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Use of hydroxychloroquine in multidrug protocols for SARS-CoV-2

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Abstract: We review the available evidence supporting the use of hydroxychloroquine-based multidrug protocols in the treatment of COVID-19, in response to a recently published editorial in the Tasman Medical Journal.

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1. Introduction

We read with interest an editorial by Millar [**1**] concerning the role of hydroxychloroquine in the treatment of COVID-19 patients. Community standard of care multidrug therapies for COVID-19 [**2–9**] were based on signals of benefit and acceptable safety. At the onset of the pandemic, there was insufficient time for large prospective randomized controlled trials (RCTs) to validate community standard of care protocols. In such studies, randomization should handle the validity threats of selection bias and both known and unknown confounders, however successful randomization requires a large number of patients with outcome events (e.g. hospitalizations, deaths), to ensure that the patients experiencing these events are also randomized [**10**]. As an example, the number n of events needed to randomize a dichotomous equiprobable confounder variable (i.e. similar to male/female) within $x = 10\%$ margin (with 95% confidence) can be obtained by bounding the ratio σ/μ of standard deviation to mean with $2(\sigma/\mu) = 2(1/n)^{1/2} \leq x = 0.1$, thus requiring $n \geq 400$ expected events. Therefore, for RCTs with a mortality endpoint, if one assumes $p = 2\%$ case fatality rate (CFR), then the estimated sample size required to achieve sufficient randomization is $N = n/p \geq 20,000$, which is impractical in the midst of an emergency.

2. Use of hydroxychloroquine for SARS-CoV-2

Clinicians understood quickly that no single drug was going to be necessary nor sufficient to treat acute COVID-19 with its three phases of viral replication, cytokine storm, and thrombosis. Hydroxychloroquine was part of the initial multidrug protocol used by Zelenko from March 2020 [**11**]. On April 28, 2020, Zelenko published a letter [**12, 13**], also reproduced in his posthumous autobiography [**14**], reporting the details of his hydroxychloroquine-based multidrug protocol and his patient outcomes. Zelenko's protocol consisted of risk stratifying patients as high or low risk, and treating the high-risk patients with hydroxychloroquine (200 mg twice daily for 5 days), azithromycin (500 mg once daily for 5 days), and zinc sulfate (50 mg elemental zinc for 5 days) [**2**]. He defined three categories of high-risk patients: (a) all patients with age $\geq$ 60 years; (b) patients that were immunocompromised or had comorbidities or whose BMI was ≥ 30 kg/m^2; (c) all patients not satisfying the previous two conditions who developed shortness of breath. By April 28, 2020, Zelenko had treated 405 high-risk patients resulting in 6 hospitalizations and 2 deaths. No hospitalizations or deaths were observed amongst the other 1,045 low-risk patients who received only supportive care. He improved on his triple drug protocol by introducing budesonide nebulization and oral dexamethasone at the beginning of May 2020, and selective use of apixaban near the end of May 2020 [**15**]. By June of that year he had treated 800 high-risk patients, resulting cumulatively in 12 hospitalizations and 2 deaths [**14, 16, 17**]. While public health policy in the United States opposed the adoption of Zelenko's protocol [**18**], the community standard of care developed from that point forward to the widely adopted McCullough protocol [**3–5, 19**] (Fig. 1).

In 2022, we proposed a statistical technique for comparing a case series (N, a) of N patients that received treatment with a negative events (e.g. hospitalizations, deaths, etc.) against historical controls that lower-bound the probability x of a negative event without treatment by an inequality $p_1 < x$ [**15**]. Our technique calculates

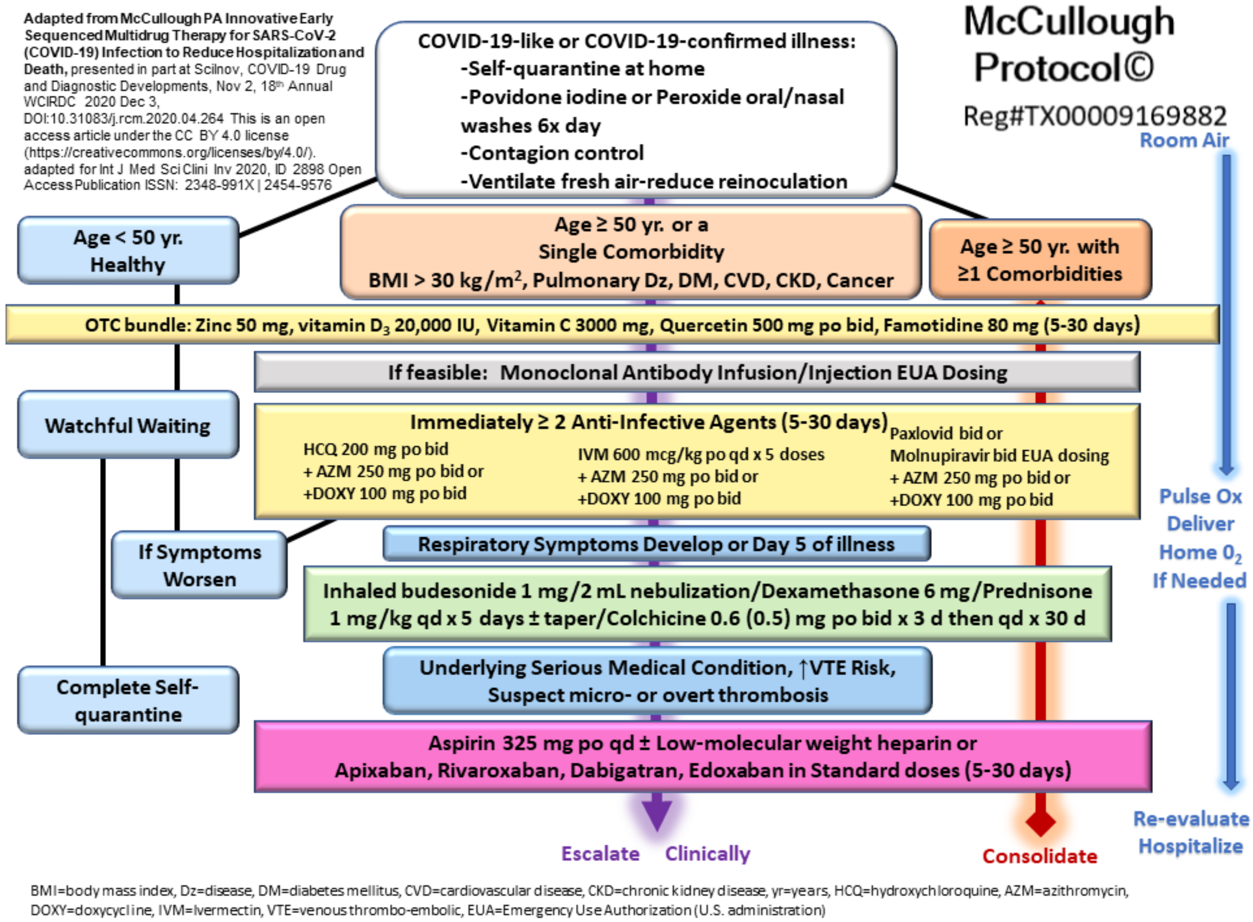

Figure 1: McCullough's protocol [**5–7, 19**] of sequenced multidrug pre-hospital treatment of the three stages of COVID-19: viral proliferation, cytokine injury, and thrombosis. No single drug is necessary nor sufficient.

from (N, a) an *efficacy threshold* x_0 and a *random selection bias threshold* x_1, both dependent on the desired level of confidence $1 - p_0$ (Fig. 2). Then, $p_1 > x_0$ implies the existence of treatment efficacy *by the preponderance of evidence*, meaning that it is more likely than not that the observed effect cannot be entirely accounted for by random selection bias, thus justifying an emergency adoption. Likewise, $p_1 > x_1$ implies that the existence of some treatment efficacy is *clear and convincing*, meaning that we can have $1 - p_0$ confidence that the observed benefit cannot be entirely accounted for by random selection bias, at which point there is no longer sufficient equipoise to ethically justify a randomized controlled trial against placebo. Here, *random selection bias* refers to any possible selection bias that can result by randomly choosing N patients out of the entire population. This analysis can be used only with regimens that have known acceptable safety, limiting its applicability to treatments using safe repurposed medications.

Because Zelenko treated only high-risk patients, with increased likelihood of death relative to the general population, we can compare his case series against observed outcomes over the entire United States population. This comparison is biased towards the null hypothesis, however a positive result that overcomes this bias is sufficient. For $(N, a) = (405, 2)$ we obtained $x_0 = 1.8\%$ and $x_1 = 4.0\%$ and for $(N, a) = (800, 2)$ we obtained $x_0 = 1.0\%$ and $x_1 = 2.0\%$, using 95% confidence [**15**]. During 2020, the CFR in the United States ranged between 2% and 6% [**20**]. Using $p_1 = 2\%$, it follows that by the end of April 2020 the mortality rate reduction benefit was supported by the preponderance of evidence, with crossover to clear and convincing by June 2020. Similar analysis shows clear and convincing hospitalization rate reduction by the end of April 2020 [**15**].

Furthermore, from a case series of 10,429 outpatients, treated in Marseilles, France by Raoult's group in the IHU Méditerranée Infection Institute with hydroxychloroquine and azithromycin, in addition to

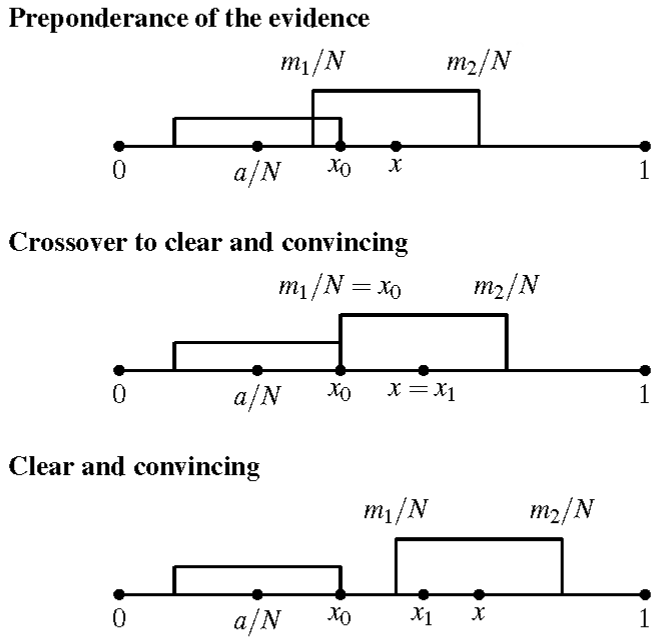

Figure 2: Statistical comparison of a case series (N, a) of N treated patients with a negative events against the population-level probability x of a negative event without treatment. A positive finding depends on the relative position between the confidence interval for the probability of negative event with treatment (on the left) and the confidence interval $[m_1/N, m_2/N]$ for the probability of a negative event without treatment for N randomly selected patients. This figure is adapted from the graphical abstract of Gkioulekas *et al.*[**15**] under the terms of the CC-BY-4.0 license.

standard of care, through the end of December 2020 [**21**], we identified a case series of 1495 high-risk patients (age $\geq$ 60 years) with 5 reported deaths, whereas no deaths were reported for the other 8,934 patients. Using $(N, a) = (1495, 5)$ gives a random selection bias threshold $x_1 = 1.4\%$ for 95% confidence which compares favorably with the CFR in France which ranged from 2% to above 14% during 2020, indicating a clear and convincing finding of mortality rate reduction [**15, 20**]. The standard of care used by Raoult's group included zinc supplementation, enoxaparin for patients older than 70 or with comorbidities, and dexamethasone, for patients with high viral roads, inflammatory pneumonopathy, or based on clinical judgment [**21**]. The mortality rate for patients receiving only this standard of care was 2.1% for high-risk patients with age $\geq$ 60 years (11 deaths out of 520 high-risk patients and no deaths reported for the other 1594 patients) [**21**], which was lower than one would have expected for untreated high-risk patients [**15**]. It was also 7-fold larger than the 0.3% mortality rate observed for high-risk patients in the $(N, a) = (1495, 5)$ case series who were treated with hydroxychloroquine and azithromycin in addition to the standard of care.

Recently, Raoult released his dataset of 30,423 COVID-19 patients treated through the end of 2021 [**22–24**]. An independent analysis of his data, using propensity score matching and logistic regression, has shown that hydroxychloroquine and azithromycin were associated with 58% reduction of the composite endpoint of ICU admissions and deaths, whereas azithromycin alone was associated with 27% reduction over the same endpoint [**25**]. This result also implies that the positive results observed, when using hydroxychloroquine and azithromycin in combination, cannot be exclusively attributed to azithromycin alone. Further evidence has been reviewed by Luzariaga and Iglesias [**26**].

The premise underlying Zelenko's protocol was to reduce the viral multiplication rate and enable the immune system to clear the virus before the infection invades the lungs [**2, 27**]. Subsequently, McCullough's protocol (Fig. 1) recognized that COVID-19 is a tri-phasic illness, with viral proliferation followed by cytokine injury and thrombosis, requiring a carefully timed sequenced treatment of each phase [**5–7, 19**]. Consequently, randomized controlled trials [**28, 29**] (RCTs) of hydroxychloroquine on hospitalized patients, at the last two stages of the illness, do not extrapolate to outpatients treated during the first stage. To prevent hospitalization, any treatment intervention should be administered early, preferably within 3 days from the onset of symptoms [**30**], unlike the 8-day window used by the TOGETHER trial [**31**]. Spivak *et al.* [**32**] is underpowered and tested hydroxychloroquine monotherapy, thus not necessarily generalizable to Zelenko's triple-drug therapy. Furthermore, although the inclusion criteria only allowed patients up to 72 hours after a positive COVID-19 test, this does not account for the unknown additional delay between onset of symptoms and testing.

We concur with Millar's skepticism [**1**] concerning meta-analyses based on studies that use a multiplicity of treatments. At minimum, outpatient studies need to be separated from inpatient studies and considered separately. The effect size obtained from a meta-analysis is quantitatively meaningful when the underlying studies investigate very similar treatment protocols. Furthermore, his comments [**1**] suggesting more evidence is needed are well taken. There remain opportunities for large clinical trials for the treatment of high-risk recurrent infections.

3. Conclusion

It is our interpretation that hydroxychloroquine played an important role in preventing hospitalizations and deaths due to COVID-19, particularly in 2020 with the more virulent strains. Widespread use of nasal sprays and gargles, aspirin, vitamin D, ivermectin, nirmatrelvir/ritonavir, molnupiravir, favipiravir, colchicine, corticosteroids, and anticoagulants (Fig. 1) in protocols all contributed to the benefits of early treatment which were widely favored over therapeutic nihilism in the pre-hospital phase. In case of a future pandemic, involving a novel disease, doctors should be encouraged to attempt treatments with repurposed medications based on biological plausibility, signals of benefit, and acceptable safety. Article 37 of the 2013 Helsinki declaration allows the use of unproven treatments if *"proven interventions do not exist or other known interventions have been ineffective"* and the unproven treatment *"offers hope of saving life, reestablishing health or alleviating suffering"* [**33**]. When these efforts result in case series of treated patients that show a large magnitude of benefit, then statistical comparison with historical controls can be used to support the strength of association between treatment and improved outcomes [**15, 34**]. As evidence accumulates, the Bradford Hill criteria framework can be used to assess the support for a causality claim [**35, 36**], as an inference to the best explanation [**37, 38**]. This evidence can be gathered rapidly and form the basis for an agile emergency response to future pandemics, if public health is willing to leverage the clinical experience of medical doctors at the front lines.

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