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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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