Clinical and microbiological effect of a combination of hydroxychloroquine and azithromycin in 80 COVID-19 patients with at least a six-day follow up: an observational study

Running title: Hydroxychloroquine-Azithromycin and COVID-19

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Abstract

We need an effective treatment to cure COVID-19 patients and to decrease the virus carriage duration. In 80 in-patients receiving a combination of hydroxychloroquine and azithromycin we noted a clinical improvement in all but one 86 year-old patient who died, and one 74 year-old patient still in intensive care unit. A rapid fall of nasopharyngeal viral load tested by qPCR was noted, with 83% negative at Day7, and 93% at Day8. Virus cultures from patient respiratory samples were negative in 97.5% patients at Day5. This allowed patients to rapidly de discharge from highly contagious wards with a mean length of stay of five days. We believe other teams should urgently evaluate this cost-effective therapeutic strategy, to both avoid the spread of the disease and treat patients as soon as possible before severe respiratory irreversible complications take hold.

Keywords

COVID-19; SARS-CoV-2; hydroxychloroquine; aithromycin; PCR; culture

Introduction

In late December 2019, an outbreak of an emerging disease (COVID-19) due to a novel coronavirus (named SARS-CoV-2 latter) began in Wuhan, China and quickly spread in a substantial number of countries (*1;2*). The epidemic was declared a pandemic by the WHO on 12 March 2020 (*3*). According to a Chinese study, 80% of patients present with mild symptoms and the overall fatality rate is about 2.3%, although this rises to 8.0% in patients between the ages of 70 to 79 years and to 14.8% in those aged 80 years and over (*4*). However, it is highly likely that there are a significant number of asymptomatic carriers in the population, and thus it is probable that the mortality rate has been overestimated. To take the example of the outbreak onboard the Diamond Princess cruise-ship, the fatality rate was 1.4%

(*5*). France is now facing the onslaught of COVID-19 with more than 25,000 cases, as March 26th, 2020 (*5*). Thus, there is a critical and urgent need for an effective treatment in order to cure symptomatic patients but also to decrease the duration of virus carriage and thus limit transmission in the community. Among the candidate drugs to treat COVID-19, repositioning old drugs for use as an antiviral treatment is an interesting strategy, because knowledge about these drugs' safety profile, side effects, posology and drug interactions are already well known (*6;7*).

Three *in vitro* studies have demonstrated that chloroquine phosphate inhibits SARS-CoV-2 (*8;9*) and two have demonstrated that hydroxychloroquine sulfate inhibits SARS-CoV-2 (*8-10*). Other studies have pointed out that drug repurposing may identify approved drugs that could be useful for the treatment of this disease including, notably, chloroquine, hydroxychloroquine and azithromycin, as well as anti-diabetics such as metformin, angiotensin receptor inhibitors such as sartans, or statins such as simvastatin (*11*). In addition, chloroquine has demonstrated its efficacy in Chinese COVID-19 patients in clinical trials by reducing fever, improving CT imaging, and delaying disease progression (*12-14*), leading Chinese experts to recommend chloroquine-based treatment (500 mg twice per day for ten days) as a first line-treatment for mild, moderate and severe cases of COVID-19 (*15*).

In a preliminary clinical trial on a small cohort of COVID-19 patients, we demonstrated that those treated with hydroxychloroquine (600 mg per day, N=20 patients) had a significant reduction in viral carriage at D6-post inclusion, with 70% of patients testing negative for the virus through nasopharyngeal PCR, compared to untreated controls (N=16) with only 12.5% patients testing negative using PCR at D6-post inclusion (16). In addition, of the twenty patients who were treated with hydroxychloroquine, six received azithromycin for five days (for the purposes of preventing bacterial super-infection) and all (100%) were virologically cured at D6-post inclusion, compared to 57.1% of the remaining 14 patients (*16*). By contrast,

a Chinese study conducted in 30 COVID-19 patients showed no significant differences between patients treated with 400 mg per day during five days (N=15) and controls (N=15) regarding pharyngeal carriage of viral RNA at day7, however, patients received multiple additional treatments including antivirals (*17*).

A recent Chinese survey revealed that the median duration of viral shedding was 20.0 days (IQR 17.0–24.0) in survivors, but SARS-CoV-2 was detectable until death in non-survivors. The shortest observed duration of viral shedding among survivors was eight days, whereas the longest was 37 days (*18*). Therefore, a treatment enabling the viral carriage to be cleared and COVID-patients to be clinically cured at an early stage would help limit the transmission of the virus.

In this report we describe our results in patients treated with hydroxychloroquine in combination with azithromycin over a period of at least three days, with three main endpoints: i) clinical outcome ii) contagiousness as assessed by PCR and culture and iii) long of stay in infectious disease (ID) unit.

Methods

Study design and participants

The study was conducted at the University Hospital Institute *Méditerranée Infection* in Marseille, France. Patients with PCR-documented SARS-CoV-2 RNA from a nasopharyngeal sample were admitted to our infectious diseases (ID) ward. It should be noted that six patients enrolled at our institute who were described in our first paper, with a six-day follow-up (N=6) (16), were also included in the present study, with a longer follow-up.

Clinical classification and clinical follow-up

Upon admission, patients were grouped into two categories: i) those with an upper respiratory tract infection (URTI) presenting with rhinitis and/or pharyngitis, and/or isolated low-grade fever and myalgia, and ii) those with lower respiratory tract infections (LRTI) presenting with symptoms of pneumonia or bronchitis. The time between the onset of symptoms and admission, and the time between the onset of symptoms and treatment was documented. Risk factors for severe COVID-19, including older age, cancer, cardiovascular disease, hypertension, and diabetes (*4*), as well as chronic obstructive pulmonary disease, obesity and any immunosuppressive treatments were documented.

The national early warning score (NEWS) for COVID-19 patients, was collected upon ward admission and during follow up. The NEWS score was calculated based on the following parameters: age, respiratory rate, oxygen saturation, temperature, systolic blood pressure, pulse rate and level of consciousness (*19*). We defined three risk categories for clinical deterioration: low score (NEWS 0-4), medium score (NEWS 5-6), and high score (NEWS≥7) for COVID patients.

The need for oxygen therapy, transfer to the intensive care unit (ICU), death, and length of stay in the ID ward were documented.

Chest computed tomography

Patients systematically underwent an unenhanced chest low-dose computed tomography (LDCT) upon admission or soon after, using a single CT machine (Revolution EVO - GE Healthcare, WI, USA). All images were analysed by experienced chest radiologists, then classified as compatible or not compatible with pneumonia. Images were considered to be compatible in the presence of peripheral multifocal ground-glass opacities with or without reticulations, or in the presence of alveolar consolidation or crazy paving pattern.

PCR assay

Nasopharyngeal swabs were collected on a daily basis until discharge with some exceptions. Some discharged patients were also sampled during follow-up at our out-patients department. SARS-CoV-2 RNA was assessed by real-time reverse transcription-PCR using a hydrolysis probe-based system that targets the gene encoding the envelope (E) protein (*20*), as previously described (*20;21*). Negative results for viral RNA detection were defined as those with a cycle threshold (Ct) value ≥35.

Culture

Cultures were attempted in a random selection of patients. A 500 µL aliquot of the liquid collected from the nasopharyngeal swab were passed through 0.22-µm pore sized centrifugal filter (Merck millipore, Darmstadt, Germany), and were then inoculated in wells on 96-well culture microplates, of which four wells contained Vero E6 cells (ATCC CRL-1586) in Minimum Essential Medium culture medium with 4% foetal calf serum and 1% glutamine. After centrifugation at 4,000 g, microplates were incubated at 37°C. Plates were observed every day for evidence of a cytopathogenic effect. The presumptive detection of the virus in supernatant was performed using SU5000 SEM (Hitachi) then confirmed by specific RT-PCR.

COVID treatment

Patients with no contraindications (Supplementary document 1) were offered a combination of 200 mg of oral hydroxychloroquine sulfate, three times per day for ten days combined with azithromycin (500mg on D1 followed by 250mg per day for the next four days). For patients with pneumonia and NEWS score≥5, a broad spectrum antibiotic (ceftriaxone) was added to hydroxychloroquine and azithromycin. Twelve-lead electrocardiograms (ECG) were performed on each patient before treatment and two days after treatment began. All ECGs were reviewed by senior cardiologists. The treatment was either not started or discontinued

when the QTc (Bazett's formula) was > 500 ms and the risk-benefit ratio was estimated to be between 460 and 500 ms. The treatment was not started when the ECG showed patterns suggesting a channelopathy and the risk-benefit ratio was discussed when it showed other significant abnormalities (i.e., pathological Q waves, left ventricular hypertrophy, left bundle branch block). In addition, any drug potentially prolonging the QT interval was discontinued during treatment. Symptomatic treatments, including oxygen, were added when needed. An ionogram and verification of serum potassium levels in particular, was systematically performed upon admission. When needed, standard blood chemistry was checked.

Criteria for discharge

Criteria for discharge changed over the course of the study. Initially, patients with two successive negative nasopharyngeal samples resulting from PCR assay (CT value ≥35) were discharged. From 18 March, patients with a single nasopharyngeal sample with a PCR CT value ≥34 were discharged to their homes or transferred to other units for continuing treatment, Ultimately, because of a crucial need to admit new, untreated inpatients, inpatients already receiving treatment with a PCR CT value <34, with good clinical outcome and good adherence to treatment were also discharged. When possible, further follow-up was continued in other units or through out-patient consultations.

Criteria for contagiousness

Patients with a PCR value of <34 were considered presumably contagious based on results of a study showing that culture are negative under this condition (https://www.mediterranee-infection.com/pre-prints-ihu/). Patients with positive culture were considered contagious.

Outcome

The primary endpoints were i) an aggressive clinical course requiring oxygen therapy or transfer to the ICU after at least three days of treatment, ii) contagiousness as assessed by PCR and culture, and iii) length of stay in the ID ward.

Statistics

Variation of culture positivity rate was assessed statistically as the proportion of variance explained by Ct value and considered adequately fitted if the coefficient of determination [R2 statistic] was >50%.

Ethics Statement

The protocol was approved by the ethical committee of the University Hospital Institute *Méditerranée Infection* (N°: 2020-01). The study was performed according to the good clinical practices recommended by the Declaration of Helsinki and its amendments.

Results

Demographics and patient status at admission (Tables 1 and 2)

A total of 80 patients with confirmed COVID-19 were hospitalised at the Méditerranée Infection University Hospital Institute (N=77) and at a temporary COVID-19 unit (N=3) with dates of entry from 3–21 March 2020. All patients who received treatment with hydroxychloroquine and azithromycin (*16*) for at least three days and who were followed-up for at least six days were included in this analysis. The median age of patients was 52 years (ranging from 18 to 88 years) with a M/F sex ratio of 1.1. 57.5% of these patients had at least one chronic condition known to be a risk factor for the severe form of COVID-19 with hypertension, diabetes and chronic respiratory disease being the most frequent. The time

between the onset of symptoms and hospitalisation was on average five days, with the longest time being 17 days. 53.8% of patients presented with LRTI symptoms and 41.2% with URTI symptoms. Only 15% of patients were febrile. Four patients were asymptomatic carriers. The majority of patients had a low NEWS score (92%) and 53.8% of patients had LDCT compatible with pneumonia. The mean PCR Ct value was 23.4.

Hydroxychloroquine and azithromycin combined treatment (Table 2 and 3)

The mean time between the onset of symptoms and the initiation of treatment was 4.9 days and most patients were treated on the day of admission or on the day after (93.7%). A total of 79/80 patients received treatment on a daily basis throughout the whole study period, which lasted a maximum of ten days. In one patient, the treatment had to be stopped on Day4 because, although it was well tolerated there was a potential risk of interaction with another drug. Adverse events were rare and minor.

Clinical course (Table 3)

The majority (65/80, 81.3%) of patients had favourable outcome and were discharged from our unit at the time of writing with low NEWS scores (61/65, 93.8%). Only 15% required oxygen therapy. Three patients were transferred to the ICU, of whom two improved and were then returned to the ID ward. One 74 year-old patient was still in ICU at the time of writing. Finally, one 86 year-old patient who was not transferred to the ICU, died in the ID ward (Supplementary Table 1).

Contagiousness as assessed by PCR Ct value and culture (Figures 1 and 2)

A rapid fall of nasopharyngeal viral load tested by qPCR was noted, with 83% negative at Day7, and 93% at Day8. The number of patients presumably contagious (with a PCR Ct value <34) steadily decreased overtime and reached zero on Day12 (Figure 1). A marked decrease

was observed after six days of treatment. After ten days, two patients only were still presumably contagious with Ct values of 32 and 29 respectively. The proportion of patients with a Ct value >34 significantly decreased overtime ($R^2 = 0.9$). Virus cultures from patient respiratory samples were negative in 97.5% patients at Day5. The number of contagious patients (with positive culture) early decreased after three days of treatment (Figure 2). After five days of treatment, two patients only were contagious. On Day8 post-treatment only one of these two patients was contagious and ceased to be contagious on Day9. The proportion of negative culture significantly decreased overtime ($R^2 = 0.8$).

Length of stay in the ID ward

Of the 65 patients who were discharged from the ID ward during the study period, the mean time from initiation to discharge was 4.1 days with a mean length of stay of 4.6 days.

Discussion

COVID-19 poses two major challenges to physicians.

The first is the therapeutic management of patients. In this context, it is necessary to avoid a negative evolution of pneumonia, which usually occurs around the tenth day and may result in acute respiratory distress syndrome, the prognosis of which, in particular in the elderly, is always poor, whatever the cause. The primary therapeutic objective is therefore to treat people who have moderate or severe infections at an early enough stage to avoid progression to a serious and irreversible condition. By administering hydroxychloroquine combined with azithromycin, we were able to observe an improvement in all cases, except in one patient who arrived with an advanced form, who was over the age of86, and in whom the evolution was irreversible. For all other patients in this cohort of 80 people, the combination of hydroxychloroquine and azithromycin resulted in a clinical improvement that appeared

significant when compared to the natural evolution in patients with a definite outcome, as described in the literature. In a cohort of 191 Chinese inpatients, of whom 95% received antibiotics and 21% received an association of lopinavir and ritonavir, the median duration of fever was 12 days and that of cough 19 days in survivors, with a 28% case-fatality rate (*18*). The favourable evolution of our patients under hydroxychloroquine and azithromycin was associated with a relatively rapid decrease in viral RNA load as assessed by PCR, which was even more rapid when assessed by culture. These data are important to compare with that of the literature which shows that the viral RNA load can remain high for about three weeks in most patients in the absence of specific treatment (*18;22*) with extreme cases lasting for more than a month. A study conducted in 76 Chinese COVID-19 in patients showed that high viral RNA load is associated with the severity of the disease (*23*). Furthermore, in a study conducted on a small group of 16 Chinese COVID-19 inpatients, viral RNA was positively detected in 50% of them, after resolution of symptoms for a median duration of 2.5 days and a maximum of eight days (*24*). Therefore, the rapid decrease in viral RNA load is one element suggesting the effectiveness of this treatment. Furthermore, to our knowledge, the measurement of viral culture during treatment was also evaluated for the first time. The fall in culture positivity from the 48th day is spectacular, although, in a relatively small number of cases, some people maintain a positive culture.

The second challenge is the rapid spread of the disease in the population through contagious individuals. The elimination of viral carriage in the human reservoir of the virus has recently been recognised as a priority (*25*). To this end, the rapid negativation of cultures from patients' respiratory samples under treatment with hydroxychloroquine plus azithromycin shows the effectiveness of this association. In addition, and in parallel to this study, we evaluated *in vitro* the association of hydroxychloroquine and azithromycin on SARS-CoV-2 infected cells, and showed that there was a considerable synergy of these two products when

they were used at doses which mimic the concentrations likely to be obtained in humans (https://www.mediterranee-infection.com/pre-prints-ihu2/). Thus, in addition to its direct therapeutic role, this association can play a role in controlling the disease epidemic by limiting the duration of virus shedding, which can last for several weeks in the absence of specific treatment. In our Institute, which contains 75 individual rooms for treating highly contagious patients, we currently have a turnover rate of 1/3 which allows us to receive a large number of these contagious patients with early discharge.

Chloroquine and hydroxychloroquine are extremely well-known drugs which have already been prescribed to billions of people. Because of anecdotical reports of heart complications with such drugs in patients with underlying conditions, it would be useful to perform an ECG before or at the very beginning of the treatment (*26*). This problem is solved by hospitalising patients at risk with multiples pathogens in continuing care units with ECG monitoring allowing for the early detection and treatment of these rare but possible cardiac side-effects.

Azithromycin is the drug that has been the most widely prescribed against respiratory infections and a recent (2010) study showed that one in eight American out-patients, has been prescribed azithromycin (*27*). Indeed, there have probably been more than a billion azithromycin prescriptions around the world since it was first discovered. The toxicity of each of these two drugs does not, therefore, pose a major problem. Their possible toxicity in combination has been suggested in a few anecdotal reports but, to our knowledge, has never been demonstrated.

In conclusion, we confirm the efficacy of hydroxychloroquine associated with azithromycin in the treatment of COVID-19 and its potential effectiveness in the early impairment of contagiousness. Given the urgent therapeutic need to manage this disease with effective and safe drugs and given the negligible cost of both hydroxychloroquine and azithromycin, we believe that other teams should urgently evaluate this therapeutic strategy both to avoid the

spread of the disease and to treat patients before severe irreversible respiratory complications take hold.

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Ref Type: Internet Communication

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Legends to figures

Figure 1. SARS-CoV-2 PCR from nasopharyngeal samples overtime. Black bars: number of patients with available results, grey bars: number of patients with PCR Ct value <34, solid line: percentage of patients with PCR Ct value <34, dashed line: polynomial regression curve.

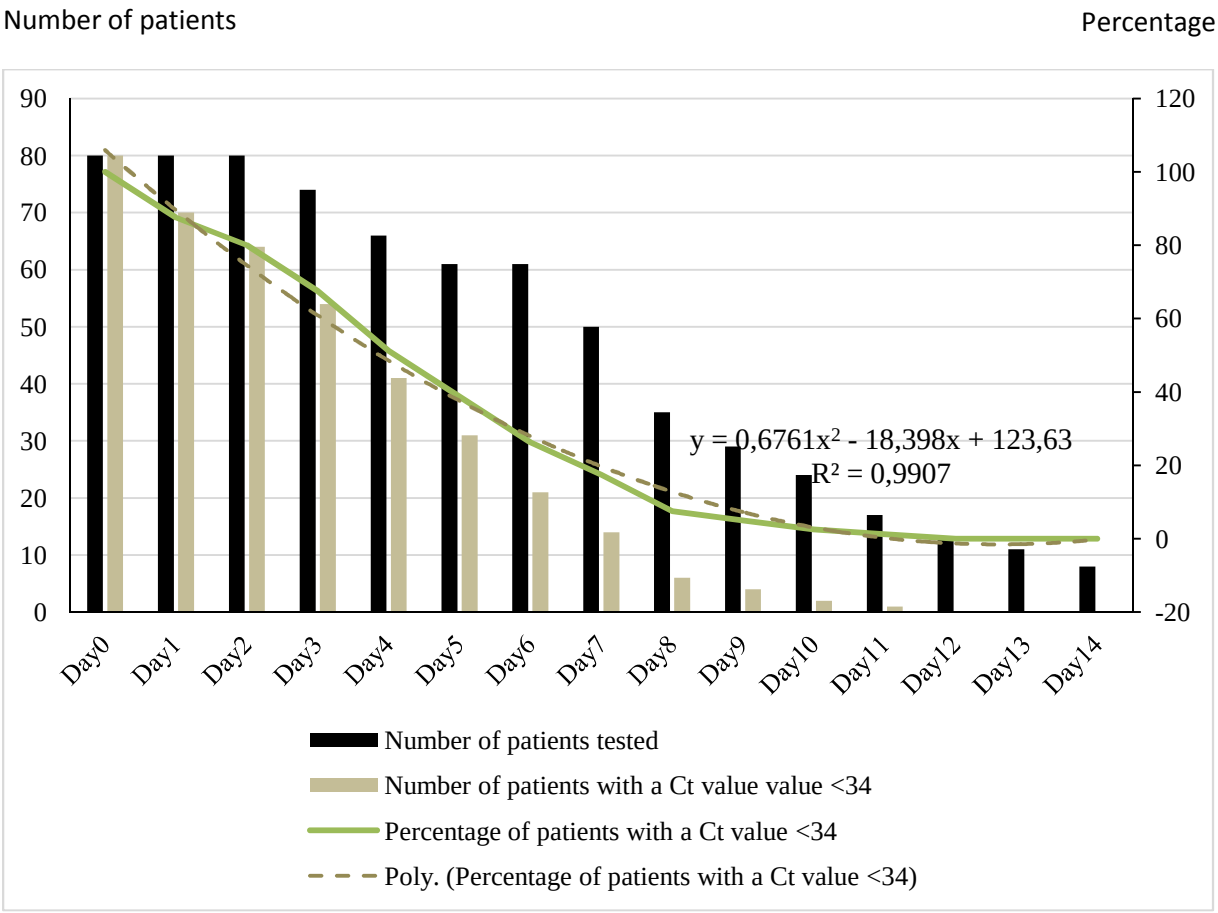

Figure 2. SARS-CoV-2 culture from nasopharyngeal samples overtime. Black bars: number of patients with available results, grey bars: number of patients with positive culture, solid line: percentage of patients with a positive culture, dashed line: polynomial regression curve.

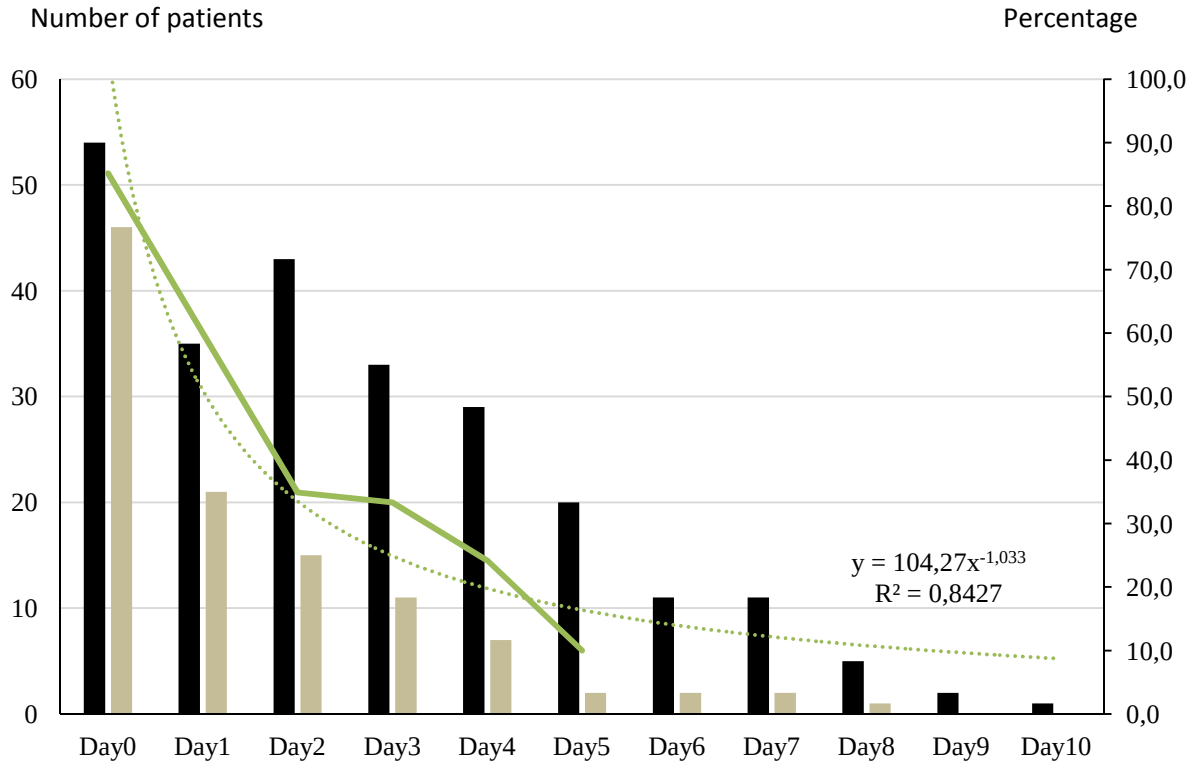

Table 1: Sociodemographic characteristics and chronic conditions

	n	%
Age (years)		
Median	52.5	
Interquartile	42	62
Min - Max	20	88
[20-45[	24	30.0
[45-50)	12	15.0
[50-60)	21	26.2
[60-70)	13	16.2
[70-80)	5	6.3
≥80	5	6.3
Male	42	52.5
Chronic conditions		
Cancer	5	6.3
Diabetes	9	11.2
Coronary artery disease	6	7.5
Hypertension	13	16.3
Chronic respiratory diseases	8	10.0
Obesity	4	5.0
Immunosuppressive treatment	4	5.0
Non-steroid anti-inflammatory treatment	2	2.5

Table 2: Clinical status at admission

	n	%
Time between onset of symptoms and hospitalisation		
Mean ± SD	4.8 ± 5,6	
Min - Max	1	17
Clinical classification		
Asymptomatic	4	5.0
Upper respiratory tract infection symptoms	33	41.2
Lower respiratory tract infection symptoms	43	53.8
Fever	12	15.0
Temperature in febrile patients		
Mean ± SD	38.6 ± 0.12	
Min - Max	38.5	38.8
Cough	47	58.8
Rhinitis	13	16.3
NEWS score (N = 75, 5 missing data)		
0 – 4 (low)	69	92.0
5 – 6 (medium)	4	5.3
≥ 7 (high)	2	2.7
Pulmonary CT-scanner within 72 hours of admission		
Not performed	16	20.0
Not consistent with pneumonia	21	26.2
Consistent with pneumonia	43	53.8
Viral load at inclusion (Ct)		

Mean ± SD	23.6 ± 4.3	
Min - Max	14	33
Time between onset of symptoms and treatment		
Mean ± SD	4.9 ± 3.6	
Min - Max	1	17
Treatment started on Day0	49	61.2
Treatment started on Day1	26	32.5
Treatment started on Day2	3	3.8
Treatment started on Day3	2	2.5

Table 3: Treatment and outcome

	n	%
Oxygen therapy	12	15.0
Transfer to intensive care unit	3	3.8
Death	1	1.2
Discharged	65	81.2
Currently hospitalised		
ICU	1	1.2
Infectious disease ward	13	16.2
Other antibiotic intake	18	22.5
Possible adverse events		
Nausea or vomiting	2	2.5
Diarrhoea	4	5.0
Blurred vision*	1	1.2
Time from treatment initiation to discharge (n = 65)		
Mean ± SD	4.1 ± 2.2	
Min - Max	1	10

Length of stay in infectious diseases ward (n = 65)		
Mean ± SD	4.6 ± 2.1	
Min - Max	1	11
NEWS score in discharged patients (N = 65)		
0 – 4 (low)	61	93.8
5 – 6 (medium)	4	6.2
≥ 7 (high)	0	-

*after five days of treatment

Supplementary document 1. Hydroxycloroquine and azithromycin contra-indications

Hydroxychloroquine

Absolute contraindications to hydroxychloroquine include known hypersensitivity to hydroxychloroquine or chloroquine, amino-4 quinolines, amodiaquine, mefloquine, glafenine, floctafenine, antrafenine, retinopathy, age < 6 years, lactation, patients taking citalopram, escitalopram, hydroxyzine, domperidone, and piperaquine because of increased risk of arrhythmia and torsades de pointes. Relative contraindications or cases in which it is not recommended include cases of hepatic porphyria, hypersensitivity to lactose, abnormalities of galactose metabolism, lactase deficiency, and digestive malabsorption / intolerance syndrome due to the presence of lactose as an excipient.

Azithromycin

Absolute contraindications to azithomycin include known hypersensitivity to azithromycin, erythromycin, chlarithromycin, dirithromycin, josamycin, midecamycin diacetate, roxithromycin, telithromycin, macrolides, ketolides, everolimus, pimecrolimus, sirolimus, temsirolimus, fidaxomicine, peanut oil and soy. Contraindications also include pseudomembranous colitis, anaphylactic shock, severe skin involvement, acute exanthematic pustulosis, DRESS syndrome, severe liver failure, patient taking colchicine, cisapride, dihydroergotamine and ergotamine. Use is not recommended for patients with cholestase, or who are taking bromocriptine, cabergoline, lisuride and pergolide. It is not recommended in cases of hypersensitivity to lactose, abnormality of galactose metabolism, lactase deficiency, and digestive malabsorption / intolerance syndrome due to the presence of lactose as an excipient.

Source: Theriaque: independent-drug database for good use of drugs by health practitioners. Husson MC. Ann Pharm Fr. 2008 Nov-Dec;66(5-6):268-77. 5. http://www.theriaque.org

Supplementary Table 1. Detail of patients who were transferred to the intensive care unit or who died

Age (years)	Sex	Comorbidities	Time between symptom onset and treatment (days)	Ct value at admission	Time between initiation of treatment and transfer to ICU (days)	Reason for ICU transfer	Time between initiation of treatment and PCR Ct value ≥34 (days)	Time in ICU (days)	Outcome
46	M	None	16	29	1	Polypnea 50 cycles/minutes	3	2	Return to infectious diseases ward
54	M	Hypertension, Diabetes	11	22	2	ARDS	Still < 34	8	Return to infectious diseases ward
75	F	None	3	25	8	Hypoxaemia (PaO_2 62mm$Hg_)$ despite non-invasive oxygenation	8	1	Still hospitalised in ICU
86	M	Hypertension, Corticosteroid medication for five days before admission	5	19	7	No ICU transfer	Still < 34	0	Died in infectious diseases ward