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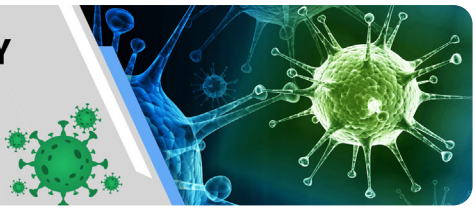

An Independent Analysis of a Retrospective Cohort of 30,423 Covid-19 Patients Treated at IHU-Mediterranean in Marseille, France: Part 1, Efficacy of early Treatment with Hydroxychloroquine and Azithromycin

Valere Lounnas*,[1], Eleftherios Gkioulekas[2], Marc Rendell[3], Alexis Lacout[4], Xavier Azalbert[5], Christian Perronne [6]

Abstract

A cohort of 30,423 Covid-19 patients treated between March 2020 and December 2021 at the IHU-Méditerranée Infection in Marseille (France) was retrospectively analyzed in terms of treatment attempted and disease worsening factors to quantify efficacy with respect to the composite endpoint of transfer to intensive care unit or death, within a couple of months (56 days) from admission. Within limitations of the data and of the models, after adjustment for sampling biases, multivariate logistic regression analyses were performed to determine unadjusted and adjusted odds ratios (ORs) for the subset of patients having received the combined treatment hydroxychloroquine plus azithromycin (HCQ-AZ) or no specific treatment (i.e. no HCQ, no AZ and no ivermectin (IVM)) (24,943 patients). An efficacy of 58% in reducing the risk of ICU transfer and death was measured (HCQ-AZ unadjusted OR = 0.499; 95%CI = [0.343; 0.727], $p < 0.001$) (HCQ-AZ adjusted OR = 0.419; 95%CI = [0.327; 0.539], $p < 0.001$). AZ without HCQ but associated with ivermectin in 31.3% of the cases was significantly active as well with respect to no specific treatment, with a measured efficacy of 27% (unadjusted OR = 0.720, 95% CI=[0.574;0.905] $p=0.005$ and adjusted OR=0.727, 95%CI=[0.608;0.870] $p < 0.001$). Interactions between HCQ-AZ and the model covariates were systematically explored. No interaction between HCQ-AZ treatment and vaccination was detected. Statistically significant favorable interactions were detected between HCQ-AZ treatment and male sex, age categories ≥ 50 years, the UK variant and when the variant was not determined, obesity, chronic obstructive pulmonary disease (COPD), cancer, immunodeficiency, confirming the high efficacy of this early treatment. No statistically significant unfavorable interaction of HCQ-AZ with any covariate was detected. Limitations of the models and their implications for the results are discussed extensively.

Keywords: Covid-19, observational cohort, hydroxychloroquine, azithromycin, multivariate analysis, logistic regression, propensity score matching, monocentric study, university medical institute, IHU-Méditerranée Infection

Introduction

In the first months of the Covid-19 pandemic, during the spring and early summer 2020, several large phase 3-like randomized controlled trials (RTC) were hastily conducted to establish whether hydroxychloroquine (HCQ), a long-known drug of widespread use for decades as antimalarial medication

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and in some rheumatic autoimmune disorders (RADs) [1], was active in Covid-19 [2-6]. The reason was that chloroquine, a closely related analog of HCQ, also exhibits antiviral properties in vitro, with strong antiviral effects on SARS-CoV infection of primate cells. These results were published in 2005 following the pandemic of 2003 [7-10]. In addition, a number of observational studies conducted on broad variety of cohorts of patients (ranging from a few tens to several thousand patients) indicated potential efficacy of hydroxychloroquine [11,14-18]. A strong reduction in nasal viral carriage was also evidenced after few days of treatment with the combination of hydroxychloroquine and azithromycin (HCQ + AZ) [19-21]. Despite opposing claims, the mechanism of action of hydroxychloroquine as antiviral agent is actually known [22-29]. It acts by concentrating in the endosomes and lysosomes (by more than 50,000-fold with respect to the cytoplasm and extracellular matrix) making them sufficiently alkaline (pH ca. 11-12) to induce denaturation of the surface proteins of the SARS-CoV-2 viruses and of the proteins of the nuclear capside, making virions irreversibly destabilized. It may also prevent the activity of furine, the optimum of which is comprised between pH 5 and 8 [25]. Although endocytosis is not the only possible route for viruses penetration in cells, blocking this mechanism may have some marked impact on the rate in cell entry and thus on the disease course. It has also been conjectured that hydroxychloroquine may increase the intracellular concentration of zinc ions which inhibit the RNA dependent RNA polymerase protein used by the virus to replicate [26].

Unfortunately, all attempted randomized clinical trials (RCTs) of hydroxychloroquine on hospitalized patients were doomed to failure for several logical reasons that are quite obvious at the present time; some were politically convoluted [27-31]. Rationality had escaped the mind of many clinicians when it was decided by prominent institutions to conduct these trials. Apart from the obvious impossibility of their implementation and completion in a time period as short as 2 to 3 months, RCTs that were initiated have failed for reasons easy to understand: (a) they were organized in a rush; (b) the variety of the Covid-19 disease parameters at inclusion precluded the standardization of the trialed treatments because their effectiveness largely depended on the quality of accompanying cares; (c) the timing of the treatment as well as the possible use of comedications that turned out to be crucial parameters impossible to define beforehand; (d) patients have flocked to hospitals at varying stages of severity with a large number of comorbid factors (age, obesity, diabetes mellitus, arterial hypertension, active cancer, immunodeficiency,...); thus some studies included patients at a late stage of the disease, with respiratory failure, just before their admission in intensive care unit (ICU), at a time where antiviral treatment is not anymore effective; (e) clinical practice was heterogeneous across hospitals; (f) the safe optimal dosage of HCQ was dramatically exceeded, at a toxic level, in *Recovery*, the large English multi-institutional trial [6]. This occurred just after a trial in Brazil [2] was officially aborted due to a dramatic number of toxic deaths resulting from an absurdly high dose of chloroquine administered to the patients. Surprisingly, this happened even though the pharmacokinetics of HCQ had been previously published in a peer-reviewed international journal [9]. In short, all of the conditions that allow randomized trials to measure a benefit in cancer research were not met for an infectious disease that can kill a person in less than 2 to 3 weeks after the onset of symptoms. The proponents of RCTs did not realize the ethical impossibility of carrying out such trials on patients at risk of rapid death. Unlike clinical cancer trials, where patients in the control arm receive the best standard treatment available, in Covid-19 no standard treatment existed beforehand, so practitioners found themselves morally bound to use the treatment they thought appropriate, consistently with article 37 of the 2013 Helsinki declaration [32] given the best medical knowledge of the time.

In the Covid-19 randomized trials, the problem of the control arm, coyly referred to as standard of care (SOC), was that basically no specific treatment was administered, as for a placebo group. This methodology is clearly against the ethical rules in conducting research in humans, including during health crisis such as epidemics. The Steering Commitee on Bioethics of the Council of Europe stated in a document issued in 2012 : “placebo may only be used as the control method under strict conditions – i.e., when there are no methods of proven effectiveness, or when withdrawal or withholding of such methods does not present an unacceptable risk or burden. Consequently, the Research ethics committees should pay particular attention to the foreseeable risk or burden. No other reasons would be ethically acceptable” [33]. Although, not legally binding, it is clearly recognized in a work document of the Council for International Organizations of Medical Sciences (CIOMS)(established jointly by the World Health Organization and UNESCO in 1949) that these ethical rules cannot be abrogated in times of natural catastrophes like an epidemic : “conducting research in these situations raises important challenges such as the need to generate knowledge quickly, maintain public trust, and overcome practical obstacles to implementing research. These challenges need to be carefully balanced with the need to ensure the scientific validity of the research and uphold ethical principles in its conduct” [34].

The IHU-Méditerranée Infection in Marseille (France) is both a university medical and research institute on infectious diseases, especially founded in 2011 to deal with the outburst of a pandemic such as Covid-19. From March 2020 to December 2021, it has provided 30,423 Covid-19

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patients with state-of-the-art level of care. This is a very large cohort treated with HCQ in combination with azithromycin (AZ), in a coherent manner at a single public institution. We rationally analyze in retrospect the data made public with statistical tools broadly used to infer efficacy of treatment in observational cohorts. The IHU-Méditerranée medical teams recently attempted to publish their results [35,36], but a form of modern medical inquisition forced them to withdraw their preprint. However, they allowed public access to their data to be freely re-analyzed by other researchers. While encouraging independent analyses, they finally published their results with an additional American author cardiologist, Peter McCullough [37]. They have since been subject to renewed pressure from the University ethical authorities. It is therefore paramount that this data set is made available and analyzed by external sources to prevent political pressures to affect scientific evidence to emerge.

Methods

The monocentric retrospective cohort of 30,423 COVID-19 patients of IHU-Méditerranée Infection was downloaded from a public depository site DRYAD [35,36,38]. A subset consisting of the patients having received either HCQ and AZ on an intent-to-treat basis (meaning that HCQ-AZ was possibly interrupted followed by subsequent ivermectin) or no specific treatment (no HCQ, no AZ and no ivermectin (ICM)) was extracted. This subset is refered to as the "HCQ-AZ" subset. A second subset, refered to as the "AZ" subset, consisting of the patient having received no HCQ but AZ, possibly associated with IVM, or no specific treatment (no HCQ, no AZ and no ivermectin (ICM)) was extracted as well.

Baseline characteristics, including all the variables (also indistinctly called covariates) at disposal, were established for the two subsets and Chi-square tests were calculated to evaluate imbalance in the percentage of occurrence of every baseline demographic variable, disease severity and disease cofactors between treatment and no treatment. Detailed analysis of the "period" and "variant" categorical variables was performed. Variables assessing treatment effect were constructed at 42, 56, 90 and 640 days following admission at the IHU-Méditerranée either as outpatient (ambulatory care) or as inpatient (hospitalized patient), the first day was the day of admission. These variables were set either to 0 by default or to 1 when death or transfer to the intensive care unit (ICU) occurred in the period of time considered.

The software package R version 4.3.1 [39] was used to perform multivariate analysis using logistic models regressed on the baseline covariates. All baselines variables were treated as binary variables, except for "Age", "Period" and "Variant" that were treated as categorical variables. "Age" categories were 1: < 50 years, 2: [50 – 69] years, 3: [70 – 89] years and 4: > 89. "Period" categories were 1: March 3, 2020 – June 15, 2020; 2 : June 16, 2020 – Sep. 20, 2020; 3 : Sep. 21, 2020 – Nov. 20, 2020; 4 : Nov. 23, 2020 – March 21, 2020; 5 : March 22, 2021 – June 21, 2021; 6 : June 28, 2021 – Sep. 21, 2021, 7 : Sep. 22. 2021 – Dec. 21, 2021."Variant" categories were 1: Wuhan, 2: Marseille 4, 3: UK, 4: Delta, 5: other variants, 6: variant not determined.

R is designed to deal with categorical variables by evaluating each category inside a categorical variable as a binary variable. All missing data for the covariates "ivermectin", "diabetes" (diabetes mellitus), "obesity", "high blood pressure", "asthma", "COPD" (chronic obstructive pulmonary disease), "cancer", "immunodeficiency", "auto-immune diseases" and "chronic cardiac diseases" were replaced by the numerical mean value of the corresponding variables as it is best recommended practice in such a situation. Unless stated otherwise, the variable "sex" was set to 0 for men and 1 for women. Imbalances between baseline covariates were adjusted using the propensity score method provided by the MatchIt package of R [39-41] with the Optimal Full Matching option "full" and with the logistic probability law (R default option: link="logit").

The independence of covariates was assessed with overlap percentage tables. Logistic regression was run with the generalized linear model 'glm' function of the 'survival' library of R [42,43]. Calculations were performed including all baseline covariates (model(a)). Unadjusted and adjusted odds ratios were calculated at 42, 56, 90 and 640 days. Sensitivity analysis was performed to assess the effect of excluding disease aggravating covariates from the logistic regression was also explored in model (b) and model (c) (sensitivity analysis). In models (b) disease aggravating factors ("asthma", "cancer", "immunodeficiency", "auto-immune diseases" and "chronic cardiac diseases") were removed and in models (c) both risk factors covariates ("diabetes", "obesity", "high blood pressure") and disease aggravating factor covariates were removed. Sensitivity analysis was also performed using reduced subsets to explore the treatment effect on age and sex. All possible interactions between treatment and covariates were tested. Complete analysis is presented only for the cutoff value set at 56 days. All R command lines used are explicitly listed in Appendix 4 (supplementary material).

Results

Baseline characteristics

Baseline characteristics of the subset "HCQ-AZ" are collected in Table 1 showing imbalances ($p < 0.001$) for most of the variables and risk factor covariates between the treatment and control groups. The detailed reasons why some patients did not receive HCQ nor AZ is explained in the supplementary material of a previously published paper of the

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IHU-Méditerranée teams [17]. Consent for the prescription of hydroxychloroquine for its legal "off-label" use was requested from each patient after possible contraindication assessed. The most frequent contraindications to AZ were allergies to macrolides and comedication with colchicine.

Analysis of the Variables (covariates) Independence

Table 2 presents the detailed analysis of the categorical variable "Variant" in the subset "HCQ-AZ". Here, "events" represents both deaths and ICU admissions within 56 days. It shows that the "Variant" variable presented a relatively homogeneous distribution across the different variants that have appeared during the pandemic. Except for the "null" category (data missing or not determined) which was multicorrelated to other categories, the variant categories were independent from each other and thus the categorical variable "Variant" is well suited for logistic regression.

Table 3 shows that the categorical variable "Period" delineating distinct periods along the 21 consecutive months, recorded in the pandemic course, is not appropriate to enter the logistic regression model because it is strongly correlated to the categorical variable "Variant". Attempts to include it resulted in the impossibility to obtain convergence of constructed models and led to aberrant results.

There was a large chunk of missing data for the covariate describing disease aggravating factors in the dataset of IHU-Méditerranée. In fact, for the "Period" category ≤ 3, all these covariates have null values. This had an impact on the logistic regression model including all variables. Constructing a model for the "HCQ-AZ" subset reduced to the "Period" covariate > 3 could not be achieved with including the "inpatient" covariate that described the severity of the disease at inclusion. Table 4a shows that these covariates were actually highly correlated among themselves. 45.6%

Table 1: Baseline patients characteristics for subset "HCQ-AZ"

	All		HCQ-AZ		no HCQ no AZ no IVM		p-value*
	N	%	N	%	N	%	
	24943	100	23172	92.9	1771	7.1	
Men	11920	47.8	11077	47.8	843	47.6	0.939
Women	13023	52.2	12095	52.2	928	52.4	0.943
Age Categories (years)							
1. < 50	14052	56.3	12981	56	1071	60.5	0.06
2. 50-69	8639	34.6	8154	35.2	485	27.4	< 0.001
3. 70-89	2118	8.5	1934	8.3	184	10.4	< 0.001
4. > 89	134	0.5	103	0.4	31	1.8	< 0.001
SARS-CoV-2 variants categories							
1. Wuhan	3790	15.2	3598	15.5	192	10.8	< 0.001
2. B.1.160 (Marseille 4)	3515	14.1	3176	13.7	339	19.1	< 0.001
3. B.1.7.7 (UK)	4162	16.7	3988	15.7	174	9.8	< 0.001
4. B.1.617.2 (Delta)	4375	17.5	4273	17.2	102	5.6	< 0.001
5. Others	2209	8.9	2000	8.6	209	11.8	< 0.001
6. Null	6892	27.6	6137	26.5	755	42.6	< 0.001
Period categories							
1: March 3, 2020 – June 15, 2020	3835	15.4	3637	15.7	198	11.2	< 0.001
2: June 16, 2020 – Sep. 20, 2020	2736	11	2292	9.9	444	25.1	< 0.001
3: Sep. 21, 2020 – Nov. 20, 2020	3216	12.9	2788	12	428	24.2	< 0.001
4: Nov. 23, 2020 – March 21, 2020	4839	19.4	4536	19.6	303	17.1	0.006
5: March 22, 2021 – June 21, 2021	4617	18.5	4393	19	224	12.6	< 0.001
6: June 28, 2021 – Sep. 21, 2021	3880	15.6	3752	16.2	128	7.2	< 0.001
7: Sep. 22. 2021 – Dec. 21, 2021	1820	7.3	1774	7.7	46	2.6	< 0.001
Outpatients only	22186	88.9	20642	89.1	1544	87.2	0.563
Inpatients only	2234	9	2037	8.8	197	11.1	0.003

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Out- and in-patients		523	2.1	493	2.1	30	1.7	0.264
Diabetes		709	2.8	674	2.9	48	2.7	0.453
High blood pressure		1513	6.1	1436	6.2	97	5.5	0.11
Obesity		2649	10.6	2553	11	112	6.3	< 0.001
Patient with ≥ 2 of the 3 factors above		854	3.4	813	3.5	41	2.3	0.194
Asthma		1014	4.1	984	4.2	30	1.7	< 0.001
COPD		154	0.6	143	0.6	11	0.6	1
Cancer		532	2.1	517	2.2	15	0.8	< 0.001
Immunodeficiency		239	1	228	1	11	0.6	0.17
Auto-immune diseases		705	2.8	683	2.9	22 1.2	-	< 0.001
Chronic cardiac diseases		241	1	230	1	11	0.6	0.161
Null		11500	46.1	10224	44.1	1276	72	< 0.001
ICU transfer		349	1.4	321	1.4	28	1.6	0.575
Death days	**42**	227	0.9	191	0.8	36	2	< 0.001
	56	237	1	196	0.8	41	2.3	< 0.001
	90	251	1	207	0.9	44	2.5	< 0.001
	640	324	1.3	270	1.2	54	3	< 0.001
Ivermectin		329	1.3	329	1.4	0	0	-
Number of events		532	2.1	467	2	65	3.7	< 0.001

*Chi-square test

Table 2: "Variant" variable analysis: "HCQ-AZ" subset at 56 days

Variant	Patients		HCQ-AZ		Events whole cohort		Events in control group
	N	(%)	N	(%)	N	(%)	N
Wuhan	3790	15.2	3598	94.1	88	2.3	5
Marseille 4	3515	14.1	3176	89.4	119	3.4	8
UK	4162	16.7	3988	95.1	83	2.4	9
delta	4375	17.5	4273	96.8	70	1.6	11
others	2209	8.9	2000	88.6	69	3.1	5
null	6892	27.6	6137	87.4	103	1.5	27
total	24943	100	23172	91.7	532	2.1	65

(77/169) of the patients in the group of patients presenting at least one aggravating factor and who experienced an event (ICU transfer or death within 56 days) had more than 1 aggravating factor. 76.2% (77/101) of the ICU transfer or death concerning patients with disease aggravating factors were actually inpatients.

Table 4b shows the intrication (overlap) between the "inpatient" variable, the risk factors and the disease aggravating factors covariates in the subset of the HCQ-AZ subset for which these covariates were informed.

Propensity Score Matching

The results of the propensity score matching (PSM) procedure using Optimal Full Matching ("full" option) to correct for imbalances between treatment and control in the number of patients in every subgroup of the subset are presented in Figure 1 for the "HCQ-AZ" and "AZ" subsets including all baseline covariates (model (a)). Figures of merit of the PSM calculations are presented in the Supplementary Material section (appendices 1a and 1b). Each subset required several minutes of central processing unit time (ca. 5 minutes). The Love plots calculated for "HCQ-AZ" and "AZ" subsets show the absolute standardized mean differences (ASMD) between treatment and control, before and after matching for each covariate (Figure 1). Ideally, ASMDs should approach 0.0 and be lower than the ASMDs for unmatched subgroups. When all covariates were included (model (a)) we noted

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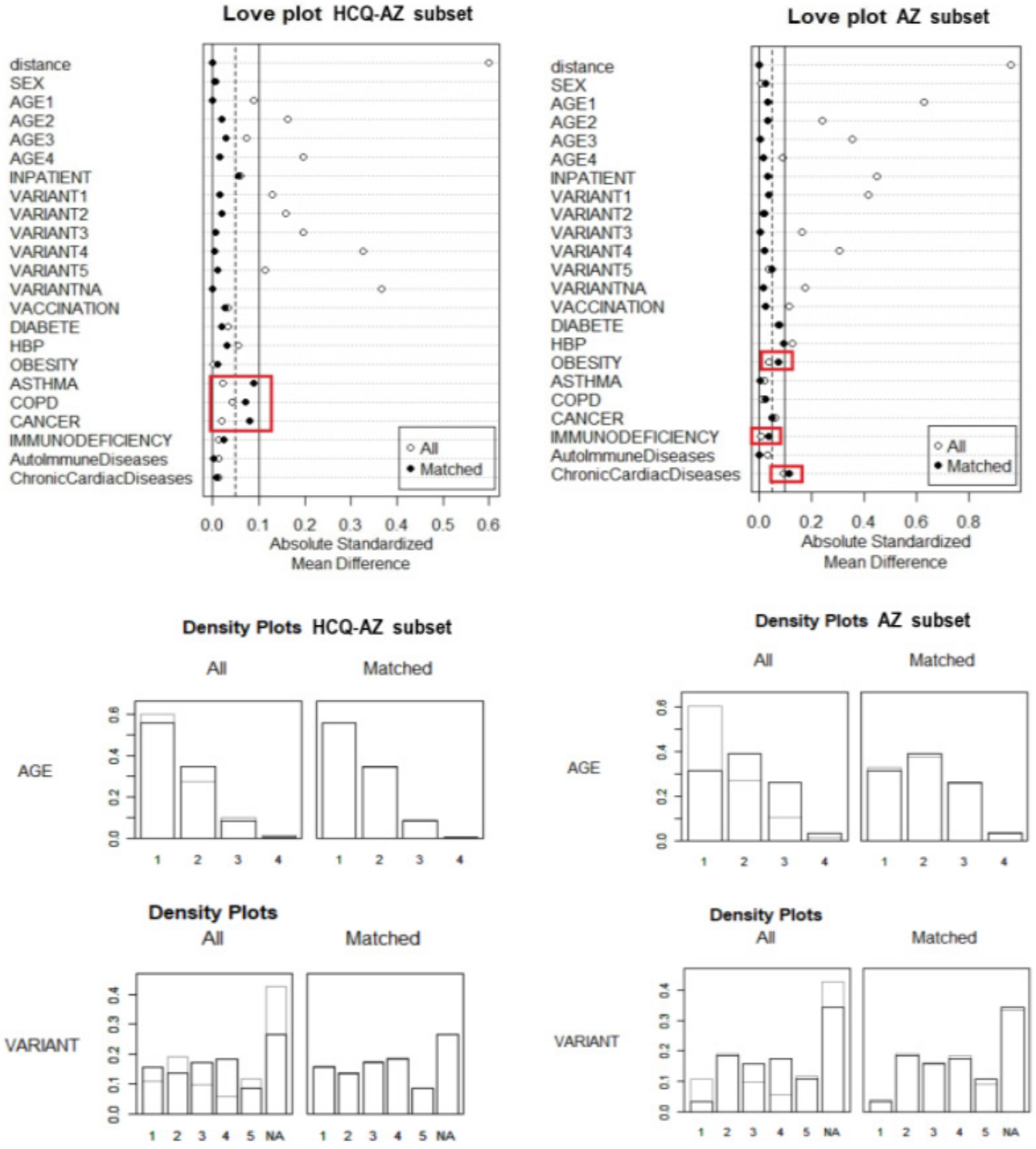

AGE1: < 50 years, AGE2: [50 - 69] years, AGE3: [70 - 89] years, AGE4: > 89 years, VARIANT1: Wuhan, VARIANT2: Marseille 4, VARIANT3: UK, VARIANT: Delta, VARIANT5; other types, VARIANTNA: not determined, HBP: High blood pressure, COPD;: Chronic obstructive pulmonary diseases

Figure 1: Propensity Score Optimal Full Matching

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that the procedure could not converge to improve balance for all covariates, some were in fact deteriorated (Asthma, COPD, cancer in the "HCQ-AZ" subset and Obesity, Immundeficiency and ChronicCardiacdiseases in the "AZ" subset). In fact, PSM allowed improvement in all covariate subgroups only when risk factors and disease aggravating were removed (data not shown). The reason is the high level of correlation between all these covariates (see previous section). Overall, all ASMDs ≤ 0.1 are usually considered as acceptable matching [42,43]. Here we have this condition fulfilled, especially for models (c) with ASMDs values < 0.05 insuring high reliability for this particular model (data not shown). Density plots are shown to illustrate the efficacy of the PSM balancing procedure for the categorical covariates "Age" and "Variant". Other covariates were well balanced as well (data not shown).

Logistic regression model - multivariate analysis

The logistic model regressed on all the IHU-Méditerranée baseline predefined covariates (except for "Period" that was excluded) converged with the full optimal matching PSM procedures. Unadjusted and adjusted ORs for evaluating treatment effect in the treated group (ATT) were calculated for model (a) at 42, 56, 90 and 640 days cutoffs, respectively, with similar results (Table 5a). A slight absolute decay of about 5% between 56 days and 640 days cutoffs is observed at 640 days cutoff (see Discussion section). Complete results at 56 days cutoffs of the multivariate logistic regressions with adjusted ORs at 56 days cutoff using are presented for models (a) of the "HCQ-AZ" and "AZ" subsets in Table 5b, and in tables 6a, 6b.

Table 3: "Period" variable analysis for the "HCQ-AZ" subset (events at 56 days cutoff)

Period[1]	Number of patients N	Variant type			Patients with HCQ-AZ		Events		Events in control
		Type	N	%	N	%	N	%	
1	3835	W	3790	98.8	3637	94.8	83	2.3	5
		Mar-04	0	0					
		UK	0	0					
		Delta	0	0					
		Others	8	0.2					
		Null	37	1					
2	2736	W	0	0	2292	83.8	51	2.2	3
		Mar-04	781	28.5					
		UK	1	0					
		Delta	0	0					
		Others	825	30.2					
		Null	1129	41.3					
3	3216	W	0	0	2788	86.7	59	2.1	12
		Mar-04	943	29.3					
		UK	0	0					
		Delta	0	0					
		Others	379	11.8					
		Null	1894	58.9					
4	4839	W	0	0	4536	93.7	122	2.7	10
		Mar-04	1684	34.8					
		UK	845	17.5					
		Delta	3	0					
		Others	608	12.6					
		Null	1699	35.1					
5	4617	W	0	0	4393	94.5	78	1.8	13
		Mar-04	107	2.3					
		UK	3276	71					
		Delta	10	0.2					
		Others	312	6.8					
		Null	912	19.8					

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6	3880	W	0	0	3752	96.7	36 1.0	11
		Mar-04	0	0				
		UK	40	1.1				
		Delta	2900	74.7				
		Others	4	0.1				
		Null	936	24.1				
7	1820	W	0	0	1774	97.5	38 1.5	11
		Mar-04	0	0				
		UK	0	0				
		Delta	1462	80.3				
		Others	73	4				
		Null	285	15.7				

[1]Period 1 (March 3, 2020 – June 15, 2020); 2 (June 16, 2020 – Sep. 20, 2020); 3 (Sep. 21, 2020 – Nov. 20, 2020); 4 (Nov. 23, 2020 – March 21, 2020); 5 (March 22, 2021 – June 21, 2021); 6 (June 28, 2021 – Sep. 21, 2021); 7 (Sep. 22. 2021 – Dec. 21, 2021); Mar 4 = Marseille 4 variant.

Table 4a: Events distribution at 56 days for disease aggravating covariates

	"HCQ-AZ" subset reduced to "Period" ≥ 4							
	HCQ-AZ				**No HCQ no AZ no IVM**			
	N	**%**	**Events**	**%**	**N**		**Events**	**%**
"HCQ-AZ" subset	14455		274	1.9	701		45	6.4
Inpatient	1346	9.3	246	18.3	207	29.5	39	18.8
Covariates								
Null	1507	10.4	154	10.2	206		36	17.5
Diabetes	674	4.7	27	4	35	5	2	5.7
Obesity	2553	17.66	58	2.3	96	13.7	4	4.2
High blood pressure	1436	9.93	43	3	77	10.9	3	3.9
Asthma	984	6.8	7	0.71	30	4.3	1	3.3
COPD[1]	143	0.99	5	3.49	11	1.6	2	18.2
Cancer	517	3.57	9	1.74	15	2.1	2	13.3
Immunodeficiency	228	1.58	4	1.75	11	1.6	2	18.2
Auto-immune diseases	683	4.72	6	0.88	22	2.1	0	NA
Chronic cardiac Diseases	230	1.59	10	4.35	11	1.6	1	9.1
Total redundant number of events[2]			169				17	
Total actual number of events for patients at risk			92				9	
Inpatient at risk[3]	495	3.42	74	14.9	25	3.6	3	12

[1]Chronic obstructive pulmonary disease

[2]A number of patients that experienced a defined event (ICU transfer or death with 56 days) had more than 1 baseline aggravating factors.

[3]Inpatients with at least one disease aggravating cofactor.

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Table 4b: Analysis of risk factors and diseases aggravating covariates overlap percentages[1] (N=13507)

		1	2	3	4	5	6	7	8	9	10	A1	A2	A3	A4
1: Inpatient	763		137	287	223	74	63	27	22	25	41	190	398	200	5
	%		19	38	29	9.7	12	17	9	4.6	16.6	24.9	52.2	26.2	19
2: Diabetes	722			282	343	65	61	22	36	45	74	155	381	182	4
	%			39	48	9	11	14	15	8.3	30	21.4	52.8	25.2	15
3: Obesity	2665				564	268	112	36	64	153	61	1434	1045	184	2
	%				37	26	21	23	26	28.3	24.7	53.8	39.2	18.4	7.4
4: HBP[2]	1533					123	120	50	52	111	94	238	880	401	14
	%					12	22	32	21	20.1	38.1	18.3	57.4	40.1	52
5: Asthma	1020						34	23	35	52	8	664	291	65	0
	%						6.3	15	14	9.6	3.2	65.1	28.5	6.5	0
6: Cancer	541							12	36	62	23	134	284	121	2
	%							7.7	15	11.5	9.3	24.8	52.5	22.4	7.4
7: COPD[3]	156								9	8	14	27	87	41	1
	%								5.8	5.1	9	17.3	57.8	26.3	3.7
8: Immuno-deficiency	244									36	12	92	117	34	1
	%									14.8	4.9	37.7	49	13.9	3.7
9: Auto-immune	540										10	299	323	85	0
	%										4	55.4	59.8	15.7	0
10: Chronic cardiac	247											25	135	81	6
	%											10.1	54.7	32.8	22

[1]Percentages calculated with respect to the minimum value of the overall number of occurrences between the two compared items (the color code is light orange for ovelap in the range [10-20]%, orange in the range [20-30]% and red above 30%); A1 = age < 50 years (n=7659), A2 : age 50 to 69 years (n=4822), A3 : age 70 to 89 (n=999); A4 : age > 89 years (n=27)

[2]High blood pressure

[3]Chronic obstructive pulmonary disease

Table 5a: Logistic regression models treatment ORs calculated for HCQ-AZ subset at different time cutoffs (PSM with Optimal Full Matching)

	HCQ-AZ subset[1] (N = 24943)					
Cutoff (days)	unadjusted after PSM			adjusted after PSM		
	OR	95%CI	p-value	OR	95%CI	p-value
42	0.52	[0.363 ; 0.744]	< 0.001	0.457	[0.353 ; 0.596]	< 0.001
56	0.499	[0.343 ; 0.727]	< 0.001	0.419	[0.327 ; 0.539]	< 0.001
90	0.506	[0.347 ; 0.737]	< 0.001	0.425	[0.332 ; 0.547]	< 0.001
640	0.542	[0.380 ; 0.771]	< 0.001	0.474	[0.372 ; 0.606]	< 0.001

[1]subset with in 1.4% of the cases HCQ-AZ interrupted and subsequent ivermectin

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Table 5b: Treatment ORs for the different logistic regression models calculated at 56 days cutoff using PSM with Optimal Full Matching

	"HCQ-AZ" subset					
Model	OR	95%CI	p-value	OR	95%CI	p-value
	unadjusted after PSM			adjusted after PSM		
HCQ-AZ[1] (N = 24943)	0.499	[0.343 ; 0.727]	< 0.001	0.419	[0.327 ; 0.539]	< 0.001
AZ[2] (N = 6349)	0.72	[0.574 ; 0.905]	0.005	0.727	[0.608 ; 0.870]	< 0.001

[1]subset with in 1.4% of the cases HCQ-AZ interrupted and subsequent ivermectin
[2]subset with ivermectin associated to AZ in 31.3% of the cases

Table 6a: Multivariate logistic regression model (a) for the "HCQ-AZ" subset[1] (N = 24943) at 56 days cutoff

	"HCQ-AZ" subset[1]		
Treatment factor	**OR[2]**	**95%CI**	**p-value**
HCQ-AZ	0.419	[0.327 ; 0.539]	< 0.001
Demography			
Sex (female/male)	0.425	[0.357 ; 0.504]	< 0.001
Age < 50 yrs (reference)			
Age 50-69 (vs ref.)	2.535	[1.981 ; 3.271]	< 0.001
Age 70-89 (vs ref.)	5.245	[4.042 ; 6.862]	< 0.001
Age > 89 (vs ref.)	9.304	[5.664 ; 14.434]	< 0.001
Disease severity			
Inpatient	55.168	[43.109 ; 71.599]	< 0.001
Variant			
A (Wuhan) (reference)			
B.1.160 (Mars 4) (vs ref.)	1.276	[0.988 ; 1.651]	0.063
B.1.7.7 (UK) (vs ref.)	1.275	[0.963 ; 1.687]	0.089
B.1.617.2 (Delta) (vs ref.)	1.216	[0.904 ; 1.633]	0.193
Others (vs ref.)	1.359	[1.010 ; 1.824]	0.042
Null (vs ref.)	0.844	[0.651 ; 1.095]	0.201
Vaccination			
Vaccinated	0.928	[0.568 ; 1.466]	0.755
Comorbidities			
Diabetes	1.144	[0.781 ; 1.652]	0.48
Obesity	1.97	[1.489 ; 2.592]	< 0.001
High blood pressure	0.995	[0.725 ; 1.353]	0.976
Asthma	0.777	[0.434 ; 1.313]	0.369
COPD	3.197	[1.730 ; 5.682]	< 0.001
Cancer	0.772	[0.434 ; 1.304]	0.353
Immunodeficiency	3.119	[1.527 ; 5.995]	0.001
Auto-immune diseases	0.792	[0.393 ; 1.470]	0.486
Chronic cardiac diseases	1.095	[0.612 ; 1.889]	0.752

[1]with interruption and subsequent ivermectin in 1.4% of the cases
[2]if not indicated otherwise ORs are with respect to covariates values set to zero

Table 6b: Multivariate logistic regression model (a) for the "AZ" subset (N = 6349) at 56 days cutoff

	"AZ" subset[1]		
Treatment factor	**OR[2]**	**95%CI**	**p-value**
AZ	0.727	[0.608 ; 0.870]	< 0.001
Demography			
Sex (female/male)	0.497	[0.419 ; 0.588]	< 0.001
Age < 50 yrs (reference)			
Age 50-69 (vs ref.)	2.359	[1.687 ; 3.359]	< 0.001
Age 70-89 (vs ref.)	5.161	[3.714 ; 7.316]	< 0.001
Age > 89 (vs ref.)	7.869	[5.238 ; 11.975]	< 0.001
Disease severity			
Inpatient	40.444	[29.130 ; 57.850]	< 0.001
Variant			
A (Wuhan) (reference)			
B.1.160 (Mars 4) (vs ref.)	1.196	[0.826 ; 1.755]	0.351
B.1.7.7 (UK) (vs ref.)	1.437	[0.955 ; 2.183]	0.084
B.1.617.2 (Delta) (vs ref.)	1.586	[1.076 ; 2.366]	0.021
Others (vs ref.)	1.349	[0.888 ; 2.804]	0.164
Null (vs ref.)	1.92	[1.334 ; 2.804]	< 0.001
Vaccination			
Vaccinated	0.472	[0.277 ; 0.781]	0.0044
Comorbidities			
Diabetes	1.055	[0.641 ; 1.706]	0.83
Obesity	1.749	[0.641 ; 1.706]	0.004
High blood pressure	0.703	[0.469 ; 1.044]	0.084
Asthma	0.935	[0.460 ; 1.768]	0.843
COPD	4.012	[1.856 ; 8.252]	< 0.001
Cancer	1.175	[0.600 ; 2.177]	0.621
Immunodeficiency	4.616	[2.038 ; 9.976]	< 0.001
Auto-immune diseases	0.321	[0.096 ; 0.828]	0.035
Chronic cardiac diseases	0.414	[0.215 ; 0.756]	0.006

[1]in association with ivermectin in 31.3% (1434/4578) of the cases
[2]if not indicated otherwise ORs are with respect to covariates values set to zero

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When the covariate "inpatient" was excluded from the calculations the logistic regression converged and results were similar to those obtained for the complete "HCQ-AZ" subset, with respect to unadjusted and adjusted ORs for the treatment effect (data not shown). However, somewhat more spurious ORs were obtained for covariate such as "age" category 4 (age > 89 yrs) (data not shown), indicating that the "inpatient" status was a variable that dominated the mathematical stability of the constructed models.

In all models, calculated ORs evaluating the average treatment effect on treated patients (ATT) were statistically significant demonstrating efficacy not only of the HCQ-AZ treatment but also of AZ possibly associated with IVM. Adjusted treatment ORs were statistically significant, 0.42 and 0.73, respectively, with model (a) at 56 days cutoff, with respect to no treatment (no HCQ, no AZ and no IVM), that is to say a reduction of the risk of being transferred to ICU or dying reduced by 58% and 27%, respectively. In this respect, although relatively sensitive to the inclusion of risk factors and comorbidities covariates in the models (see OR results for models (b) and (c) in sensitivity analysis, Table 8), the general trend of the HCQ-AZ treatment effect administered as intent-to-treat, taking into account ORs calculated at 42 days and 56 days cutoiff (Table 5a), is clearly around 54% to 58% risk reduction of ICU transfer or death (keeping in mind that model(a) that includes all confounders is the more accurate).

Analysis of the covariates ORs (calculated with respect to their reference value and independently of other covariates values) showed that the variables "sex", "age" and "inpatient", describing the patient demographic characteristics and disease severity, all had a statistically significant impact ($p < 0.001$). Of note, for women ("sex =1") a statistically significant reduction in the risk of experiencing an event (either being transferred to ICU or dying) was observed with respect to men (not with respect to no treatment) with an OR = 0.43 which corresponds to a risk reduction of 57%. For age categories ≥ 50 years, a statistically significant increase in the risk of event (OR > 1) was observed for each category with respect to patients of age < 50 years.

In the HCQ-AZ subset, OR = 55.2 for the covariate "inpatient" in model (a) means that hospitalized patients had 55 times more chances to be transferred to ICU or to die than ambulatory (day hospital) patients. As for the "variant" categorical variable, relative instability was observed between models. Results for models (b) and (c) are presented in appendices 2a and 2b in supplementary material. Instability in the models reflects the lack of independence between covariates (presence of partially confounding variables). Model (a) that includes all potential confounders must be considered the more reliable one in interpreting the results.

The calculated models consistently detected statistically significant interactions of some covariates with the treatment in the "HCQ-AZ" subset : favorable for men (with respect to women), for age categories ≥ 50 years, variant not determined ("Variant" category "null"), COPD, cancer.

Sensitivity analysis

To assess the uncertainty pertaining to our logistic regression models, we performed additional sensitivity calculations by excluding comorbidities (model (b)) and comorbidities and risk factors (model (c)). Complete results for this two models are provided in appendices 3a and 3b of supplementary material. We observed that removing the comorbidities (model (b)) doesnot markedly change the treatment OR nor the remaining covariate ORs. Interestingly, a favorable interaction between HCQ-AZ and Inpatient appeared in model (b), reflecting the fact that this covariate is a confounders for comorbidity covariates. Reducing the HCQ-AZ subset to different age < 50 years showed that efficacy of HCQ-AZ cannot be measured for this age category due to to few events recorded. Finally, an absolute difference of 6% was recorded between men and women in terms of treatment OR reflecting the favorable interaction observed between the treatment and male sex.

Table 7: Logistic regression model (a) – treatment: covariate interactions detected in "HCQ-AZ" subset[2]

Interaction[1]	OR	95%CI	p-value
	"HCQ-AZ" subset[2]		
HCQ-AZ:SEX (men/women)	0.391	[0.196 0.748]	0.006
HCQ-AZ:AGE2	0.25	[0.078 0.690]	0.012
HCQ-AZ:AGE3	0.061	[0.018 0.182]	< 0.001
HCQ-AZ:AGE4	0.096	[0.015 0.557]	0.009
HCQ-AZ:VARIANT3	0.194	[0.059 0.618]	0.006
HCQ-AZ:VARIANT6	0.125	[0.046 0.323]	< 0.001
HCQ-AZ:OBESITY	0.105	[0.027 0.398]	< 0.001
HCQ-AZ:COPD	0.024	[0.004 0.143]	< 0.001
HCQ-AZ:CANCER	0.076	[0.010 0.865]	0.019
HCQ-AZ:IMMUNODEFICIENCY	0.038	[0.003 0.664]	0.022

[1]calculations performed at 56 days cutoff; HCQ = hydoxychloroquine, HCQ:SEX interaction value calculated for men with respect to women; AGE3 = age in the interval [70 – 89] years; AGE4 = age ≥ 90 years; COPD = Chronic Obstructive Pumonary Diseases ; VARIANT3 = UK ; VARIANT6 = Null.
[2]with interruption and subsequent ivermectin in 1.4% of the cases.

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Table 8: Sensitivity analysis: Logistic regression treatment ORs for different subsets calculated at 56 days cutoff

Subset	OR	95%CI	p-value	OR	95%CI	p-value
	unadjusted after PSM			adjusted after PSM		
HCQ-AZ[1] model (b)	0.536	[0.408 ; 0.703]	<0.001	0.392	[0.301 ; 0.516]	< 0.001
HCQ-AZ[1] model ©	0.524	[0.362 ; 0.760]	<0.001	0.433	[0.330 ; 0.572]	<0.001
HCQ-AZ[1] (age < 50 years) (N = 14052)	0.573	[0.055 ; 5.97]	0.641	na	na	na
HCQ-AZ[1] (age ≥ 50 years) (N = 10891)	0.467	[0.055 ; 5.97]	<0.001	0.379	[0.274 ; 0.582]	<0.001
HCQ-AZ[1] men (N = 11920)	0.48	[0.385 ; 0.599]	<0.001	0.389	[0.281 ; 0.542]	<0.001
HCQ-AZ[1] women (N = 13023)	0.435	[0.295 ; 0.643]	<0.001	0.45	[0.278 ; 0.750]	0.002
HCQ-AZ[2] (N = 24614)	0.528	[0.407 ; 0.686]	< 0.001	0.409	[0.313 ; 0.537]	< 0.001
AZ[3] (N = 4915)	0.693	[0.561 ; 0.857]	< 0.001	0.609	[0.490 ; 0.757]	< 0.001

[1]subset with in 1.4 of the cases HCQ-AZ interrupted with subsequent ivermectin
[2]subset excluding patients having received ivermectin subsequent to HCQ-AZ
[3]subset excluding patients having received ivermectin in association with AZ (31.3% of the cases)

Discussion

Despite some imperfections inherent to the nature of the collected data and of the statistical model used, detailed analysis of this exceptionally large single institution cohort has provided us with a unique opportunity to cast a close look on a coherent corpus of data in terms of medical practice. The main concern in this article is the evaluation of the IHU-Méditerranée treatment which was the combined treatment HCQ + AZ. In the analysis, we kept the intent-to-treat aspect of the data by not excluding the 1.4% of the patients who received ivermectin as a second intention, subsequent to HCQ-AZ interruption. This way, we have avoided to exclude the situation where the disease was aggravated. All univariate and multivariate models we constructed showed a significant efficacy of HCQ-AZ in male patients for categories of age ≥ 50 years. For age < 50 no effect could be measured due to too few events. There were only 0.7% and 0.2% of events recorded in the men and women subsets, respectively, for the category of age < 50 years. That does not mean that the treatment may be without effect in preventing long Covid, reducing the length of symptoms, and accelerate healing in this patient population prone to recover from Covid-19 infection without treatment. In the studied subsets, vaccination was not associated with a reduction of the risk of event and no interaction was detected between vaccination and the HCQ-AZ treatment nor the AZ treatment. A favorable statistically significant interaction with HCQ-AZ was measured when the variant type was not determined ("Variant" category "null"). In fact, the variant category "null" may have actually reflected a category of patients with milder diseases, which was not an incentive for determining the variant type. Importantly, no interaction was detected between HCQ-AZ and vaccination in all the subsets analyzed. The respective merits of these approaches for combined therapy or vaccination must be assessed taking into account their costs, their easiness to administrate and rapidity of access, as well as their short term and long term side effects (benefit/risk ratio).

Results of interactions detected between treatment and covariates must be considered with some caution (possibility of model bias or instability due to partially confounding covariates) unless they consistently appear across different models. It seems HCQ-AZ had a favorable interaction for age categories > 49 years, for patients with obesity, COPD, cancer, immunodeficiency and for male patients. The latter interaction possibly results from the fact that women are more careful to their health condition and treat themselves generally earlier than men. Especially, young men have a tendency to delay their search for treatment when they are sick and thus, receive treatment on average later than women or older men. Overall they survive by themselves better than men. Thus, the HCQ-AZ treatment is more prone to show efficacy in men provided it is administered before the disease is irreversibly aggravated. It was reported that AZ may lead to untoward side effects in the elderly women indicating that this antibiotic should perhaps be administered with additional precaution to this category of patients [44]. However, sensitivity analysis performed on the HCQ-AZ subset reduced to women shows a statistically very significant treatment effect in women. The "doubly robust" method we have used (propensity score matching plus multivariate regression on baseline covariates) is particularly well suited the assert causal treatment inference in observational studies which by nature are non randomized [40,45,46]. The IHU-Méditerranée Infection dataset insured that some of the conditions of applicability were fulfilled: i.e. the dataset was sufficiently large to be commensurate with the use of quite a number of baseline covariates (16

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covariates were considered) known for their potential effects on the disease outcome. Propensity score matching (PSM) is well suited for observational studies as it is aimed at reducing the treatment assignment bias, eliminating the effect of confounding variables, and mimic randomization. One difficulty of applying PSM resides in the dimensionality of the problem to be solved computationally, especially when covariates are quite intricated (multicorrelated to some relatively high degree). It increases exponentially with the number of covariates, some of which may be categorical. This often precludes the 'full' matching option of the R software package from being performed because of the limited RAM of laptop computers. Beyond the lack of computational power, the impossibility of acceptably converging PSM exists due to variables correlation. There exists a fast "quick" matching option of R, using Fast Full Generalized Matching, designed to partially palliate the problem of computational power [47,48]. However, in our case this option produced quite different results, with treatment ORs shifted by an absolute non negligible difference of about 4%, and differing interactions as well, compared with Optimal Full Matching ("full" option). It is thus advocated to use the more accurate Full Optimal Matching, modern computers with large RAM memory allowing it.

Another potential drawback is that the propensity scores may not approximate accurately enough the real probabilities for each subgroup of patients considered to have received treatment. Additionally, covariates potentially describing effects on the treatment outcome should vary monotonically. Whereas it is the case for binary variables such as "Sex" and "ICU", it is not necessarily the case with covariates such as "Age", "Period" and "Variant" which can in fact be treated as separated categorical covariates using the first category as reference. The two variables "Period" and "Variant" were strongly correlated to each other and including both of them in the model did not allow its convergence. The main problem hampering PSM is the fact that some covariates may not be sufficiently independent from others causing instability in the calculated covariate ORs. In our case, the risk factors and other disease aggravating covariates are strongly correlated among themselves and with the "inpatient" status. Their effect may be seriously intricated with regard to the production and interpretation of a multivariate regression model. This issue is reflected in the poor propensity score matching observed with Optimal Full Matching in the Love plot for "vaccination", "obesity", "asthma", "cancer". The main drawback in the IHU dataset is its incompleteness. Data for the period prior to Nov. 23, 2020 are missing for the 9 covariates describing the disease potential aggravating factors (comorbidities). Despite this problem, the procedure of replacing missing data by the mean values of their associated covariates alleviated efficiently the difficulty and allowed not only the "match it" algorithm of R to produce an acceptable result but also the convergence of the logistic regression using the 'glm' package. We provide in Appendix 4 in supplementary material the complete set of instructions we have used to perform the calculations with the R software package. Anyone willing to reproduce our results may do so very easily or be able to conduct further analysis and provide additional information.

Our approach followed the state-of-the-art practice broadly used for evaluating treatment in observational studies. It pleads in favor of the intent-to-treat ethical position of the physicians of the IHU-Méditerranée in managing Covid-19 patients. One limitation of our study is that all patients treated at the IHU-Méditerranée, who did not receive HCQ and AZ, due to not consenting or due to contraindications, were still treated [17] with zinc supplementation, anti-inflammatory medications, and anti-coagulants based on their risk profile, even on an outpatient basis, consistently with the recommendations by the McCullough protocol [49], but not consistently with the NIH guidelines for outpatients [50]. Thus, neutral results for HCQ and AZ for age < 50 years, with respect to the composite endpoint of ICU admission or death, do not necessarily extrapolate to a comparison between HCQ-AZ treatment group outcomes and the outcomes that would have happened in a counterfactual scenario where the same patients are treated in accordance with the NIH guidelines for outpatients. Many will argue that the level of confidence of our analysis cannot be considered at the same value of proof as for results from randomized clinical trials. But randomized trials have many pitfalls and cannot be conducted to completion in times of urgency for the reasons presented in the Introduction section. In general, weaknesses of RCTs can threaten their external validity [18,51]. Empirical evidence has previously shown that retrospective studies tend to give consistent effect size estimates with randomized controlled trials [52,53], thanks to the development of modern techniques that allow statistical adjustment for the known confounders. Randomized controlled trials are particularly well suited in the evaluation of new medications with unknown safety profiles, where the expected benefit for the patient requires a very strict statistical control due to its narrow margin of benefit and the fact that it is not obvious whether adverse outcomes are caused by the illness itself or the very deletereious safety profile of a toxic drug administered at repeated high doses (eg. cancer treatment evaluation). In contrast, the IHU protocol is based on medications with known acceptable safety, ICU admissions and deaths are clearly caused by the COVID-19 disease and not the attempted treatment (see Introduction section), and the risk factors for poor prognosis are well-known. The very large size of the IHU cohort further increases the level of confidence in our findings. All things being considered, the ORs we have calculated are reliably evidencing of a true treatment effect.

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It is important to note that many studies which failed to show the benefit of HCQ-based treatments did not follow the same protocol as that of the IHU-Méditerrranée, with either too low or too high a dose of hydroxychloroquine. A too high a dose could be toxic, as in the *Recovery* trial, by inhibiting interferon secretion and stimulating severe damage through pulmonary shunting [28,31]. Many cases of failure of HCQ-AZ treatment were the consequence of a prescription given too late in the course of the disease. It is indisputable that HCQ-AZ exhibit efficacy independently of vaccination in multivariate analysis with a sufficiently improved survival benefit for the category of age ≥ 50 years to preclude doubts on the reality of the measurement.

Conclusion

State-of-the-art statistical analysis of the IHU-Méditerranée data demonstrated the efficacy of the empirical treatment using a combination of hydroxychloroquine and azithromycin, given as an early treatment. Taking into account the very large size of the observational single-institution cohort of patients coherently treated, together with the quality of the statistical approach we used, these results pose a serious challenge to those who have continuously denied the potential efficacy of hydroxychloroquine-based treatment of Covid-19 patients during the pandemic. We have confirmed the validity of the intent-to-treat approach, in times of urgency, with a combination of medications reasonably thought, in early 2020, as having a potential efficacy on the disease at hand. This work should provide an incentive for other independent researchers to conduct further analysis, possibly with more advanced methods.

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