Effect of combination therapy of hydroxychloroquine and azithromycin on mortality in COVID-19 patients.

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Abstract

Conflicting evidence regarding the use of hydroxychloroquine and azithromycin for the treatment of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection do exist.

We performed a retrospective single-center cohort study including 377 consecutive patients admitted for pneumonia related to coronavirus disease (COVID-19). Of these 297 were in combination treatment, 17 were on hydroxychloroquine alone and 63 did not receive any of these two drugs because of contraindications. The primary endpoint was in-hospital death.

Mean age was 71.8±13.4 years and 34.2% were women. We recorded 146 deaths: 35 in no treatment, 7 in hydroxychloroquine and 102 in hydroxychloroquine + azithromycin group (log-rank test for Kaplan-Meier curve $p<0.001$).

At multivariable Cox proportional hazard regression analysis, age (hazard ratio [HR] 1.057, 95% confidence interval [CI] 1.035-1.079, $p<0.001$), mechanical ventilation/CPAP (HR 2.726, 95%CI 1.823-4.074, $p<0.001$), C Reactive Protein above the median (HR 2.191, 95%CI 1.479-3.246, $p<0.001$) were directly associated with death, whilst use of hydroxychloroquine + azithromycin (vs. no treatment) (HR 0.265, 95%CI 0.171-0.412, $p<0.001$) was inversely associated.

In this study, we found a reduced in-hospital mortality in patients treated with a combination of hydroxychloroquine and azithromycin after adjustment for comorbidities. A large randomized trial is necessary to confirm these findings.

Keywords. COVID-19, SARS-CoV-2, mortality, hydroxychloroquine, azithromycin.

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is spreading worldwide since December 2019 and still no proven effective therapy has been found. First therapy proposed to treat coronavirus disease 2019 (COVID-19) has been the association of lopinavir-ritonavir, a protease inhibitor approved for HIV infection. However, Cao et al. observed no benefit comparing lopinavir-ritonavir treatment of hospitalized patients with severe COVID-19 [1], and this treatment is currently not recommended. Currently, only Remdesivir has been approved for COVID-19 treatment, as it reduced recovery time by 4 days in 1063 patients randomized to either remdesivir 200 mg loading dose followed by 100 mg daily or placebo for up to 10 days[2] with a similar rate of adverse events between the two groups[3]. However, no effect on in-hospital mortality was found.

Chloroquine and its derivative hydroxychloroquine, based on few preclinical studies, have been also proposed as therapies for COVID-19. A Chinese randomized trial in patients with mild disease showed a significantly shorter recovery time in the group treated with hydroxychloroquine versus the standard of care along with a radiological improvement[4]. Differently, a retrospective study performed in the United States Veterans Health Administration medical centres found an increased mortality associated with the treatment with hydroxychloroquine [5]. Moreover, an observational study has shown no significant association between hydroxychloroquine use and risk of intubation or death [6]. Furthermore, a recent randomized controlled trial has found no differences between patients treated with hydroxychloroquine plus the standard of care versus the standard of care alone in terms of virus elimination [7].

On the basis of a very small non-randomized study, azithromycin has been proposed as possible treatment in association with hydroxychloroquine [8]. Azithromycin, is an antibiotic belonging to the class of macrolides, with some proven efficacy in acute respiratory distress syndrome (ARDS) [9, 10]. It is known to have immunomodulatory properties through the polarization of macrophages towards the reparative state [11] and the reduction in the production of pro-inflammatory cytokines such as IL-8, IL-6, TNF alpha [12] and iNOS expression [13]. Recently two large retrospective studies evaluating in-hospital mortality associated with the use of the combination of hydroxychloroquine and azithromycin (or another macrolides such as clarithromycin), have shown no benefits [14]

Despite the lack of a proven clinical efficacy and some concerns regarding the possible Qt prolongations caused by the association of hydroxychloroquine and azithromycin [15], given the low price and the wide availability, the association of these two drugs has become the most used treatment in patients with moderate-severe COVID-19.

Since the urgent need to find answers to the many questions posed by the fight to SARS-CoV2 infection and some negative evidences regarding the use of hydroxychloroquine, we here propose a retrospective observational study to assess the efficacy of the combination of hydroxychloroquine plus azithromycin for hospitalized patients with medium/severe COVID-19.

Methods

Study design and participants

We conducted this study at the Policlinico of Monza, a highly specialized hospital based in Lombardy which have been designated as one of the centres dedicated to treat COVID-19 patients during the emergency. We have enrolled all consecutive adult patients diagnosed with COVID-19 aged at least 18 years admitted to our hospital from 27th of February 2020 to the 20th of April 2020. The only exclusion criteria applied were the presence of contraindications to hydroxychloroquine or azithromycin (see below).

Patients have been considered enrolled if they had positivity to SARS-CoV-2 RT-PCR testing of oropharyngeal or nasopharyngeal swab specimen (according to WHO guidance); radiographic evidence of pulmonary infiltrates at CT scan and clinical documentation of lower respiratory symptoms, or SpO2 $\leq$ 94% on room air. Specimens from the upper respiratory tract, lower respiratory tract, or both and CT scan were obtained on the first day of hospitalization. The results of this clinical trial are reported in accordance with STROBE guidelines.

Data regarding demographic, clinical, pharmacological, biochemical (including C reactive protein [CRP] and white blood cells), microbiological information have been manually abstracted independently by three investigators from medical records ML, SM, MM. Every discordance has been discussed and adjudicated according to the majority.

Treatments

We have divided our cohort in three groups according to the COVID-19 treatment received: 1) no treatments, 2) hydroxychloroquine alone, 3) combination of hydroxychloroquine and azithromycin. All treatments have been initiated the first day of hospitalization. According to institutional clinical guidelines all patients has been administered hydroxychloroquine and azithromycin except those with a contraindication such as: history of G6PD deficiency, cirrhosis, long QT syndrome, or porphyria of any classification, ECG with QTc interval ≥500 msec, known hypersensitivity to hydroxychloroquine or 4-aminoquinoline derivative or macrolides. Patients with contraindication received hydroxychloroquine alone or no treatment according to clinical judgment. Hydroxychloroquine has been administered at the dose of 200 mg TD (alone or in combination) and azithromycin at the dose of 500 milligrams OD for 10 days. Patients have been closely monitored during treatment. EKG has been performed before and after the treatment and in case of electrolytic imbalances and treatment interruption was considered if QTc >500 ms. Patients with cardiologic comorbidities and risk factors have been monitored with cardiac telemetry. Since hypokalaemia and hypomagnesemia are associated with increased risk of serious arrhythmia, correction of hypokalaemia to a level >4 mEq/l and hypomagnesemia to a level of >2 mg/dl was performed when necessary [16].

Patients have been followed up throughout the hospitalization. The primary endpoint was death.

Statistical analysis

Categorical variables were reported as percentage and compared by the chi-squared test. Continuous variables were expressed as mean ± standard deviation and Student t-test was used to compare means. ANOVA test was used to compare groups. A first descriptive analysis of the study cohort according to different treatments has been performed.

Survival Kaplan-Meier curves were run to investigate the primary endpoint of in-hospital death according to treatment groups and compared by the log-rank test. Univariable and multivariable proportional hazard Cox regression analysis was used to estimate the relative hazard ratio (HR) with 95% confidence interval (95%CI) for each variable. All available variables were entered as covariates in the final model. For the Cox regression analysis, CRP and white blood cells were

dichotomized according the median value. Only p-values below 0.05 were considered statistically significant. All the tests used are two-sided and the analyses were performed using electronic software packages (SPSS-25.0, IBM SPSS Inc. and MedCalc).

Results

Table 1 reports patients' characteristics according to treatment groups. Patients treated with hydroxychloroquine and azithromycin were younger than those treated with hydroxychloroquine alone or untreated, and were more likely to be treated with CPAP (**Table 1**).

Conversely these patients had a lower prevalence of cardiovascular and cerebrovascular disease (**Table 1**). A similar value of CRP and White Blood Cells was present among groups. Length of stay was higher in combination therapy group (**Table 1**).

In-hospital mortality

We recorded 146 deaths: 35 in no treatment, 7 in hydroxychloroquine and 102 in hydroxychloroquine + azithromycin group (log-rank test $p<0.001$, **Figure 1**).

Univariable Cox Regression analysis showed that age, use of CPAP or mechanical ventilation, hypertension, prevalent cardiovascular and cerebrovascular disease, COPD, CRP and white blood cells above the median were associated with death (**Figure 2**).

At multivariable Cox proportional hazard regression analysis (**Table 2**), age (hazard ratio [HR] 1.057, 95% confidence interval [CI] 1.035-1.079, $p<0.001$), mechanical ventilation/CPAP (HR 2.726, 95%CI 1.823-4.074, $p<0.001$), C Reactive Protein above the median (HR 2.191, 95%CI 1.479-3.246, $p<0.001$) were directly associated with death, whilst use of hydroxychloroquine + azithromycin (vs. no treatment) (HR 0.265, 95%CI 0.171-0.412, $p<0.001$) was inversely associated.

No fatal arrhythmias have been observed during treatment.

Discussion

This is the first study which founds a beneficial effect of combination therapy of hydroxychloroquine + azithromycin on in-hospital mortality in COVID-19 patients. This result is in contrast with the study by Rosenberg and reasons accounting for these differences may be several. First our cohort is younger with a lower prevalence of diabetes; furthermore, in the study Rosenberg et al. it is not reported proportion of patients treated with CPAP/mechanical ventilation, which may affect mortality rate. In our study, a similar value of C-reactive protein and white blood cells was found among groups, which were therefore homogenous. Similarly, to other studies [5-7], we did not find a reduction of mortality in patients administered with hydroxychloroquine alone.

The main problem of efficacy of HCQ against SARS-CoV2 is probably linked to drug dosing. Hydroxychloroquine has shown to have *in vitro* activity against SARS-CoV2, but half maximal effective concentration (EC50) needed to explicate an antiviral activity is 20 times higher than concentrations used for malaria treatment [17-19]. A PBPK Model based on *in vitro* and *in vivo* PK data developed by Yao et al. [19] suggested an effective dose of hydroxychloroquine of 400 mg BD for first four treatment days, followed by 200 mg BD for other four days. According to the pharmacokinetic evaluation performed by Perinel et al. [20] in critical patients, a dose of 800 mg OD should be more appropriate to rapidly achieve therapeutic concentrations. In studies reporting non-efficacy of hydroxychloroquine, doses used to treat patients have not being declared [5, 6]. However, it should be pointed out that hydroxychloroquine PBPK models and simulations to predict lung hydroxychloroquine concentrations, have been based exclusive on rat *preclinical in vivo* studies. Furthermore, the PK data has been obtained only with healthy population. Thus, the predictive value of proposed models should be improved with more specific data reflecting hydroxychloroquine disposition in tissues, plasma and immunity cells [21]. Anyway, despite its limitations, PBPK modelling is one of the most valuable approach to estimate tissue specific drug concentration [22].

Because of the risk of arrhythmias, the dosage used in many patients was the one approved in malaria and autoimmune diseases. Similarly, in our study HCQ has been administered with the schedule dose 200 mg TD. With high probability this dose is insufficient to reach an appropriate therapeutic concentration.

Conversely, the combination therapy with azithromycin significantly increases efficacy. This effect may be due to both the early start of treatment and the relative high dose of azithromycin

used, but also to the synergy between azithromycin and HCQ due to the different binding sites. Azithromycin interacts with the ganglioside-binding domain of SARS-CoV-2 spike protein, while HCQ molecules can saturate virus attachment sites on gangliosides in the vicinity of the primary coronavirus receptor ACE-2[23]. According to this model, azithromycin is directed against the virus, whereas hydroxychloroquine is directed against cellular adhesion cofactors. Binding to these two sites can lead to a synergistic antiviral mechanism at the plasmatic membrane level [23]. Furthermore, azithromycin may be responsible for less bacterial complications in these patients, leading to a less severe disease.

In fact, in contrast with other studies [24-27] we have recorded fewer drug-related adverse effects. This is due both to the exclusion of patients with risk factors and to a strict monitoring protocol of adverse effect, included careful prevention of electrolytes imbalances. This is an important point in the management of patients with COVID-19, because the severity of this disease is inherently associated with lower serum concentrations of sodium, potassium and calcium [28]. Despite this apparent safety profile in a strictly monitored cohort in which we excluded patients with contraindications and corrected electrolytes imbalance during in-hospital staying, we cannot exclude that these drugs may have side effects in some high-risk subgroups of patients, such as those with pre-existing cardiovascular disease, or those taking drugs already causing a QT interval prolongation. In these cases, the risk-benefit ratio of using these drugs should be carefully considered.

As far as no effective drugs to reduce mortality in COVID-19 patients has been found, our findings show a beneficial effect of this combination therapy may have clinical implications for the management of these patients. It is also noteworthy that azithromycin and hydroxychloroquine are inexpensive drugs that might be easily available to use on large samples of population with a potential benefit also in low-income countries.

There are also several limitations to acknowledge. A first limitation relates to the study design, as we performed a single-center observational study, which does not allow to completely correct for confounders. Only a randomized double-blind clinical trial would provide more solid evidence. However, similar characteristics of patients among groups were found along with a similar inflammation degree as shown by WBC and CRP. Furthermore, we included Caucasian patients, thus the generalizability of our findings to other populations is uncertain. Finally, despite in-

hospital death is a strong endpoint, it may be sometimes challenging to identify the exact cause of death in these patients.

In conclusion, this study founds a lower mortality rate in COVID-19 patients treated with a combination of hydroxychloroquine and azithromycin. Reasons for this association need to be further investigated.

Study Highlights

What is the current knowledge on the topic?

Few clinical evidences are available regarding the use of hydroxychloroquine and azithromycin for patients with COVID-19 despite this combination has been the most used worldwide so far.

What question did this study address?

This study evaluates the relationship between combination therapy of hydroxychloroquine and azithromycin and inhospital mortality in patients with SARS-CoV2 infection related pneumonia.

What does this study add to our knowledge?

This study reveals an inverse relation between hydroxychloroquine and azithromycin use and in-hospital mortality, when compared with hydroxychloroquine alone or no treatment. The combination was safe as patients with contraindications were excluded.

How might this change clinical pharmacology or translational science?

This is the first report on a large sample that shows the potential efficacy of the combination of hydroxychloroquine and azithromycin in COVID-19

Author Contributions

M.L., A.P., G.I., A.M., S.M., M.M., G.P. D.P., P.G., F.S. wrote the manuscript; A.P., F.S. M.L. designed the research; M.L., A.P., G.I., A.M., S.M., M.M., P.G., collected data; D.P. analyzed the data.

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Table 1. Characteristics of patients according to treatments.

	Overall (n=377)	Treatment groups			p*
		None (n=63)	HCQ (n=17)	HCQ + azithromycin (n=297)	
Age	71.8±13.4	75.4±11.9	76.3±13.1	70.8±13.6	0.018
Women (%)	34.2	33.3	52.9	33.3	0.250
CPAP (%)	13.0	3.2	5.9	15.5	0.021
Mechanical Ventilation (%)	12.5	12.7	11.8	12.5	0.995
CPAP+ Mechanical ventilation (%)	22.8	15.9	17.6	24.6	0.285
Hypertension (%)	63.4	66.7	64.7	62.6	0.827
Diabetes (%)	18.8	20.6	23.5	18.2	0.794
Cancer (%)	14.1	14.3	23.5	13.5	0.509
Cardiovascular disease (%)	34.2	47.6	23.5	32.0	0.038
COPD (%)	13.0	20.6	17.6	11.1	0.105
Cerebrovascular disease (%)	16.2	28.6	29.4	12.8	0.003
Autoimmune disease (%)	7.4	7.9	11.8	7.1	0.762
Obesity (%)	8.0	4.8	11.8	8.4	0.522
C-reactive protein**	106.1±89.3	108.3±87.4	92.1±82.6	106.5±90.3	0.796
White Blood Cells	7783.0±3801.2	8181.0±4767.1	8423.5±4216.9	7662.0±3544.2	0.480

Length of stay (days)	13.3±9.7	7.1±6.9	6.8±4.6	14.9±9.8	<0.001

*among groups

**data missing in 2 patients.

COPD: Chronic obstructive pulmonary disease; CPAP: Continuous Positive Airway Pressure; HCQ: Hydroxychloroquine.

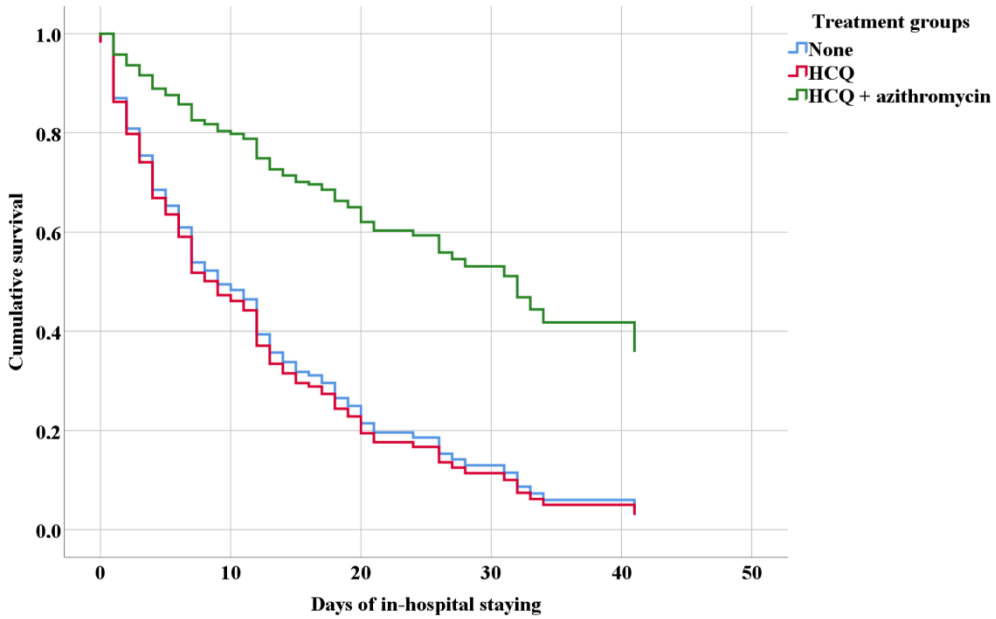
Table 2. Multivariable Cox proportional hazard regression analysis of factors associated with in-hospital death.

	Hazard ratio	95.0% Confidence Interval		p value
		Lower	Upper	
Female sex	0.715	0.482	1.059	0.094
Age	1.057	1.035	1.079	0.000
MV+CPAP	2.726	1.823	4.074	0.000
Hypertension	1.492	0.997	2.232	0.052
Diabetes	1.220	0.813	1.830	0.337
Cancer	1.186	0.736	1.912	0.483
Cardiovascular disease	1.249	0.869	1.795	0.229
COPD	1.322	0.828	2.110	0.243
Cerebrovascular disease	1.037	0.671	1.603	0.869
Autoimmune disease	0.851	0.410	1.765	0.664
Obesity	1.329	0.779	2.268	0.297
WBC above the median	1.074	0.761	1.516	0.684
CRP above the median	2.191	1.479	3.246	0.000
HCQ (vs. no treatment)	1.108	0.536	2.293	0.782
HCQ + azithromycin (vs. no treatment)	0.265	0.171	0.412	0.000

Figure Legends

Figure 1. Kaplan Meier curves for in-hospital mortality according to different treatments.

Figure 2. Forest plot of univariable hazard ratio of factors associated with in-hospital death.



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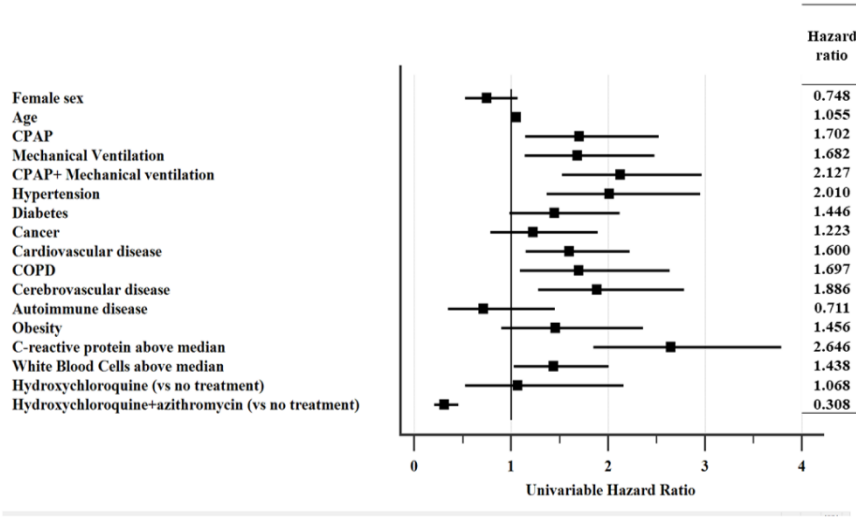

	Hazard ratio	95%CI low	95%CI high	p value
Female sex	0.748	0.524	1.068	0.110
Age	1.055	1.038	1.072	<0.001
CPAP	1.702	1.148	2.524	0.008
Mechanical Ventilation	1.682	1.141	2.479	0.009
CPAP+ Mechanical ventilation	2.127	1.526	2.966	<0.001
Hypertension	2.010	1.369	2.951	<0.001
Diabetes	1.446	0.984	2.123	0.060
Cancer	1.223	0.789	1.895	0.369
Cardiovascular disease	1.600	1.153	2.222	0.005
COPD	1.697	1.092	2.638	0.019
Cerebrovascular disease	1.886	1.279	2.783	0.001
Autoimmune disease	0.711	0.348	1.452	0.349
Obesity	1.456	0.898	2.363	0.128
C-reactive protein above median	2.646	1.849	3.787	<0.001
White Blood Cells above median	1.438	1.029	2.008	0.033
Hydroxychloroquine (vs no treatment)	1.068	0.528	2.162	0.854
Hydroxychloroquine+azithromycin (vs no treatment)	0.308	0.208	0.457	<0.001

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