Hydroxychloroquine (HCQ) used to treat malaria and autoimmune diseases such as rheumatoid arthritis gained significant attention in early 2020 as a potential treatment for COVID-19 based on its in vitro antiviral properties against SARS-CoV and SARS-CoV-2

#Our reply

A reference supporting the claim of the *in vitro* antiviral properties would have allowed the reader to assess this claim. For example, no inhibitory effects on anti-SARS-CoV-2-mediated cytotoxicity or on viral load has been reported at "concentrations achievable by usual doses" (1).

and its favorable safety profile at recommended usual doses in acute and chronic use.

#Our reply

A safety profile might be "favorable" only when there is a demonstrated clinical benefit for the patient. Indeed, in the absence of a demonstrated clinical benefit, even a small risk of harm leads to an unfavorable risk benefit ratio (2,3).

HCQ was selected by the World Health Organization (WHO) and other medicines regulatory bodies for repurposing for COVID-19. For instance, in Belgium, off-label use of HCQ in monotherapy was recommended for hospitalized COVID-19 patients at a carefully selected dose based on limited pharmacokinetic models suggesting that a dosage of 400mg twice daily for 1 day, followed by 200 mg twice daily for another 4 days (i.e. a total of 2,400 mg in total over 5 days) should have sufficient antiviral activity.[1, 2] Several trials of different designs were conducted to investigate the efficacy and safety of HCQ for the prevention and/or the treatment of COVID-19 patients.[3] As the pandemic unfolded, conflicting findings emerged from clinical trials and observational studies. Some early studies indicated potential benefits, while others raised issues about the drug's safety and efficacy.[4]

#Our reply

The reference [4] has been published in 2023 and reported no benefit regarding the risk of hospitalization and the viral load in outpatients with confirmed COVID-19. It is unclear how this reference supports this sentence.

Indeed, concerns about potential adverse drug reactions, including heart rhythm abnormalities and increased mortality,[5, 6] had prompted regulatory agencies to caution against its use outside of controlled settings.[6] The WHO and other health authorities reversed their recommendations of the use of HCQ in COVID-19 patients based on accumulating evidence.[7-9]

#Our reply

The WHO did not recommend using HCQ in COVID-19 (see for example (4)).

Several manuscripts aimed at gathering the information available in published and unpublished data to demystify these conflicting results and claims.[3, 6-9] Recently, Pradelle et al. estimated the in-hospital mortality attributable to HCQ during the first wave of COVID-19 by combining the mortality rate, HCQ exposure, number of hospitalized patients, and the increased relative risk of death with HCQ.[10] The main finding of their study is that HCQ might have been associated with an excess of 16,990 deaths during the first wave of the COVID-19 pandemic in the 6 countries for which data were available. Such attributable risk analysis is associated with many limitations, some of which being identified by the authors.[10] However,

we want to point out the major limitation that their study did not adequately address dose-subgroup and sensitivity analyses which precludes any overall firm conclusions on in-hospital mortality attributable to HCQ.

The authors utilized the odds ratio (OR) published by Axfors et al., which encompasses 14 published and 15 unpublished trials as the estimator for HCQ-related mortality.[6] This meta-analysis reported an OR of 1.11 (95% CI 1.02; 1.20) and was based on 4,316 patients treated with HCQ and 5,696 controls. The outcomes reported by Pradelle et al. were heavily influenced by this effect size.[10]

#Our reply

This comment is not specific to our study; this assertion holds true for any modeling.

However, the significance of this effect size of 1.11 should be interpreted with caution and specific estimates based on the doses administered should be used. Indeed, two studies, namely WHO SOLIDARITY and RECOVERY, contribute to 88.9% of the weights in their overall model.[6] In essence, the pooled OR obtained from the meta-analysis is heavily influenced by these two specific trials. As highlighted by the authors themselves, RECOVERY and WHO SOLIDARITY employed HCQ in comparatively higher doses than all other trials, which may explain the increased OR observed while including them in the model.

#Our reply

In our study, we clearly highlighted the limitations in the precision of the estimation of HCQ effect by meta-analysis of Axfors et al. (5). However, this meta-analysis was based on an updated database of the worldwide available evidence of the treatment effect of hydroxychloroquine on mortality for COVID-19. It relied on randomized trials, enabling a relative estimate of the treatment effect with a lower risk of bias compared to non-randomized studies. Moreover, the weights of RECOVERY and WHO SOLIDARITY trials highlight that lower HCQ doses have not been assessed with reliable power in other trials.

To provide a nuanced analysis of the impact of this aspect on the overall results, we reiterated their meta-analysis and conducted a dose-subgroup analysis to assess whether using lower doses of HCQ (e.g., ≤2400mg/5 days or ≤4800mg/5 days) also significantly increased the risk of mortality across trials. As suggested above, the 'low-dose' HCQ regimen (2400 mg in total over 5 days) was recommended and used as a reasonable regimen for hospitalized patients.[1] Our analyses revealed that when pooling studies employing HCQ doses ≤2400mg/5 days (i.e., k=12, n patients treated with HCQ=947, n controls=745), an OR of 0.94 (95%CI 0.56; 1.59) was found (Figure 1A), indicating no increase in the mortality rate anymore. Importantly, there was no significant reduction in mortality rate with HCQ at ≤2400mg/5 days neither. The same observation held true when pooling studies employing HCQ doses ≤4800mg/5 days (i.e., k=25, n patients treated with HCQ=1672, n controls=1479) with an OR of 0.97 (95% CI 0.73; 1.29). Only high dose regimens of HCQ are associated with a significant increase in mortality (Figure 1A & Figure 1B).

#Our reply

This section is about the meta-analysis of Axfors et al. (5), not about our article. Nevertheless, we can comment that the authors mix up the absence of evidence with the evidence of absence in their interpretation. Indeed, the confidence interval they provided is compatible with an increased risk of death. Moreover, they reported non-significant p-value for the subgroup difference testing ($p_{interaction}$: 0.40 and 0.29 for

Figure 1A and 1B, respectively), which would suggest that the effect of HCQ on mortality is not significantly modified by its doses (the power of this test being probably low, however), therefore allowing to use the overall estimate.

Besides this methodological concern of applying an effect size found exclusively for high-dose studies to all patients, regardless of the dose they might have received, this OR of 1.11 has not been demonstrated to be robust. Indeed, Axfors et al.[6] did not conduct a leave-one-out analysis despite this sensitivity analysis is considered as a crucial methodological step to assess the robustness of a model. Interestingly, upon excluding either the WHO SOLIDARITY or the RECOVERY study from the model in leave-one-out analysis, the significance of the results is annulled (omitting WHO SOLIDARITY: OR 1.08 (95%CI:0.99; 1.19), omitting RECOVERY: OR 1.11 (95%CI:0.95; 1.30), plot available in Open Science Framework https://osf.io/ewudy/). The robustness of a meta-analytic model should be ensured through sensitivity analyses, and the significance of an effect size should not be attributable to solely one single trial. Furthermore, Axfors et al. ran additional sensitivity analyses to assess the robustness of their results across four different meta-analytic approaches (reported in their Appendix).[10]

#Our reply

Again this is about the article of Axfors et al. (5), it is unclear why the authors referred to our article (Ref [10] in their comment) for the appendix of Axfors et al.

From these results, it is noteworthy that only one of the meta-analytic approaches tested (i.e. the Hartung-Knapp- Sidik-Jonkman (HKSJ) model with the Paule-Mandel estimator for tau2) yielded to a statistically significant OR of 1.11, while the three other statistical approaches failed to demonstrate the statistical significance of the effect size.

#Our reply

Again this is about the article of Axfors et al. (5).

When embarking on a study of such public health interest, Pradelle et al.[10] should have ensured that the main effect size on which they based their analysis,[6] and which was consistently employed across their models to estimate the number of excess deaths, was robust and unbiased. Our reanalysis points out that this is not the case, rendering their results unreliable.[10]

#Our reply

See previous reply about the non-significant subgroup difference testings that the authors provided.

As a more general comment, the dosing regimen is critical in the development of new medicines and mainly for drug repurposing in the absence of already available robust clinical data. Underdosing and overdosing may lead to a lack of efficacy, and adverse drug reactions, respectively, which could potentially impact the net clinical benefit. Understanding the dose/concentration-dependent efficacy and toxicity of HCQ, as well as their determinants is therefore essential in assessing the risk-benefit trade- off in COVID-19 clinical trials. When a risk such as QTc prolongation is identified with HCQ, additional measures and warning should be implemented including QTc determination in all admitted patients and close cardiac monitoring to minimize such a risk.

In conclusion, applying an excess of mortality in the population treated with doses where no increase of mortality is found

#Our reply

It is unclear which doses the authors are referring to. Their reanalysis did not show the absence of increase of mortality even at lower doses, as previously commented.

creates a misleading overestimation of deaths associated with the use of HCQ in hospitalized patients with COVID-19.

#Our reply

It is unclear what is misleading in the estimate we provided.

On the other hand, even at low doses HCQ regimen, no reduction in mortality with HCQ was observed suggesting that, when it comes to mortality as the outcome, HCQ did not show a benefit in hospitalized patients suffering from COVID-19. This mainly justifies the past and still up-to-date recommendations and guidelines to not use HCQ in this indication.

#Our reply

It is unclear how "past [...] recommendations and guidelines to not use HCQ in this indication" is consistent with previous comments of the authors "in Belgium, off-label use of HCQ in monotherapy was recommended for hospitalized COVID-19 patients" and "The WHO and other health authorities reversed their recommendations of the use of HCQ in COVID-19 patients".

#Overall reply

The concerns of the authors were mostly related to the article of Axfors et al. (5). We suggest the authors contact the journal in which the meta-analysis of Axfors et al. has been published if they want to comment on it. For reference, the meta-analysis of Axfors et al. has not received any comments or critiques on the Nature Communications website.

REFERENCE

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