

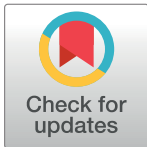
RESEARCH ARTICLE

Hydroxychloroquine and tocilizumab therapy in COVID-19 patients—An observational study

Andrew Ip¹, Donald A. Berry^{2,3}, Eric Hansen⁴, Andre H. Goy⁵, Andrew L. Pecora⁵, Brittany A. Sinclair⁵, Urszula Bednarz⁵, Michael Marafelias⁵, Scott M. Berry², Nicholas S. Berry², Shivam Mathura⁴, Ihor S. Sawczuk⁶, Noa Biran⁵, Ronaldo C. Go⁶, Steven Sperber⁶, Julia A. Piwoz⁶, Bindu Balani⁶, Cristina Cicogna⁶, Rani Sebti⁶, Jerry Zuckerman⁶, Keith M. Rose⁶, Lisa Tank⁶, Laurie G. Jacobs⁶, Jason Korcak⁶, Sarah L. Timmapuri⁶, Joseph P. Underwood⁶, Gregory Sugalski⁶, Carol Barsky⁶, Daniel W. Varga⁶, Arif Asif⁶, Joseph C. Landolfi⁶, Stuart L. Goldberg^{1*}

1 Division of Outcomes and Value Research, John Theurer Cancer Center at Hackensack University Medical Center, Hackensack, New Jersey, United States of America, **2** Berry Consultants LLC, Austin, Texas, United States of America, **3** M.D. Anderson Cancer Center of the University of Texas, Houston, Texas, United States of America, **4** COTA, Boston, Massachusetts, United States of America, **5** John Theurer Cancer Center at Hackensack University Medical Center, Hackensack, New Jersey, United States of America, **6** Hackensack Meridian Health, Hackensack New Jersey and Hackensack Meridian School of Medicine, Nutley, New Jersey, United States of America

* Stuart.Goldberg@hackensackmeridian.org



OPEN ACCESS

Citation: Ip A, Berry DA, Hansen E, Goy AH, Pecora AL, Sinclair BA, et al. (2020) Hydroxychloroquine and tocilizumab therapy in COVID-19 patients—An observational study. *PLoS ONE* 15(8): e0237693. <https://doi.org/10.1371/journal.pone.0237693>

Editor: Chiara Lazzeri, Azienda Ospedaliero Universitaria Careggi, ITALY

Received: May 21, 2020

Accepted: August 1, 2020

Published: August 13, 2020

Copyright: © 2020 Ip et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: The study involves a review of the electronic medical records of patients infected with SARS-CoV-2. The research data set has removed most, but not all protected health information (for example: actual dates of infection were included as needed for analysis, but would be considered PHI). The dataset is also considered property of Hackensack Meridian Health and not owned by the investigators. Therefore, for both reasons, the dataset cannot be made openly available. However, upon request we are willing to share portions of the data for appropriate review.

Abstract

Hydroxychloroquine has been touted as a potential COVID-19 treatment. Tocilizumab, an inhibitor of IL-6, has also been proposed as a treatment of critically ill patients. In this retrospective observational cohort study drawn from electronic health records we sought to describe the association between mortality and hydroxychloroquine or tocilizumab therapy among hospitalized COVID-19 patients. Patients were hospitalized at a 13-hospital network spanning New Jersey USA between March 1, 2020 and April 22, 2020 with positive polymerase chain reaction results for SARS-CoV-2. Follow up was through May 5, 2020. Among 2512 hospitalized patients with COVID-19 there have been 547 deaths (22%), 1539 (61%) discharges and 426 (17%) remain hospitalized. 1914 (76%) received at least one dose of hydroxychloroquine and 1473 (59%) received hydroxychloroquine with azithromycin. After adjusting for imbalances via propensity modeling, compared to receiving neither drug, there were no significant differences in associated mortality for patients receiving any hydroxychloroquine during the hospitalization (HR, 0.99 [95% CI, 0.80–1.22]), hydroxychloroquine alone (HR, 1.02 [95% CI, 0.83–1.27]), or hydroxychloroquine with azithromycin (HR, 0.98 [95% CI, 0.75–1.28]). The 30-day unadjusted mortality for patients receiving hydroxychloroquine alone, azithromycin alone, the combination or neither drug was 25%, 20%, 18%, and 20%, respectively. Among 547 evaluable ICU patients, including 134 receiving tocilizumab in the ICU, an exploratory analysis found a trend towards an improved survival association with tocilizumab treatment (adjusted HR, 0.76 [95% CI, 0.57–1.00]), with 30 day unadjusted mortality with and without tocilizumab of 46% versus 56%. This observational cohort study suggests hydroxychloroquine, either alone or in combination with azithromycin, was not associated with a survival benefit among hospitalized COVID-19 patients. Tocilizumab demonstrated a trend association towards reduced mortality among ICU patients. Our findings

Requests can be made through the corresponding author or directly to representatives of Hackensack Meridian Health (Dr Ihor Sawczuk, Chief Research Officer HMM; Email: Ihor.Sawczuk@hackensackmeridian.org). We have included in the Supporting Information the statistical output that may assist interested readers in understanding the data more fully and could be utilized to assist in independent confirmation.

Funding: The authors received no specific funding for this work.

Competing interests: I have read the journal's policy and the authors of this manuscript have the following competing interests: DAB, SMB, and NSB are employed by Berry Consultants LLC which provided statistical support for the study. EH, AHG, ALP, SM, and SLG are employed or have ownership interest in COTA Inc which provided statistical support for the study. AHG discloses consulting fees from Physicians' Education Resource, LLC for his contributions to COVID-19 and Cancer Care. This does not alter our adherence to PLOS ONE policies on sharing data and materials.

are limited to hospitalized patients and must be interpreted with caution while awaiting results of randomized trials.

Trial Registration: [Clinicaltrials.gov Identifier: NCT04347993](https://clinicaltrials.gov/ct2/show/study/NCT04347993)

Introduction

The global pandemic caused by a novel coronavirus [severe acute respiratory syndrome (SARS)-CoV-2] and its disease, COVID-19, has led to infection in over 15.8 million individuals and more than 640,000 deaths as of July 25, 2020 [1, 2]. As there are no approved treatments, management of COVID-19 is largely supportive [3, 4].

One empirical treatment for COVID-19 which has received attention is hydroxychloroquine, an antimalarial drug repurposed in recognition of its anti-inflammatory properties in the treatment of autoimmune conditions. Hydroxychloroquine and its analogue, chloroquine, demonstrate suppression of SARS-CoV-2 replication in vitro, with hydroxychloroquine demonstrating greater potency [5, 6]. Studies from the original SARS-CoV virus suggest a mechanism of action involving impairment of the terminal glycosylation of angiotensin converting enzyme 2 (ACE2), inhibition of SARS-CoV viral entry, and rapid elevation of endosomal pH that prevents endosome-mediated viral entry [7–10]. The immunomodulatory effects are thought to be due to the accumulation of the drug in lymphocytes and macrophages leading to reduction of proinflammatory cytokines, including type I interferons, tumor necrosis factor alpha, and interleukin-6 [9]. Other anti-inflammatory effects may be related to inhibition of signaling pathways [11].

Several early small clinical reports have shown conflicting evidence regarding the efficacy of hydroxychloroquine in COVID-19 [12, 13]. Subsequently, an observational cohort study of 1376 hospitalized patients from a New York hospital using propensity modeling found no significant association between hydroxychloroquine use and intubation or death (hazard ratio, 1.04, 95% confidence interval, 0.82 to 1.32) [14]. A second observational cohort study of 1438 hospitalized patients throughout the New York metropolitan region also found a lack of survival association with hydroxychloroquine with or without concomitant azithromycin (HR 1.35 and 1.08 respectively) [15]. A recently reported randomized Brazilian trial enrolling 504 hospitalized SARS-CoV-2 confirmed patients with mild-to-moderate disease (defined as not requiring significant supplemental oxygen support) found that a 7-day course of hydroxychloroquine either with azithromycin or alone did not result in better clinical outcomes as measured by a seven-level ordinal scale at 15 days [16].

As the clinical course of COVID-19 progresses, patients enter a hyperinflammatory phase with dysregulation of adaptive immune responses and a cytokine storm with elevation in plasma levels of pro-inflammatory cytokines including interleukins (IL) 2, 6, 7, and 10, granulocyte-colony stimulating factor (G-CSF), interferon-gamma-inducible protein-10 (IFN-gamma, IL-10), and tumor necrosis factor alpha (TNF-alpha). This cytokine storm results in a pro-thrombotic milieu, cardiomyopathy, and ultimately multi-organ failure [17, 18]. Tocilizumab, a monoclonal antibody against membrane bound IL-6 receptor inhibiting binding of soluble IL-6 and subsequent signal transduction, has been proposed as a therapeutic candidate for impeding cytokine storm [19]. Small single institution series have suggested benefit among severely ill patients [20, 21]. Preliminary results from a press release for the French CORIMUNO-TOCI trial (NCT04331808), an open-label randomized trial of hospitalized patients with COVID-19 (n = 129), noted a reduction in the proportion of participants who died or needed

ventilation in the tocilizumab group, although full results of the trial have not yet been released [22]. An Italian observational study involving 544 COVID-19 patients (with 16% requiring mechanical ventilator support) reported that tocilizumab treatment was associated with a reduced risk of subsequent invasive mechanical ventilation or death [23]. A Michigan USA observational study also noted an associated reduction in mortality among intubated COVID-19 patients [24].

In the absence of RCTs, observational studies may provide useful early insights into effective treatment strategies [25, 26]. However in an observational study, treatment allocations are based upon physician judgement, rather than random assignment, increasing the risk of bias and not accounting for known and unknown risk factors. Thus, causal inferences on effectiveness of treatments are challenging, but confounding effects can be partially mitigated via statistical methods [27, 28].

Understanding these limitations, but with the urgency for evaluating potential therapeutic approaches during the current COVID-19 pandemic, we established an observational database within a 13-hospital network spanning New Jersey using an integrated electronic health record (EHR) system (EPIC; Verona, WI). In this observational cohort study we report our survival outcomes with hydroxychloroquine and tocilizumab among hospitalized patients with COVID-19.

Methods

Study design and cohort selection

This retrospective, observational, multicenter cohort within the Hackensack Meridian Health network (HMH) used EHR-derived data to study hospitalized COVID-19 patient outcomes. Our primary objective was to analyze the effect of hydroxychloroquine in hospitalized patients. A secondary, exploratory objective was to investigate the effect of tocilizumab in the ICU population.

Patients were included in the database based on the following inclusion and exclusion criteria: 1) Positive SARS-CoV-2 diagnosis by reverse-transcriptase polymerase chain reaction, 2) Hospitalized within the time frame of March 1, 2020 until May 5, 2020, 3) Non-pregnant, 4) Not on a randomized clinical trial, and 5) Did not die during first day of hospitalization, and 5) Were not discharged to home within 24 hours (Fig 1).

Hackensack Meridian Health Institutional Review Board approved this study on March 27, 2020 under study # Pro2020-0342. Waiver of consent and HIPAA authorization was granted as this retrospective research was a non-interventional protocol utilizing electronic health records to abstract data. No patients were contacted for this study. Protected health information was not made available except to investigators. De-identified information was provided to statisticians. The study period was March 1, 2020 until May 5, 2020.

Data sources and baseline variables

We collected data from HMH's EHR (Epic) which is utilized throughout the network. Hospitalized patients throughout HMH were flagged by the EHR if SARS-CoV-2 testing was positive. These EHR-generated reports served as our eligible cohort to sample. Demographic, clinical characteristics, treatments, and outcomes were manually abstracted by research nurses and physicians from the John Theurer Cancer Center at Hackensack University Medical Center. Assigning patients to our data team occurred in real-time, and not randomized. To reduce sampling bias, we abstracted 3325 of the 3438 (97%) possible hospitalized patients by April 22, 2020 (with follow up until May 5, 2020), and performed stratification as discussed in our analytic approach. Data abstracted by the team was entered using REDCap (Research Electronic Data Capture) hosted at HMH [29, 30]. Data abstraction occurred daily from March 28, 2020

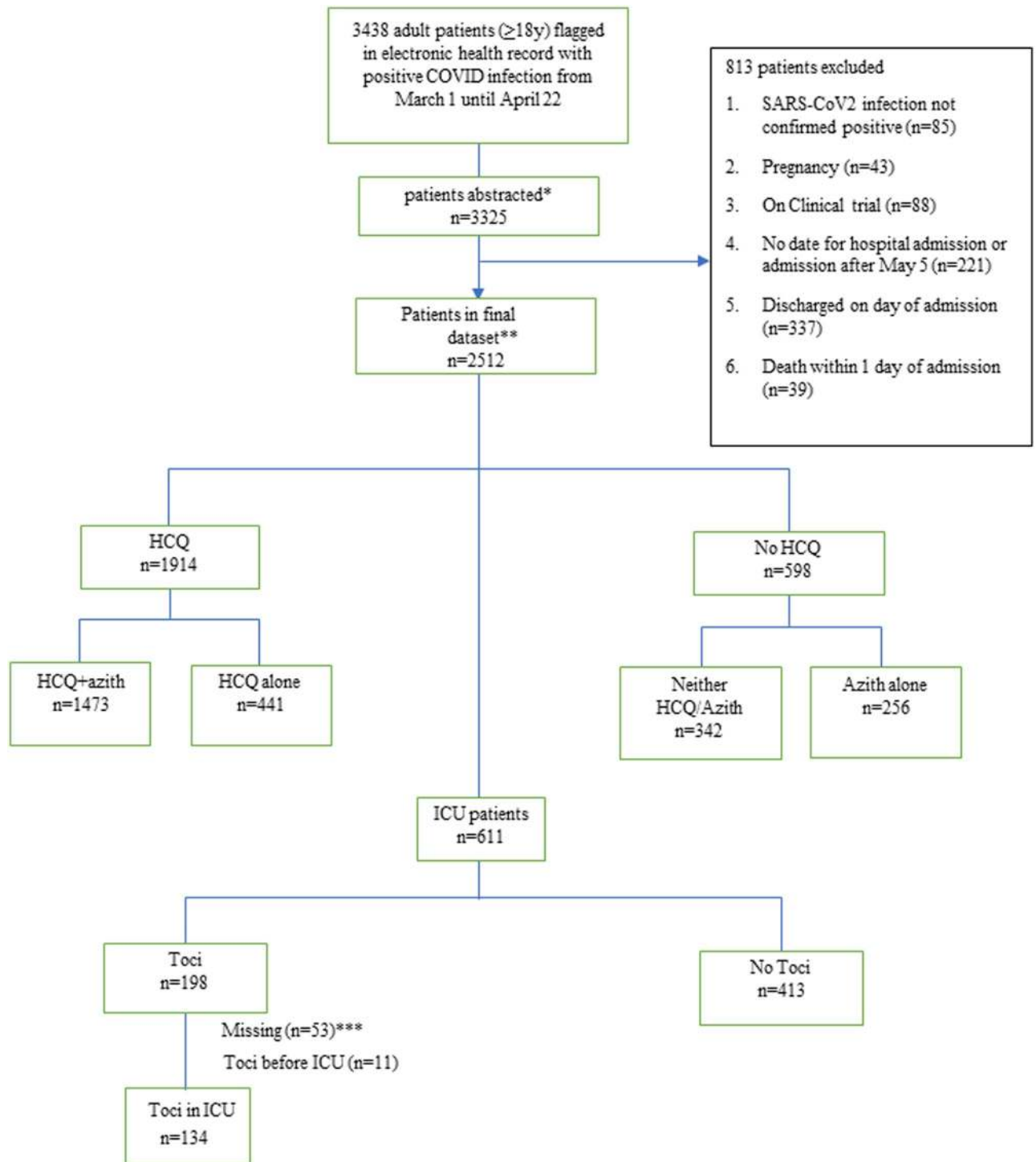


Fig 1. Cohort selection flow diagram. Flow Diagram of patient sampling strategy of hospitalized COVID-19 patients in Hackensack Meridian Health network. *Convenience sampling was performed when assigning patients to our data team, and sampling bias is possible. 3325 of the 3438 (97%) available records were abstracted. **Follow-up until final study cut-off date of May 5, 2020. *** 53 patients who received toci did not have sufficient data (date of administration) to analyze Tocilizumab (toci); Hydroxychloroquine (HCQ).

<https://doi.org/10.1371/journal.pone.0237693.g001>

until May 5, 2020. Quality control was performed by physicians (AI, SLG) overseeing nurse or physician abstraction.

Demographic information was collected by an electronic face sheet, with gender, race or ethnicity self-reported. Academic centers were defined as quaternary referral centers with accredited residency, fellowship, and medical student programs. Nursing home or rehabilitation patients, if diagnosed prior to hospital admission, were defined as ambulatory patients. Comorbidities were defined as diagnosed prior to hospitalized for COVID-19. History of hypertension, diabetes, chronic lung disease (COPD or asthma), hypertension, cancer, coronary artery disease, cerebrovascular disease, renal failure, and rheumatologic disorder were abstracted from provider notes or medical history sections found within the EHR. If not listed in the patient's record, the comorbidity was recorded as absent.

Presenting clinical data was abstracted from thorough review of unstructured notes as well as structured data. Hospital readmissions were counted as the same admission, with baseline data used from the initial hospitalization. If multiple positive or indeterminate results were found in a patient's record for SARS-CoV-2, the first initial positive test was used as the date of diagnosis.

Exposures

For the effect of hydroxychloroquine, we separated patients into 4 different groups— 1) Hydroxychloroquine, 2) Hydroxychloroquine in combination with Azithromycin, 3) Azithromycin alone, and 4) neither drug. Exposure to hydroxychloroquine or azithromycin was defined as documentation of drug administration in the EHR. Dosing, duration, and timing in relation to symptom onset and admission were also collected. If no evidence of administration of drug was found, this was recorded as not having received the drug.

For tocilizumab, exposure was defined as receipt of the drug within the ICU setting as found in the EHR. If no date of administration was found, this was labeled as insufficient missing data for analysis. If no evidence of administration of the drug was found, this was recorded as not having received the drug.

Outcome measures

The primary outcome measurement was death with follow-up through May 5, 2020. Mortality was identified on chart review by a provider note announcing time of death or if the EHR labeled the patient as deceased. As death certificates were not readily available, cause of death was identified using the EHR by identifying the most immediate cause(s) documented [31]. Respiratory cause of death included any hypoxic condition related to COVID-19. Cardiac cause of death included cardiac arrest, myocardial infarction, or arrhythmias. Infectious cause of death included bacterial sepsis or secondary infections not including COVID-19. Other cause of death included multi-organ failure as well as alternative causes. Follow-up occurred until the study cut-off date of May 5.

Adverse drug events related to hydroxychloroquine were also described, including discontinuation due to arrhythmia or QT prolongation. This was obtained by provider documentation as well as EKG reports within the EHR.

Statistical analyses

The statistical plan is available in the (S1 Appendix). Descriptive analyses of baseline characteristics by hydroxychloroquine exposure were performed using chi-square tests for categorical variables. Dose, frequency, timing, and duration of treatment were also summarized.

We used propensity-score stratification for the remaining statistical analyses [32, 33]. We fit a logistic regression model to the probability of being assigned to the experimental arm

(tocilizumab, hydroxychloroquine, or hydroxychloroquine plus azithromycin) compared with the control population (not assigned to the respective treatment). Patients are stratified into propensity-score quintiles and these strata are used to adjust treatment effects in a proportional hazards model.

The model for selecting factors to be included in propensity scores was a two-stage backward selection approach. We evaluated each of the factors as univariate predictors with factors having p-value less than 0.10 included for further consideration. We removed factors sequentially and one at a time from the multivariate model if their p-values were less than 0.50, with largest p-values considered first. We fit the final propensity-scores model using multivariate logistic regression of the selected factors. We then stratified the propensity scores for the entire population into quintiles and used these quintiles as an ordinal (4-degree-of-freedom) variable to adjust the relative treatment comparison in a proportional hazards model (see [S2 Appendix](#), for output).

We evaluated the following factors for all propensity-score models: gender, coronary disease, stroke, heart failure, arrhythmia, African American, COPD, renal failure, rheumatologic disorder, inflammatory bowel disease, advanced liver disease, age, diabetes mellitus, insulin use prior to hospitalization, asthma, HIV/hepatitis, any cancer, and log ferritin. The final propensity-score model for hydroxychloroquine included the first 15 of these factors. That for hydroxychloroquine plus azithromycin included the first 15 of these factors plus cancer. That for tocilizumab included age, gender, COPD, and renal failure.

Tocilizumab was assigned preferentially for patients in the ICU. Tocilizumab patients included in our analyses received their first dose of the drug in the ICU. Control patients were those who were admitted to the ICU and who never received tocilizumab either before or after admission to the ICU. The start time for analysis was the day of admission to the ICU. Hydroxychloroquine and hydroxychloroquine plus azithromycin were evaluated from day of hospital admission, whether initially in the ICU or not. The control population consisted of patients who never received the respective treatment.

We also sought to address the factorial nature of treatment with combination hydroxychloroquine and azithromycin. Propensity scores are based on predicting particular an individual therapy and so do not naturally generalize to a factorial setting. For these analyses we averaged the two propensity scores calculated separately for hydroxychloroquine and hydroxychloroquine plus azithromycin. We then stratified into propensity quintiles based on that average and proceeded as indicated above. The raw results of our proportional hazards analyses adjusting for propensity scores is provided ([S2 Appendix](#)).

Patients still alive and in the hospital were censored as of May 5, 2020. Patients who had been discharged from the hospital were censored as of day 36 following hospital admission.

We provide hazard ratios of treatment in comparison with control together with corresponding p-values and confidence intervals based on Wald tests. We show the unadjusted survival data using Kaplan-Meier plots from which we identify 30-day mortality for each treatment. Statistical calculations used JMP[®] Pro 15.0.0. Confidence intervals and p-values in this study are descriptive measures of distance between outcomes of treatment groups or distance from hazard ratio 1.00. These measures do not have the same inferential interpretations that are possible for primary end point analyses of RCTs.

Results

Unadjusted baseline characteristics of hospitalized patients

There were 3,438 patients flagged in our EHR with positive COVID19 infection within the 13-hospital network spanning New Jersey, and data was abstracted on 3,325 (97%). 2512

hospitalized patients met inclusion criteria for this study (Fig 1). As indicated in Table 1, the median age of the cohort was 64 years (IQR 52–76) with a male predominance (62%). Nursing home and rehabilitation patients comprised 16% of the cohort. Co-morbidities were common with 55% having hypertension, 41% obesity (BMI \geq 30), 32% diabetes, 16% coronary arterial disease, 15% COPD/asthma, 12% cancer, and 31% having 3 or more chronic conditions.

At the time of hospital presentation, fever was present in 72%, 69% with dyspnea, 68% with cough, 23% gastrointestinal complaints and 12% had altered mental status. The median time from self-reported onset of symptoms to hospitalization was 5 days (IQR 3–7). 611 (24%) patients required intensive care unit support during their hospitalization, of which 150 patients were admitted to the ICU within the first day. Oxygen saturation below 94% was identified in 44%. When measured and recorded in the electronic health record, inflammatory markers were elevated with serum ferritin >1500 ng/mL in 23% and d-dimer >1 mcg/mL in 16% of patients.

Treatment with hydroxychloroquine

In this non-randomized observational cohort 1914 hospitalized patients (76%) received at least one dose of hydroxychloroquine during the study timeframe (23% as single agent and 77% in combination with azithromycin) (Fig 1). Dosing and duration was at prescribers' discretion. The majority of patients received 800 mg on day 1, and 400 mg on day 2–5 (80%, $n = 1533$), followed by 200 mg TID (4%, $n = 71$) and other (15%, $n = 299$), and missing dosing information (1%, $n = 11$). Median duration of hydroxychloroquine was 5 days (IQR 4–5). The median days of symptoms before hydroxychloroquine administration was 5 (IQR 2–9), and median days in hospital before first dose 1 was 1 (IQR 0–2).

Patients receiving hydroxychloroquine at any time during their hospitalization were younger, less likely to live in a nursing home, but presented later in their clinical course (5 days vs 3 days of symptoms prior to hospital admission) with more symptomatic disease (higher incidences of fever, cough, dyspnea, and lower oxygen saturation) (Table 1). After the United States Food and Drug Administration Emergency Use Authorization on March 28, 2020, prescriptions of hydroxychloroquine in our cohort temporally increased [569 (30%) prior to March 28 compared to 1314 (68%) post announcement, 42 (2%) dates missing] [34].

Discontinuation of hydroxychloroquine due to prolongation of QTc or arrhythmias was recorded in the electronic health records in 76 (4%) and 33 (2%) patients. During the entire hospital course, arrhythmias were noted in 101 (5%) hydroxychloroquine patients, compared to 22 (4%) in the non-hydroxychloroquine cohort. Cardiomyopathy was described in 20 (1%) treated patients compared to 7 (1%) patients without hydroxychloroquine.

Survival outcomes and hydroxychloroquine therapy

Among 2512 hospitalized patients with COVID-19 there have been 547 deaths (22%), 1539 (61%) discharges and 426 (17%) remain hospitalized as of the study cut-off date. In the entire cohort, causes of death included 319 (58%) respiratory, 108 (20%) cardiac, 42 (8%) other, 41 (7%) infectious, and 37 (7%) other. Within the hydroxychloroquine treated cohort, 89 of 432 (21%) of deaths were attributed to cardiac causes, compared to 19 of 115 (16%) deaths in the non-hydroxychloroquine cohort.

As shown in Table 2, using propensity modeling as described above, there was no significant association between survival and any use of hydroxychloroquine during the hospitalization (HR, 0.99 [95% CI, 0.80–1.22]), hydroxychloroquine alone (HR, 1.02 [95% CI, 0.83–1.27]), or hydroxychloroquine in combination with azithromycin (HR, 0.98 [95% CI, 0.75–1.28]). The unadjusted 30-day mortality for patients receiving hydroxychloroquine alone,

Table 1. Unadjusted baseline characteristics of hospitalized COVID-19 patients.

	All (n = 2512)	No HCQ (n = 598)	HCQ* (n = 1914)	P-value
Demographics, n (%)				
Age Median (Iqr)	64 (52–76)	66 (52–80)	64 (53–75)	0.011
Age Distribution				
[0,10]	16	12 (75%)	4 (25%)	<.001
[10,20]	8	3 (38%)	5 (62%)	
[20,30]	54	20 (37%)	34 (63%)	
[30,40]	153	42 (27%)	111 (73%)	
[40,50]	314	71 (23%)	243 (77%)	
[50,60]	490	90 (18%)	400 (82%)	
[60,70]	570	117 (21%)	453 (79%)	
[70,80]	477	106 (22%)	371 (78%)	
[80,90]	332	97 (29%)	235 (71%)	
[90,100]	98	40 (41%)	58 (59%)	
Gender				0.09
Female	945	242 (26%)	703 (74%)	
Male	1,565	354 (22%)	1,211 (78%)	
Missing	2	2 (100%)	0 (0%)	
Race/Ethnicity				<.001
African American	285	80 (28%)	205 (72%)	
Asian	105	10 (10%)	95 (90%)	
Caucasian	1,308	339 (26%)	969 (74%)	
Hispanic	483	89 (18%)	394 (82%)	
Other*	255	55 (22%)	200 (78%)	
Missing	76	25 (33%)	51 (67%)	
Nursing Home/Rehab**				<.001
No	2,082	447 (21%)	1,635 (79%)	
Yes	396	143 (36%)	253 (64%)	
Missing	34	8 (24%)	26 (76%)	
Academic vs. Community				0.24
Academic	777	197 (25%)	580 (75%)	
Community	1,735	401 (23%)	1,334 (77%)	
Smoking History				0.69
Current	77	20 (26%)	57 (74%)	
Former	448	112 (25%)	336 (75%)	
Neither (Non-Smoker)	1,620	378 (23%)	1,242 (77%)	
Missing	367	77 (21%)	290 (79%)	
Comorbidities, n (%)				
Comorbidity Count				0.001
0	444	134 (30%)	310 (70%)	
1	665	142 (21%)	523 (79%)	
2	634	132 (20%)	502 (80%)	
≥3	769	190 (24%)	579 (76%)	
Diabetes				0.09
No	1,699	422 (25%)	1,277 (75%)	
Yes	813	176 (22%)	637 (78%)	
COPD/asthma				0.80
No	2,136	506 (24%)	1,630 (76%)	

(Continued)

Table 1. (Continued)

	All (n = 2512)	No HCQ (n = 598)	HCQ* (n = 1914)	P-value
Yes	376	92(24%)	284 (76%)	
Hypertension				0.67
No	1,126	263 (23%)	863 (77%)	
Yes	1,386	335 (24%)	1,051 (76%)	
Coronary Disease				0.04
No	2,114	487 (23%)	1,627 (77%)	
Yes	398	111 (28%)	287 (72%)	
Cerebrovascular Disease				0.002
No	2,389	554 (23%)	1,835 (77%)	
Yes	123	44(36%)	79(64%)	
Cancer				0.86
No	2,221	527 (24%)	1,694 (76%)	
Yes	291	71(24%)	220 (76%)	
Renal Failure				0.93
No	2,325	553 (25%)	1,772 (75%)	
Yes	187	45 (24%)	142 (76%)	
Rheumatologic Disorder				0.04
No	2,437	588(24%)	1,849 (76%)	
Yes	75	10 (13%)	65 (87%)	
Obesity (BMI>30)				<0.001
No	1630	427 (26%)	1203 (74%)	
Yes	882	171 (19%)	711 (81%)	
Presenting Symptoms, n (%)				
Fever				<0.001
No	704	214 (30%)	490 (70%)	
Yes	1,808	384 (21%)	1,424 (79%)	
Cough				<0.001
No	805	260 (32%)	545 (68%)	
Yes	1,707	338 (20%)	1,369 (80%)	
Shortness of Breath				<0.001
No	778	279 (36%)	499 (64%)	
Yes	1,734	319 (18%)	1,415 (82%)	
GI***				0.33
No	1,940	471 (24%)	1,469 (76%)	
Yes	572	127 (22%)	445 (78%)	
Altered Mental State				0.007
No	2,210	507(23%)	1,703 (77%)	
Yes	302	91(30%)	211 (70%)	
Disease severity, n (%)				
Oxygenation < 94%				<0.001
No	1,370	403 (29%)	967 (71%)	
Yes	1,107	178 (16%)	929 (84%)	
Missing	35	17 (49%)	18 (51%)	
Ferritin >1500 ng/mL				0.62
No	1313	214 (16%)	1099 (84%)	
Yes	400	70 (18%)	330 (82%)	
Missing	799	314 (39%)	485 (61%)	

(Continued)

Table 1. (Continued)

	All (n = 2512)	No HCQ (n = 598)	HCQ* (n = 1914)	P-value
D-Dimer > 1 mcg/mL				0.30
No	872	131 (15%)	741 (85%)	
Yes	172	20 (12%)	152 (88%)	
Missing	1468	447(30%)	1021 (70%)	
ICU Admission****				0.88
Admitted	150	37 (25%)	113 (75%)	
Not Admitted	2,362	561 (24%)	1,801 (76%)	

*Hydroxychloroquine (HCQ) administration of at least one dose, and at any point during hospitalization.

**Includes multi-racial, and not specified in other choices

*** Admitted from a nursing home or rehabilitation facility

**** Includes nausea, vomiting, diarrhea, or other Gastrointestinal complaint obtained from medical record.

***** Admission to ICU on first day of hospitalization, as defined by chart review of medical record.

<https://doi.org/10.1371/journal.pone.0237693.t001>

azithromycin alone, the combination or neither drug was 25%, 20%, 18%, and 20%, respectively (Fig 2).

Toci Tocilizumab; HCQ hydroxychloroquine; AZI azithromycin. Toci in ICU includes all patients whose first dose of tocilizumab was in the ICU or controls who were admitted to the ICU and known to not have received tocilizumab during their hospital stay. “In hospital” includes all patients who received the drug at any time during their stay in the hospital. “Main effect” is the estimated benefit of the drug whether added to the other drug or not. A drug given “only” means not with the other drug. 30-day mortality rates are estimated using the corresponding Kaplan-Meier survival curve at 30 days (See Figs 2 and 3).

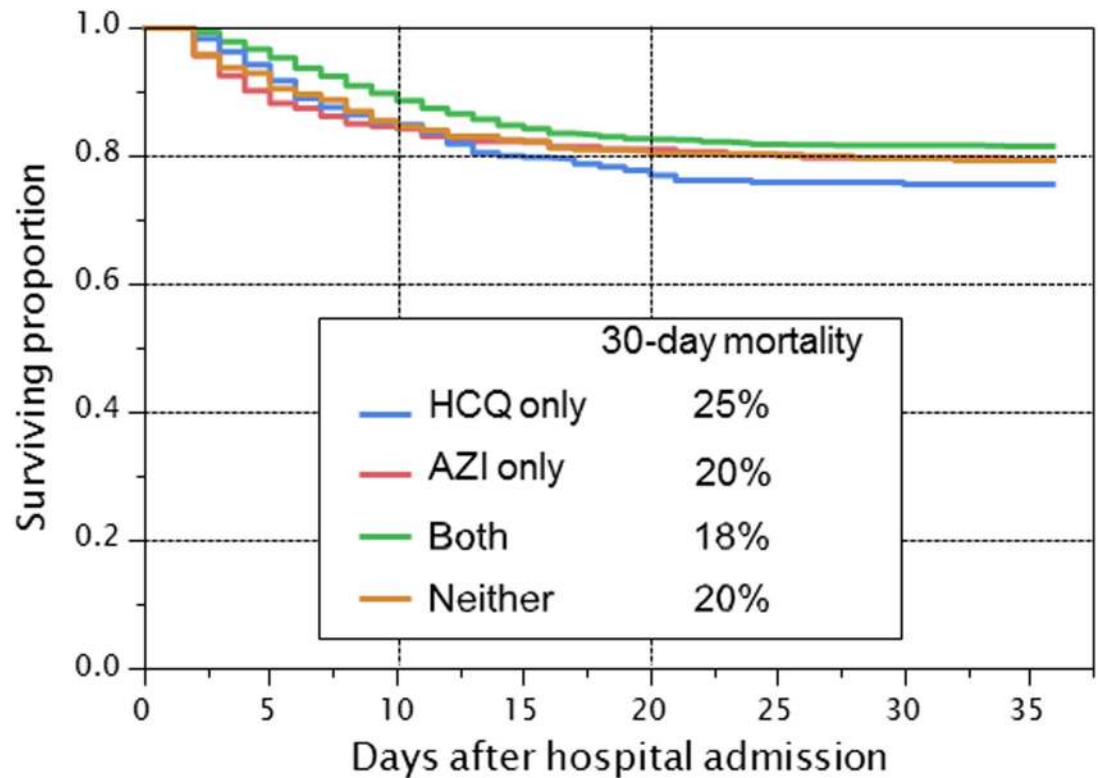
Exploratory analysis on tocilizumab therapy

198 (8%) patients received tocilizumab therapy during their hospitalization. Table 3 shows baseline characteristics. 11 patients received their first dose prior to admission to the ICU and 53 patients had unknown timing or insufficient data. Thus 134 patients with documentation of their first dose of tocilizumab within an ICU setting represented the exploratory treatment cohort. 413 patients in the ICU never received tocilizumab as of May 5, 2020 and serve as the control cohort. Tocilizumab was administered as a single dose in 104 (78%), with the majority receiving 400 mg (96%), followed by 800 mg (1%), 8 mg/kg (1%), 4 mg/kg (1%), and missing

Table 2. Propensity-score adjusted hazard ratios, confidence intervals.

	HR	95% conf. interval		P-value	30-day mortality rate	
		Lower	Upper		Experimental	Control
Any HCQ in hospital	0.99	0.80	1.22	0.92	0.20	0.20
HCQ+AZI in hospital	0.98	0.75	1.28	0.89	0.18	0.20
Factorial main effects and interaction of HCQ and AZI						
HCQ main effect	1.02	0.83	1.27	0.83	0.25 (HCQ only)	0.20 (Neither)
AZI main effect	0.89	0.72	1.10	0.28	0.20 (AZI only)	0.20 (Neither)
Interaction				0.091	0.18 (Both)	0.20 (Neither)
Tocilizumab therapy in the Intensive Care Unit						
Toci in ICU	0.76	0.57	1.00	0.053	0.46	0.56

<https://doi.org/10.1371/journal.pone.0237693.t002>



	Admission	Day 10	Day 20	Day 30
HCQ	441	370	294	221
AZI	256	212	191	173
Both	1473	1310	1053	893
Neither	342	286	251	222

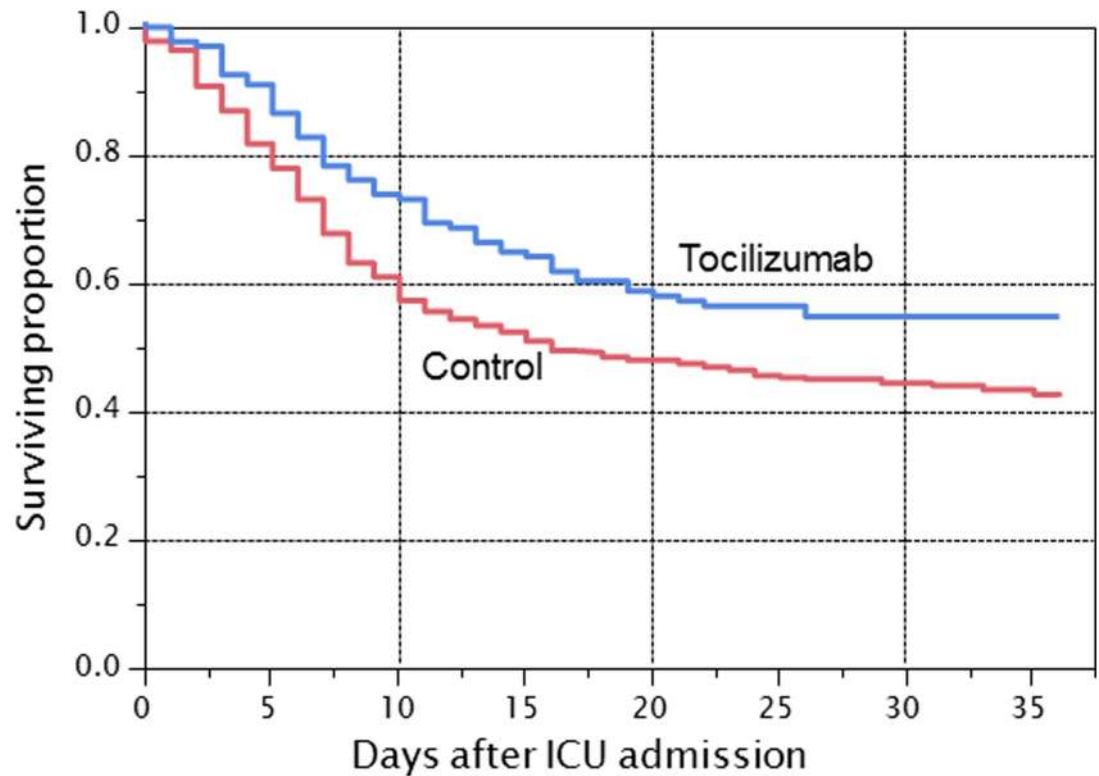
Fig 2. Unadjusted association of treatment with hydroxychloroquine on overall survival among hospitalized COVID-19 patients. Unadjusted Kaplan-Meier estimates of survival by treatment allocation to hydroxychloroquine (HCQ), azithromycin (AZI), both, or neither. Patients at risk at admission, day 10, day 20, and day 30 are shown. Patients still alive and in the hospital were censored as of May 5, 2020. Patients who had been discharged from the hospital were censored as of day 36 following hospital admission. 30-day mortality rates are shown for each treatment.

<https://doi.org/10.1371/journal.pone.0237693.g002>

dosing (1%). Secondary bacteremia occurred in 44 of the 413 (11%) patients in the non-treated group, compared to 18/134 (13%) in the treated group. Secondary pneumonia occurred in 25 of the 413 (6%) patients in the non-treated group, compared to 12 of the 134 (9%) in the treated group. As shown in Table 2, in the analysis using propensity modeling, there was a trend association between survival and treatment with tocilizumab within the ICU setting (HR, 0.76 [95% CI, 0.57–1.00]). The unadjusted 30-day mortality favored tocilizumab (46% versus 56%) (Fig 3).

Discussion

This retrospective observational cohort study of 2512 hospitalized COVID-19 patients within a 13-hospital network did not find the empirical use of hydroxychloroquine with or without



	Admission	Day 10	Day 20	Day 30
Toci	134	98	75	64
Control	413	248	188	133

Fig 3. Unadjusted association of treatment with tocilizumab on overall survival among hospitalized ICU COVID-19 patients. Unadjusted Kaplan-Meier estimates of survival by treatment allocation to tocilizumab (toci) or no toci. Patients at risk at admission, day 10, day 20, and day 30 are shown. Patients still alive and in the hospital were censored as of May 5, 2020. Patients who had been discharged from the hospital were censored as of day 36 following hospital admission. 30-day unadjusted mortality with and without tocilizumab is 46% versus 56%.

<https://doi.org/10.1371/journal.pone.0237693.g003>

co-treatment with azithromycin to be associated with a reduction in mortality (adjusted HR, 0.99 for any hydroxychloroquine during hospitalization [95% CI, 0.80–1.22]). Our multi-center findings confirm the recent observational studies from New York [14, 15]. Our results are also in agreement with the results of a recent randomized controlled study that enrolled patients with only mild-to-moderate disease, a cohort with less severe than our study [16]. Collectively these studies do not support the routine use of hydroxychloroquine outside a clinical trial. Furthermore, none of the reported observational studies have addressed the role of hydroxychloroquine among individuals with minimally symptomatic disease in the pre-hospital setting.

Unlike randomized controlled trials, observational studies have inherent biases in patient allocations that cannot be fully adjusted for during statistical analyses. Although we utilized propensity modeling to mitigate known imbalances it is possible that unmeasured confounding factors may still be important. For example, in our series we observed a change in the prescribing patterns of hydroxychloroquine during the study timeframe. Similarly dosing and timing of hydroxychloroquine varied throughout the 13-hospital network. These factors

Table 3. Unadjusted baseline characteristics of evaluable ICU patients receiving tocilizumab.

	All Patients (n = 547)	No Toci (n = 413)	Toci* (n = 134)	P-value
Demographics, n (%)				
Age Median (Iqr)	67 (58–76)	69 (58–77)	62 (53–70)	<0.0001
Age Distribution, n (%)				0.0008
[0,10]	1	1 (100%)	0 (0%)	
[10,20]	4	4 (100%)	0 (0%)	
[20,30]	9	5 (56%)	4 (44%)	
[30,40]	19	11 (58%)	8 (42%)	
[40,50]	42	29 (69%)	13 (31%)	
[50,60]	101	66 (65%)	35 (35%)	
[60,70]	155	114 (74%)	41 (26%)	
[70,80]	133	108 (81%)	25 (19%)	
[80,90]	72	64 (89%)	8 (11%)	
[90,100]	11	11 (100%)	0 (0%)	
Gender				0.016
Female	191	156 (82%)	35 (18%)	
Male	356	257 (72%)	99 (28%)	
Missing	0			
Race/Ethnicity**				0.19
African American	47	35 (74%)	12 (26%)	
Asian	29	17 (59%)	12 (41%)	
Caucasian	316	239 (76%)	77 (24%)	
Hispanic	95	74 (78%)	21 (22%)	
Other	43	36 (84%)	7 (16%)	
Missing	17	12 (71%)	5 (29%)	
Nursing Home/Rehab***				<0.0001
No	471	344 (73%)	127 (27%)	
Yes	64	60 (94%)	4 (6%)	
Missing	12	9 (75%)	3 (25%)	
Academic vs. Community				<0.0001
Academic	177	159 (90%)	18 (10%)	
Community	370	254 (69%)	116 (31%)	
Smoking History				0.26
Current	18	13 (72%)	5 (28%)	
Former	92	74 (80%)	18 (20%)	
Neither (Non-Smoker)	326	235 (72%)	91 (28%)	
Missing	111	91 (82%)	20 (18%)	
Comorbidities, n (%)				
Comorbidity Count				0.003
0	70	51 (73%)	19 (27%)	
1	144	94 (65%)	50 (35%)	
2	141	118 (84%)	23 (16%)	
≥3	192	150 (78%)	42 (22%)	
Diabetes				0.61
No	343	256 (75%)	87 (25%)	
Yes	204	157 (77%)	47 (23%)	
COPD/asthma				0.69
No	458	344 (75%)	114 (25%)	

(Continued)

Table 3. (Continued)

	<u>All Patients (n = 547)</u>	<u>No Toci (n = 413)</u>	<u>Toci* (n = 134)</u>	<u>P-value</u>
Yes	89	69 (78%)	20 (22%)	
Hypertension				0.04
No	207	146 (71%)	61 (29%)	
Yes	340	267 (79%)	73 (21%)	
Coronary Disease				0.80
No	447	336 (75%)	111 (25%)	
Yes	100	77 (77%)	23 (23%)	
Cerebrovascular Disease				0.35
No	521	391 (75%)	130 (25%)	
Yes	26	22 (85%)	4 (15%)	
Cancer				0.18
No	479	357 (75%)	122 (25%)	
Yes	68	56 (82%)	12 (18%)	
Renal Failure				0.097
No	492	366 (74%)	126 (26%)	
Yes	55	47 (85%)	8 (15%)	
Rheumatologic Disorder				0.59
No	528	397 (75%)	131 (25%)	
Yes	19	16 (84%)	3 (16%)	
Obesity (BMI >30)				
No	344	261 (76%)	83 (24%)	
Yes	203	152 (75%)	51 (25%)	
Presenting Symptoms, n (%)				
Fever				0.011
No	154	128 (83%)	26 (17%)	
Yes	393	285 (73%)	108 (27%)	
Cough				0.018
No	173	142 (82%)	31 (18%)	
Yes	374	271 (72%)	103 (28%)	
Shortness of Breath				0.042
No	144	118 (82%)	26 (18%)	
Yes	403	295 (73%)	108 (27%)	
GI****				0.33
No	434	332 (76%)	102 (24%)	
Yes	113	81 (72%)	32 (28%)	
Altered Mental State				0.89
No	461	347 (75%)	114 (25%)	
Yes	86	66 (77%)	20 (23%)	
Disease severity, n (%)				
Oxygenation < 94				0.69
No	229	175 (76%)	54 (24%)	
Yes	317	237 (75%)	80 (25%)	
Missing	1	1 (100%)	0 (0%)	
ICU Admission*****				0.36
Admitted	137	108 (79%)	29 (21%)	
Not Admitted	410	305 (74%)	105 (26%)	

(Continued)

Table 3. (Continued)

	<u>All Patients (n = 547)</u>	<u>No Toci (n = 413)</u>	<u>Toci* (n = 134)</u>	<u>P-value</u>
Treatments, n (%)				
Steroids				0.60
No	195	150 (77%)	45 (23%)	
Yes	352	263 (75%)	89 (25%)	
Hydroxychloroquine				0.004
No	61	55 (90%)	6 (10%)	
Yes	486	358 (74%)	128 (26%)	
Azithromycin				<0.0001
No	128	120 (94%)	8 (6%)	
Yes	419	293 (70%)	126 (30%)	
Hydroxychloroquine+azithromycin				<0.0001
No	156	145 (90%)	14 (10%)	
Yes	391	268 (69%)	123 (31%)	

*Tocilizumab (toci) administration of at least one dose, and if given after entering the ICU. Those who were given toci prior to ICU (n = 11) were excluded. Those who had insufficient dates of administration or ICU admission were also excluded (n = 53).

**Includes multi-racial, and not specified in other choices.

***Admitted from a nursing home or rehabilitation facility.

**** Includes nausea, vomiting, diarrhea, or other Gastrointestinal complaint obtained from medical record.

***** Admission to ICU on first day of hospitalization, as defined by chart review of medical record.

<https://doi.org/10.1371/journal.pone.0237693.t003>

(dosing, schedules, and changes in treatment patterns over time) are difficult to quantify in large observational studies. Nonetheless, our primary analysis is concordant with the other recent studies.

A potentially clinically important finding from an exploratory analysis revealed a favorable association between tocilizumab administration and survival among COVID-19 patients requiring ICU support. Our study represents one of the largest report of tocilizumab during this pandemic but is subject to all of the concerns of observational studies. However, if confirmed by ongoing randomized clinical trials, tocilizumab would represent the first successful therapy to reduce mortality.

Limitations

Multiple ongoing RCT's will ultimately determine the efficacy of hydroxychloroquine and tocilizumab in COVID-19. This observational study has limitations. First, observational studies cannot draw causal inferences given inherent known and unknown confounders. We attempted to adjust for known confounders using our propensity model approach. Second, misclassification is possible as we performed manual abstraction of EHR data. Third, hydroxychloroquine is a drug that likely has a therapeutic window that requires appropriate dosing, duration, and timing of administration [35]. We acknowledge our results should be interpreted with caution as significant heterogeneity existed within these factors, although a majority of our patients received the same dose, completed 5 days of treatment, and were given the drug within 24 hours of hospitalization. Fourth, low sample size limited our exploratory tocilizumab analysis. Fifth, our study focused on patients in New Jersey, limiting the applicability to other geographic regions, although the state's population is diverse, and the network included 13-hospitals (both academic and community) all with differing COVID-19 treatment

protocols. Lastly, we acknowledge the possibility of sampling bias as we collected data from a convenience sample in attempts to conduct the investigation quickly during this pandemic.

Conclusions

This observational cohort study from a multi-hospital system spanning New Jersey, when taken together with other studies, does not support the routine (off label and outside of clinical trial) use of hydroxychloroquine to reduce mortality among hospitalized COVID-19 patients. We cannot comment about the efficacy of this agent in the pre-hospital setting. Our exploratory review of IL-6 blockade with tocilizumab among ICU patients is encouraging and warrants further study. Common concerns regarding the limitations of observational studies should be applied until results of randomized trials are available.

Supporting information

S1 Appendix. Statistical analysis plan for Hackensack Meridian Health COVID-19 cases.
(DOCX)

S2 Appendix. Statistical output: Pages 1 to 7: Tocilizumab; Pages 8 to 14: Hydroxychloroquine (HCQ); Pages 15 to 21: HCQ + azithromycin (AZI); Pages 22 to 30: HCQ & AZI factorial.
(DOCX)

Acknowledgments

The authors wish to thank the nurses, data managers, and physicians who after caring for their patients assisted in the abstraction of the clinical data.

Author Contributions

Conceptualization: Andrew Ip, Eric Hansen, Andre H. Goy, Andrew L. Pecora, Brittany A. Sinclair, Urszula Bednarz, Michael Marafelias, Scott M. Berry, Nicholas S. Berry, Shivam Mathura, Ihor S. Sawczuk, Noa Biran, Ronaldo C. Go, Steven Sperber, Julia A. Piwoz, Bindu Balani, Cristina Cicogna, Rani Sebti, Jerry Zuckerman, Keith M. Rose, Lisa Tank, Laurie G. Jacobs, Jason Korcak, Sarah L. Timmapuri, Joseph P. Underwood, Gregory Sugalski, Carol Barsky, Daniel W. Varga, Arif Asif, Joseph C. Landolfi, Stuart L. Goldberg.

Data curation: Andrew Ip, Andre H. Goy, Brittany A. Sinclair, Michael Marafelias, Noa Biran, Ronaldo C. Go, Julia A. Piwoz, Bindu Balani, Keith M. Rose, Lisa Tank, Laurie G. Jacobs, Jason Korcak, Sarah L. Timmapuri, Joseph P. Underwood, Gregory Sugalski, Carol Barsky, Daniel W. Varga, Arif Asif, Joseph C. Landolfi, Stuart L. Goldberg.

Formal analysis: Andrew Ip, Donald A. Berry, Eric Hansen, Andre H. Goy, Andrew L. Pecora, Michael Marafelias, Scott M. Berry, Nicholas S. Berry, Shivam Mathura, Noa Biran, Cristina Cicogna, Stuart L. Goldberg.

Investigation: Andrew Ip, Andre H. Goy, Andrew L. Pecora, Urszula Bednarz, Ihor S. Sawczuk, Noa Biran, Ronaldo C. Go, Steven Sperber, Julia A. Piwoz, Bindu Balani, Rani Sebti, Jerry Zuckerman, Keith M. Rose, Lisa Tank, Laurie G. Jacobs, Jason Korcak, Sarah L. Timmapuri, Joseph P. Underwood, Gregory Sugalski, Carol Barsky, Daniel W. Varga, Arif Asif, Joseph C. Landolfi, Stuart L. Goldberg.

Methodology: Andrew Ip, Donald A. Berry, Eric Hansen, Scott M. Berry, Nicholas S. Berry, Shivam Mathura, Steven Sperber, Rani Sebti, Jerry Zuckerman, Stuart L. Goldberg.

Project administration: Andrew Ip, Brittany A. Sinclair, Urszula Bednarz, Michael Marafelias, Ihor S. Sawczuk, Joseph C. Landolfi, Stuart L. Goldberg.

Resources: Andrew Ip, Brittany A. Sinclair, Urszula Bednarz, Joseph P. Underwood, Daniel W. Varga, Stuart L. Goldberg.

Software: Donald A. Berry, Michael Marafelias, Shivam Mathura.

Supervision: Andrew Ip, Brittany A. Sinclair, Ihor S. Sawczuk, Stuart L. Goldberg.

Validation: Andrew Ip, Donald A. Berry, Eric Hansen, Andre H. Goy, Scott M. Berry, Nicholas S. Berry, Shivam Mathura, Bindu Balani, Cristina Cicogna, Sarah L. Timmapuri, Stuart L. Goldberg.

Visualization: Andrew Ip, Steven Sperber, Stuart L. Goldberg.

Writing – original draft: Andrew Ip, Donald A. Berry, Eric Hansen, Andre H. Goy, Andrew L. Pecora, Brittany A. Sinclair, Urszula Bednarz, Michael Marafelias, Scott M. Berry, Nicholas S. Berry, Shivam Mathura, Ihor S. Sawczuk, Noa Biran, Ronaldo C. Go, Steven Sperber, Julia A. Piwoz, Bindu Balani, Cristina Cicogna, Rani Sebti, Jerry Zuckerman, Keith M. Rose, Lisa Tank, Laurie G. Jacobs, Jason Korcak, Sarah L. Timmapuri, Joseph P. Underwood, Gregory Sugalski, Carol Barsky, Daniel W. Varga, Arif Asif, Joseph C. Landolfi, Stuart L. Goldberg.

Writing – review & editing: Andrew Ip, Donald A. Berry, Eric Hansen, Andre H. Goy, Andrew L. Pecora, Brittany A. Sinclair, Urszula Bednarz, Michael Marafelias, Scott M. Berry, Nicholas S. Berry, Shivam Mathura, Ihor S. Sawczuk, Noa Biran, Ronaldo C. Go, Steven Sperber, Julia A. Piwoz, Bindu Balani, Cristina Cicogna, Rani Sebti, Jerry Zuckerman, Keith M. Rose, Lisa Tank, Laurie G. Jacobs, Jason Korcak, Sarah L. Timmapuri, Joseph P. Underwood, Gregory Sugalski, Carol Barsky, Daniel W. Varga, Arif Asif, Joseph C. Landolfi, Stuart L. Goldberg.

References

1. Dong E, Du H and Gardner L, "An interactive web-based dashboard to track COVID-19 in real time," *Lancet Infect Dis*, 2020; 20(5); p. 533–534. [https://doi.org/10.1016/S1473-3099\(20\)30120-1](https://doi.org/10.1016/S1473-3099(20)30120-1) PMID: [32087114](https://pubmed.ncbi.nlm.nih.gov/32087114/)
2. Holshue M, DeBolt C, Lindquist S, et al. "First Case of 2019 Novel Coronavirus in the United States," *N Engl J of Med* 2020, 382:929–936.
3. Guan W, Ni Z, Hu Y, et al. "Clinical Characteristics of Coronavirus Disease 2019 in China," *N Engl J of Med*, 2020. 382: 1708–1720.
4. COVID-19 Treatment Guidelines Panel. Coronavirus Disease 2019 (COVID-19) Treatment Guidelines. National Institutes of Health. <https://www.covid19treatmentguidelines.nih.gov/>. Accessed [May 12, 2020].
5. Yao X, Ye F, Zhang M, et al. "In Vitro Antiviral Activity and Projection of Optimized Dosing Design of Hydroxychloroquine for the Treatment of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)," *Clin Infect Dis*. 2020; ciaa237. <https://doi.org/10.1093/cid/ciaa237> PMID: [32150618](https://pubmed.ncbi.nlm.nih.gov/32150618/)
6. Liu J, Cao R, Xu M, et al. "Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitro." *Cell Discov* (2020). 6: 16; 1–4. <https://doi.org/10.1038/s41421-020-0156-0> PMID: [32194981](https://pubmed.ncbi.nlm.nih.gov/32194981/)
7. Wang M., Cao R., Zhang L, et al. "Remdesivir and Chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro," *Cell Res*. 30; 269–271. <https://doi.org/10.1038/s41422-020-0282-0> PMID: [32020029](https://pubmed.ncbi.nlm.nih.gov/32020029/)
8. Vincent M, Bergeron E, Benjannet S, et al. "Chloroquine is a potent inhibitor of SARS coronavirus infection and spread," *Virology* 2005; 2:69 <https://doi.org/10.1186/1743-422X-2-69> PMID: [16115318](https://pubmed.ncbi.nlm.nih.gov/16115318/)

9. Savarino A, Boelaert J, Cassone A, Majori G, and Cauda R. "Effects of chloroquine on viral infections: an old drug against today's diseases?," *Lancet Infect Dis*. 2003 Nov; 3(11):722–727. [https://doi.org/10.1016/s1473-3099\(03\)00806-5](https://doi.org/10.1016/s1473-3099(03)00806-5) PMID: 14592603
10. Mauthe M, Orhon I, Rocchi C, et al. "Chloroquine inhibits autophagic flux by decreasing autophagosome-lysosome fusion," *Autophagy*, 2018. 14(8); 1435–1455. <https://doi.org/10.1080/15548627.2018.1474314> PMID: 29940786
11. Schrezenmeier E, and Dornier T, "Mechanisms of action of hydroxychloroquine and chloroquine: implications for rheumatology," *Nat Rev Rheumatol*. 2020 Mar; 16(3):155–166. <https://doi.org/10.1038/s41584-020-0372-x> PMID: 32034323
12. Chen J., Liu D., Liu L., et al. "A pilot study of hydroxychloroquine in treatment of patients with common coronavirus disease-19 (COVID-19)," *Journal of Zhejiang University (Medical Sciences)*, 2020; 49(1): 1–6.
13. Gautret P., Lagier JC, Parola P., et al. "Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial." *International Journal of Antimicrobial Agents* (2020), <https://doi.org/10.1016/j.ijantimicag.2020.105949>
14. Geleris J, Sun Y, Platt J, et al. Observational Study of Hydroxychloroquine in Hospitalized Patients with Covid-19 [published online ahead of print, 2020 May 7]. *N Engl J Med*. 2020; <https://doi.org/10.1056/NEJMoa2012410> PMID: 32379955
15. Rosenberg ES, Dufort EM, Udo T, et al. Association of Treatment With Hydroxychloroquine or Azithromycin With In-Hospital Mortality in Patients With COVID-19 in New York State. *JAMA*. Published online May 11, 2020. <https://doi.org/10.1001/jama.2020.8630> PMID: 32392282
16. Cavalcanti AB, Zampieri FG, Rosa RG, et al. Hydroxychloroquine with or without Azithromycin in Mild-to-Moderate Covid-19. *N Engl J Med* July 23, 2020; <https://doi.org/10.1056/NEJMoa2019014> PMID: 32706953
17. Channappanavar R, Perlman S. Pathogenic human coronavirus infections: causes and consequences of cytokine storm and immunopathology. *Semin immunopathol* 2017; 39:529–39. <https://doi.org/10.1007/s00281-017-0629-x> PMID: 28466096
18. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020; 395:497–506. [https://doi.org/10.1016/S0140-6736\(20\)30183-5](https://doi.org/10.1016/S0140-6736(20)30183-5) PMID: 31986264
19. Xu Z, Shi L, Wang Y, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *The Lancet Respir med* 2020; 8:420–2 [https://doi.org/10.1016/S2213-2600\(20\)30076-X](https://doi.org/10.1016/S2213-2600(20)30076-X) PMID: 32085846
20. Xu X, Han M, Li T, Sun W, Wang D, Fu B, et al: Effective treatment of severe COVID-19 patients with tocilizumab. *Proc National Acad Sci Apr* 2020, 202005615; <https://doi.org/10.1073/pnas.2005615117> PMID: 32350134
21. Luo P, Liu Y, Qiu L, Liu X, Liu D, Li J. Tocilizumab treatment in COVID-19: A single center experience. *J Med Virol*. 2020; 1–5. <https://doi.org/10.1002/jmv.25801> PMID: 31502247
22. Tocilizumab improves significantly clinical outcomes of patients with moderate or severe COVID-19 pneumonia [press release]. April 2020. [Online]. <https://www.aphp.fr/content/tocilizumab-improves-significantly-clinical-outcomes-patients-moderate-or-severe-covid-19>. [Accessed May 12, 2020].
23. Guaraldi G, Meschiari M, Cozzi-Lepri A, et al. Tocilizumab in patients with severe COVID-19: a retrospective cohort study. *Lancet Rheumatol* June 24, 2020 [https://doi.org/10.1016/S2665-9913\(20\)30173-9](https://doi.org/10.1016/S2665-9913(20)30173-9)
24. Somers EC, Eschenauer GA, Troost JP, et al. Tocilizumab for treatment of mechanically ventilated patients with COVID-19, *Clinical Infectious Diseases*, ciaa954, <https://doi.org/10.1093/cid/ciaa954> PMID: 32651997
25. Sherman R., Anderson SDPG., Gray G, et al. "Real-World Evidence—What is it and What can it tell us?," *N Engl J of Med*, 2016. 375: 2293–2297.
26. Food and Drug Administration, "Use of Electronic Health Record Data in Clinical Investigations Guidance for Industry," July 2018. [Online]. <https://www.fda.gov/media/97567/download>. [Accessed 8 April 2020].
27. Food and Drug Administration, "Use of real-world evidence to support regulatory decision-making for medical devices: draft guidance for industry and Food and Drug Administration staff," 31 August 2017. [Online]. <https://www.fda.gov/media/120060/download>. [Accessed 8 April 2020].
28. Food and Drug Administration, "Framework for FDA's Real-World Evidence Program," December 2018. [Online]. <https://www.fda.gov/media/120060/download>. [Accessed 8 April 2020].
29. Harris PA, Taylor R., Thielke R., Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)—A metadata-driven methodology and workflow process for providing translational research

- informatics support, *J Biomed Inform.* 2009 Apr; 42(2):377–81 <https://doi.org/10.1016/j.jbi.2008.08.010> PMID: [18929686](https://pubmed.ncbi.nlm.nih.gov/18929686/)
30. Harris PA., Taylor R, Minor BL, et al. The REDCap consortium: Building an international community of software partners, *J Biomed Inform.* 2019 May 9 <https://doi.org/10.1016/j.jbi.2019.103208> PMID: [31078660](https://pubmed.ncbi.nlm.nih.gov/31078660/)
 31. Anderson RN, Miniño AM, Hoyert DL, Rosenberg HM. Comparability of cause of death between ICD-9 and ICD-10: preliminary estimates. *Natl Vital Stat Rep.* 2001 May 18; 49(2):1–32. PMID: [11381674](https://pubmed.ncbi.nlm.nih.gov/11381674/)
 32. Rosenbaum PR, Rubin DB. Reducing bias in observational studies using subclassification on the propensity score. *J Am Stat Assoc.* 1984. 79:516–524.
 33. Austin PC. An introduction to propensity score methods for reducing the effects of confounding in observational studies. *Multivariate Behav Res.* 2011. 46:399–424. <https://doi.org/10.1080/00273171.2011.568786> PMID: [21818162](https://pubmed.ncbi.nlm.nih.gov/21818162/)
 34. Food and Drug Administration, “Re: Request for Emergency Use Authorization for Use of Chloroquine Phosphate or Hydroxychloroquine Sulfate Supplied from the Strategic National Stockpile for Treatment of 2019 Coronavirus Disease.” March 2020. [Online]. <https://fda.gov/media/136534/download>. [Accessed 8 April 2020]
 35. Garcia-Cremades M, Solans BP, Hughes E, et al. Optimizing Hydroxychloroquine Dosing for Patients with COVID-19: An Integrative Modeling Approach for Effective Drug Repurposing. *Clin. Pharmacol. Ther.* 2020 April 12; 108(2): 253–63. <https://doi.org/10.1002/cpt.1856> PMID: [32285930](https://pubmed.ncbi.nlm.nih.gov/32285930/)