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ORIGINAL ARTICLE

Systemic exposure to hydroxychloroquine and its relationship with outcome in severely ill COVID-19 patients in New York City

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Aim: To investigate the relationship between systemic exposure to hydroxychloroquine (HCQ) and its metabolite desethylhydroxychloroquine (DHCQ) and clinical outcome in severely ill patients treated with a standard oral dose regimen of HCQ during the first wave of COVID-19 in New York City.

Methods: We correlated retrospective clinical data with drug exposure prospectively assessed from convenience samples using population pharmacokinetics and Bayesian estimation. Systemic exposure was assessed in 215 patients admitted to ICU or COVID-ward for whom an interleukin-6 level was requested and who were still alive 24 hours after the last dose of HCQ. Patients received oral HCQ 600 mg twice daily on day 1 followed by 4 days of 400 mg daily.

Results: Fifty-three precent of the patients were intubated at 5.4 ± 6.4 days after admission and 26.5% died at an average of 32.2 ± 19.1 days. QTc at admission was 448 ± 34 ms. Systemic exposure to HCQ and DHCQ demonstrated substantial variability. Cumulative area under the serum concentration–time curve up to infinity for HCQ was 71.4 ± 19.3 h mg/L and for DHCQ 56.5 ± 28.3 h mg/L. Variability in systemic exposure was not clearly explained by renal function, liver function or inflammatory state. In turn, systemic exposure did not correlate with intubation status, survival or QTc prolongation.

Conclusion: This study in severely ill patients was not able to find any relationship between systemic exposure to HCQ and DHCQ and clinical outcome at a routine dose regimen and adds to the growing body of evidence that oral HCQ does not alter the course of disease in COVID-19 patients.

KEYWORDS
clinical outcome, COVID-19, hydroxychloroquine, pharmacokinetics, pharmacodynamics

1 | INTRODUCTION

During the first wave of COVID-19 in 2020 hydroxychloroquine (HCQ) was used extensively in the USA, advocated by the government based on preclinical observations that suggested a potential role of this drug in the treatment of the disease.[1] Modelling and simulation papers corroborated this potential by suggesting that 5-day regimens would reach sufficient concentrations to demonstrate efficacy.[2–4] However, a great number of papers subsequently demonstrated a lack of efficacy, and if anything, potential cardiotoxicity, although the latter

has remained controversial.[1,5–9] Since a placebo-controlled study was never conducted, the exact role of HCQ in the treatment of COVID-19 remains unclear.

Efficacy and toxicity of drugs are determined by many factors, including extent of disease, comorbidity, comedication, genetic predisposition as well as viral susceptibility. Interindividual differences in systemic exposure to a drug is, however, often underappreciated but often does explain efficacy and side effects of a drug or lack thereof. HCQ is absorbed well after oral administration and shows linear pharmacokinetics (PK) with a very high volume of distribution. It is metabolized to various metabolites, is partly excreted into the urine, and has a very long half-life, ranging from 5–40 days in the literature.[2,10] Surprisingly little is known about the interindividual differences in PK of HCQ in patients with COVID-19 and if such differences would translate into efficacy and side effects in subgroups of patients on standard dose regimens.[3]

We investigated the individual systemic exposure to HCQ and its major metabolite desethylhydroxychloroquine (DHCQ) in a large number of patients with COVID-19 who were treated in our hospital between March and June of 2020. Individual systemic exposure was assessed in each patient by measuring the HCQ and DHCQ concentration in each serum sample that was sent to the laboratory for interleukin-6 (IL6) assessment. This data, which could be a single or multiple measurements per patient, was combined with a newly developed population PK model for HCQ and DHCQ using empirical Bayes estimates (EBEs) of PK parameters to determine the individual systemic exposure to HCQ and DHCQ in each patient. Subsequently we investigated the relationship between systemic exposure and clinical outcome in this population.

What is already known about this subject

- Hydroxychloroquine (HCQ) has been used for the treatment of patients with COVID-19 infections, especially during the early phase of the pandemic.
- There are ample data suggesting that HCQ has little to no effect in patients with COVID-19, which might be related to the systemic exposure to the drug.
- The clinical pharmacology of HCQ has not been widely investigated in patients with COVID-19.

What this study adds

- This study investigated the pharmacokinetics of HCQ and its major metabolite in very sick patients with COVID-19 during the first wave of the pandemic in a large inner-city university medical centre in New York City.
- Systemic exposure to HCQ and its metabolite was assessed in individual patients using a combination of randomly collected samples and extensive pharmacokinetic modelling and simulation and the relationship with clinical outcome was explored.
- Clinical outcome was not correlated to systemic exposure to HCQ or its metabolite in a group of very sick COVID-19 patients.

2 | METHODS

From March to June 2020, a total of $n = 3256$ patients with COVID-19 were admitted to Columbia University Irving Medical Center (CUIMC); $N = 2929$ of these patients were adults and admitted to either 1 of the COVID-wards or 1 of the intensive care units (ICUS) and $n = 1419$ of these received HCQ treatment (with and without azithromycin) while at the hospital. HCQ was determined in 1 or more serum samples of $n = 389$ of these patients, $n = 215$ of whom received the oral standard dose regimen consisting of a loading dose of 600 mg twice daily (bid) for 1 day, followed by 400 mg daily for 4 days. Details of the various groups are listed in Table 1. HCQ and its metabolite DHCQ were quantified in serum using a laboratory-developed liquid chromatography tandem mass spectrometry method. Sensitivity of the assay was 10 ng/mL for HCQ and 10 ng/mL for DHCQ and intra- and interday precision was 5.5 and 5.6%, respectively. HCQ and its metabolite were determined in a total number of 877 serum samples. Samples were convenience samples left-over after quantification of IL6 in support of patient care. Samples were aliquoted immediately after IL6 determination and stored at −80°C until analysis for HCQ and DHCQ. Medical and drug administration data were retrieved from the patients' electronic medical record (Epic). Efficacy outcome data collected were intubation, days to intubation, death and days to death. Side effect outcome data were QTc interval, which was determined as part of routine patient care.[7] The study was approved by CUIMC's Institutional Review Board (IRB). Informed consent was waived by the IRB. M.T.Y. was the principal investigator of this study. Patients and public were not involved in the design, conduct or reporting of this study.

2.1 | PK

A population PK model was developed for oral HCQ and DHCQ on a total of $n = 877$ samples from $n = 421$ patients who had received HCQ.

The population PK model was developed using the nonlinear mixed effect model software NONMEM (NONMEM 7.4, ICON Development Solution, USA). The ADVAN2, DVAN4, and ADVAN6 user-defined subroutine were used. The structural model was explored sequentially for parent drug and metabolite, but the parameters for the final model were fitted simultaneously (ADVAN6).

For the HCQ serum data, based on various published models,[11–13] a 1-compartment and a 2-compartment model with first order absorption and elimination were explored. We also evaluated

TABLE 1 Characteristics of analysed cohorts

Characteristic	All[a]	And adults admitted Ward or ICU[b]	And received HCQ	And with PK	And HCQ standard regimen[c]
Sample size	3256	2929	1419	382	215
Characteristics					
Age (y)	(3256) 62.7 ± 18.1	(2929) 63.5 ± 18.0	(1419) 64.5 ± 16.0	(382) 63.2 ± 13.6	(215) 63.1 ± 13.0
Sex (female)	(1454) 44.7%	(1311) 44.8%	(590) 41.6%	(132) 34.6%	(70) 32.6%
Race (nonwhite)	(2469) 75.8%	(2232) 76.2%	(1093) 77.0%	(297) 77.8%	(172) 80.0%
Ethnicity (Hispanic)	(1616) 49.6%	(1472) 50.3%	(720) 50.7%	(190) 49.7%	(104) 48.4%
BMI	(2749) 29.2 ± 8.5	(2593) 29.2 ± 8.5	(1327) 29.8 ± 8.8	(369) 29.7 ± 8.2	(210) 30.2 ± 8.4
Admission profile					
Days Sx to admit	(2603) 7.2 ± 7.4	(2417) 7.1 ± 7.3	(1272) 7.2 ± 5.8	(360) 7.5 ± 5.4	(196) 8.0 ± 5.8
Days Sx to HCQ	n/a	n/a	(1272) 8.8 ± 5.9	(360) 9.1 ± 5.9	(196) 9.5 ± 6.4
Days admit to HCQ	n/a	n/a	(1419) 1.6 ± 2.2	(400) 1.5 ± 2.5	(215) 1.5 ± 2.9
Ward	(2317) 71.2%	(2291) 78.2%	(1010) 71.2%	(149) 39.0%	(95) 44.2%
ICU	(688) 21.1%	(638) 21.8%	(409) 28.8%	(233) 61.0%	(120) 55.8%
Initial temp	(3198) 99.2 ± 1.6	(2905) 99.2 ± 1.6	(1414) 99.5 ± 1.6	(381) 99.5 ± 1.6	(215) 99.6 ± 1.7
AST	(2765) 67 ± 170	(2765) 67 ± 170	(1413) 66 ± 95	(382) 74 ± 92	(215) 76 ± 114
ALT	(2756) 47 ± 124	(2756) 47 ± 124	(1413) 46 ± 66	(382) 53 ± 72	(215) 56 ± 88
MDRD	(2898) 65 ± 39	(2898) 65 ± 39	(1419) 65 ± 37	(382) 67 ± 35	(215) 69 ± 34
IL6[d]	(223) 106 ± 97	(223) 106 ± 97	(470) 124 ± 109	(379) 134 ± 112	(212) 124 ± 114
QTc	(2399) 455 ± 40	(2399) 455 ± 40	(1296) 449 ± 32	(342) 452 ± 35	(198) 448 ± 34
Clinical outcome					
Intubated	(552) 17.0%	(508) 17.3%	(381) 26.9%	(218) 57.1%	(113) 52.6%
Days to Intub	(552) 4.0 ± 13.1	(508) 3.7 ± 11.3	(381) 4.3 ± 10.0	(218) 4.9 ± 6.6	(113) 5.4 ± 6.4
Discharged living	(2449) 75.2%	(2204) 75.3%	(1030) 72.6%	(262) 68.6%	(158) 73.5%
Days to discharge	(2449) 10.7 ± 15.5	(2204) 11.9 ± 15.9	(1030) 16.4 ± 19.1	(262) 32.7 ± 25.6	(158) 32.2 ± 26.9
Expired	(730) 22.4%	(725) 24.7%	(389) 27.4%	(120) 31.4%	(57) 26.5%
Days to death	(730) 12.1 ± 13.3	(725) 12.2 ± 13.4	(389) 15.9 ± 14.4	(120) 26.3 ± 18.5	(57) 31.1 ± 19.1

Abbreviations: admit, day of admission; ALT, alanine aminotransferase (U/L); AST, aspartate aminotransferase (U/L); HCQ, hydroxychloroquine; ICU, intensive care unit; IL6, interleukin 6 (pg/mL); Initial temp, initial body temperature (F); Intub, intubation; MDRD, modification of diet in renal diseases clearance (mL/min); QTc, corrected QT interval (ms); Sx, start of symptoms.

Continuous variable presented as mean ± standard deviation.

[a]All recorded encounters between 29 February 2020 and 1 June 2020 includes outpatient, discharged from emergency department and paediatrics.

[b]All admitted age ≥18 years, discharge status known.

[c]600/600 same day, followed by 400 on each of 4 subsequent days.

[d]Pre-HCQ administration IL6 sample available.

whether fixing the lag time or absorption rate would improve the performance of the model. The DHCQ serum data were then modelled simultaneously with the parent drug. Diagnostic plots were used to confirm that the serum concentrations of HCQ and DHCQ were well described by this model.

The interindividual variability with a log-normal distribution was explored for all the PK parameters:

$$P = TVP \cdot \exp(\eta_P) \quad \eta_P \sim N(0, \omega_P^2)$$

Where P represents the individual value of the parameter P, *TVP* represents the typical value of the parameter P, and η_P denotes the interindividual variability, which is assumed to have a normal distribution with mean equals to 0 and variance equals to ω_P^2.

The combined additive and proportional error model was used to describe the residual unexplained variability:

$$C_{ij} = \widehat{C_{ij}} \cdot (1 + \varepsilon_{1ij}) + \varepsilon_{2ij} \quad \varepsilon_{1ij} \sim N(0, \sigma_1^2) \text{ and } \varepsilon_{2ij} \sim N(0, \sigma_2^2)$$

Where the C_{ij} represents the observed concentration of subject i at time j, the $\widehat{C_{ij}}$ represents the predicted concentration, ε_{1ij} and ε_{2ij} represent the proportional and additive error. They were assumed to have a normal distribution with mean 0 and variances σ_1^2 and σ_2^2. Due to large numbers of missing covariate values (23.3% of BMI, 20.9% of

sex, and 20.9% of age), we did not conduct a covariate search in this population PK model.

The population PK model provided EBEs (from NONMEM estimation) for individual model PK parameters based on patient's serum HCQ and DHCQ concentration data and each patient's dose regimen. Individualized model PK parameters were used to simulate serum concentrations over time from which the area under the serum concentration–time curve from the first administration until 48 hours after the final dose (AUC_{0-144h}), the AUC from the first administration until infinity (AUC_{0-inf}) were calculated for each patient using the PKNCA package in R (version 4.0.2 and PKNCA version 0.9.4).[14] Cmax and Cmin were the simulated maximum serum concentration of HCQ and DHCQ reached after the final dose administration and the concentration 24 hours after the final dose administration, respectively.

2.2 | Statistical analysis

Data describing patients' admission and treatment in hospital, their demographics, clinical and laboratory data and clinical outcomes from both manual chart review of the electronic medical record (Epic) and data from the clinical data warehouse, were merged with PK analysis data (AUC_{0-144h}, AUC_{0-inf}, CL and $CL_{[m]}$, Cmax and Cmin). Data were categorized and rescaled to facilitate interpretation. The chronology of events were calculated as offsets, in days, from the following milestones: date of first symptoms, date of admission, date of intubation, date of first HCQ dose, date of last HCQ dose, date of discharge. Categorical variables are presented as counts and percent of group and analysed with χ^2 or Fisher's exact test, as appropriate. Continuous variables are presented as means and standard deviations and between group comparisons analysed with independent *T*-tests. When unequal variances were encountered, a comparison was made with the Satterthwaite correction and with the Wilcoxon signed rank test and the more conservative of the 2 analyses reported. Analysis of PK data used both *T*-tests of geometric mean ratios with 90% confidence intervals for group differences, and quintiles of PK values to assess ordinal trends in PK values. Kaplan–Meier survival analysis and the log rank test was used to assess differences in quintiles of PK strata in post-HCQ regimen survival rates. Cox proportional hazards regression models were used to assess time-to-event analysis of discharge survival status predicted by PK quintiles with time-independent adjustment for patient age. Data processing and statistical analysis used SAS (SAS Institute, Cary, NC, USA).

3 | RESULTS

3.1 | Patients and outcome

Characteristics of all patients considered for the study are listed in Table 1. Of the 3256 patients admitted during the study period data, from a total of 421 adult patients were used for PK model building and data from a total of 215 adult patients were used to investigate the relationship between systemic exposure and outcome. We chose to restrict the analysis of the relationship of HCQ PK to clinical outcome to those patients who received the standard HCQ dose regimen and were alive at the end of 1 day following the day they received the last HCQ administration.

Of these 215 patients, 55.8% were admitted to the ICU, 52.6% were intubated with an average of 5.4 days from admission to intubation, 73.5% survived with an average period of stay in the hospital of 32.2 days, and 26.5% died on average 31.1 days after admission.

3.2 | Pharmacokinetics

The final dataset for developing the PK model contained data from 421 patients. The total number of serum concentration data points available for modelling was 860 and 817 for HCQ and DHCQ. The average number of concentration data points per patient were 2.04 and 1.94 for HCQ and DHCQ, respectively. Model building steps are listed in Appendix 1. HCQ and DHCQ PK were best described using a 2-compartment PK model for HCQ combined with a 2-compartment PK model for DHCQ as schematically described in Figure 1. Population PK parameters as determined in the $n = 421$ patients are listed in Table 2. The lag time of absorption was fixed according to a previously published PK model for HCQ.[12] The fraction of metabolism is described by parameter Fm. In the final model, the between-subject variability was supported on all the parameters. Two combined (additive and proportional) error models were supported for HCQ and DHCQ respectively.

Systemic exposure using noncompartmental PK parameters was determined in $n = 421$ patients and demonstrated a remarkable variability. Mean ± SD AUC_{0-inf} was 54.1 ± 25.9 h mg/L for HCQ (CV = 48%). This was partly due to the wide variety in dose regimens that patients ended up getting during this initial quite hectic period of the pandemic. There were $n = 215$ patients who had received the

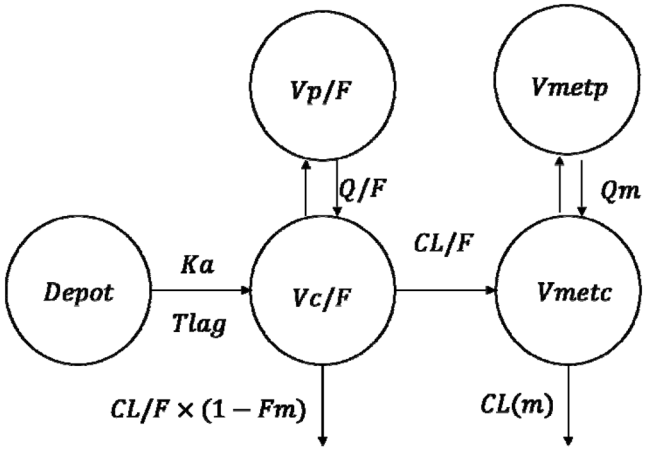

FIGURE 1 Pharmacokinetic model for hydroxychloroquine (HCQ) and desethylhydroxychloroquine (DHCQ) in COVID-19 patients.

TABLE 2 HCQ and metabolite DHCQ pharmacokinetic parameters, between subject variability, and residual variability

Parameter	Estimate	RSE%
Ka(1/h)	0.564	33
Tlag(h)	0.39	FIXED
CL/F(L/h)	39.6	5
Vc/F(L)	3170	8
Vp/F(L)	10 500	12
Q/F(L/h)	68.7	8
Fm	0.657	27
CL(m)/F(L/h)	36.6	26
Vmetc/F(L)	1010	31
Vmetp/F(L)	1710	18
Qm/F(L/h)	37.4	39
BSV on CL	37.9%	9
BSV on Vc	116.2%	7
BSV on Vp	223.4%	7
BSV on Q	100.8%	9
BSV on Ka	1189.9%	16
BSV on Fm	57.2%	11
BSV on CL(m)	33.8%	22
BSV on Vmetc	71.6%	31
BSV on Vmetp	190.2%	23
BSV on Qm	60.5%	112
σ 1(prop)(parent)	25.7%	9
σ 2(add)(parent)	1.24	40
σ 1(prop)(metabolite)	27.1%	10
σ 2(add)(metabolite)	3.01	27

Ka: First-order absorption rate constant; Tlag: absorption lag time; CL/F: apparent clearance; Vc/F: apparent volume of distribution of the central compartment for the parent drug; Vp/F: apparent volume of distribution of the peripheral compartment for the parent drug; Q/F: apparent intercompartmental clearance; Fm: fraction metabolized; CLm/F: apparent clearance for the metabolite; Vmetc/F: apparent volume of distribution of the central compartment for the metabolite; Vmetc/F: apparent volume of distribution of the peripheral compartment for the metabolite; Qm/F: apparent intercompartmental clearance for the metabolite; BSV: between-subject variability; RUV: residual unexplained variability.

standard regimen and who were all still alive at least 24 h after the last dose of HCQ. PK parameters (CL/F and $CL_{[m]}/F$) and systemic exposure (AUC_{0-144h} and AUC_{0-inf}) in this group demonstrated a smaller but still substantial variability as shown in Table 4 (HCQ mean ± SD AUC_{0-inf} was 71.4 ± 19.3 h mg/L; CV = 27%). An illustrative example of individualized serum concentration time curves of HCQ and DHCQ is given in Figure 2. Scatter plots of observed (measured) *vs*. individualized serum concentrations for the entire dataset are provided in Appendix 2. Visual predictive checks are provided in Appendix 3. Table 3 provides patient and dosing information for all 3 groups (i.e. all 1419 patients who received HCQ, the $n = 382$ patients who had PK data available and the $n = 215$ patients who received the standard dose regimen).

Table 4 and Appendix 4 show the PK and systemic exposure data stratified by renal function, liver enzymes, QTc and IL6, with the biochemistries and ECGs assessed nearest to the first and nearest to the last HCQ dose. Lower levels of renal function seem to be associated with higher systemic exposure to HCQ and lower DHCQ, albeit that differences in systemic exposure between normal and abnormal, while statistically significant, are modest. The differences are also reflected in the clearance values of HCQ and DHCQ. Liver function as reflected by ALT and AST seemed to have little to no influence on systemic exposure to HCQ and DHCQ. Systemic exposure to HCQ and DHCQ did not differ between patients with a normal QTc and an abnormal QTc interval. Systemic exposure also did not appear to differ between patients with normal and abnormal IL6 serum concentrations.

3.3 | PK and outcome

There was no clear difference in systemic exposure to HCQ and DHCQ according to IL6 serum concentrations either at near the beginning or near the end of treatment. In addition, there was no clear difference in systemic exposure to HCQ and DHCQ according to QTc time, either at the start of treatment of at the end of a routine dose regimen of HCQ.

There were also no clear differences for the systemic exposure to HCQ and DHCQ between intubation and nonintubation nor were there clear differences between survival and death, with the possible exception of slightly higher DHCQ AUCs in survivors (Table 4). Cmin and Cmax on day 5 of treatment gave similar results (Appendix 4). The potential relationships between AUCs and Clearance values of HCQ and DHCQ were further investigated using Cox regression analysis according to quintiles of systemic exposure and clearance values. As illustrated in Figure 3 for DHCQ AUC_{0-inf} and Cl_m/F and survival, we found again no clear relationships between systemic exposure of HCQ and DHCQ and either intubation or survival using probability analysis, and there was also no difference in these relationships according to sex. Statistics for Figure 3 are provided in Appendix 5.

4 | DISCUSSION

Our study shows a substantial variability in the systemic exposure to HCQ and its metabolite DHCQ in a population of severely ill COVID-19 patients in a New York City hospital during the first wave of the COVID-19 pandemic. The variability in systemic exposure was not clearly explained by renal function, liver function or inflammatory state. In turn, the variability in systemic exposure also did not appear to explain intubation or survival nor did it seem to explain QTc prolongation. This study therefore corroborates with earlier studies that demonstrated that oral HCQ does not alter the course of disease in COVID-19 patients.

The COVID-19 pandemic has been keeping the world in its grip for 2 years now. During this period the global community has made

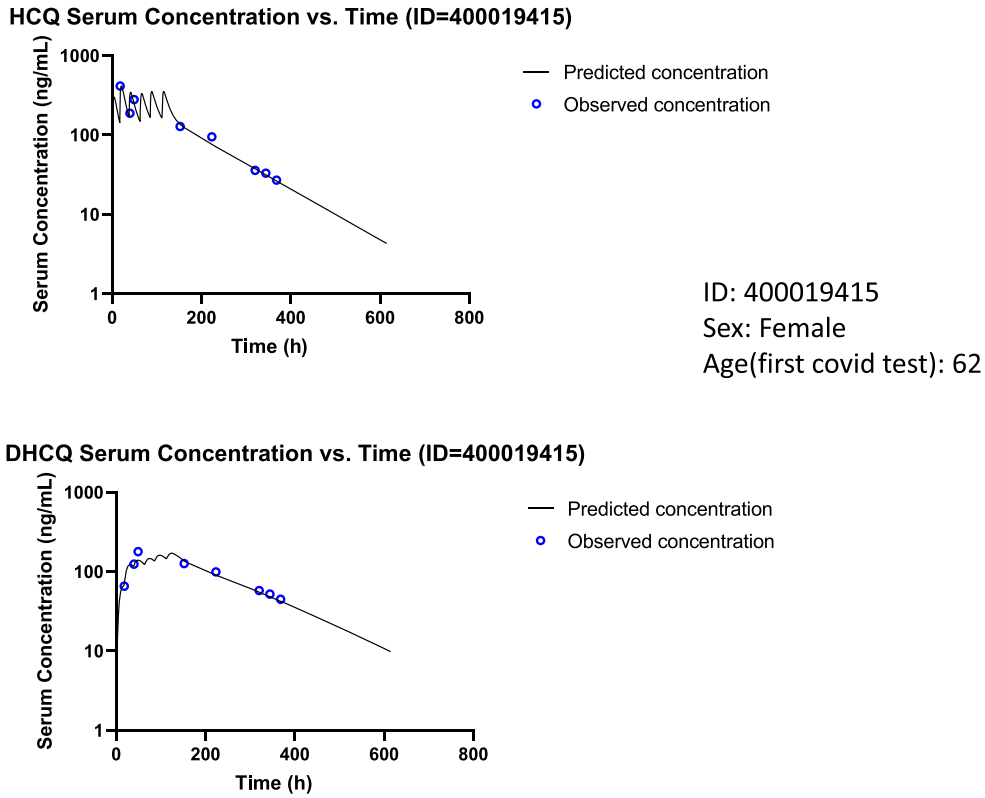

FIGURE 2 Illustrative example of individualized serum concentration–time curves of hydroxychloroquine (HCQ) and desethylhydroxychloroquine (DHCQ) in a 62-year-old female patient who received the standard oral regimen of HCQ.

TABLE 3 Details of HCQ administration

Characteristic	All who received HCQ	And with PK available	And HCQ standard regimen
Sample size (patients)	1419	382	215
Prior to 1st HCQ			
Nearest			
MDRD	69 ± 41	71 ± 40	72 ± 41
ALT	46 ± 66	46 ± 66	56 ± 88
AST	66 ± 95	74 ± 92	76 ± 114
QTc	454 ± 39	450 ± 44	446 ± 48
IL6	124 ± 109	130 ± 112	124 ± 114
Intubation status	26.9%	57.1%	52.6%
Days to 1st HCQ	1.65 ± 2.25	1.58 ± 2.62	1.49 ± 2.93
Average dose/day	580 ± 67	568 ± 52	560 ± 0
Average days given	3.9 ± 2.2	4.4 ± 2.9	5 ± 0
Days from 1st dose to discharge			
All	21.9 ± 27.2	34.5 ± 27.0	37.1 ± 28.0
Survived	25.3 ± 29.7	39.4 ± 28.7	40.2 ± 30.1
Expired	13.0 ± 15.8	23.6 ± 18.5	28.4 ± 19.0

Continuous variable presented as mean ± standard deviation.

tremendous progress in our understanding of the virus and the disease and has discovered various drugs and vaccines to successfully prevent and treat the disease. None of these data were available in March–May 2020 when the virus was wreaking havoc in New York City and therefore patients were treated based on low-level evidence-based medicine. Generating evidence for any drugs normally involves randomized controlled phase 3 trials, preceded by phase 1 and 2 studies, which in turn are preceded by rigorous preclinical and translational pharmacological and toxicological studies. In March–May 2020 no such studies had been conducted with HCQ in COVID-19

TABLE 4 Details of pharmacokinetic analysis in HCQ standard regimen and survived to day 7 ($n = 215$ AUC) geometric means

Days to 1st HCQ	1.49 ± 2.93					
	HCQ AUC inf	HCQ AUC 0–144	DHCQ AUC inf	DHCQ AUC 0–144	CL/F	CLm/F
All $n = 215$	69.7 (68.0–71.5)	31.4 (30.3–32.6)	50.5 (47.8–53.3)	15.3 (14.3–16.4)	39.0 (38.1–39.9)	36.1 (35.6–36.6)
Nearest 1st HCQ						
MDRD (abnl) $n = 84$	**74.0 (70.7–77.6)**	**33.3 (31.4–35.4)**	48.0 (44.0–52.3)	14.7 (13.1–16.4)	**36.8 (35.4–38.3)**	36.6 (35.8–37.4)
(nrml) $n = 123$	**66.9 (65.1–68.8)**	**30.2 (28.8–31.5)**	52.5 (48.9–56.4)	15.8 (14.4–17.2)	**40.5 (39.5–41.6)**	35.7 (35.0–36.4)
ALT (abnl) $n = 86$	68.2 (65.3–71.3)	31.2 (29.5–32.9)	53.3 (48.9–58.1)	16.2 (14.5–18.0)	39.8 (38.4–41.5)	35.7 (34.9–36.6)
(nrml) $n = 111$	70.7 (68.4–73.0)	31.6 (30.1–33.3)	49.5 (45.9–53.4)	15.0 (13.6–16.5)	38.4 (37.3–39.5)	36.1 (35.5–36.8)
AST (abnl) $n = 157$	69.3 (67.3–71.4)	31.4 (30.0–32.7)	51.7 (48.5–55.1)	15.8 (14.5–17.1)	39.2 (38.2–40.2)	36.0 (35.3–36.5)
(nrml) $n = 40$	70.8 (66.8–75.0)	31.7 (29.3–34.3)	48.9 (43.2–55.3)	14.4 (12.2–17.1)	38.5 (36.5–40.6)	36.2 (35.1–37.3)
QTc (abnl) $n = 20$	76.6 (69.4–83.8)	36.5 (29.2–43.8)	55.5 (42.0–69.0)	18.4 (12.8–23.9)	**36.6 (34.2–39.0)**	38.7 (34.6–38.8)
(nrml) $n = 293$	71.9 (69.4–74.4)	33.3 (32.1–34.5)	57.1 (53.6–60.6)	18.4 (17.2–19.7)	**39.9 (39.0–40.9)**	36.4 (35.9–37
IL6 (abnl) $n = 71$	68.9 (65.7–72.1)	31.1 (29.1–33.3)	47.8 (43.0–53.1)	14.8 (13.0–16.9)	39.4 (37.7–41.1)	37.0 (35.9–38.1)
(nrml) $n = 142$	70.0 (67.8–71.9)	31.4 (30.2–32.7)	51.9 (48.8–55.2)	15.5 (14.3–16.8)	38.9 (37.9–39.9)	35.6 (35.1–36.1)
Nearest last HCQ						
MDRD (abnl) $n = 98$	70.6 (67.6–73.7)	31.7 (30.1–33.3)	**46.0 (42.4–50.0)**	**14.3 (12.9–15.7)**	38.5 (37.0–40.0)	**36.9 (36.2–37.7)**
(nrml) $n = 105$	68.5 (66.6–70.5)	31.4 (30.0–32.8)	**55.2 (51.2–59.5)**	**16.6 (15.0–18.3)**	39.6 (38.6–40.7)	**35.2 (34.5–35.9)**
ALT (abnl) $n = 106$	**66.2 (64.2–68.2)**	30.6 (29.3–32.0)	49.4 (45.8–53.3)	15.6 (14.4–17.0)	**40.9 (39.7–42.0)**	36.1 (35.3–36.8)
(nrml) $n = 90$	**73.8 (70.7–77.0)**	32.5 (30.8–34.4)	51.1 (46.7–55.9)	14.8 (13.1–16.7)	**37.0 (35.6–38.4)**	36.1 (35.3–36.8)
AST (abnl) $n = 129$	69.0 (66.6–71.6)	31.8 (30.5–33.2)	50.1 (46.7–53.7)	15.6 (14.4–16.9)	39.4 (38.1–40.7)	36.1 (35.5–36.8)
(nrml) $n = 67$	70.6 (68.3–72.9)	30.9 (29.0–32.8)	50.4 (45.5–55.9)	14.6 (12.7–16.8)	38.4 (37.3–39.6)	35.9 (35.0–36.9)
QTc (abnl) $n = 15$	71.4 (60.9–81.9)	35.3 (27.8–42.8)	75.6 (49.5–101)	24.6 (15.1–34.0)	40.6 (35.1–46.0)	**34.0 (31.8–36.2)**
(nrml) $n = 115$	72.4 (68.1–76-6)	32.4 (30.7–34.1)	55.4 (50.1–60.7)	17.5 (15.7–19.3)	39.7 (38.2–41.1)	**36.4 (35.6–37.3)**
IL6 (abnl) $n = 67$	67.3 (64.3–70.5)	30.3 (28.7–31.9)	**45.1 (40.5–50.2)**	13.9 (12.3–15.8)	40.1 (38.5–41.9)	**37.3 (36.2–38.5)**
(nrml) $n = 84$	69.0 (65.9–72.2)	32.4 (31.1–33.8)	**56.8 (52.0–62.1)**	17.7 (16.1–19.3)	39.4 (37.8–41.0)	**34.9 (34.1–35.7)**
Intubated $n = 113$	67.2 (64.6–70.0)	30.3 (28.8–31.8)	47.0 (43.2–51.1)	14.5 (13.1–15.9)	**40.2 (38.8–41.7)**	**36.6 (35.8–37.4)**
Not intubated $n = 102$	72.1 (70.2–74.1)	32.5 (31.0–34.2)	55.0 (51.8–58.5)	16.3 (14.8–17.9)	**37.9 (37.1–38.7)**	**35.5 (35.0–36.0)**
Died $n = 57$	71.3 (67.0–75.8)	31.8 (29.5–34.4)	**44.4 (40.2–49.1)**	**13.5 (11.9–15.3)**	38.1 (36.0–40.3)	**37.5 (36.4–38.7)**
Lived $n = 158$	68.9 (67.2–70.6)	31.1 (30.0–32.4)	**53.1 (49.9–56.5)**	**16.0 (14.8–17.4)**	39.4 (38.6–40.3)	**35.5 (35.0–36.1)**

Bolded comparison statistically different by Satterthwaite unequal variance adjusted T-test ($P < .05$).
Normal ranges: MDRD >60 mL/min, AST 8 to 33 U/L, ALT 7 to 40 U/L, EKG QTc < 500 ms, bottom 2 tertiles of IL6 recorded values.
Areas under the curve (AUCs) in h mg/L.

and therefore the evidence-based medicine was not just missing a comparative clinical trial but also an appropriate dose-finding study, which often identifies a dose–effect relationship, or, rather, a systemic exposure–effect relationship. Clinical studies have meantime demonstrated a lack of effect of HCQ on COVID-19 while cardiotoxicity of the drug in this population remains unclear.[1,5–7] Given the absence of routine clinical pharmacology dose-finding studies we therefore hypothesized that efficacy and side effects of HCQ could be related to systemic exposure to the drug and its metabolite. In other words, it could be that the drug would be effective and/or have side effects at higher systemic exposure, while the drug would not demonstrate efficacy in the overall group. Convenience samples from IL6 assays combined with population PK and Bayesian estimation allowed us to assess the systemic exposure to HCQ and its metabolite in each individual patient, which in turn enabled us to explore the relationship between systemic exposure and clinical outcome.

Systemic exposure was assessed by first developing a population PK model that simultaneously describes the serum concentrations of HCQ and DHCQ during HCQ administration. The model was developed based on several earlier described models for HCQ and DHCQ and adequately described the serum concentrations over time. For the development of the model, we used all available patient HCQ and DHCQ data, which means that patients ranged from those who only had a single HCQ administration to those who had received HCQ for >5 days. About half of the patients only had a single data point but the other half had more, ranging from 2 to 10 data points. As mentioned, the model adequately described the serum concentration–time data of HCQ and DHCQ as assessed by comparison of individualized and observed concentration of the entire population as well as

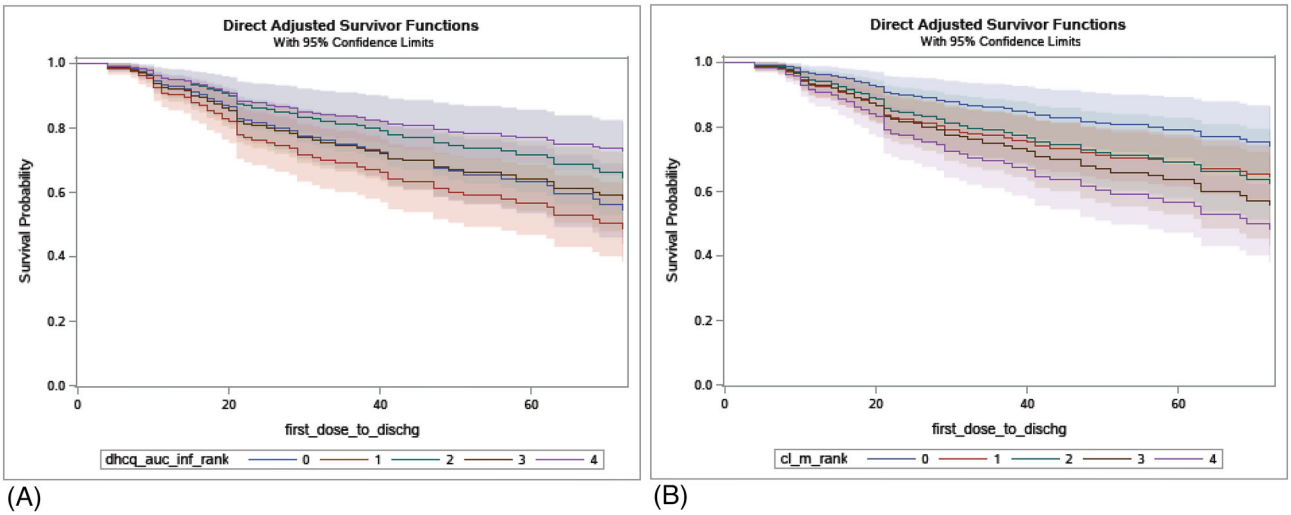

FIGURE 3 Cox-regression models for survival according to quintiles of desethylhydroxychloroquine (DHCQ) area under the serum concentration–time curve from the first administration until infinity (AUC_{0-inf} and clearance of the metabolite (CLm).

individual patient data. Normally, the predictive performance of a PK model, combined with limited samples, would include a comparison of the AUC as determined with the model *vs*. a gold-standard, which is usually a trapezoidal-rule-based AUC determined from many sampling points for each patient (e.g. 10 samples per dose interval). In the absence of such data, however, the validation of our model strongly suggests that the AUC is determined with acceptable accuracy and precision, regardless of the number of samples for each patient and regardless of the time these samples were collected. Indeed, this approach is quite normal and has been applied to many different studies.[15–19] Comparisons of our PK model with other models developed for HCQ is difficult, given the differences in sampling strategies, assays, incorporated metabolites, dose regimens, modelling and simulation software and strategies, and diseases and severity of disease between our study and those described in the literature.[13,20–23] However, corrected for the dose regimen, our serum concentration data were similar to earlier described plasma concentrations in COVID-19 patients, which were in turn lower than those described in patients with malaria.[13,20] We used the model, combined with limited sampling and EBEs to determine several PK parameters, the AUC_{0-144h}, the AUC_{0-inf}, the CL/F and $CL_{(m)}$/F. Other parameters of systemic exposure such as C_{min} and C_{max} were also explored and gave similar results as AUC. Both clearances were explored because they are the most physiological parameter in PK. Any effect of liver function, renal function or inflammation on clearance would result in a correlation between the respective biochemical parameters and CL/F and $CL_{(m)}$/F. HCQ is excreted unchanged into the urine and is metabolized by cytochrome P450 iso-enzymes and an influence of renal impairment and liver impairment may therefore be expected.[10,24] However, our results suggest that there was no substantial influence of any of these parameters on the clearance of HCQ or its metabolite, which seems in line with previous reports. An absence of a correlation between serum HCQ concentrations and inflammation determined by C-reactive protein levels was described earlier for COVID-19 patients.[25] Simulations using a physiologically based PK model described substantial increases in HCQ lung concentrations but relatively small increases in HCQ serum concentrations in COVID-19 patients with renal impairment.[26]

In terms of clinical outcome, our data also did not show any significant correlation between systemic exposure and QTc prolongation, inflammation, intubation or survival. We did not see a difference in systemic exposure between patients with normal QTc and abnormal QTc interval, neither did we observe a change in QTc interval potentially correlated with systemic exposure. We did not see a specific change in QTc interval in this population at all. These findings are similar to previous findings such as those recently reported by Eveleens Maarse *et al*. who, in a randomized controlled trial in healthy volunteers, did not find an effect at plasma concentrations up to 200 ng/mL.[27] The use of sex-specific reference ranges for QTc might have revealed additional information but for our current analysis a reference range for QTc of <500 ms was used for all patients.

The population was restricted to only those patients who had completed a full course of 600 mg bid for the first day followed by 400 mg daily for 4 days and who were still alive 24 hours after the last HCQ administration. The latter was chosen to minimize the heterogeneity in the patient and outcome dataset, especially with respect to timing and frequency of data, which varied strongly between these real-time patients. In this standardized dataset we did not observe a difference in systemic exposure between those patients who ended up intubated and not. Neither did we observe a difference in systemic exposure between those who survived and those who died. Subsequent Cox regression analysis with quintiles of AUC and CL/F also did not show any correlation between systemic exposure (or clearance) and outcome. Recently, Alvarez *et al*. reported a relationship between Cmin and length of stay in the hospital,[28] a finding that we were not able to reproduce in our study (data not shown), whether exploring this relationship in all 215 patients, or in subpopulations according to intubation or mortality status. Differences in populations, disease and

sample matrix and other factors might explain these different observations. Other outcome parameters, such as the World Health Organization scale for clinical improvement, would also have been interesting, but they were not investigated in our present study, partly because our observation period preceded the publication of this scale on 20 June 2020.[29] Interestingly, the geometric mean of the Cmin and Cmax on day 5 in our study were 121 and 330 ng/mL, respectively. Yao *et al*. reported that HCQ possesses antiviral activity against SARS-CoV-2 in vitro with an EC50 of 240 ng/mL (0.72 uM) on Vero-Cells.[4] And while it is challenging to compare total serum concentrations with EC50 assessed in vitro, the relatively low serum concentrations observed in our study might help explain the lack of effect and lack of correlation between systemic exposure and effect of HCQ treatment.

The patients in whom we investigated the correlation between systemic exposure and outcome, were sicker than the average COVID-19 population, with increased risk of intubation and death. This is explained by the fact that we measured HCQ and its metabolites in samples originally sent to the laboratory for IL6 measurements, which, during the first wave, was believed to be a potential marker for disease activity, and was used in our institution for the sickest patients.[30] Our findings of absence of any correlation between systemic exposure to HCQ and its metabolite and clinical outcome are therefore only applicable to very sick patients. In addition, we limited our patient population to those on the 600 mg bid and 400 mg daily regimen, and again, our correlative findings therefore only apply to those who received this standard regimen and who were still alive 24 hours after the last dose. Despite these limitations, we think that these data do provide additional insight into why HCQ does not seem to work in COVID-19. In the absence of any relationship between systemic exposure and outcome one could conclude 1 of 2 things: either (i) the systemic exposure–effect curve is very flat at this dose in this population; or (ii) HCQ does not change the course of the disease at any exposure because it simply has no effect on the virus in very sick patients. Start of treatment was 9.5 ± 6.4 days after patient-reported onset of symptoms, which might also play a role in the lack of correlation between systemic exposure and outcome as HCQ might only be active in the earliest phase of an infection.

Another limitation of our study is that we did not specifically investigate the role of azithromycin, although most patients also received azithromycin at the time of HCQ. In addition, we did not specifically look into the influence of dialysis and extracorporeal membrane oxygenation. An influence of both on the PK of HCQ can be expected but neither has been specifically investigated and the former was expected to be handled by MDRD-based renal function assessments.[24]

In conclusion, we describe the outcome of HCQ treatment in severely ill COVID-19 patients in a New York City hospital during the first wave of the pandemic in relation to the systemic exposure to HCQ and its metabolite. We found no potential factors that could explain the remarkable variability in systemic exposure to HCQ and DHCQ. We also did not find any correlations between systemic exposure to HCQ and DHCQ and either QTc prolongation or risk for intubation or death. This finding adds to the growing body of evidence suggesting that HCQ does not alter the course of COVID-19 and should therefore not be used for the treatment of patients with COVID-19.

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COMPETING INTERESTS

None of the authors has a conflict of interest for this work.

CONTRIBUTORS

A.L., Y. Y, D.J.M, R.B., M.T.Y. and S.C. designed and conducted the study, analysed the data and wrote and revised the manuscript.

DATA AVAILABILITY STATEMENT

The data used in this study are available from the corresponding authors upon request and in compliance with New York State Law and US Federal Law. Any request will need to be approved by Columbia University Irving Medical Center's Institutional Review Board before data can be shared.

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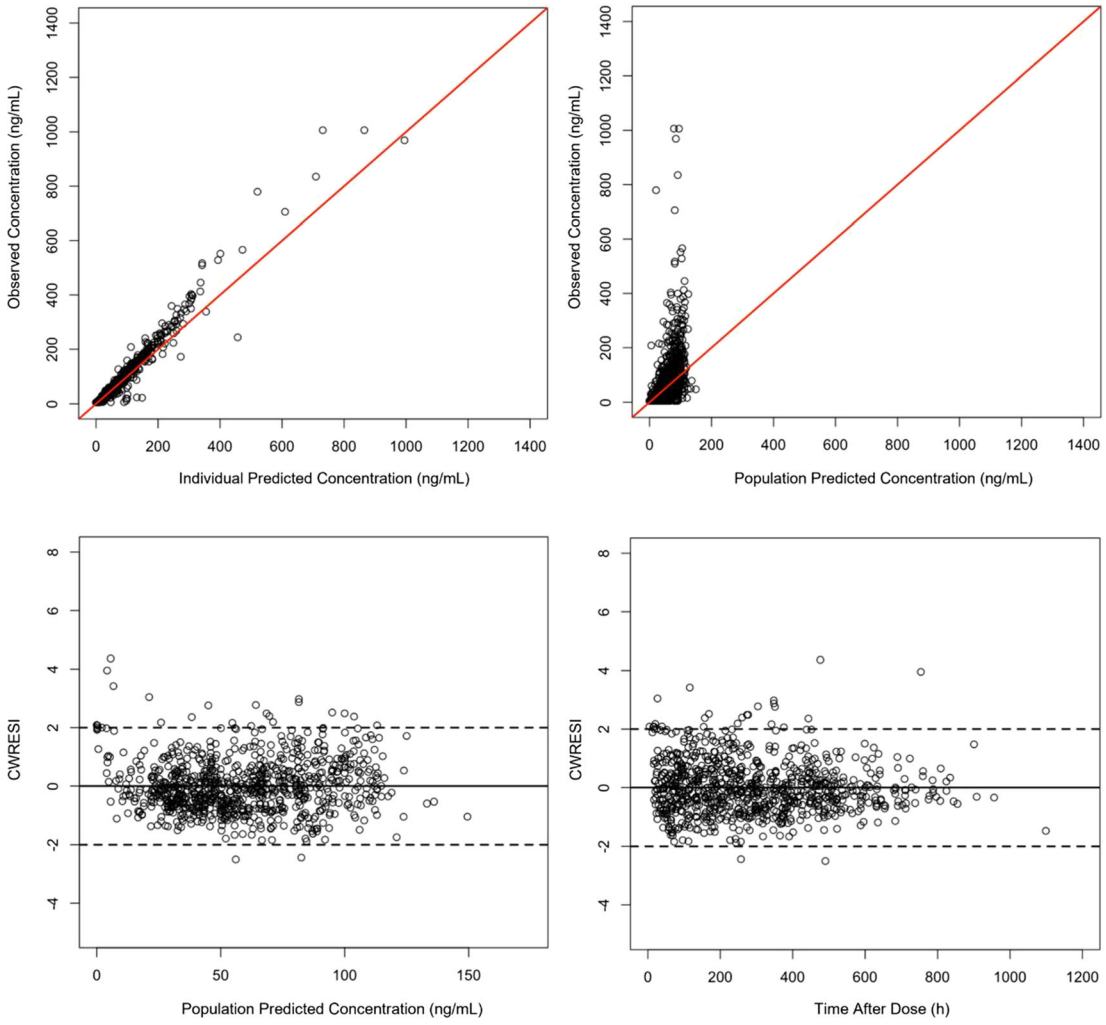
APPENDIX 1

Model-building steps				
Parent drug				
	Model	**ETA**	**OFV**	**Change in OFV**
1	1 CMT, first order absorption (KA, Tlag fixed)	CL/F, V/F	7610.83	
2	2 CMT, first order absorption (KA, Tlag fixed)	CL/F, V2/F, V3/F, Q/F	7255.622	−355.208
3	2 CMT, first order absorption (Tlag fixed)	CL/F, V2/F, V3/F, Q/F, KA	7201.263	−54.359
Parent drug + metabolite				
	Model	**ETA**	**OFV**	**Change in OFV**
1	Base model + 1CMT for metabolite (Vmet = Vc)	CL/F, Vc/F, Vp/F, Q/F, Ka, Fm, CL(m)/F	14110.399	
2	Base model + 1CMT for metabolite	CL/F, Vc/F, Vp/F, Q/F, Ka, Fm, CL(m)/F, Vmet/F	14059.435	−50.964
3	Base model + 2CMT for metabolite	CL/F, Vc/F, Vp/F, Q/F, Ka, Fm, CL(m)/F, Vmetc/F, Vmetp/F, Qm/F	14045.993	−13.442
4	Base model + 2CMT for metabolite (dual RUV model)	CL/F, Vc/F, Vp/F, Q/F, Ka, Fm, CL(m)/F, Vmetc/F, Vmetp/F, Qm/F	13996.652	−49.341

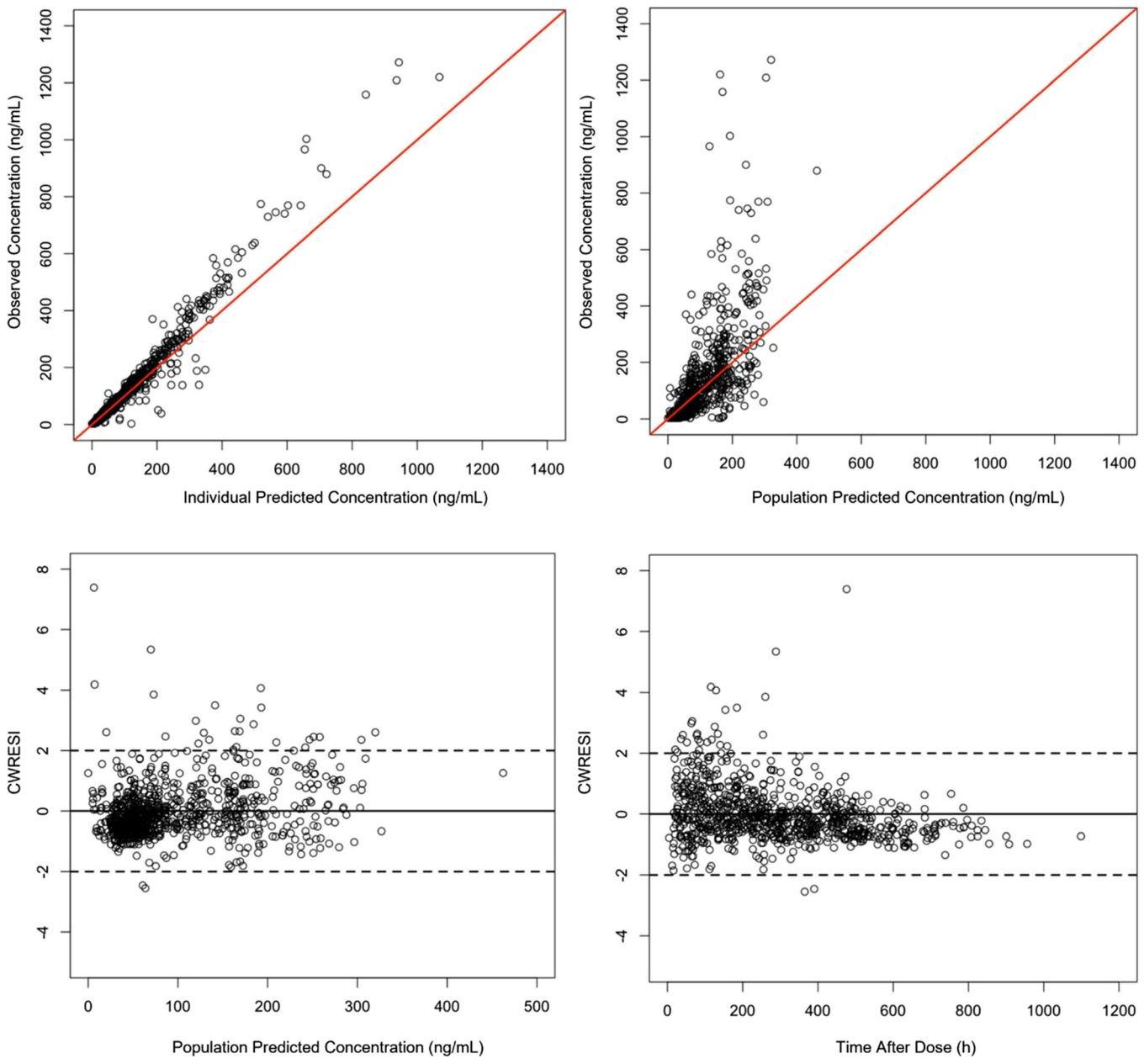
APPENDIX 2

Individual and population predicted serum concentration *vs.* observed (measured) concentration of HCQ (A) and DHCQ (B)

A.

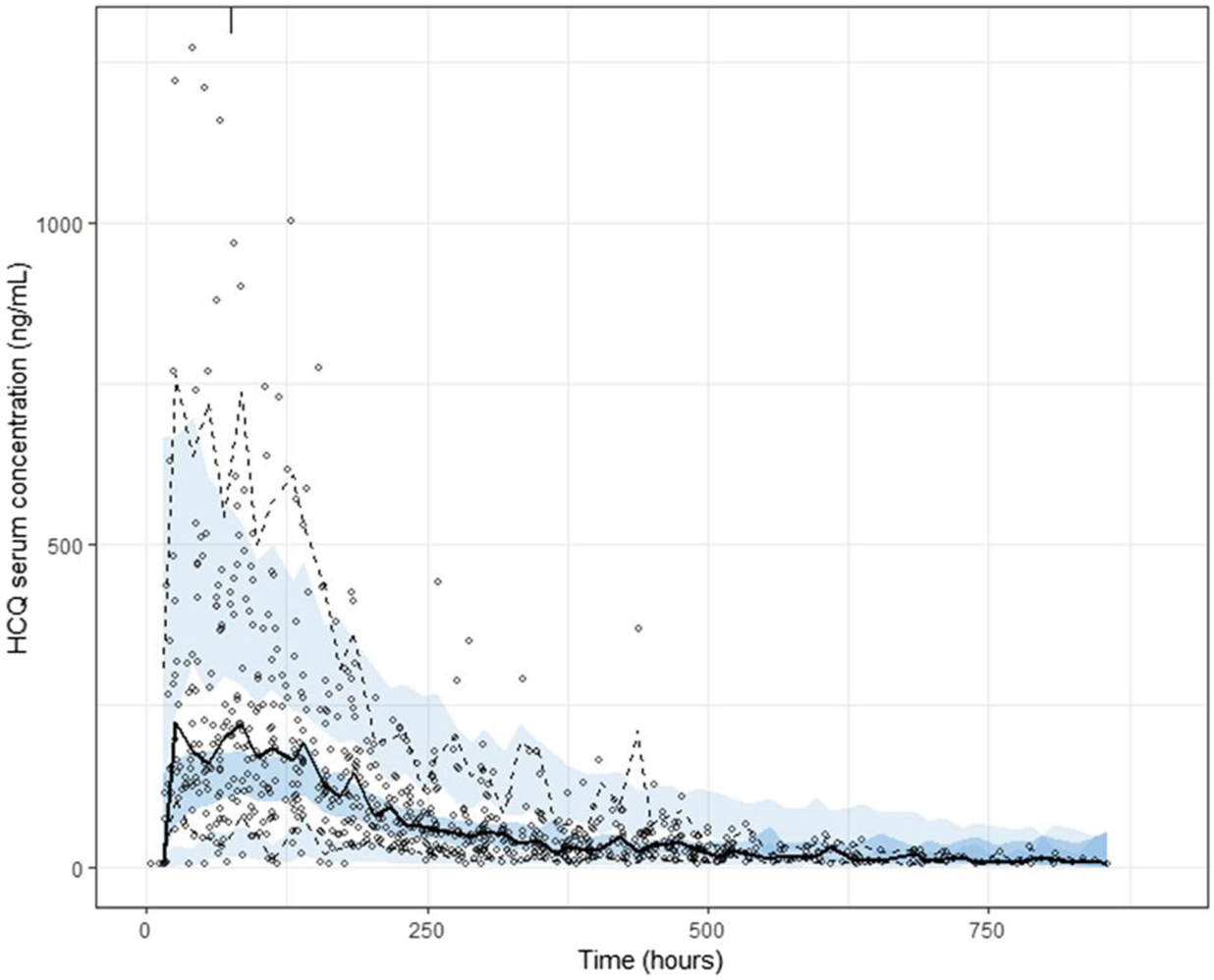


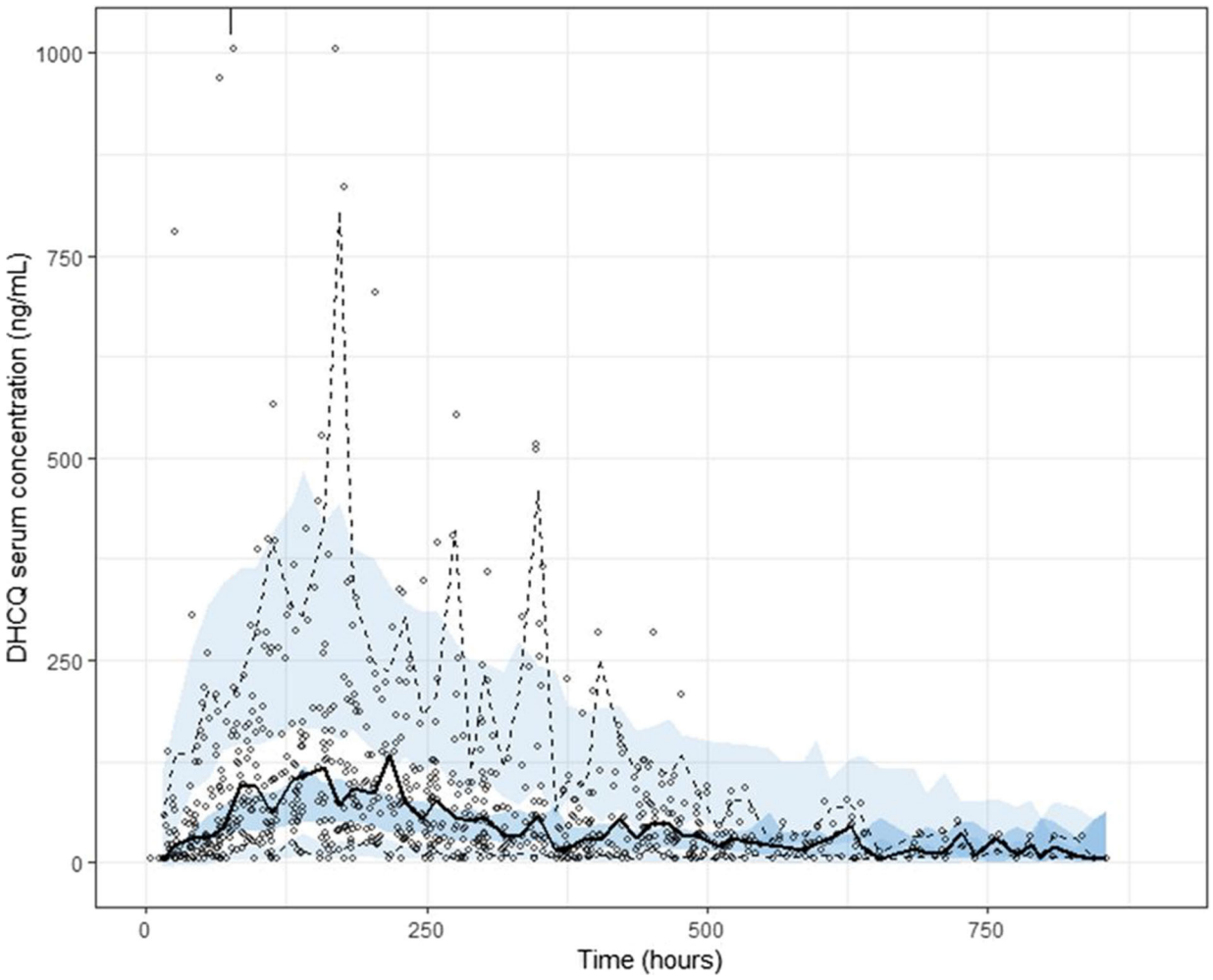
B.


Observed Concentration (ng/mL)
Individual Predicted Concentration (ng/mL)
Observed Concentration (ng/mL)
Population Predicted Concentration (ng/mL)
CWRESI
Population Predicted Concentration (ng/mL)
CWRESI
Time After Dose (h)

APPENDIX 3

Visual predictive checks (VPCs) for HCQ and DHCQ using the final model





A visual predictive check (VPC) was performed with 1000 simulation using stratification of parent drug and metabolite. The observed HCQ and DHCQ concentration, and the 5th, 50th, and 95th percentiles, were plotted with the corresponding percentiles for the simulated value. The VPC was generated using R package vpc (1.2.2).

APPENDIX 4

Details of pharmacokinetic analysis in HCQ standard regimen and survived to day 7 ($n = 215$)

Geometric means.

Days to 1st HCQ	1.49 ± 2.93 Cmax HCQ	Cmin HCQ	Cmax DHCQ	Cmin DHCQ
All $n = 215$	330 (318–343)	121 (114–128)	138 (128–148)	27 (24–29)
Nearest 1st HCQ				
MDRD (abnl) $n = 84$	347 (326–369)	126 (115–139)	133 (119–149)	26 (22–31)
(nrml) $n = 123$	319 (305–334)	118 (110–126)	141 (129–154)	27 (25–30)
ALT (abnl) $n = 86$	336 (316–358)	117 (108–127)	145 (130–162)	28 (24–32)
(nrml) $n = 111$	327 (311–344)	122 (112–133)	135 (123–149)	27 (23–31)
AST (abnl) $n = 157$	328 (314–343)	119 (111–128)	142 (131–154)	28 (25–31)
(nrml) $n = 40$	342 (316–370)	123 (110–138)	131 (110–156)	24 (19–31)
QTc (abnl) $n = 20$	357 (305–417)	111 (84–146)	133 (100–177)	18 (11–32)
(nrml) $n = 293$	335 (325–345)	124 (118–129)	141 (133–149)	28 (26–30)
IL6 (abnl) $n = 71$	332 (312–353)	126 (116–138)	133 (116–152)	26 (22–29)
(nrml) $n = 142$	327 (313–342)	119 (110–127)	139 (129–151)	27 (24–31)
Nearest last HCQ				
MDRD (abnl) $n = 98$	336 (319–355)	119 (108–131)	**127 (115–140)**	25 (21–28)
(nrml) $n = 105$	322 (307–337)	125 (118–133)	**150 (136–166)**	29 (26–33)
ALT (abnl) $n = 106$	325 (311–340)	118 (109–128)	139 (128–151)	27 (24–31)
(nrml) $n = 90$	331 (312–351)	127 (117–137)	135 (119–152)	26 (22–30)
AST (abnl) $n = 129$	326 (311–341)	126 (119–134)	139 (127–151)	27 (25–31)
(nrml) $n = 67$	331 (312–351)	114 (101–128)	134 (116–153)	25 (20–30)
QTc (abnl) $n = 15$	341 (307–378)	133 (114–155)	**193 (147–253)**	**34 (28–42)**
(nrml) $n = 115$	325 (312–339)	124 (115–133)	**132 (119–146)**	**26 (23–30)**
IL6 (abnl) $n = 67$	319 (302–336)	116 (104–129)	**125 (109–142)**	**22 (18–26)**
(nrml) $n = 84$	321 (308–334)	133 (126–139)	**158 (143–174)**	**31 (27–35)**
Intubated $n = 113$	320 (307–333)	116 (106–126)	129 (117–143)	25 (22–28)
Not intubated $n = 102$	339 (319–360)	128 (120–137)	148 (134–163)	29 (26–33)
Died $n = 57$	343 (320–367)	120 (107–136)	123 (109–140)	25 (21–31)
Survived $n = 158$	324 (310–337)	122 (115–129)	143 (132–155)	27 (25–30)

Bolded comparison statistically different by Satterthwaite unequal variance adjusted T-test ($p < .05$).

Normal ranges: MDRD >60 mL/min, AST 8 to 33 U/L, ALT 7 to 40 U/L, EKG QTc < 500 ms, bottom 2 tertiles of IL6 recorded values.

Cmin and Cmax in ng/mL.

APPENDIX 5

Cox proportional hazards mode: time to death ($n = 215$, 57 events, 158 right-censored)

Parameter	Estimate	StdErr	HR	95% CI	*P*-value
DHCQ CL_m age overall model					.07
Age (y)	0.034	0.014	1.034	1.006–1.063	.02
Cmin rank 0 (lowest)	−0.968	0.447	0.380	0.158–0.913	.04
Cmin rank 1	−0.317	0.390	0.728	0.339–1.565	.42
Cmin rank 2	−0.464	0.426	0.629	0.273–1.448	.28
Cmin rank 3 (highest)	−0.324	0.378	0.723	0.345–1.517	.74
DHCQ AUC age overall model					.04
Age (years)	0.033	0.013	1.034	1.007–1.061	.02
DHCQ AUC rank 0 (lowest)	0.643	0.470	1.902	0.757–4.780	.18
DHCQ AUC rank 1	0.892	0.439	2.441	1.033–5.767	.05
DHCQ AUC rank 2	0.175	0.540	1.191	0.413–3.433	.75
DHCQ AUC rank 3 (highest)	0.760	0.485	2.138	0.827–5.529	.12