JC, Parola P, Meddeb L, Mailhe M, Doudier B, Courjon J, Giordanengo V, Vieira VE, Dupont HT, Honoré S. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. *Int J Antimicrob Agents* 2020;56(1):105949.
- [10] Mallucci L. Effect of chloroquine on lysosomes and on growth of mouse hepatitis virus (MHV-3). *Virology* 1966;28(3):355–62.
- [11] Ohkuma S, Poole B. Fluorescence probe measurement of the intralysosomal pH in living cells and the perturbation of pH by various agents. *Proc Natl Acad Sci Unit States Am* 1978 Jul 1;75(7): 3327 LP – 3331.
- [12] Glaumann H, Ahlberg J. Comparison of different autophagic vacuoles with regard to ultrastructure, enzymatic composition, and degradation capacity—formation of crinosomes. *Exp Mol Pathol* 1987;47(3):346–62.
- [13] Savarino A, Boelaert JR, Cassone A, Majori G, Cauda R. Effects of chloroquine on viral infections: an old drug against today's diseases? *Lancet Infect Dis* 2003 Nov;3(11):722–7.
- [14] Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, Somasundaran M, Sullivan JL, Luzuriaga K, Greenough TC, Choe H. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* 2003 Nov;426(6965): 450–4.
- [15] Keyaerts E, Vijgen L, Maes P, Neyts J, Van Ranst M. In vitro inhibition of severe acute respiratory syndrome coronavirus by chloroquine. *Biochem Biophys Res Commun* 2004;323(1):264–8.
- [16] Vincent MJ, Bergeron E, Benjannet S, Erickson BR, Rollin PE, Ksiazek TG, Seidah NG, Nichol ST. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. *Virology* 2005 Dec;2(1): 1–0.
- [17] Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M, Shi Z, Hu Z, Zhong W, Xiao G. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Res* 2020 Mar;30(3):269–71.
- [18] Fantini J, Di Scala C, Chahinian H, Yahi N. Structural and molecular modelling studies reveal a new mechanism of action of chloroquine and hydroxychloroquine against SARS-CoV-2 infection. *Int J Antimicrob Agents* 2020;55(5):105960.
- [19] Liu J, Cao R, Xu M, Wang X, Zhang H, Hu H, Li Y, Hu Z, Zhong W, Wang M. Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitro. *Cell Discov* 2020 Mar 18;6(1):1–4.
- [20] Touret F, Gilles M, Barral K, Nougairède A, van Helden J, Decroly E, de Lamballerie X, Coutard B. In vitro screening of a FDA approved chemical library reveals potential inhibitors of SARS-CoV-2 replication. *Sci Rep* 2020;10(1):13093.
- [21] Arshad U, Pertinez H, Box H, Tatham L, Rajoli RK, Curley P, Neary M, Sharp J, Liptrout NJ, Valentijn A, David C. Prioritization of anti-SARS-CoV-2 drug repurposing opportunities based on plasma and target site concentrations derived from their established human pharmacokinetics. *Clin Pharmacol Ther* 2020 Oct; 108(4):775–90.
- [22] Fantini J, Chahinian H, Yahi N. Synergistic antiviral effect of hydroxychloroquine and azithromycin in combination against SARS-CoV-2: what molecular dynamics studies of virus-host interactions reveal. *Int J Antimicrob Agents* 2020;56(2): 106020.
- [23] Andreani J, Le Bideau M, Dufloy I, Jardot P, Rolland C, Boxberger M, Wurtz N, Rolain JM, Colson P, La Scola B, Raoult D. In vitro testing of combined hydroxychloroquine and azithromycin on SARS-CoV-2 shows synergistic effect. *Microb Pathog* 2020;145:104228.
- [24] Gordon DE, Jang GM, Bouhaddou M, Xu J, Obernier K, White KM, O'Meara MJ, Rezelj VV, Guo JZ, Swaney DL, Tummino TA. A SARS-CoV-2 protein interaction map reveals targets for drug repurposing. *Nature* 2020 Jul;583(7816):459–68.
- [25] Savarino A, Buonavoglia C, Norelli S, Trani L Di, Cassone A. Potential therapies for coronaviruses. *Expert Opin Ther Pat* 2006 Aug 31;16(9):1269–88.
- [26] de Salud del Perú Ministerio. Resolución ministerial No 139-2020-MINSA. Ministerio de Salud del Perú. 2020 Mar. Available from, <https://cdn.www.gob.pe/uploads/document/file/574295/resolucion-ministerial-139-2020-MINSA.PDF>.
- [27] McCullough PA, Kelly RJ, Ruocco G, Lerma E, Tumlin J, Wheeler KR, Katz N, Lepor NE, Vijay K, Carter H, Singh B. Pathophysiological basis and rationale for early outpatient treatment of SARS-CoV-2 (COVID-19) infection. *Am J Med* 2021 Jan 1;134(1):16–22.
- [28] Million M, Lagier JC, Gautret P, Colson P, Fournier PE, Amrane S, Hocquart M, Mailhe M, Esteves-Vieira V, Doudier B, Aubry C. Early treatment of COVID-19 patients with hydroxychloroquine and azithromycin: a retrospective analysis of 1061 cases in Marseille, France. *Trav Med Infect Dis* 2020;35:101738.
- [29] Worldometer. COVID-19 coronavirus pandemic [Internet]. 2020 Sept. Available from, <https://www.worldometers.info/coronavirus/>.
- [30] de Salud del Perú Ministerio. Sala situacional COVID-19 Perú [Internet]. Ministerio de Salud del Perú; 2020. Available from: https://covid19.minsa.gob.pe/sala_situacional.asp.
- [31] Pedersen SF, Ho Y-C. SARS-CoV-2: a storm is raging. *J Clin Invest* 2020 May;130(5):2202–5.
- [32] Prodromos C, Rumschlag T. Hydroxychloroquine is effective, and consistently so when provided early, for COVID-19: a systematic review. *New Microbes New Infect* 2020;38:100776.
- [33] Hiba V, Chowdhury M, Levi-Vinograd I, Rubinovitch B, Leibovici L, Paul M. Benefit of early treatment with oseltamivir in hospitalized patients with documented 2009 influenza A (H1N1): retrospective cohort study. *J Antimicrob Chemother* 2011 May 1;66(5):1150–5.
- [34] Rodríguez A, Díaz E, Martín-Loeches I, Sandiumenge A, Canadell L, Díaz JJ, Figueira JC, Marques A, Alvarez-Lerma F, Vallés J, Baladrín B. Impact of early oseltamivir treatment on outcome in critically ill patients with 2009 pandemic influenza A. *J Antimicrob Chemother* 2011 May 1;66(5):1140–9.
- [35] Zarychanski R, Stuart TL, Kumar A, Doucette S, Elliott L, Kettner J, Plummer F. Correlates of severe disease in patients with 2009 pandemic influenza (H1N1) virus infection. *Can Med Assoc J* 2010 Feb 23;182(3):257–64.
- [36] Guisado-Vasco P, Valderas-Ortega S, Carralón-González MM, Roda-Santacruz A, González-Cortijo L, Sotres-Fernández G, Martí-Ballesteros EM, Luque-Pinilla JM, Almagro-Casado E, La Coma-Lanusa FJ, Barrena-Puertas R. Clinical characteristics and outcomes among hospitalized adults with severe COVID-19 admitted to a tertiary medical center and receiving antiviral, antimalarials, glucocorticoids, or immunomodulation with tocilizumab or cyclosporine: a retrospective observational study (COQUIMA cohort). *EclinicalMedicine* 2020 Nov 1;28: 100591.
- [37] Derwand R, Scholz M, Zelenko V. COVID-19 outpatients – early risk-stratified treatment with zinc plus low dose hydroxychloroquine and azithromycin: a retrospective case series study. *Int J Antimicrob Agents* 2020;56(6):106214.
- [38] Heras E, Garibaldi P, Boix M, Valero O, Castillo J, Curbelo Y, Gonzalez E, Mendoza O, Anglada M, Miralles JC, Llull P. COVID-19 mortality risk factors in older people in a long-term care center. *Eur Geriatr Med* 2021 Jun;12(3):601–7.
- [39] Chen L, Zhang Z.Y., Fu J.G., Feng Z.P., Zhang S.Z., Han Q.Y., et al. Efficacy and safety of chloroquine or hydroxychloroquine in moderate type of COVID-19: a prospective open-label randomized controlled study. *MedRxiv* 2020.06.19.20136093. doi:10.1101/2020.06.19.20136093.
- [40] Guérin V, Lévy P, Thomas JL, Lardenois T, Lacrosse P, Sarrazin E, Regensberg de Andreis N, Wonner M. Azithromycin and hydroxychloroquine accelerate recovery of outpatients with mild/moderate. *Asian J Med Heal* 2020;18(April):45–55.
- [41] Su Y, Ling Y, Ma Y, Tao L, Miao Q, Shi Q, Pan J, Lu H, Hu B. Efficacy of early hydroxychloroquine treatment in preventing COVID-19 pneumonia aggravation, the experience from Shanghai, China. *Biosci trends* 2020 Dec 31;14(6):408–14.
- [42] Mokhtari M, Mohraz M, Gouya MM, Tabar HN, Tabrizi JS, Tayeri K, Aghamohamadi S, Rajabpoor Z, Karami M, Raeisi A, Rahmani H. Clinical outcomes of patients with mild COVID-19 following treatment with hydroxychloroquine in an outpatient setting. *Int Immunopharm* 2021 Jul 1;96:107636.
- [43] Ip A, Ahn J, Zhou Y, Goy AH, Hansen E, Pecora AL, Sinclair BA, Bednarz U, Marafelias M, Sawczuk IS, Underwood JP. Hydroxychloroquine in the treatment of outpatients with mildly symptomatic COVID-19: a multi-center observational study. *BMC Infect Dis* 2021 Dec;21(1):1–2.
- [44] Fonseca SN, de Queiroz Sousa A, Wolkoff AG, Moreira MS, Pinto BC, Takeda CF, Rebouças E, Abdon AP, Nascimento AL, Risch HA. Risk of hospitalization for COVID-19 outpatients treated with various drug regimens in Brazil: comparative analysis. *Trav Med Infect Dis* 2020 Nov 1;38:101906.
- [45] Sulaiman T, Mohana A., Alawdah L, Mahmoud N., Hassanein M., Wani T., et al. The effect of early hydroxychloroquine-based therapy in COVID-19 patients in ambulatory care settings: a nationwide prospective cohort study. *medRxiv* 2020.09.09.20184143. doi:10.1101/2020.09.09.20184143.
- [46] Padhy BM, Mohanty RR, Das S, Meher BR. Therapeutic potential of ivermectin as add on treatment in COVID 19: a systematic review and meta-analysis: ivermectin in COVID-19: a meta-analysis. *J Pharm Pharmacol* 2020 Nov 23;23:462–9.
- [47] Tardif JC, Bouabdallaoui N, L'Allier PL, Gaudet D, Shah B, Pillinger MH, Lopez-Sendon J, da Luz P, Verret L, Audet S, Dupuis J. Colchicine for community-treated patients with COVID-19 (COLCORONA): a phase 3, randomised, double-blinded, adaptive, placebo-controlled, multicentre trial. *Lancet Respir Med* 2021 Aug;9(8): 924–32.
- [48] Lenze EJ, Mattar C, Zorunski CF, Stevens A, Schweiger J, Nicol GE, Miller JP, Yang L, Yingling M, Avidan MS, Reiersen AM. Fluvoxamine vs placebo and clinical deterioration in outpatients with symptomatic COVID-19: a randomized clinical trial. *J Am Med Assoc* 2020 Dec 8;324(22):2292–300.
- [49] Chananan N, Palmo T, Sharma K, Kumar R, Graham BB, Pasha Q. Sex-derived attributes contributing to SARS-CoV-2 mortality. *Am J Physiol Endocrinol Metab* 2020 Sep;319(3):E562–7.
- [50] Scotland RS, Stables MJ, Madalli S, Watson P, Gilroy DW. Sex differences in resident immune cell phenotype underlie more efficient acute inflammatory responses in female mice. *Blood* 2011 Nov;118(22):5918–27.
- [51] Ghosh S, Klein RS. Sex drives dimorphic immune responses to viral infections. *J Immunol* 2017 Mar 1;198(5):1782–90.
- [52] Wei X, Xiao YT, Wang J, Chen R, Zhang W, Yang Y, Lv D, Qin C, Gu D, Zhang B, Chen W. Sex differences in severity and mortality among patients with COVID-19: evidence from pooled literature analysis and insights from integrated bioinformatic analysis. *arXiv preprint arXiv* 2003:13547. 2020 Mar.
- [53] Conrad CH, Brooks WW, Hayes JA, Sen S, Robinson KG, Bing OH. Myocardial fibrosis and stiffness with hypertrophy and heart failure in the spontaneously hypertensive rat. *Circulation* 1995 Jan 1;91(1):161–70.

- [54] Stelzig KE, Canepa-Escaro F, Schilero M, Berdnikovs S, Prakash YS, Chiarella SE. Estrogen regulates the expression of SARS-CoV-2 receptor ACE2 in differentiated airway epithelial cells. *Am J Physiol Lung Cell Mol Physiol* 2020 Jun;318(6):L1280–1.
- [55] Gkogkou E, Barnasas G, Vougas K, Trougakos IP. Expression profiling meta-analysis of ACE2 and TMPRSS2, the putative anti-inflammatory receptor and priming protease of SARS-CoV-2 in human cells, and identification of putative modulators. *Redox Biol* 2020 Sep 1;36:101615.
- [56] Lin B, Ferguson C, White JT, Wang S, Vessella R, True LD, Hood L, Nelson PS. Prostate-localized and androgen-regulated expression of the membrane-bound serine protease TMPRSS2. *Cancer Res* 1999 Sep 1;59(17):4180–4.
- [57] Meftahi GH, Jangravi Z, Sahraei H, Bahari Z. The possible pathophysiology mechanism of cytokine storm in elderly adults with COVID-19 infection: the contribution of “inflamm-aging”. *Inflamm Res* 2020 Sep;69(9):825–39.
- [58] Yang J, Zheng Y, Gou X, Pu K, Chen Z, Guo Q, Ji R, Wang H, Wang Y, Zhou Y. Prevalence of comorbidities and its effects in patients infected with SARS-CoV-2: a systematic review and meta-analysis. *Int J Infect Dis* 2020 May 1;94:91–5.
- [59] Ritter A, Kreis N-N, Louwen F, Yuan J. Obesity and COVID-19: molecular mechanisms linking both pandemics. *Int J Mol Sci* 2020 Aug;21(16).
- [60] Nandy K, Salunke A, Pathak SK, Pandey A, Doctor C, Puj K, et al. Coronavirus disease (COVID-19): a systematic review and meta-analysis to evaluate the impact of various comorbidities on serious events. *Diabetes Metab Syndr* 2020;14(5):1017–25.
- [61] Callender LA, Curran M, Bates SM, Mairesse M, Weigandt J, Betts CJ. The impact of pre-existing comorbidities and therapeutic interventions on COVID-19. *Front Immunol* 2020;11:1991.
- [62] China National Health Commission. Chinese clinical guidance for COVID-19 pneumonia diagnosis and treatment. seventh ed. China National Health Commission; 2020 Mar Available from: <http://kjfy.meetingchina.org/msite/news/show/cn/3337.html>.
- [63] de Salud del Perú Ministerio. Directiva Sanitaria N° 135-MINSA/CDC-2021. Directiva sanitaria para la vigilancia epidemiológica de la enfermedad por coronavirus (COVID-19) en el Perú. Ministerio de Salud del Perú. 2020 July. Available from, <https://cdn.www.gob.pe/uploads/document/file/2024343/Directiva%20Sanitaria%20N%C2%B0%20135-MINSA/CDC-2021.pdf>.
- [64] Xie Y, Wang Z, Liao H, Marley G, Wu D, Tang W. Epidemiologic, clinical, and laboratory findings of the COVID-19 in the current pandemic: systematic review and meta-analysis. *BMC Infect Dis* 2020 Aug;20(1):640.
- [65] Fang Y, Zhang H, Xie J, Lin M, Ying L, Pang P, Ji W. Sensitivity of chest CT for COVID-19: Comparison to RT-PCR. *Radiology* 2020 Aug;296(2):E115–7.
- [66] Guo C-C, Mi J-Q, Nie H. Seropositivity rate and diagnostic accuracy of serological tests in 2019-nCoV cases: a pooled analysis of individual studies. *Eur Rev Med Pharmacol Sci* 2020 Oct;24(19):10208–18.
- [67] de Salud del Perú Ministerio. Prevención, Diagnóstico y Tratamiento de personas afectadas por COVID-19 en el Perú. Resolución Ministerial N° 270-2020-MINSA. Ministerio de Salud del Perú. 2020 May. Available from, <https://www.gob.pe/institucion/minsa/normas-legales/563764-270-2020-minsa>.
- [68] Sociedad Peruana de Medicina Interna. COMUNICADO CONJUNTO SPMI, SPN, SOPEMI, SOPEHE, SPEIT SOBRE USO DE MEDICAMENTOS PARA EL COVID-19 A LA OPINIÓN PÚBLICA. 2020 Jun. Available from: <http://www.medicinainterna.net.pe/?q=node/493>.
- [69] Chirinos JA, Corrales-Medina VF, Heresi-Dávila G, Hernandez AV, Málaga G, Mallea JM, Miranda JJ, Morey OO, Rodríguez-Mori JE, Salinas-Gamero JE, Serpa-Alvarez J. Sobre las recomendaciones del Ministerio de Salud para el tratamiento farmacológico de la COVID-19 en el Perú. *Acta Méd Peru* 2020 Apr;37(2):231–5.
- [70] Huerta E. El manejo ambulatorio del COVID-19. *Diario El Comercio*. 2020 Oct. available from: <https://elcomercio.pe/tecnologia/ciencias/elmer-huerta-coronavirus-salud-hidroxycoloroquina-ivermectina-el-manejo-ambulatorio-del-covid-19-noticia>.