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Abstract: Background: Hydroxychloroquine (HCQ) dosage required to reach circulating levels that inhibit SARS-Cov-2 are extrapolated from pharmacokinetic data in non-COVID-19 patients. Methods: We performed a population-pharmacokinetic analysis from 104 consecutive COVID-19 hospitalized patients (31 in intensive care units, 73 in medical wards, n=149 samples). Plasma HCQ concentration were measured using high-performance liquid chromatography with fluorometric detection. Modelling used Monolix-2019R2. Results: HCQ doses ranged from 200 to 800 mg/day administered for 1 to 11 days and median HCQ plasma concentration was 151 ng/mL. Among the tested covariates, only bodyweight influenced elimination oral clearance (CL) and apparent volume of distribution (Vd). CL/F (F for unknown bioavailability) and Vd/F (relative standard-error, %) estimates were 45.9L/h (21.2) and 6690L (16.1). The derived elimination half-life (t1/2) was 102h. These parameters in COVID-19 differed from those reported in patients with lupus, where CL/F, Vd/F and t1/2 are reported to be 68L/h, 2440L and 19.5h, respectively. Within 72h of HCQ initiation, only 16/104 (15.4%) COVID-19 patients had HCQ plasma levels above the in-vitro half maximal effective concentration of HCQ against SARS-CoV-2 (240ng/mL).. HCQ did not influence inflammation status (assessed by C-reactive protein) or SARS-CoV-2 viral clearance (assessed by real-time reverse transcription-PCR nasopharyngeal swabs). Conclusion: The inter-individual variability of HCQ pharmacokinetic parameters in severe COVID-19 patients was important and differed from that previously reported in non-COVID-19 patients. Loading doses of 1600mg HCQ followed by 600mg daily doses are needed to reach concentrations relevant to SARS-CoV-2 inhibition within 72 hours in ≥60% (95% confidence-interval: 49.5-69.0%) of COVID-19 patients

Keywords: Hydroxychloroquine; Covid-19; Pharmacokinetics; Pharmacodynamics

82   **Abbreviations**

ALAT	Alanine aminotransferase
ASAT	Aspartate aminotransferase
β	Covariate effect parameter
BMI	Body Mass Index
BW	Bodyweight
CL/F	Apparent elimination Clearance
CNIL	National Commission on Informatics and Liberties
COVID-19	Novel coronavirus disease 2019
CRP	C-Reactive Protein
CYP	Cytochrome P450
EC50	Half maximal effective concentration
F	Bioavailability
η	Between-subject variability
HCQ	Hydroxychloroquine
Ht	Hematocrit
Ka	Absorption rate constant
MDRD	Modification of Diet in Renal Disease equation
QTc	Corrected QT interval
RE	Rheumatoid Arthritis
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SARS-CoV-2	Severe acute respiratory syndrome coronavirus
SLE	Systemic Lupus Erythematosus
T.i.d	Ter in die
U-HPLC	Ultra-High Performance Liquid Chromatography
V/F	Apparent Volume of Distribution
σ	Proportional residual variability

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1. Introduction

A new human respiratory-tropic coronavirus, SARS-CoV-2, has spread rapidly worldwide. COVID-19, the disease caused by this virus, has a very variable clinical presentation, ranging from pauci-symptomatic to acute respiratory distress syndrome. Several drugs are being evaluated for the treatment of covid-19 including hydroxychloroquine ($C_{18}H_{26}ClN_3O$) (HCQ). Some observational, non-randomized studies have suggested the possible efficacy of HCQ associated or not with azithromycin in COVID 19 patients contrasting with other studies [1-5]. Recent randomized controlled trials showed that HCQ was not effective in hospitalized or non-hospitalized patients with Covid-19 [6-9]. The results of over 120 randomized controlled trials for the treatment and prevention of COVID-19 are pending. Doses of HCQ tested were highly variable, ranging from 400mg/day for few weeks up to 2.4g on day 1 as a loading dose followed by 400 mg/day for few days, based on extrapolation from pharmacokinetics properties of HCQ derived from its approved indications (malaria, auto-immune diseases) [10]. Yao et al. reported that HCQ possesses anti-viral activity, against SARS-CoV2 in vitro [11] with an EC50 (half maximal effective concentration) of 0.72μM (240ng/mL) of HCQ on Vero-cells. The antiviral effect of HCQ, has been suggested to result from increasing intracellular pH leading to decreased phago-lysosome fusion, and impaired viral receptor glycosylation. Moreover, HCQ has immune-modulating effect by inhibiting toll-like receptor signaling, decreasing production of cytokines, especially IL-1 and IL-6, potentially mitigating the cytokine release syndrome induced by SARS-CoV-2 infection [12-14].

Steady-state pharmacokinetics of HCQ has previously been reported in healthy volunteers, adult patients with malaria [15], systemic lupus erythematosus (SLE) [16, 17] and rheumatoid arthritis (RA) [18-20] and are summarized in Table-1. Herein, we analyzed plasma and blood concentration data in a cohort of consecutive patients hospitalized with COVID-19 who received HCQ. The aim of this work was to characterize HCQ pharmacokinetics in the setting of COVID-19 and to identify its main influencing covariates. The pharmacokinetic model developed from COVID-19 patients then allowed us to determine the best HCQ dosing regimen to rapidly reach relevant theoretical antiviral concentrations, i.e. higher than HCQ EC50 on SARS-CoV-2. We finally analyzed if there was any HCQ dose-efficacy relationship on SARS-CoV-2 clearance and inflammation parameters.

2. Materials and Methods

We conducted a monocenter study in consecutive patients with confirmed COVID-19 (positive for SARS-CoV-2 with reverse transcription polymerase chain reaction (RT-PCR), sampled for HCQ therapeutic drug monitoring left at the discretion of the treating physicians. Patients were treated with oral hydroxychloroquine sulfate (Plaquenil, Sanofi-Winthrop, Paris, France). Concentrations of HCQ and its metabolites in whole blood and plasma were assayed by Ultra-High Performance Liquid Chromatography (U-HPLC) with fluorometric detection [21]. This retrospective study was based on data extracted from medical records, in strict compliance with the French reference methodology MR-004, established by French National Commission on Informatics and Liberties (CNIL) and was approved by Sorbonne University ethics Committee (CER-2020-14-JOCOVID).

2.1. Pharmacokinetic-dynamic modelling

Hydroxychloroquine time-courses were analyzed using the nonlinear mixed effect modelling software program Monolix 2019R2 (www.lixoft.eu). To ensure full convergence of the program, the iteration number was fixed to 1000 with 50 Markov Chain Monte Carlo. The effect of the demographic and clinical characteristics which were thought to influence pharmacokinetics were evaluated for the following covariates: bodyweight (BW), height, age, sex, hepatic function using ALAT (alanine aminotransferase), creatinine clearance using MDRD (modification of diet in renal disease equation), CRP (c-reactive protein) level, serum albumin, co-prescription with azithromycin or other macrolides, intensive care unit vs. medical wards patients, platelets/white

141 cells counts and hematocrit. Parameter estimates were standardized for a mean standard covariate
142 using an allometric model:

$$Pi = P_{STD} \times (COVi/COVSTD)^{PWR}$$

144 where PSTD is the standard value of parameter and Pi and COVi are the parameter and covariate
145 values of the ith individual. The superscript PWR denotes an exponent power.

146   For bodyweight, allometric scaling theory dictates that PWR are typically 1 and 0.75 for
147 volumes and clearance terms, respectively [22]. The goodness-of-fit of each model was evaluated by
148 the observed-predicted (population and individual) concentration scatter plots, by the visual
149 inspection of the individual concentration-time courses, and the prediction-corrected visual
150 predictive checks.

151   A one-compartment open model best described HCQ pharmacokinetics, whatever the sampling
152 reference, blood or plasma. The parameters of the model were the elimination oral clearance (CL/F),
153 the apparent volume of distribution (V/F) and the absorption rate constant, Ka (with F, as the
154 unknown bioavailability). Given the lack of data on the absorption phase, Ka was fixed to 0.75 and
155 1.15 h^{-1} in blood and plasma respectively as previously reported [22]. Between-subject variabilities
156 were estimated for CL/F and V/F parameters and the residual variability was described by a
157 proportional model. F stands for unknown bioavailability.

158 *2.2. HCQ and Viral clearance*

159   Different covariates, including HCQ concentration, thought to influence the time-to-PCR
160 negativation were tested using the R-program [23] and the survival package [24]. The Kaplan-Meier
161 method and log-Rank test were used for this purpose. Patients were split according to their
162 individual model-predicted HCQ plasma concentration at 48h using the 1st, 50th or 75th quartile.
163 Thereafter, two Kaplan-Meyer curves were generated for each splitting factor. The time to
164 negativation was the first occurrence when two successive RT-PCR were negative.

165 *2.3. HCQ effect on CRP*

166   The CRP time-courses were modelled as a function of time and plasma HCQ Concentration
167 (Cp) as:

$$CRP = CRP_0 * \{1 - f_{HCQ} * Cp/(Cp_{50} + Cp) - (1 - f_{HCQ}) * t/(t + t_{50})\}$$

169 where CRP$_0$, f$_{HCQ}$, Cp$_{50}$ and t$_{50}$ denote the initial CRP concentration, fractional effect of HCQ, HCQ
170 concentration or time that produce a 50% decrease in the CRP$_0$ level. The model stands for the effect
171 of HCQ (f$_{HCQ}$ and Cp$_{50}$) plus an independent time-related effect ([1 - f$_{HCQ}$] and t$_{50}$) which
172 simultaneously decrease the initial CRP$_0$ level.

173 **3. Results**

174 *3.1. Demographic and biological characteristics*

175   A total of 149 plasma samples were obtained from 104 COVID-19 patients, (n=31 in intensive
176 care units and n=73 in medical wards). Time point of drug sampling was performed at various times
177 after HCQ dosing, i.e., mean 16.2 h (SD 30 h). Characteristics of included COVID-19 patients are
178 detailed in Table-2. At the time of HCQ blood sampling, 10/104 patients (9.6%) had severe renal
179 failure with a glomerular filtration rate <30 mL/min/1.73m2 and 34/104 (32.7%) had ALAT levels 3
180 times higher than the upper normal limit. In all patients, SARS-CoV-2 was confirmed by a positive
181 (RT-PCR) assay on a nasopharyngeal sample. All patients were treated with HCQ and 75/104 (72%)
182 had a post-treatment follow-up with RT-PCR on nasopharyngeal samples. HCQ was combined with
183 a macrolide antibiotic in 29 patients (n=6 with azithromycin). The mean time between the
184 introduction of HCQ and the onset of symptoms was 8.6 ± 5 days. The usual HCQ dosage was

185 200mg t.i.d (78/104 patients) for 1 to 11 days (3 patients received an 800mg loading dose). Figure-1
186 shows plasma and blood HCQ concentrations available in our cohort.

187 **Table 1.** Median plasma and blood pharmacokinetics parameters of HCQ in several pathologies.

Plasma	COVID-19***	Lupus*	Malaria¤	Healthy+
V/F, L	6696	2440	2363	2851
CL/F, L/h	45.5	68.2	15.5	12.0
t1/2, h	102	19.5	106	172.3

Blood	COVID-19**	LUPUS*	RA¥	RA§
V/F, L	1990	903	605	2283
CL/F, L/h	14.7	18.6	9.9	15
t1/2, h	93.8	25.9	43.3	124.3

188 *HCQ sulfate 400 mg/day [16], §HCQ sulfate 400, 800, or 1,200 mg/day [19], ¥HCQ sulfate 200 or
189 400mg/day [20], ¤HCQ sulfate dose 800mg then 400 mg at 6, 24, and 48 h afterward [15], **96 patients
190 (135 samples), ***104 patients (149 samples) in our study were assessed.

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209 **Table 2.** Demographic and biological characteristics of 104 patients.

|	Mean ± standard deviation	Minimum	Maximum
Age, years	63.0 ± 14.4	25	99
Weight, kg	79 ± 16	40	150
Height, cm	169 ± 11	146	192
BMI, kg/m²	27.3 ± 5.0	17.8	51.9
Sex, (Female) %	32	NA	NA
Patient in intensive care, %	23	NA	NA
MDRD, mL/min/1.73m2.	86.0 ± 33.6	5	194
Creatinine, μmol/L	98 ± 82	34	808
Albumin, g/L.	29.0 ± 6.9	12	63
HT, %.	35.0 ± 6.5	18	49
Platelet, 10^9 L	313 ± 134	52	753
White blood cells, 10^9 L	7.5 ± 4.7	2	32.4
C-reactive protein (CRP), mg/L	86 ± 98	2	469
ALAT, U/L	69 ± 74	11	486
ASAT, U/L	63 ± 49	13	252
Dose HCQ, mg/day	563 ± 99	200	800
Observation duration, Days	5.3 ± 2.3	1	12
Blood concentration			
HCQ, ng/mL	586 ± 457	50	2792
Plasma concentration			
HCQ, ng/mL	193 ± 152	12	795
HCQBlood/HCQPlasma	4.0 ± 2.3	1	15

210 BMI: Body Mass Index, NA: non-applicable, MDRD: (modification of diet in renal disease equation),
211 HT: hematocrit, ALAT: alanine aminotransferase, ASAT: aspartate aminotransferase, HCQ:
212 hydroxychloroquine. All data were collected at the time of HCQ sampling.
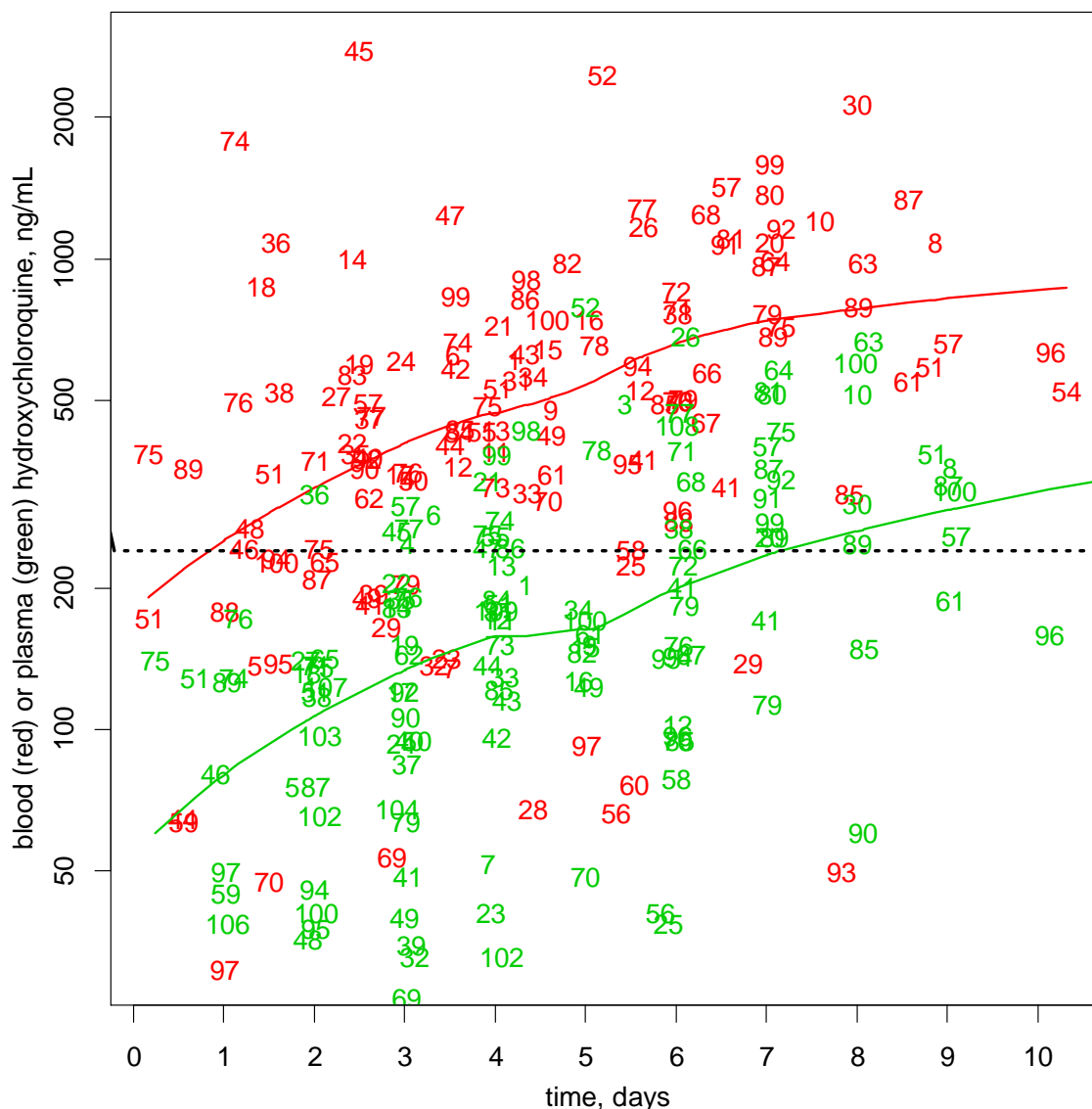
Figure 1. Observed blood (red) and plasma (green) hydroxychloroquine concentrations. Numbers stand for the patient identity and lines for the corresponding spline describing the overall trend for each matrix.

3.2. Pharmacokinetic modelling

The population plasma and blood HCQ pharmacokinetic parameter estimates and their influencing covariates are summarized in Table-3, and supplementary Table-1, respectively. These parameters estimates were different from those reported in other diseases (lupus, malaria, rheumatoid arthritis) or in healthy volunteers (Table-1). Figure-2A shows the visual predictive checks for the HCQ plasma final model in COVID-19 (for blood final model, see Figure-2B). The observed concentrations percentiles are well included in the corresponding model-predicted 90% confidence interval bands. Among the tested covariates (age, bodyweight, gender, hepatic and renal function, CRP, intensive care vs. medical wards care, macrolide/azithromycin co-prescription, platelet count), bodyweight (based on allometry principles) was the sole variable having an effect on plasma or blood HCQ CL/F and V/F prediction that improved the model. Platelet count had an additional significant effect on V/F estimation for blood HCQ (supplementary Table-1).

230 **Table 3.** Median plasma hydroxychloroquine population pharmacokinetic parameters in 104
231 COVID-19 adult patients.

| Parameter	Estimate	%res
ka, h-1	1.15	fixed
V/F, L	6690	16.1
β, V/F*(BW/70)$^\beta$	1	fixed
CL/F	45.9	21.2
β, CL/F*(BW/70)$^\beta$	0.75	fixed
ηV/F	0.61	18.9
ηCL/F	0.69	25.1
σ, ng/mL	64.1	9.76

232 CL/F, apparent elimination clearance; V/F, apparent volume of distribution; Ka, absorption rate
233 constant; F, unknown bioavailability; β, covariate effect parameter; η, between-subject variability; σ,
234 proportional residual variability; BW, bodyweight (CL/F and V/F estimates are normalized to a 70 kg
235 BW, i.e., for the ith patient $CL/F_i = CL/F*(BW_i/70)^{0.75}$.

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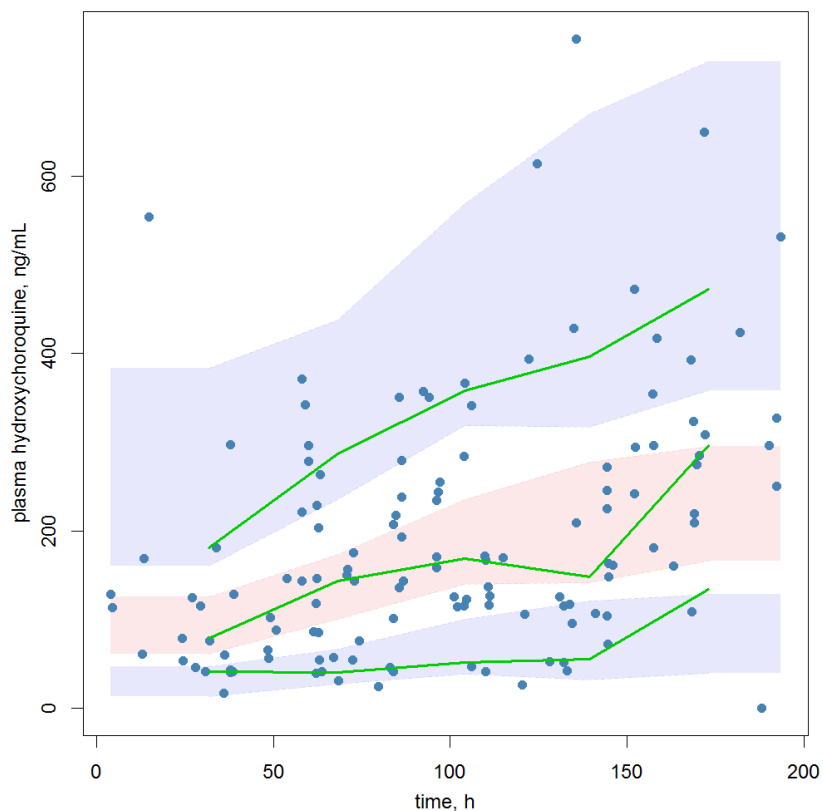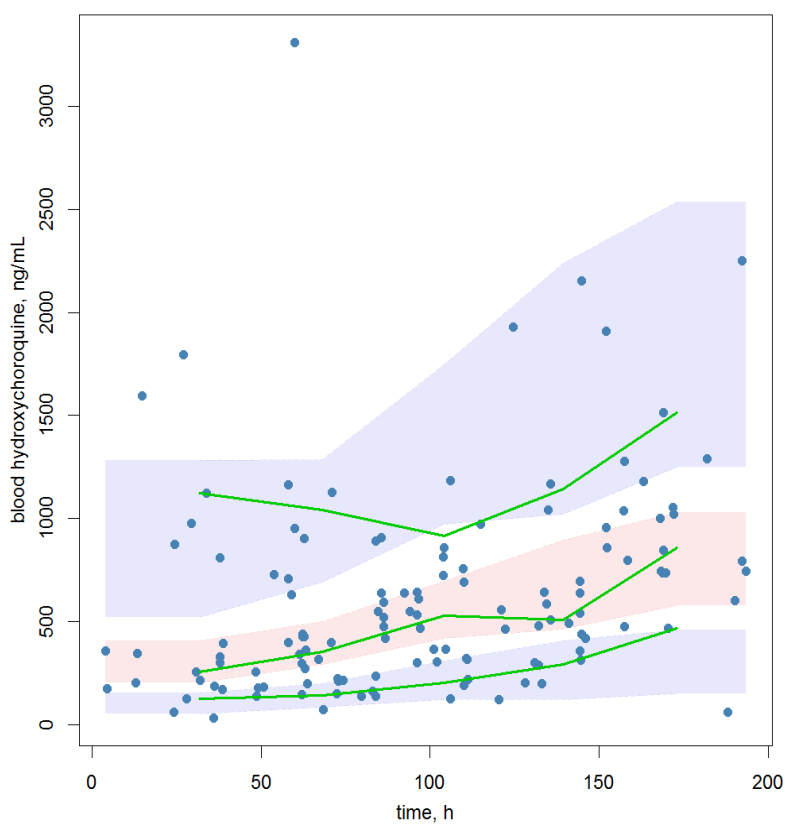
Figure 2. Prediction-corrected visual predictive check for plasma (A) and blood (B) hydroxychloroquine population pharmacokinetics. Plain (●) and green lines stand for prediction-corrected observed concentrations and their 5th, 50th and 95th percentiles. Light blue and red bands stand for the corresponding model predicted 90% confidence intervals.

Relying on our final pharmacokinetics parameters modelling, we generated representative plasma HCQ concentrations-time courses using various dosing regimens of major COVID-19 prospective trials testing HCQ (Figure-3A). Concentration vs. time profiles were also drawn according to documented plasma HCQ pharmacokinetics parameters estimates (Table-1) derived from healthy volunteers, lupus and malaria patients (Figure-3B, 3C, 3D, respectively). Depending on the diseases-specific estimates used, results were dramatically different. Figure-4 shows 4 dosing regimens based on our COVID-19 plasma HCQ pharmacokinetics estimates leading to HCQ plasma concentration above the HCQ EC50 against SARS-CoV-2 value 48 to 72h after treatment initiation. Day 1 loading doses of HCQ ≥1600mg followed by daily dose ≥600mg reached theoretical concentrations in ≥40% (95% confidence interval 30-50%) and ≥60% (95% confidence-interval: 49.5-69.0%) of COVID-19 patients within 48 and 72hours, respectively, assuming a distribution of body weights generally similar to that of our population. For a selected dosing scheme, effect of 1st and 3rd body weight quartiles on CL and Vd population parameters are shown in Figure-5A and HCQ plasma concentration-times courses for patients weighing 79kg (median bodyweight) using their individualized pharmacokinetic parameters are depicted in Figure-5B. An important between patient's variability, leading to low or unexpectedly high (potentially toxic) HCQ plasma concentrations, ensues despite administering a standardized HCQ dosing (Figure-5B).
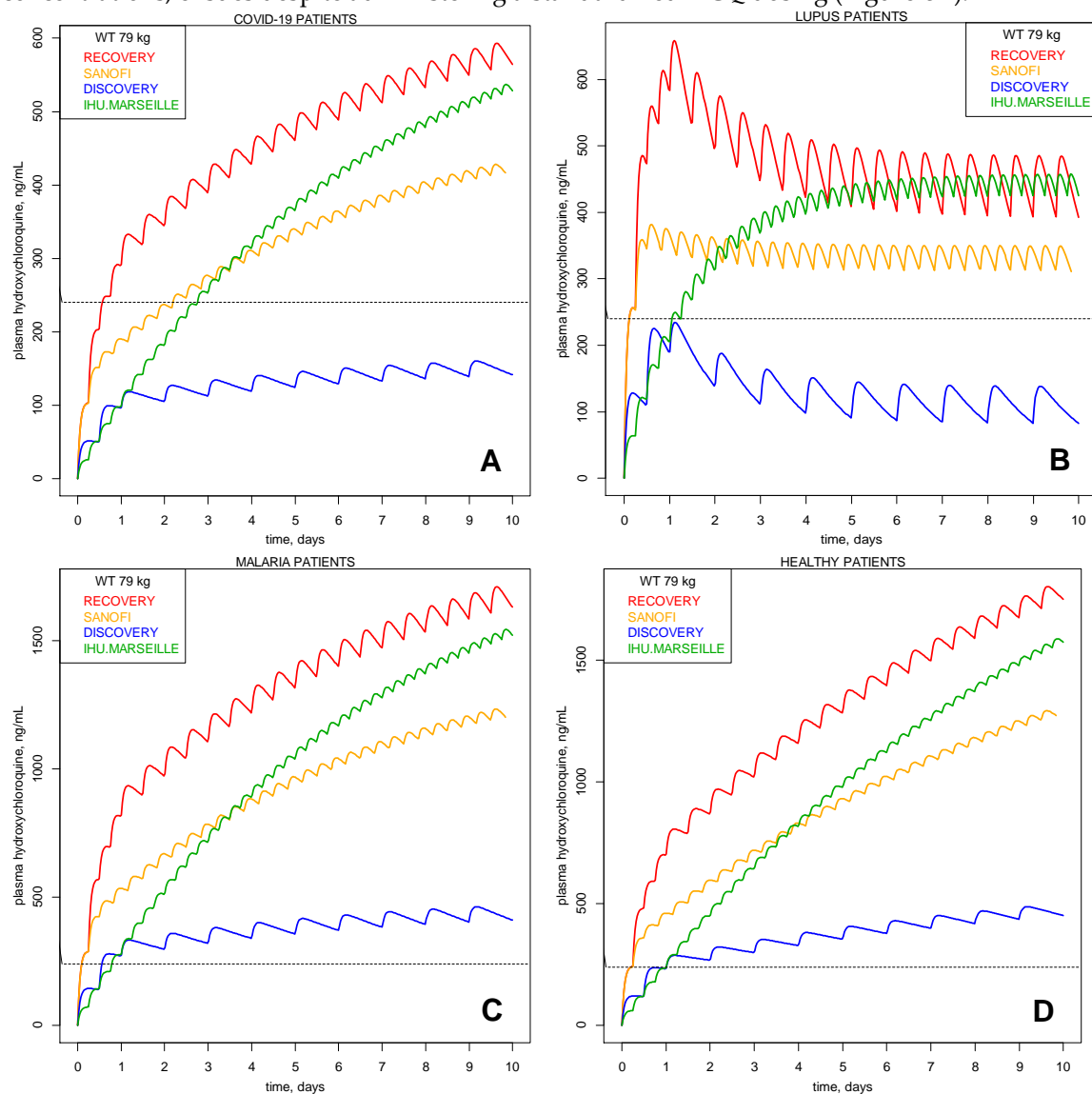

Figure 3. Representative predicted plasma HCQ concentrations-time courses as a function of the dosing regimen evaluated in major prospective trials testing HCQ for COVID-19. Curves are drawn according to our final parameters for a typical patient weight (WT) of 79 kg (observed median).

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274 Dosing schedules are 2.4 g loading dose then 400 mg/12h (RECOVERY), 1.2 g loading dose then 200
275 mg/8h (SANOFI), 200mg/8h (IHU.Marseille) and 800 mg loading dose then 400 mg/24h
276 (DISCOVERY). Curves shown are using Covid-19 patients (A), lupus patients (B), malaria patients
277 (C), and healthy subjects (D) parameters.
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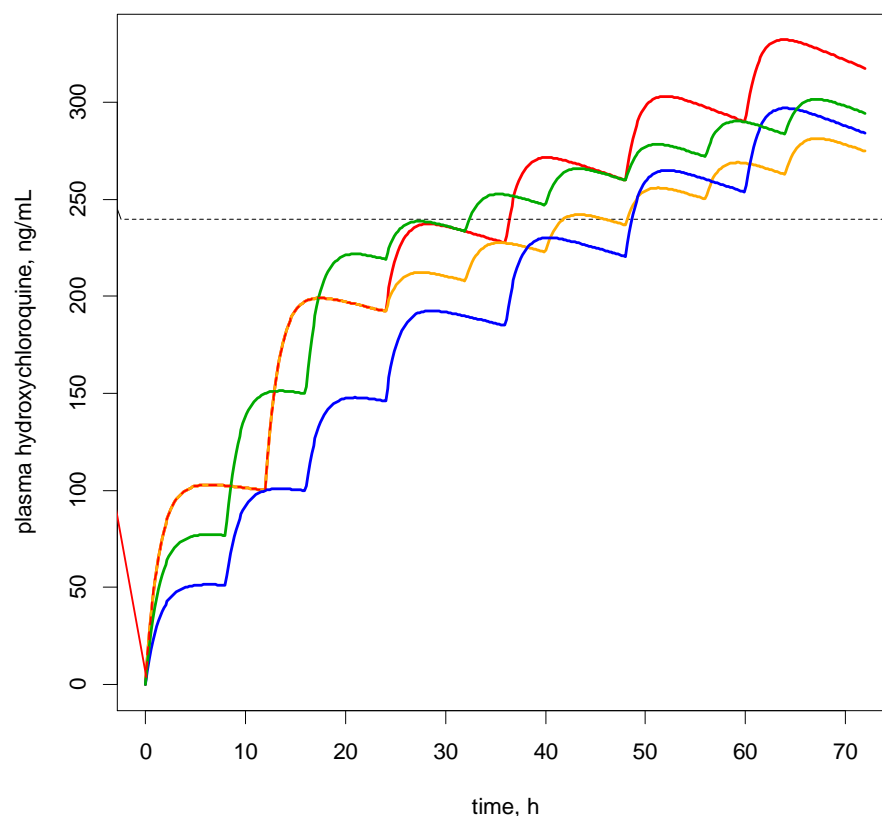
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280 **Figure 4.** Possible dosing regimen in COVID-19 patients (weighing 79 kg) according to our final model.
281 Dosing schedules represented are 800mg/12h (total 1600 mg) the 1st day, then 400mg/12h (RED);
282 800mg/12h (total 1600 mg) the 1st day, then 200mg/8h (ORANGE); 400mg/8h (total 1200mg) loading dose
283 the 1st day, then 400mg/12h (BLUE); 600mg/8h (total 1800mg) loading dose the 1st day, then 200mg/8h
284 (GREEN).
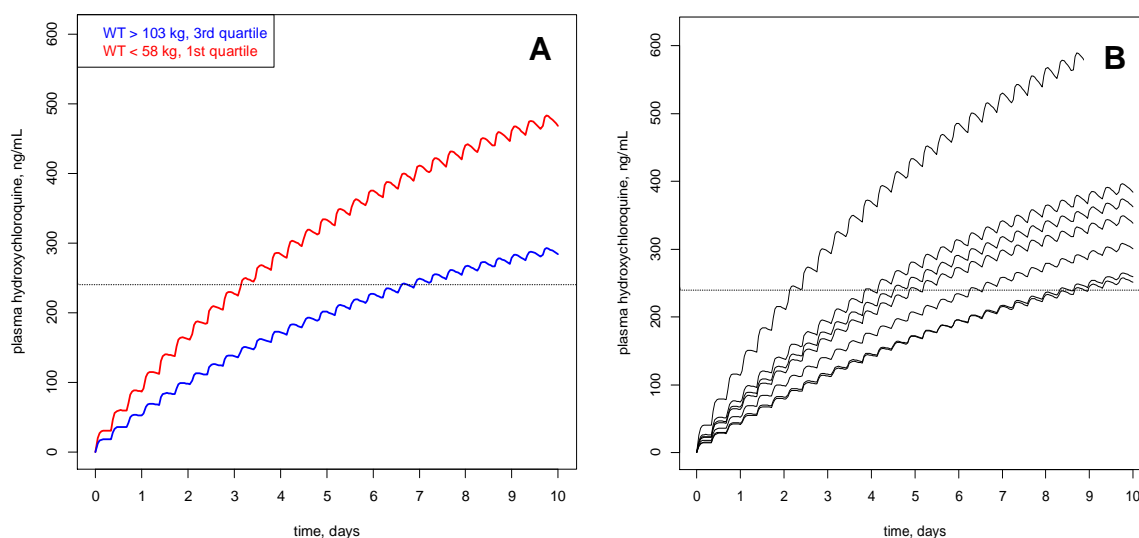
Figure 5. Mean plasma hydroxychloroquine concentration-time courses for a patient with bodyweight (WT) <58Kg or >103Kg and half-life >70h, red and blue curves, respectively (A) and for typical patients with 79kg WT and clearance (CL) ranging between 30-68 L/h and volume of distribution (Vd) between 4765-13470 L, black curves drawn from variable CL and Vd Bayesian estimates (B). Dosing regimen is 200 mg HCQ/8h, with no loading dose.

3.3. Pharmacodynamic effects of HCQ in COVID-19

A total of 75 patients were available for a SARS-CoV-2 viral status analysis using nasopharyngeal swab. PCR follow-up was negative in 40 (53%). To assess the effect of plasma HCQ concentration on time-to-PCR negativation, patients were grouped as follows: individual predicted plasma HCQ concentration at 48h below versus above 25th (72 ng/mL), 50th (95.5 ng/mL), 75th quantile (129 ng/mL). There were no significant differences in time-to-PCR negativation for all tested comparisons (Figure-6). In our cohort, only 4 and 16 patients among 104 had observed or imputed (in patients with data available after 72 hours) HCQ plasma levels >240ng/mL, the in-vitro half maximal effective concentration of HCQ against SARS-CoV-2, at 48 and 72 hours, respectively. There was also no significant effect of HCQ plasma concentration on the CRP time-course. All attempts gave non-significant values for fHCQ, or Cp50 parameters that stand for the effect of HCQ on CRP time-course, meaning that the effect of HCQ on the inflammation status could not be demonstrated.
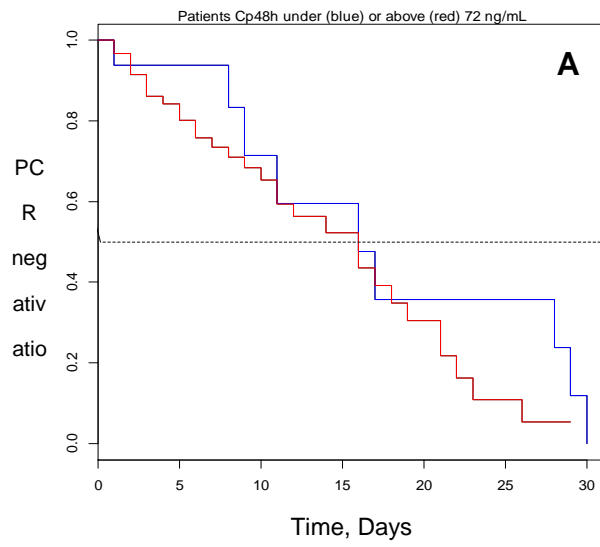
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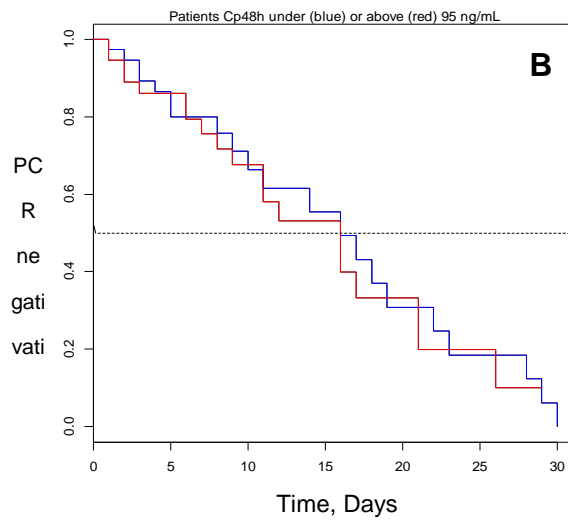
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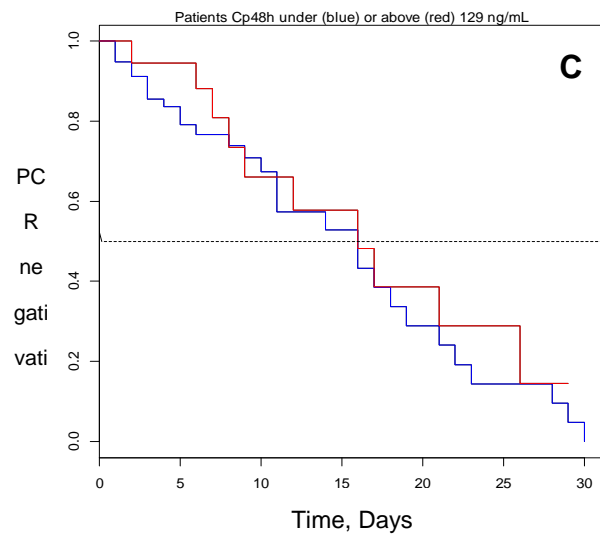
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309   **Figure 6.** Time-to-Sars-Cov-2 PCR negativation curves as a function of HCQ plasma levels within 48
310   hours of HCQ start. Blue and red curves represent patients with an HCQ plasma concentration at 48
311   h below or above the 1st HCQ plasma concentration quartile observed in our cohort, respectively (72
312   ng/mL, A), median (95 ng/mL, B) and 3rd quartile (129 ng/mL, C).

313 **4. Discussion**

314   In this study, we developed a plasma and blood population pharmacokinetics models of HCQ
315 based on data obtained in hospitalized COVID-19 patients in intensive care units and in medical
316 wards. The blood and plasma pharmacokinetics were described by a one-compartment model with
317 first-order absorption. Body weight had a significant effect on CL and Vd in both matrices. HCQ
318 pharmacokinetic parameters in COVID-19 patients are different from those of other pathologies
319 (lupus, malaria, rheumatoid arthritis) and healthy volunteers [15, 16, 20]. The theoretical ideal lowest
320 dose to achieve a target plasma concentration >EC50 (240ng/ml) within 48/72 hours in most patients
321 was 1600mg as a loading dose, followed by 200mg/8h thereafter. Nevertheless, plasma
322 concentrations of HCQ showed a high interindividual variability (see Figure-1) mainly influenced
323 by body weight. In COVID 19, either HCQ dosage adjusted on body weight or HCQ plasma
324 therapeutic drug monitoring may be useful options if HCQ is clinically effective on COVID-19.
325 However, in our cohort study, there was no significant influence of HCQ plasma concentrations on
326 inflammation (CRP) or on viral clearance (RT-PCR).
327   Interestingly, recent studies used HCQ pharmacokinetics parameters derived from
328 autoimmune diseases, to propose dosing regimen of HCQ to be used in COVID-19 patients [25-27].
329 Thus, our data suggest that relevance of these type of modelling might be toned down given the
330 importance of difference observed between HCQ pharmacokinetic parameters in COVID-19 versus
331 other settings (Table-1). Supporting our findings, preliminary pharmacokinetics data from a small
332 cohort of 7 hospitalized COVID-19 patients treated with HCQ as part of the RECOVERY trial (2.4g as
333 loading dose then 400 mg/12h) have recently been pre-published [28]. The results indicate that HCQ
334 concentrations are lower than those expected based on previous modelling, even though a high dose
335 regimen was used.
336   Of note, our PK blood parameters estimates were concordant with those estimated by Thémans
337 *et al* [29] and other groups [27, 29, 30] providing evidence that a high HCQ loading dose is needed to
338 reach circulating levels in COVID-19 patients theoretically relevant as compared to in-vitro
339 SARS-CoV-2 inhibitory concentrations.
340   In our cohort including over 100 COVID-19 patients, subjects had different profiles ranging
341 from hospitalization in medicine to intensive care unit, with variable renal and hepatic functions, as
342 well as co-prescription with macrolides, most of which are cytochrome P-450 inhibitors [31]. None
343 influenced HCQ plasma and blood pharmacokinetics in COVID-19 except weight, or weight and
344 platelet count, respectively. This finding is concordant with other HCQ pharmacokinetic studies in
345 lupus and malaria settings, in which body mass index and platelet count were also significant
346 contributing covariates in the model [16, 32].
347   Of note, the relationship between circulating concentrations of HCQ and clinical efficacy has
348 been demonstrated in rheumatoid arthritis and systemic lupus erythematosus [17-19, 33]. However,
349 our study did not show any association between plasma HCQ concentration and time to
350 negativation of SARS-CoV-2 viral load in hospitalized patients, or resolution of inflammation
351 (assessed by CRP). We are currently studying the association between the blood and plasma
352 concentration of HCQ and QTc (i.e the duration of ventricular repolarization corrected for heart rate,
353 a predictor of ventricular arrhythmias) [26] in patients with COVID-19 to further assess
354 cardiovascular safety of HCQ in COVID-19 setting [34]. Indeed, the risks of cardiotoxicity associated
355 with HCQ during the COVID-19 pandemic might increase for several reasons. Patients with
356 COVID-19 have multiple risk factors for drug-induced QT prolongation and proarrhythmia:
357 hypokalemia; fever amplifying drug-induced IKr blockade; and an increase in interleukin-6, as seen
358 in COVID-19 infection which has been suggested as a mechanism of the QT prolongation associated
359 with inflammation [35]. The French Pharmacovigilance Network has reported 103 notifications of

360 cardiac adverse drug reactions associated with "off-label" use of hydroxychloroquine since March
361 2020 up to April 2020 [36]. These observations, on top of its lack of efficacy, justified limiting the
362 prescription of HCQ in COVID-19 patients [37].

363 The retrospective, observational design of our work is the main limitation. The blood and
364 nasopharyngeal samples were not systematically assessed for all patients during the treatment
365 period. This may have biased our results by precluding to demonstrate that there was an association
366 between plasma HCQ levels and negative viral loads. Unfortunately, the detailed time course of
367 viral load was unknot available, precluding further analysis. However, multiple lines of evidence
368 are emerging against HCQ efficacy in hospitalized COVID-19, even with theoretically effective high
369 dosing regimen such as in the RECOVERY randomized controlled trial [38-40]. In that study,
370 patients received a loading dose of 2.4g then 400mg every 12 hours. HCQ was not associated with
371 reduced mortality but was associated with an increased length of hospital stay and a trend towards
372 increased risk of progression to invasive mechanical ventilation or death. [35, 41] Indeed, the dosing
373 regimen used in the RECOVERY trial was even higher than the adapted dosing regimen that we can
374 recommend based on in vitro HCQ EC50 on SARS-CoV-2 and HCQ human pharmacokinetic
375 parameters in COVID-19, identified in this work.

376 **5. Conclusions**

377 Interindividual variability of HCQ pharmacokinetics parameters in hospitalized COVID-19
378 patients was important and parameters differed from those identified in non-COVID-19 patients. No
379 effect of HCQ was found on SARS-CoV-2 (nasopharyngeal) viral clearance nor on inflammation
380 resolution. Loading doses of 1600mg HCQ followed by 600mg daily doses reached within 72 hours,
381 concentrations relevant to SARS-CoV-2 inhibition in ≥60% (95% confidence-interval: 49.5-69.0%) of
382 COVID-19 patients.

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410 **Table S1**. Median blood Hydroxychloroquine population pharmacokinetic parameters in 98 COVID-19 adult
411 patients
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Parameter	Estimate	%res
ka, h-1	0.75	fixed
V/F, L	1,990	15.9
β, V/F*(BW/70)$^\beta$	1	fixed
β, V/F*(PLAT/300,000)$^\beta$	-0.726	37
CL/F	14.7	13.5
β, CL/F*(BW/70)$^\beta$	0.75	fixed
ηV/F		
ηCL/F		
σ, proportional	0.272	12.2

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414 CL/F, apparent elimination clearance; V/F , apparent volume of distribution; Ka, absorption rate constant; F,
415 unknown bioavailability; β, covariate effect parameter; η, between-subject variability;   σ, proportional residual
416 variability; BW, bodyweight (CL/F and V/F estimates are normalized to a 70 kg BW plus V/F to a 300,000
417 platelets count, i.e., for the ith patient $CL/F_i = CL/F*(BW_i/70)^{0.75}$ and $V/F_i = V/F*(BW_i/70)*(PLAT_i/300,000)^{-0.726}$
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