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Abstract:	Background: Off-label use of hydroxychloroquine in the SARS-CoV-2 positive population has become widespread with only empirical evidence on its efficacy. This study addresses the efficacy of hydroxychloroquine on serological and supportive care measures in a hospitalized population. Methods: Consecutive adult subjects admitted for viral pneumonia secondary to SARS-CoV-2 (by polymerase chain reaction) during the last two weeks of March, 2020 were included. Those that were started on hydroxychloroquine and supportive care were compared to supportive care alone. The primary end points were effect of hydroxychloroquine usage on the need to escalate respiratory support, change in lymphocyte count, and change in neutrophil-to-lymphocyte ratio. Results: A total of 63 patients were included with 32 in the hydroxychloroquine arm. Hydroxychloroquine administration was associated with a need for escalation of respiratory support level compared to those that did not receive hydroxychloroquine at 5 days (p=0.013). The same findings were observed in a baseline-matched subgroup analysis. Absolute lymphocyte change in the hydroxychloroquine group was no different than supportive care alone (p=0.413). Hydroxychloroquine use trended towards worsening neutrophil-to-lymphocyte ratio compared to supportive care alone (+9.59 vs +1.58, p=0.51) as well as a higher risk for intubation (p=0.051). Conclusion: Hydroxychloroquine administration to the hospitalized SARS-CoV-2 positive population was associated with an increased need for escalation of respiratory support. There were no benefits of hydroxychloroquine on mortality, lymphopenia, or neutrophil-to-lymphocyte ratio improvement.

Clinical Outcomes of Hydroxychloroquine in Hospitalized Patients with COVID-19: A Quasi-Randomized Comparative Study

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Conclusion: Hydroxychloroquine administration to the hospitalized SARS-CoV-2 positive population was associated with an increased need for escalation of respiratory support. There were no benefits of hydroxychloroquine on mortality, lymphopenia, or neutrophil-to-lymphocyte ratio improvement.

Introduction:

Coronavirus, formally known as severe acute respiratory syndrome coronavirus -2 (SARS-CoV-2), has resulted in a global pandemic despite drastic measures to avoid contagious spread.[1] Accordingly, direct treatment options for infected individuals are presently being explored with an emerging therapeutic agent being hydroxychloroquine.[2] A biologically plausible mechanism of action of hydroxychloroquine in SARS-CoV-2 hosts is suppression of aberrant interferon and tumor necrosis factor levels responsible for corollary clinical findings such as lymphopenia and elevated neutrophil-to-lymphocyte ratio (NLR). Although case series have demonstrated a potential benefit in decreasing the upper respiratory viral load, no studies to date have addressed key clinical outcomes such as mortality, effect on escalation of respiratory support, and hematology benefits. Herein, we report on the therapeutic effect of hydroxychloroquine on the above clinical outcome measures, absolute lymphocyte count and NLR in patients diagnosed with SARS-CoV-2.

Methods:

The investigators found themselves in a fortuitous position to evaluate the effects of hydroxychloroquine in the Detroit, Michigan SARS-CoV-2 cohort. Our respective hospitals are 6.9 miles apart and service the same Wayne County population with extensive overlap between patients. Institutional differences resulted in one system establishing diagnostic polymerase chain reaction (PCR) for SARS-CoV-2 with a turnaround time within 24 hours, while the other institution had result times approaching one week after testing during the last two weeks of March, 2020. Both institutions treated patients confirmed as positive with an initial loading dose of 400mg by mouth bi-daily of off label hydroxychloroquine for one to two days and three to four subsequent days of a 200mg to 400mg once daily dose. As a result, study subjects in one institution received hydroxychloroquine shortly after

admission, and in the other institution did not receive hydroxychloroquine until days later when PCR resulted. This created a unique situation in which natural randomization occurred depending on which hospital they were admitted to. There were no major differences between the insurances each hospital accepts.

Institutional Review Board approval for the retrospective review of these patients was obtained (IRB#20-03-1953). Consecutive charts with a diagnosis of SARS-CoV-2 by nasal swab PCR from March 19th to March 26th, 2020 were reviewed for off-label hydroxychloroquine treatment, age, sex, comorbid conditions, absolute lymphocyte and neutrophil count both on admission and at 24 hours, and respiratory supportive care level on admission, at 24 hours and at 5 day follow-up. Comorbid conditions considered high risk throughout analysis included asthma, chronic obstructive pulmonary disease, heart failure of any variety, diabetes, a hematological malignancy, or immune compromised state in which the patient was taking a prednisone equivalent of 20mg by mouth daily. Respiratory supportive care requirements were quantified by a grading system: Room air=1; supplemental oxygen from 1L to 15L without intubation =2; intubation and mechanical ventilation=3; and failure to support respiratory needs resulting in death=4. A special note was made for death in the hydroxychloroquine arm and the final rhythm reviewed for *torsade de pointes*.

An independent, two-tailed t-test was used to compare change in absolute lymphocyte count, NLR, and respiratory support requirement level between subjects administered hydroxychloroquine and those in which treatment was withheld. Fisher's exact test was used to compare mortality rates between the groups. A logistic regression was used to analyze the effect of treatment, age, sex, comorbid conditions, baseline absolute lymphocyte count, baseline neutrophil count, and baseline supportive care needs on

whether or not respiratory supportive care needs increased throughout a 5 day hospital course or shorter in the event of death or safe discharge. All variables were included in the model *a prior*.

A subgroup analysis was conducted on patients with no high-risk comorbid conditions and was not intubated on admission. The same end points were analyzed in the subgroup analysis. In addition, the rates of intubation at 5 days were compared between the groups in this subgroup analysis. Post hoc, we evaluated whether satisfying the afore-studied neutrophil to lymphocyte ratio of 3.13 in the setting of an age equal to or greater than 50 years was predictive of increasing supportive care needs during admission using a Fischer Exact test.[4] SPSS Software (IBM, version 26) was used throughout analysis and a p-value less than 0.05 considered significant.

Results:

A total of 63 consecutive, PCR positive, subjects were included in our analysis with 32 in the hydroxychloroquine group and 31 in the standard supportive care group. 26 (41.3%) of the study population were female and the mean age of subjects at the time of study inception was 62.7 ± 15.1 years. Baseline demographics between subjects receiving hydroxychloroquine and supportive care to those receiving supportive care alone were comparable across study arms except for baseline respiratory support requirement (See Table 1). The key clinical and hematologic outcomes for the entire cohort are summarized in Table 2. The hydroxychloroquine group had a significantly higher respiratory support need at 5 days compared to the support only group (p=0.013). The hydroxychloroquine group also had a strong but not statistically significant trend towards worsening NLR (p=0.051). The logistic regression for increasing respiratory support had a Hosmer and Lemeshow Goodness of Fit Test of 0.237. Hydroxychloroquine treatment and age were independent predictors of escalation of respiratory

support needs throughout admission with odds ratios of 7.18 [CI95: 1.50 to 34.51] (p=0.014) and 1.05 [CI95: 1.01 to 1.10] (p=0.026) respectively. Baseline respiratory support requirements were inversely related to the likelihood of requiring increased level of supportive care throughout admission with an odds ratio of 0.27 [CI95: 0.08 to 0.97] (p=0.044).

A total of 38 patients were included in the matched subgroup analysis with 17 in the hydroxychloroquine group and 21 in the supportive care only group. 15 of the 38 were female (39.47%). The baseline characteristics, including baseline respiratory support, of these two groups are comparable (Table 3). The key clinical and hematologic outcome comparison results in these matched subgroups are summarized in table 4. Those receiving hydroxychloroquine still escalated respiratory support level compared to the supportive only group (p=0.041). There was a strong but not statistically significant trend towards increased rate of escalation to intubation and increase of NLR in the hydroxychloroquine group (p=0.051 and 0.053, respectively).

Post hoc, we evaluated the previously cited criteria of a neutrophil to lymphocyte ratio greater than 3.13 and age greater or equal to 50 years[4] on increasing supportive care needs in our population and found that those subjects meeting the criteria were indeed more likely to have increasing supportive care needs throughout admission (46.3% versus 18.2%, p-value 0.031). No patients throughout the study timeframe experienced *torsade de pointes*.

Discussion:

The current data on the utility of hydroxychloroquine in the SARS-CoV-2 population are limited and mostly empiric. Although not truly randomized, the 7-day difference in PCR result times between the two major hospitals serving most of the Detroit population created a unique situation for a "quasi-randomized" study when outcome measures are obtained at 24 hours or 5 days. Baseline demographics and clinical variables between study subjects were comparable. The lone exception was baseline respiratory support level was higher in the hydroxychloroquine group. Although it was generally preferred to obtain confirmatory PCR results prior to initiating hydroxychloroquine, individual physicians were not mandated to do so. It is likely that a portion of patients in respiratory distress were empirically started on hydroxychloroquine. To compensate for this confounding effect, we decided to do a matched subset analysis of patients with minimal comorbidities that were not intubated on initial presentation. The two groups in the subset analysis well balanced in terms of initial clinical variables. The elevated need for higher respiratory support of the hydroxychloroquine group in both the overall cohort and matched subset analysis is particularly concerning. This is likely unrelated to potential cardiac electrophysiologic effects of hydroxychloroquine.

The previously described NLR of 3.13 correlated with a strong cytokine response and along with an age greater or equal to 50 were predictors of worsening condition in our study as documented by others.[4] The elevation of NLR in the hydroxychloroquine groups together with the recently described fever lowering effects begs the question whether hydroxychloroquine can have a parallel effect as NSAIDS.[5] Although not the main mechanism of action, high dose hydroxychloroquine in vitro can exhibit mild cyclo-oxygenase inhibition effects.[6]

The authors understand that conclusions cannot be made based on “strong trends” in which p-values were slightly higher than the predetermined 0.05. However, considering the statistically significant elevation of respiratory support requirement in the hydroxychloroquine groups, these “strong trends” of NLR change and intubation risks are towards the expected direction and thus become harder to dismiss. As a larger dataset is analyzed, these trends may become significant.

There are major weaknesses with any retrospective study. The lack of true randomization is the most glaring weakness of this study. As mentioned previously, significant confounders still existed despite the “quasi-randomized” nature of this study. The subgroup analysis was designed to minimize the most obvious confounder of initial respiratory status. Despite the retrospective nature, the numerous safety signals in this study are alarming. New treatments should not be recommended based on retrospective studies, but safety concerns of a treatment are often identified first in a retrospective fashion.

Unfortunately, our Detroit dataset is growly rapidly, but the authors decided to publish these preliminary results because of the concerning safety signals. Practically, with the rapidly evolving treatment pattern for SARS-CoV-2, clinical medicine can only be guided by retrospective evidence in the short run. The true answer to whether hydroxychloroquine has a beneficial effect for hospitalized patients can only be obtained with a prospective randomized clinical study. However, when troubling safety concerns are emerging from retrospective studies, we as a medical community may be wise to pause and look at the data further before prescribing with the assumption that hydroxychloroquine at a minimum does no harm.

In summary, from our Detroit inpatient cohort, hydroxychloroquine at best did not appear to have a beneficial effect on meaningful clinical outcome measures of mortality, lymphopenia reconstitution, neutrophil-to-lymphocyte ratio, or risk for intubation. Patients receiving hydroxychloroquine appeared to have a worse clinical outcome in terms of requirement for escalation of respiratory support even when confounders were controlled for. We recommend more judicious prescription of hydroxychloroquine in the setting of SARS-CoV-2 before a larger analysis can be completed.

Acknowledgements: "In the end these three remain faith, hope and love…but the greatest of these is love". To all the healthcare professionals of Detroit at our respective institutions working tirelessly to care and love the sick among us, thank you. The authors have no conflicts of interest to declare.

References:

1. Cucinotta D, Vanelli M. WHO declares COVID-19 a pandemic. *Acta Biomed* 2020;91:157-60.

2. Gautret P, Lagier J, Parola P, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: Results of an open-label non-randomized clinical trial. *Int J Antimicrob Agents* 2020 [Ahead of press].

3. Chen T, Wu D, Chen H, et al. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. *BMJ* 2020 [Ahead of press].

4. Liu J, Liu Y, Xiang P, et al. Neutrophil-to-lymphocyte ratio predicts severe illness patients with 2019 novel coronavirus in the early stage. *MedRxiv* 2020 [Ahead of press].

5. Chen Z, Hu J, Zhang Z, et al. Efficacy of hydroxychloroquine in patients with COVID-19: results of a randomized clinical trial. MedRxiv 2020 [Ahead of press].

6. Ben-Chetrit E, Fischel R, Hinz B, Levy M. The effect of colchicine and hydroxychloroquine on the cyclo-oxygeases COX-1 and COX-2. Rheumatology Int. 2005.

Table 1. Baseline Demographics

Variable	Combined	Hydroxychloroquine Arm (total 32)	Standard Care Arm (total 31)	p-value
Sex, Female	26 (41.3%)	17 (53.1%)	9 (29.0%)	0.052
Age (years)	62.7 ± 15.1	61.8 ± 15.0	63.7 ± 15.4	0.625
Number of High Risk Comorbid Conditions	1.22 ± 0.96	1.34 ± 0.94	1.10 ± 0.98	0.310
Baseline Absolute Lymphocyte Count (K/µL)	0.95 ± 0.51	0.89 ± 0.47	1.02 ± 0.55	0.314
Baseline Absolute Neutrophil Count (K/µL)	5.68 ± 3.75	6.43 ± 4.58	4.91 ± 2.50	0.106
Baseline Neutrophil to Lymphocyte Ratio	7.27±6.15	8.36±7.02	6.15±4.97	0.154
Baseline Respiratory Support Requirement*	1.73±0.68	1.94±0.67	1.52±0.63	**0.012**

*Defined as 1=Room Air, 2=Non-intubated supportive oxygenation, 3=Intubation, 4=Expired after intubation/ventilation.

Table 2. Outcome of the entire cohort.

Outcome Variables	Hydroxychloroquine Arm	Supportive Care Arm	p-value
Mortality Rate	4/31 (12.90%)	1/32 (3.13%)	0.196
Change in Respiratory Support Level	+0.63± 0.79	+0.16± 0.64	**0.013**
Change in Absolute Lymphocyte Count (K/µL)	-0.16 ± 0.52	-0.61 ± 0.38	0.413
Change in Neutrophil to Lymphocyte Ratio	+9.55 ± 21.5	+1.58 ± 6.26	0.051

Table 3. Baseline Demographics in Matched Subgroups

Variable	Combined (total 38)	Hydroxychloroquine Arm (total 17)	Standard Care Arm (total 21)	p-value
Age (years)	62.11 ± 17.21	59.76 ± 18.92	64.00 ± 15.92	0.458
Number of High Risk Comorbid Conditions	0.66 ± 0.48	0.76 ± 0.44	0.57 ± 0.51	0.223
Baseline Absolute Lymphocyte Count (K/µL)	1.07 ± 0.54	1.04 ± 0.56	1.10 ± 0.54	0.710
Baseline Absolute Neutrophil Count (K/µL)	5.46 ± 3.87	6.31 ± 4.97	4.77 ± 2.63	0.228
Baseline Neutrophil to Lymphocyte Ratio	5.61±3.27	6.12±3.00	5.18±3.48	0.385

Baseline Respiratory Support Requirement*	1.50±0.51	1.59±0.51	1.43±0.51	0.341

*Defined as 1=Room Air, 2=Non-intubated supportive oxygenation, 3=Intubation, 4=Expired after intubation/ventilation.

Table 4. Outcomes of the matched subgroup analysis.

Outcome Variables	Hydroxychloroquine Arm	Supportive Care Arm	p-value
Mortality Rate	2/17 (11.76%)	1/21 (4.77%)	0.577
Rate of Intubation	7/17 (41.18%)	2/21 (9.52%)	0.051
Change in Respiratory Support Level	+0.76 ± 0.83	+0.24 ± 0.70	**0.041**
Change in Absolute Lymphocyte Count (K/µL)	+0.80 ± 0.46	1.00 ± 0.49	0.207
Change in Neutrophil to Lymphocyte Ratio	15.34±20.00	6.33 ± 4.77	0.053