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ORIGINAL RESEARCH

Outcomes of 2111 COVID-19 Hospitalized Patients Treated with Hydroxychloroquine/Azithromycin and Other Regimens in Marseille, France, 2020: A Monocentric Retrospective Analysis

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Objectives: We evaluated the 6-week mortality of SARS-CoV-2 hospitalized patients treated using a standardized protocol in 2020 in Marseille, France.

Methods: A retrospective monocentric cohort study was conducted in the standard hospital wards at the Institut Hospitalo-Universitaire Méditerranée Infection, between March and December 2020 in adults with SARS-CoV-2 PCR-proven infection.

Results: Of the 2111 hospitalized patients (median age, 67 [IQR 55–79] years; 1154 [54.7%] men), 271 were transferred to the intensive care unit (12.8%) and 239 died (11.3%; the mean age of patients who died was 81.2 (±9.9)). Treatment with hydroxychloroquine plus azithromycin (HCQ-AZ), used in 1270 patients, was an independent protective factor against death (0.68 [0.52 – 0.88]). This effect was consistent for all subgroups of age, comorbidities, severity of the disease and comedications with zinc or corticosteroids. Zinc was independently protective against death (0.39 [0.23 – 0.67]), in a subgroup analysis of patients treated with HCQ-AZ without dexamethasone. The use of high-flow oxygen therapy in elderly patients who were not eligible for intensive care unit transfer saved 19 patients (33.9%).

Conclusions: In our 2020 cohort, treating COVID-19 with HCQ-AZ was associated with lower mortality. These results need to be analyzed in the context of academic discussions about observational studies versus randomized clinical trials. More data will deserve to be analyzed in the SARS-Cov 2 variants, vaccination and post-vaccination era.

Keywords: SARS-CoV-2, COVID-19, hydroxychloroquine, azithromycin

Introduction

By January 17th, 2022, SARS-CoV-2 outbreak had infected 328 million people and killed more than five million people.[1] For 2 years, worldwide management of the disease varied significantly in terms of indications for SARS CoV-2 testing of patients, therapeutic options and follow-up. Starting March 2020, and based on preliminary Chinese data,[2,3] at our hospital in Marseille, France, we decided upon a strategy including early massive screening by PCR and early treatment with hydroxychloroquine (HCQ) and azithromycin (AZ).[4–7]

At that time, among the candidate treatments, only four main drugs (remdesivir, lopinavir-ritonavir, HCQ and dexamethasone) had been tested in large randomised studies. Lopinavir-ritonavir and remdesivir were associated with

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several and sometimes severe adverse events but did not demonstrate reproducible clinical efficacy.[8,9] Corticosteroids (mainly dexamethasone) were also widely used to treat patients.[10]

The first in vitro evidence of the efficacy of chloroquine on SARS-CoV-1 was published in 2004 by several Belgian and American teams.[11,12] In 2014, Dutch scientists screened 348 FDA-approved molecules and identified 4 molecules effective on SARS-CoV-1, including chloroquine.[13] In February 2020, Wang et al replicated these results in China on SARS-CoV-2 and clarified that chloroquine inhibited the virus both upon entry and during the intracellular stage.[9] Several other studies have then reproduced the in vitro anti-viral effect on SARS-CoV-2 of chloroquine and its derivative, HCQ.[14,15]

Different mechanisms have been proposed to explain the antiviral effect of chloroquine. This molecule is a weak base that alkalinizes intracellular acidic vesicles and interferes with microbes using the endolysosomal pathway, such as SARS-CoV -2.[16,17] In addition, coronaviruses, including SARS-CoV-2, activate and utilize endoplasmic reticulum stress and replicate in a modified endoplasmic reticulum-derived compartment.[18] Recent interactomics studies identified that chloroquine interferes with two non-structural viral protein-host protein interactions: nsp6 and the sigma-1 receptor, and Orf9c and the sigma-2 receptor.[14,15] Strikingly, both these sigma receptors are known to act as endoplasmic reticulum stress "gatekeepers".[19] Furthermore, specific agonists of the sigma-1 receptor showed an anti-viral effect but antagonists of this receptor did not.[18] Taken together, these results suggest that the antiviral effect of chloroquine on SARS-CoV-2 relies on several mechanisms but at least in part on agonist binding to sigma receptors, which is responsible for endoplasmic reticulum resistance to virus.

After the first Chinese publications about the antiviral effects of chloroquine and its derivatives against SARS-CoV-2, and our preliminary trial showing reducing viral shedding persistence when associated with azithromycin,[5] we have adopted HCQ with azithromycin (AZ) to treat confirmed COVID-19 cases, despite other published or retracted studies claiming that this regimen would not be effective or toxic.[20–24] Indeed, we have a long-time experience of the of HCQ for the management of infectious diseases such as *Coxiella burnetii* and *Tropheryma whipplei*infections.[25,26] With our experience on the follow-up of more than 2000 patients treated with long-term treatment (>1 year) of HCQ with a dosage of 600 mg/day, we reach a concentration of 1 µg/mL with a full safety. So, we chose this dosage for COVID-19 treatment.

More evidence for us to support HCQ-AZ use came with the demonstration of a synergistic effect in vitro of the HCQ-AZ combination on SARS-CoV-2 at concentrations compatible with that obtained in the human lung.[4] In addition, both HCQ and AZ are known to have immunomodulator effects, which may prevent the "cytokine storm" of COVID-19. Also, in the context of COVID-19-associated pulmonary embolism, HCQ antithrombotic effects might have been of interest.[16]

Interestingly, although no statistically significant effect of HCQ was observed in large randomized studies,[27–30] many observational studies consistently supported positive effects of HCQ early treatment.[7,31–33]

In June 2020, we retrospectively reported the comparative clinical management of 3737 outpatients and inpatients treated with HCQ-AZ or other treatments.

HCQ-AZ was associated with a decreased risk of transfer to the ICU or with death, a decreased risk of hospitalisation ≥10 days and shorter duration of viral shedding, with potential public health effects by reducing the duration of contagiousness.[34]

However, outpatients and inpatients were mixed in this past study.[35] Thereafter, we decided to analyse outpatient and inpatient cohorts separately. Accordingly, we have recently reported the data of 10,429 outpatients seen in our daycare hospital. The global mortality rate was 0.15%. It was 0.06% among the 8315 patients treated with HCQ-AZ in 2020.[35]

Here, we report the management of 2111 inpatients treated in conventional hospital wards and observed by us, between 3 March and 31 December 2020, including only 673 previously reported.[34] In comparison with previous studies, we have also analyzed here for the first time in our center the impact of zinc in combination with hydroxychloroquine and azithromycin,[36] making the present study unique in several ways.

Materials and Methods

Patients and Study Design

Our study was conducted at the Institut Hospitalo-Universitaire (IHU) Méditerranée Infection (https://www.mediterranee-infection.com/), which is home to the infectious and tropical diseases department of the Assistance Publique-

Hôpitaux de Marseille (AP-HM), France.[34] Our institute includes 75 hospital beds. Since the beginning of the outbreak, we performed early massive PCR screening both on patients suspected of having COVID-19 and their contacts.[37,38] In addition, we proposed standardised treatment and follow-up for all individuals ≥18 years of age, with PCR-documented SARS-CoV-2 RNA from a nasopharyngeal sample in our outpatient ward. The most severe patients could be hospitalised in five different ways at our institute: 1) directly after screening in our day clinic, 2) outpatients initially followed in our day clinic and then requiring hospitalisation,[35] 3) from the emergency department, 4) from other hospital wards or nursing homes, 5e) from intensive care units. Data were collected from the patients hospitalised between 3 March and 31 December 2020 and were retrospectively analysed.

Clinical, Biological and Radiological Data and Follow-Up

Demographic information (sex, age) and information on chronic conditions including cancer, diabetes mellitus, chronic heart disease, hypertension, chronic respiratory disease, obesity, hypothyroidism, asthma, obstructive sleep apnoea, and concomitant medications were recorded. The Charlson index was recorded, as previously described.[39] Clinical symptoms, including anosmia, ageusia, rhinitis, fever, cough, dyspnoea and thoracic pain, were systematically documented. Clinical severity was assessed using the National Early Warning Score adapted to COVID-19 patients (NEWS-2) upon hospital admission.[40] Three categories of clinical deterioration were defined, as previously described: low score (NEWS-2 = 0–4), medium score (NEWS-2 = 5–6), and high score (NEWS-2 ≥7).

We recorded biological parameters including haemoglobin, lymphocyte, eosinophil and platelet counts; fibrinogen; D-dimer and other coagulation factors; electrolytes; zinc; lactate dehydrogenase (LDH); creatine phosphokinase (CPK); and C-reactive protein. We had no data on vitamin D and nicotinamide. Viral load was analysed by qPCR from nasopharyngeal swabs on admission and during the follow-up, and an indirect immunofluorescence quantitative assay was used to assess the serological status against SARS-CoV-2.[41] Viral culture was attempted for PCR-positive patients.[42] A low dose CT-scan (LDCT) was proposed for all patients. Radiological lung lesions were classified into three categories: minimal, intermediate and severe involvement.[43]

COVID-19 Management

The first-line treatment consisted of the combination of HCQ (200 mg of oral salt HCQ, three times daily for ten days) and AZ (500 mg on Day 1 followed by 250 mg daily for the next four days). This regimen was proposed as a standard treatment for all patients without contraindications to these drugs. As previously detailed,[34,35] patients were informed of the off-label nature of the prescription of HCQ and AZ prior to receiving treatment. All patients underwent electrolyte analysis and an electrocardiogram (EKG) with corrected QT measurement (Bazett's formula) before starting treatment. EKGs with any abnormalities were systematically referred to a cardiologist for further assessment. In addition, from March to June 2020, we systematically performed another EKG from Day 2 to Day 5. After analysis of the first results confirming the drug safety, we only performed a control of EKG (D2 to D5) for patients with previous EKG abnormalities, other drugs potentially increasing QT concomitantly used or in the cases of ionic disorders. From 15 April 2020 following the preliminary results in the international literature,[36] we added the prescription of elemental zinc (15 mg, three times a day for 10 days).

In addition, broad-spectrum antibiotics (ceftriaxone or ertapenem) were included in the regimen for patients with pneumonia and/or NEWS scores ≥5. Since 5 April 2020, if they presented no contraindication, all patients were treated with an anticoagulant agent. The use of anticoagulant was decided according to the guidelines of the Société française d'anesthésie et de réanimation,[44] with stratification according to the level of oxygen administration, the patient's weight, D-dimers and fibrinogen dosage. For patients with a body mass index under 30 kg/m^2, we prescribed enoxaparin 4000 UI a day. If the body mass index was higher than 30 kg/m^2, or if high-flow oxygen was used, we prescribed enoxaparin 4000 UI bid or 6000 UI bid. In cases of hypercoagulability marked by D Dimers higher than 3 µg/mL or fibrinogen higher than 8 g/L, we prescribed tinzaparin 175 UI/kg/d or enoxaparin 100 UI/kg/bid (regardless of weight or level of oxygen administration). In cases of renal impairment, sodic or calcic heparin was used. If patients were already receiving treatment with an anticoagulant agent upon admission, treatment was continued or adjusted for heparin, according to the recommendations of the clinician in charge.[44]

Standard care included systematic oxygen supplementation. From June 2020 we used dexamethasone 6 mg for ten days, for patients outside the acute phase of the disease who required increased oxygen. Finally, from 15 September 2020, we used high-flow oxygen therapy devices for patients who were not eligible for intensive care due to their age and/or their comorbidities and for whom transfer to the ICU was not possible.[45]

Outcomes

The primary outcome was six-week mortality from admission date. Regarding the endpoint for clinical efficacy treatment analysis, we used two methods. Firstly, we performed an "intention-to-treat" analysis. Secondly, as previously described, we analysed the per protocol outcome, selecting 72 hours after beginning the treatment for the evaluation.[34] As a clinical outcome, we also evaluated transfer to the ICU as a secondary outcome.

Statistical Analysis

Categorical variables were presented as n (%). We used the Wilcoxon Mann Whitney test, Student's *t*-test, $\chi 2$ test, or Fisher's exact test to compare differences between groups of patients where appropriate. We performed multiple correspondence analysis (MCA) to investigate the associations between clinical data, biological data, radiological data, and the treatment received. In order to control for selection bias in comparing mortality between treatment groups, we used a propensity score weighting approach. The propensity score was calculated using a logistic regression with sex, age groups, NEWS-2 score, comorbidities and in-hospital treatment(s) (HCQ, AZ, zinc and/or corticosteroids when appropriate) as covariates. The predicted probabilities from the propensity-score model were then used to calculate the stabilised inverse-probability-weighting weights.[46] The association between treatment groups and mortality was then assessed using weighted multivariable Cox models. Cox models were adjusted on the following variables: sex, age groups, NEWS-2 score, comorbidities and in-hospital treatment (HCQ, AZ, zinc and/or corticosteroids where appropriate). Adjusted hazard ratios with 95% confidence intervals were calculated from the Cox regression coefficient estimates. Sensitivity analyses were performed by assessing whether observed effects were reproducible and consistent across subgroups according to age class, sex, comorbidities, disease severity, co-medications, and reasons for non-treatment. A two-sided α value of less than 0.05 was considered to be statistically significant. Analyses were carried out using SAS 9.4 statistical software (SAS Institute, Cary, NC).

Ethics Statement

The data presented in this study were collected retrospectively from the routine care setting using the hospital's electronic health recording system. In France, at the time the study was conducted, treatment of COVID-19 with HCQ was approved off-label for hospital delivery only. For all patients, HCQ-AZ was prescribed either during complete hospitalisation or at day-care clinic by one of the physicians, after collegial decision based on their analysis of the most recent scientific data available and after assessment of the benefit/harm ratio of the treatment. In line with the European General Data Protection Regulation No 2016/679, patients were informed of the potential use of their medical data and that they could refuse the use of their data. The analysis of collected data followed the MR-004 reference methodology registered under No. 2020–152 in the AP-HM register. The non-interventional, retrospective nature of the study was approved by our institute's review board committee (Méditerranée Infection No.: 2021–015).

Results

Overall Characteristics of Patients

From 3 March to 31 December 2020, 2111 patients were hospitalised in our institute; 1155 (54.7%) of them were male. The median age was 67 years, 682 patients (32.3%) were over 75 years of age and 146 (6.9%) were over 89 years of age. Baseline clinical and biological characteristics are reported in Table 1, Tables S1 and S2, respectively. Most of the patients were hospitalised from the emergency department (1.114, 52.8%), 496 patients (23.5%) directly after evaluation in our day clinic. A total of 270 (12.8%) were first outpatients treated in our day clinic and then hospitalised, 193 patients (9.1%) came from other hospital wards and 38 patients (1.8%) were referred from the intensive care unit. A total of 1270

Table 1 Baseline Clinical Characteristics of 2111 COVID-19 Hospitalized Patients Treated with Hydroxychloroquine/Azithromycin and Other Regimens in Marseille, France, 2020

	All		ICU Transfer		Deaths	
	n	%	n	%	n	%
n	2111		271		239	
Sex - men	1154	54.7	200	73.8	148	61.9
Age – mean (std) Q1-median-Q3	65.8 (17.2) 55-67-79		63.2 (11.0) 56-64-72		81.2 (9.9) 75-83-89	
Age 18–29	67	3.2	1	0.4	0	0
Age 30–39	118	5.6	6	2.2	0	0
Age 40–49	168	8	27	10	2	0.8
Age 50–59	380	18	60	22.1	7	2.9
Age 60–69	451	21.4	91	33.6	22	9.2
Age 70–79	401	19	73	26.9	56	23.4
Age 80–89	380	18	13	4.8	105	43.9
Age >89	146	6.9	0	0	47	19.7
Charlson index V1[a]- mean (std) Q1-median-Q3	4.5 (2.7) 2-4-6		4.0 (2.1) 2-4-5		6.9 (2.2) 5-7-8	
Charlson index V2[b]- mean (std) Q1-median-Q3	1.4 (1.7) 0-1-2		1.3 (1.5) 0-1-2		2.4 (2.0) 1-2-3	
Chronic condition(s)						
Hypertension	956	45.3	129	47.6	150	62.8
Diabetes mellitus	571	27	90	33.2	81	33.9
Cancer disease	246	11.7	32	11.8	42	17.6
Chronic respiratory diseases	393	18.6	47	17.3	62	25.9
Chronic heart diseases	520	24.6	59	21.8	116	48.5
Obesity	495	23.4	103	38	39	16.3
Hypothyroidism	210	9.9	22	8.1	31	13
Asthma	159	7.5	19	7	16	6.7
Obstructive sleep apnoea	112	5.3	21	7.7	15	6.3
Other inflammatory disease	97	4.6	12	4.4	16	6.7
Medications						
Metformin	336	15.9	50	18.5	34	14.2
Beta blocking agents	404	19.1	55	20.3	74	31.0
Verapamil	28	1.3	3	1.1	4	1.7
HMG CoA reductase inhibitors	418	19.8	57	21.0	64	26.8
Fibrates	26	1.2	3	1.1	6	2.5
Dihydropyridine derivatives	557	26.4	89	32.8	96	40.2
Angiotensin II receptor blockers	357	16.9	54	19.9	44	18.4
ACE inhibitors	251	11.9	34	12.5	30	12.6
Tobacco consumption	210	9.9	34	12.5	24	10.0
Pulmonary CT-scanner						
Missing	208	9.9	16	5.9	33	13.8
Normal	229	10.8	10	3.7	13	5.4
Minimal	496	23.5	22	8.1	31	13
Intermediate	717	34	90	33.2	69	28.9
Severe	461	21.8	133	49.1	93	38.9
Clinical symptoms						
Fever	601	28.5	112	41.3	67	28
Cough	1023	48.5	146	53.9	79	33.1
Rhinitis	127	6	8	3	3	1.3

(*Continued*)

Table 1 (Continued).

	All		ICU Transfer		Deaths	
	n	%	n	%	n	%
Anosmia	258	12.2	39	14.4	9	3.8
Ageusia	255	12.1	42	15.5	10	4.2
Dyspnoea	942	44.6	171	63.1	134	56.1
Thoracic pain	172	8.1	13	4.8	5	2.1
NEWS score – mean (std) Q1-median-Q3	5.7 (2.8) 4-6-8		7.0 (2.5) 5-7-9		8.3 (2.4) 7-8-10	
NEWS 0–4	735	34.8	41	15.1	11	4.6
NEWS 5–6	580	27.5	75	27.7	48	20.1
NEWS ≥7	796	37.7	155	57.2	180	75.3
Mode of hospitalisation						
Other wards	193	9.1	8	3	20	8.4
Firstly outpatient then hospitalisation	270	12.8	20	7.4	6	2.5
Directly from day clinic	496	23.5	58	21.4	23	9.6
From ICU	38	1.8	38	14	0	0
From emergency department	1114	52.8	147	54.2	190	79.5
Treatments						
HCQ-AZ	1270	60.2	158	58.3	93	38.9
Zinc	1302	61.7	170	62.7	161	67.4
Dexamethasone	530	25.1	169	62.4	121	50.6

Notes: [a]Charlson index with age. [b]Charlson index without age.

(60.2%) patients received the combination of HCQ-AZ. Of the 841 patients not treated with this combination, 529 patients (62.9%) had a contraindication, the treatment was not proposed by the physician for 251 patients (29.9%), 33 refused the treatment (3.9%), and data were not available for 28 patients (3.3%) (Table 2). In addition, 1302 (61.7%) patients were treated with zinc and 530 (25.1%) patients received dexamethasone.

Clinical, Biological and Radiological Characteristics

Underlying conditions and clinical symptoms are comprehensively described in Table 1. The mean Charlson index was 4.5 (±2.7). Most of the patients (796, 37.7%) had a NEWS-2 score ≥7 at the admission. A cough was the most frequent symptom (1023, 48.5%), followed by dyspnoea (942, 44.6%), fever (601, 28.5%), anosmia (258, 12.2%), ageusia (255,

Table 2 COVID-19 Hospitalized Patients Not Prescribed with Hydroxychloroquine and Azithromycin Combination (n = 841), Marseille, France, 2020

	n	%
Not proposed by the physician	251	29.9
Refused the combined treatment	33	3.9
Contraindication	529	62.9
Prolonged QTc	90	10.7
Other cardiac disorder	126	15.0
Risk of drug interactions	201	23.9
Ophthalmologic	5	0.6
Other contraindication	107	12.7
Other	28	3.3
Total	**841**	**100.0**

12.1%), thoracic pain (172, 8.1%) and rhinitis (127, 6%). Patients' biological characteristics upon admission of patients are comprehensively detailed in Table 3. The QT value was higher in the "No HCQ+AZT" (419.4 ms ±40.2) rather than in HCQ-AZ group (400.5 ms ±35.6). The multiple correspondence analysis (MCA) allowed for the identification of different groups of patients depending on the outcome and highlighted the main clinical, biological and radiological involvement associated with death (Figure 1).

Adverse Events Associated with Treatments

We listed 224 adverse events (Table 3). All adverse events were mild and included mostly gastrointestinal symptoms (74 cases of diarrhoea, 35 cases of nausea/vomiting and 29 cases of abdominal pain). We paid specific attention to QTc prolongation, which was observed in 38 patients (1.8%). Among them, only 11 patients had a QT > 500ms (0.52%). Among the 27 patients with QT < 500 ms, 13 patients (0.62%) had a QT expansion higher than 60 ms and 14 lower (0.66%). Thirty patients were treated with combination HCQ-AZ, 7 with AZ and 1 with HCQ. No cases of *torsade de pointe* or sudden death were observed.

Clinical outcomes

Of the 2111 hospitalised patients, 271 (12.8%) were transferred into ICU (male, 73.8%). The mean age was 63.2 (±11.0) years old (Table 1, Figure S1). A total of 239/2111 (11.3%) patients, including those who were transferred to the ICU, died within six weeks (male, 61.9%). Their mean age was 81.2 (±9.9) years old. Almost two-thirds of patients with a fatal outcome were 80 years of age or older (152 patients, 63.6%, Tables 1 and S1). Nine patients with a fatal outcome were under 60 years old. Of these nine patients, six had severe underlying conditions: two had Down's Syndrome with restrictive pulmonary syndrome, one had a mislabelled mental disability and chronic pulmonary insufficiency, one had late stage multiple sclerosis rendering him bedridden, one had a late stage inflammatory neurological disease, and one patient suffered from vasculitis, cardiomyopathy, renal chronic insufficiency, diabetes mellitus and chronic obstructive

Table 3 List of Adverse Events (n = 224) Among 2111 COVID-19 Hospitalized Patients Treated with Hydroxychloroquine/Azithromycin and Other Regimens in Marseille, France, 2020

	n	%
At least one adverse event	224	10.6
Diarrhoea	74	3.51
Prolonged QTc	38	1.8
-QT > 500 ms	11	0.52
-Expansion > 60 ms and QT < 500 ms	13	0.62
-Expansion < 60 ms and QT < 500 ms	14	0.66
Nausea/vomiting	35	1.66
Abdominal pain/other digestive troubles	29	1.37
Acute renal failure	21	0.99
Cytolysis/cholestasis	20	0.95
Neuropsychiatric signs (mood disorder, insomnia, nervousness)	17	0.81
Skin disorders	16	0.76
Oral candidiasis	14	0.66
Headache	13	0.62
Anorexia	12	0.57
Fainting	9	0.43
Blurred vision and other visual disturbance	5	0.24
Dizziness	4	0.19
Palpitations/tachycardia	4	0.19
Paraesthesia	2	0.09
Trembling	1	0.05

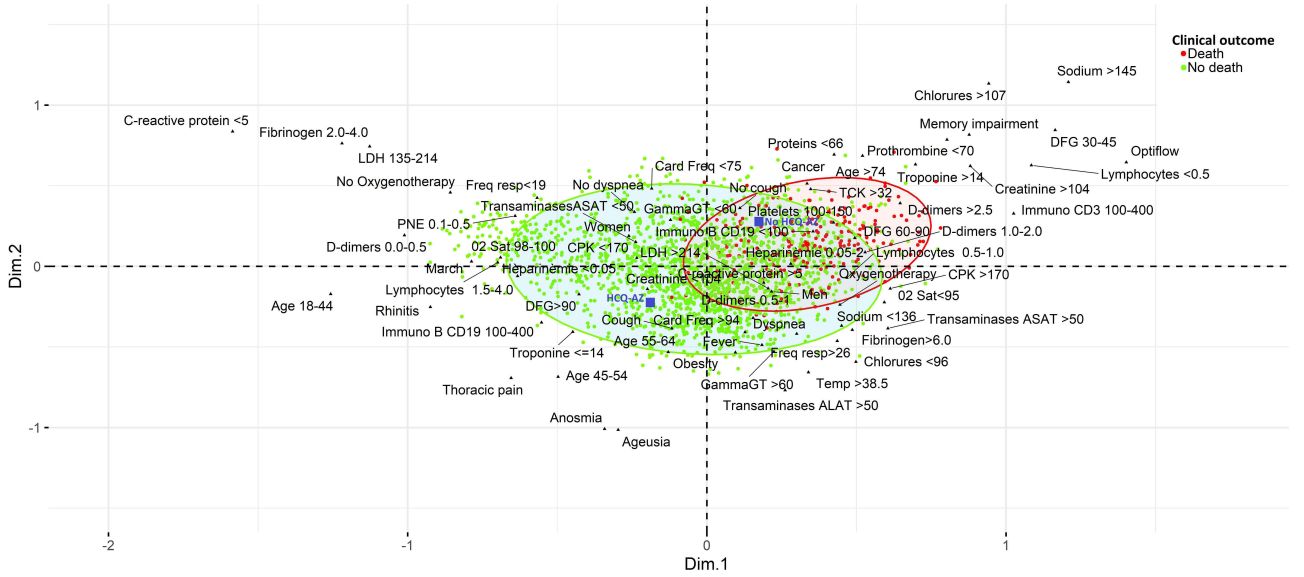

Figure 1 Baseline clinical and biological characteristics of 2111 COVID-19 hospitalized patients treated with hydroxychloroquine/azithromycin and other regimens in Marseille, France, 2020 - multiple correspondence analysis. For the multiple correspondence analysis, all variables were active except for clinical outcome (death status) and treatment groups (HCQ-AZ/No HCQ-AZ). The red dots represents patients who died and green dots patients who did not die. Blue squares represent the treatment groups variable. Ellipses: 90% confidence ellipses around clinical outcome categories (death/no death).

pulmonary disease. Only three patients who died had only moderate underlying conditions: one patient was a 49-year-old migrant with poorly stabilised type 1 diabetes, one 54-year-old patient was morbidly obese, and one 59-year-old patient had hypertension.

No patients under the age of 39 died, and the mortality rate was 1.2% for the 40–49 age group, 1.8% for 50–59, 4.9% for 60–69, 14% for 70–79, 27.6% for 80–89 and 32.2% for patients over the age of 89. Interestingly, the 90-day mortality rate of patients hospitalised in our institute was lower than national data in all age groups for the period from 1 March to 15 June 2020 (Figure S2). Finally, mortality rates differed significantly depending on the mode of admission in our institute (2.2% for those who were first outpatients and were then hospitalized; 4.6% for patients who were directly hospitalized from our day clinic; 10.4% for patients transferred from other wards, and 17.1% for patients hospitalized from the emergency department (Table S1)).

HCQ-AZ Combination

The duration of hospitalization has been significantly shorter in the HCQ-AZ group (6.6 days vs 7.4 days in the No HCQ-AZ group, $p < 0.001$). The virus load at inclusion was not statistically different between the "HCQ-AZ" (24.4 ±5.3 CT) and the "No HCQ-AZ" groups (24.5 ± 5.7 CT). The six-week mortality rate of patients treated with combination of HCQ-AZ was significantly lower than patients treated with other regimen whether in intention-to-treat (7.3% versus 17.4%, $p < 0.001$) or per protocol including patients treated ≥3 days (5.9% versus 16.6%, $p < 0.001$). In a weighted multivariate Cox proportional hazards model, HCQ-AZ was an independent protective factor against death (death hazard ratio (HR) 0.68, 95% confidence interval (95% CI) (0.52–0.88)) (Figures 2–3, Tables 4–5). This effect was consistent for all subgroups of age, comorbidities, severity of the disease and comedications with zinc or corticosteroids (Figure 2). Reasons for non-treatment (contraindication, non-proposition and refusal) were not confounding factors, as subgroup analyses excluding or including only these patients highlighted a similar protective effect (Figure 2). This independent protective factor was confirmed in a 10-year age-stratified multivariable Cox proportional-hazards models from 55 to >80 years with hazard ratio ranging from 0.12 to 0.97 (Figure S3).

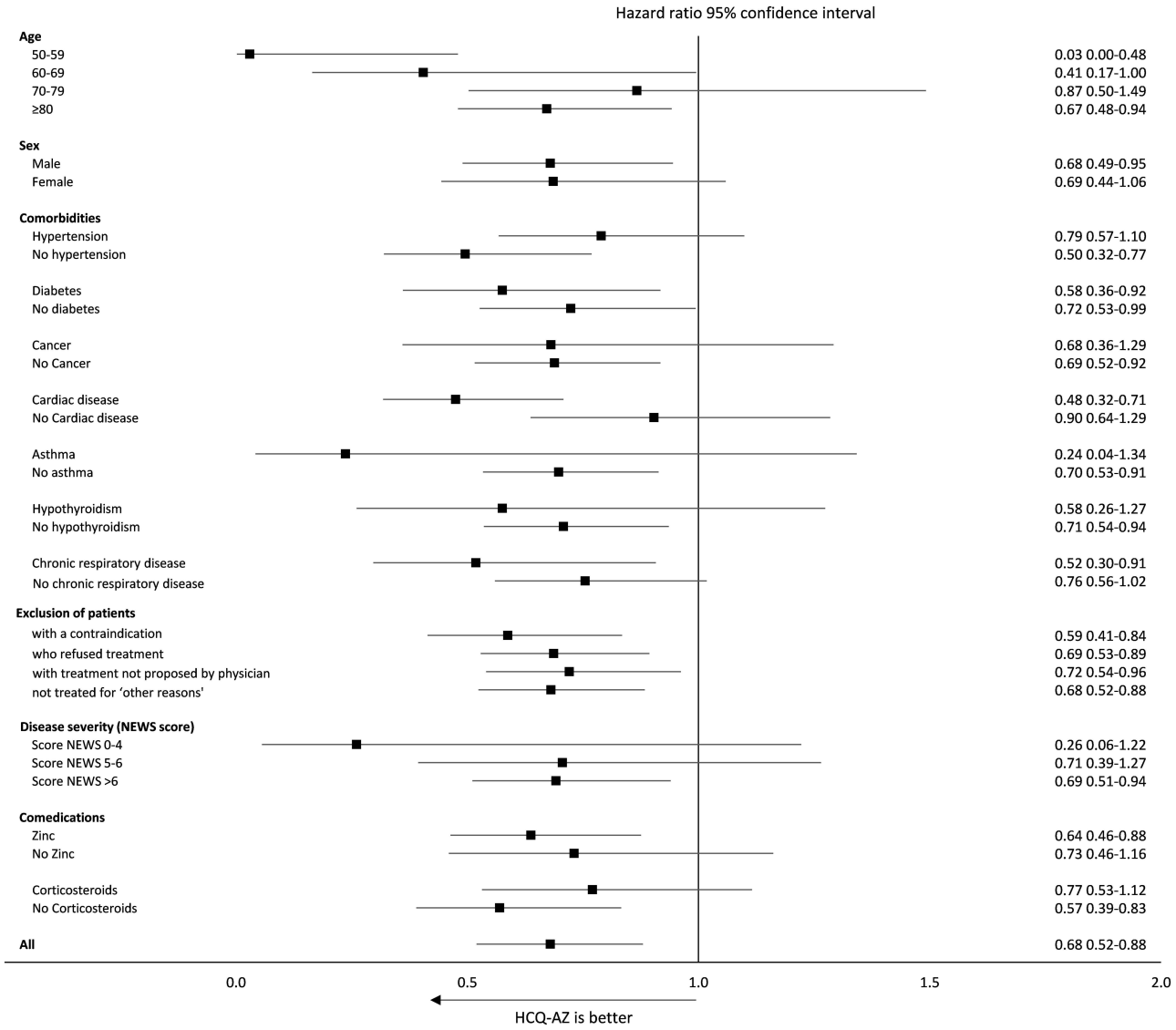

Figure 2 Association between treatment group (HCQ-AZ vs No HCQ-AZ) and death according to age, sex, comorbidities, severity and co-medications - stratified multivariable Cox proportional-hazards models (n=2111 COVID-19 hospitalized patients treated with hydroxychloroquine/azithromycin and other regimens in Marseille, France, 2020).

Zinc

Comparing the 1302 patients treated with zinc to the 809 other patients not treated with zinc, using propensity weighted analysis, we did not demonstrate a reduction in death independently of age, comorbidities, severity of the diseases and other treatment (Figure S4 Table S3). Nevertheless, subgroup analyses evidenced that zinc was an independent protective factor against death among patients treated with HCQ-AZ without dexamethasone (n = 1018, death hazard ratio (HR), 0.39, 95% CI 0.23–0.67, p = 0.0011; weighted multivariate Cox proportional hazards model) (Figure 4) and a trend for beneficial effect was observed in those treated with AZ only (n = 435, death hazard ratio (HR), 0.64, 95% CI 0.39–1.06, p = 0.0813).

Dexamethasone

Patients treated with dexamethasone were significantly older, more frequently male, had more severe symptoms and were significantly more likely to die (Table S4). Using a propensity weighted score to compare them, corticosteroids remained an independent factor associated with death for patients with CRP <100 mg/L (death

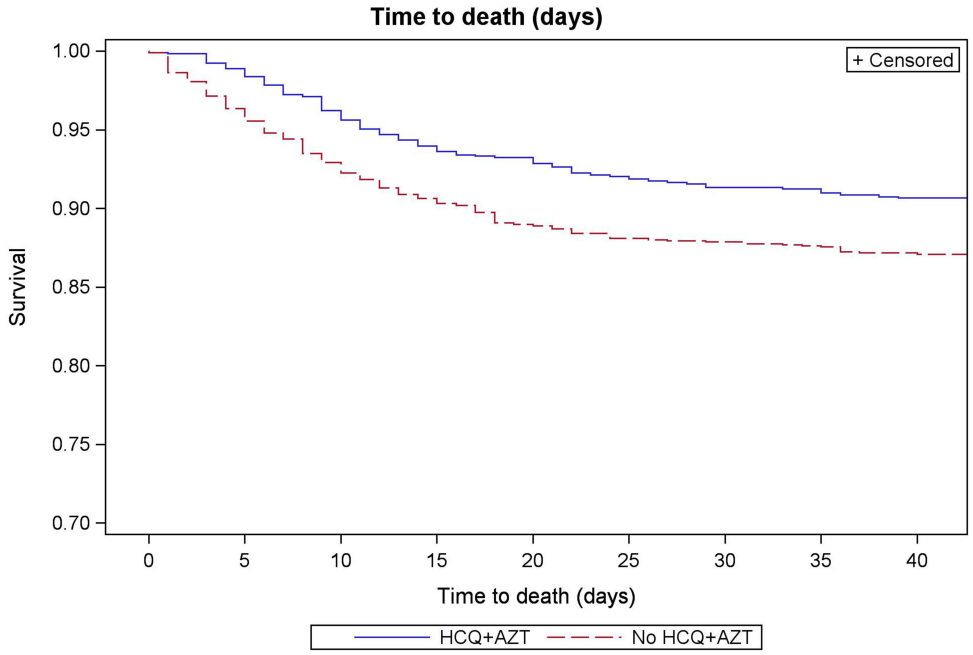

Figure 3 Kaplan–Meier curve of survival according to treatment groups (propensity weighted sample, n = 2111 COVID-19 hospitalized patients treated with hydroxychloroquine/azithromycin and other regimens in Marseille, France, 2020). Log rank test: p = 0.0135.

hazard ratio (HR) 3.36, 95% confidence interval (2.09–5.40)) (Table S4, Figure S5). Conversely, for patients with CRP > 100mg/L, no difference in death outcome was observed between patients treated with or without corticosteroids (Table S6, Figure S6).

Table 4 Comparison of Treatment Groups (HCQ-AZ vs No HCQ-AZ, n = 2111 COVID-19 Hospitalized Patients Treated with Hydroxychloroquine/Azithromycin and Other Regimens in Marseille, France, 2020)

	Unweighted Sample			Propensity Weighted Sample		
	HCQ-AZ N = 1270	No HCQ-AZ N = 841	P*	HCQ-AZ N = 1270	No HCQ-AZ N = 841	P*
Age mean (std)	63.0 (16.7)	70.0 (17.2)	<0.001	65.6 (15.0)	65.1 (21.4)	0.558
Men (%)	54.8%	54.5%	0.876	55.0%	55.6%	0.778
NEWS score						
0–4	38.3%	29.5%	<0.001	35.0%	35.5%	0.963
5–6	27.8%	27.0%		27.3%	26.8%	
>6	33.9%	43.5%		37.7%	37.7%	
Comorbidities						
Hypertension	40.3%	52.8%	<0.001	45.0%	44.8%	0.912
Diabetes mellitus	26.0%	28.7%	0.176	26.9%	26.5%	0.861
Cancer disease	11.3%	12.2%	0.489	12.0%	12.2%	0.853
Chronic respiratory diseases	16.2%	22.2%	0.001	18.6%	19.0%	0.820
Chronic heart diseases	17.4%	35.6%	<0.001	24.4%	24.5%	0.980
Obesity	22.9%	24.3%	0.476	23.2%	23.3%	0.969
Hypothyroidism	8.4%	12.2%	0.004	9.7%	9.6%	0.912
Asthma	7.3%	7.8%	0.655	7.6%	7.8%	0.875
Other inflammatory disease	3.9%	5.7%	0.047	4.6%	4.6%	0.977
Treatments (other than HCQ-AZ)						
Zinc	57.2%	68.5%	<0.001	61.9%	61.6%	0.888
Corticosteroids	19.8%	33.1%	<0.001	25.5%	25.6%	0.970

Note: *Chi-square/Fisher's exact or Student's *t*-test where appropriate.

Table 5 Association Between Treatment Groups (HCQ-AZ vs No HCQ-AZ) and Death – Multivariable Cox Proportional-Hazards Model (n = 2111 COVID-19 Hospitalized Patients Treated with Hydroxychloroquine/Azithromycin and Other Regimens in Marseille, France, 2020)

	HR 95% CI[a]	p
Treatment group (ref. No HCQ-AZ)	0.68 0.52–0.88	0.0037
Age (ref 18–54)		
55–64	2.59 0.83–8.09	0.1023
65–74	4.71 1.62–13.68	0.0044
>74	12.70 4.49–35.96	<0.0001
Sex men (ref. women)	1.31 0.99–1.74	0.0566
NEWS score (ref. 0–4)		
5–6	3.28 1.65–6.55	0.0007
>6	6.13 3.15–11.95	<0.0001
Number of comorbidities		
Hypertension	1.11 0.84–1.47	0.4697
Diabetes mellitus	1.01 0.76–1.35	0.9374
Cancer disease	1.10 0.78–1.55	0.5923
Chronic respiratory diseases	1.33 0.95–1.85	0.0925
Chronic heart diseases	1.56 1.19–2.04	0.0012
Obesity	0.66 0.45–0.95	0.0260
Hypothyroidism	1.15 0.77–1.71	0.4971
Asthma	1.14 0.64–2.03	0.6668
Other inflammatory disease	2.01 1.21 −3.35	0.0071
Treatments (other than HCQ-AZ)		
Zinc	0.63 0.47–0.84	0.002
Corticosteroids	2.56 1.92–3.40	<0.0001

Note: [a]Hazard ratio 95% CI.

High-Flow Oxygen Therapy

Fifty-six elderly patients who were not eligible for transfer to the ICU due to their age and comorbidities were treated in our institute using high-flow oxygen therapy. The mean age of these patients was 80.5 years (median 82.5) and 32 (57.1%) were male. These patients suffered from several underlying conditions (mean Charlson index: 6.8). Upon admission to our wards, clinical involvement was severe, with 80.4% of the patients having NEWS-2 score ≥7 (Table S7). Ultimately, 19 patients (33.9%) were weaned off HFNO and survived thanks to this technique.

Discussion

In our institute, and during the first year of the SARS-CoV-2 pandemic, we implemented a widespread strategy of PCR screening of patients and early treatment. This led us to perform more than 600,000 PCRs, for 400,000 patients, of which 45,000 were positive. More than 20,000 were treated in our institute as inpatients or outpatients.[38]

When we have reported our 2020 outpatients' study,[35] the need for early treatment using HCQ was demonstrated on a large Iranian outpatient study (28,759 outpatients) and a Saudi Arabian study (5541 outpatients).[31,32] Moreover, the setting of a daycare hospital allowing for an early access to healthcare may have contributed to the low fatality rate in our cohort. Indeed, patients admitted from the emergency ward had a 10-fold higher risk of death compared to patients initially treated as outpatients in our center (17 versus 2%), and a 4-fold higher risk compared to patients directly admitted the day they come to the daycare hospital (17 vs 5%).

Herein, in a monocentric cohort of 2111 patients hospitalized in 2020, we noted a beneficial effect of HCQ-AZ after controlling for age, comorbidities and severity of the disease. This effect was consistent for all subgroups analyzed, and

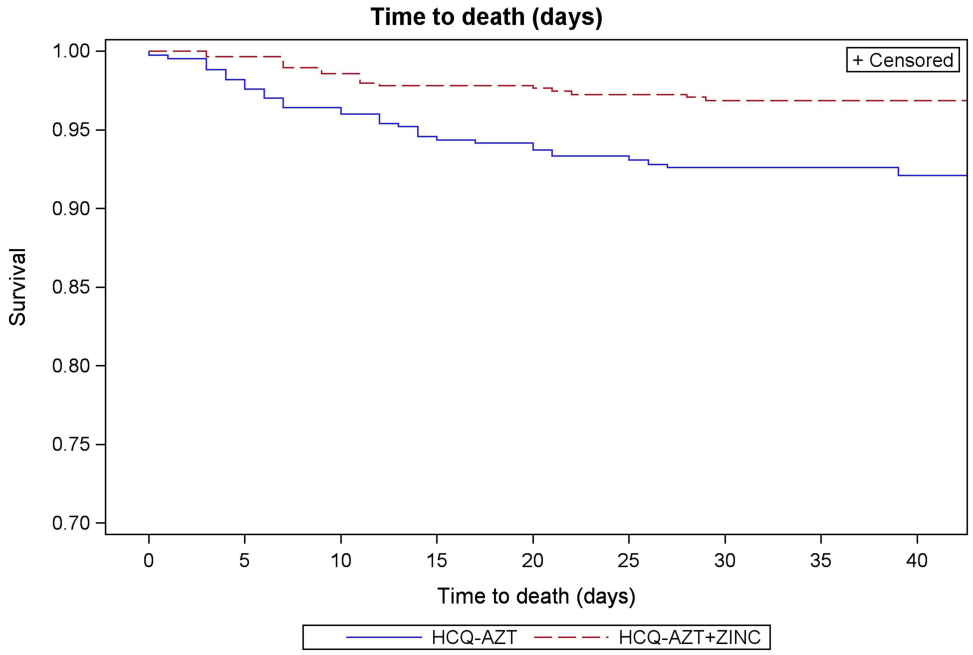

Figure 4 Kaplan–Meier curve of survival according to treatment groups (propensity weighted sample, n = 1018 COVID-19 patients treated with HCQ-AZ ± zinc (no corticosteroid) in Marseille, France 2020). Log rank test: p=0.0011. Adjusted hazard ratio: 0.39 0.23–0.67 (p<0.001).

reasons for non-treatment (contraindication, non-proposition by the physician and refusal by the patient) were not confounding factors, as shown with subgroup analyses. Our work was performed on hospitalized patients treated in a unique institute using drugs at a dosage already used in other indications. For the first time in our center, we evidenced the beneficial effect of zinc when added to HCQ and AZ. We performed a stringent follow-up to assess the condition of patients and consequently we are certain of the veracity of these observations. Overall, the data set contains 1.7 million items that is accessible to everyone (10.35081/mm67-dj74). This large cohort allows us to confirm the absence of significant cardiotoxicity when HCQ and AZ are used in hospitalized patients carefully using a standardized protocol. Indeed, we did not observe any torsades de pointe nor sudden death.

In this study, undoubtedly, the mortality rate that we observed was lower than in most studies including only hospitalized patients.[20,27–29] The risk of death in patients was the same as that previously described in other series, and patients over 80 years of age or with severe underlying conditions are particularly vulnerable. Conversely, the risk of death is extremely rare in patients under the age of 60 without comorbidities, as soon as they have access to care.

However, the use of HCQ-AZ for COVID-19 treatment has resulted in academic discord and even political issues.[47] Passionate debates have occurred in the media and scientific journals about the possible toxicity of CQ or HCQ.

Moreover, the discussion about the need randomized controlled trials (RCTs) to support therapeutic choice and public health decision is an issue and may be considered as limitation of our study. However, a Cochrane Library publication stated that observational studies and randomized controlled trials (RCTs) should give the same results.[48] Interestingly, most observational studies reporting that early HCQ with or without treatment shows positive results, whereas it is not effective when used very late and/or with high dosage over a long period. On the other side, studies based on big data have not shown such results.

Anyway, our goal here is not to be part of the discussion about RCTs versus observational studies. We think that controversies are part of science and that such monocentric experience can help with the management of future outbreaks or new outbreaks linked to COVID-19. When patients are grouped in cohorts, daily observations allow standard care to be adjusted, such as the early use of anticoagulation for COVID-19 in patients. Finally, the equipment in the HFNO allowed us to propose a therapeutic treatment to patients who were not eligible for transfer to the ICU due to their age or comorbidities,

which enabled us to save 19 lives in 2020. For us, this series also supports that protocols and recommendations must be established and modified as knowledge of the disease increases. This approach is difficult in randomised trials.

Finally, we did not find any benefit of corticosteroids, as reported in the Recovery trial,[10] and which may have been part of the basic recommendations on the treatment of this disease. The Simpson effect cannot be excluded in the evaluation of corticosteroids, because the patients treated with corticosteroids had significantly more severe condition and were hospitalized at different stages of the disease.[10,49] However, caution is essential, especially in the acute phase of the disease or when there is no inflammatory syndrome during which the effect may be harmful.

In meta-analyses, the choice of the selected studies influences dramatically the results that may be biased.[50] We continue to believe that monocentric studies are highly valuable due to the homogeneity of standard care (the "in our hands" phenomenon). Moreover, the concentration in any given institute leads to a progression in the quality of care, which is linked to medical experience, the importance of which should not be neglected.

Conclusion

We think that drug repurposing or repositioning is an important field in drug discovery that identifies new therapeutic possibilities for existing drugs. In addition to HCQ-AZ, other possible drug candidates for Covid-19 treatment might be identified.[51] Also, access to care and the quality of care remains a major element in patient care and observation remains a major element in reflecting on that care, particularly when it comes to new diseases. Our series focused on patients hospitalized in 2020, at which time there were no credible oral therapeutic alternatives. Since then, other oral alternatives have been proposed (paxlovid, molnupiravir …).[52] However, based on our experience and the results reported here, we will continue to use HCQ-AZ in hospitalized COVID-19 patients. We will continue our observations in the SARS-Cov 2 variants,[53] vaccination and post-vaccination era.[54]

The IHU Task Force Includes Clinicians, Microbiologists, Statisticians, Pharmacologists, Hematologists, Epidemiologists Who Contributed to the Care and the Diagnostic in the Patients of This Cohort, and to the Writing of This Paperand/or The Care of the Patients

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Disclosure

Prof. Dr Didier Raoult reports personal fees from Scientific board member of Eurofins company, Founder and shareholder of a microbial culture company (Culture Top), received personal fees from Hitachi High-Technologies Corporation, Tokyo, Japan, from 2018 to 2020, Founder and shareholder of Biotechnology "Techno-jouvence", Founder and shareholder of a Biotech company "Gene and Green TK, Founder and shareholder of rapid diagnosis of infectious diseases company "Pocramé", outside the submitted work. The authors declare no other competing interests.

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