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Pharmacokinetics and Pharmacodynamics of Hydroxychloroquine in Hospitalized Patients with COVID-19

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45 **Abstract:** Background: Hydroxychloroquine (HCQ) dosage required to reach circulating levels that
46 inhibit SARS-Cov-2 are extrapolated from pharmacokinetic data in non-COVID-19 patients.
47 Methods: We performed a population-pharmacokinetic analysis from 104 consecutive COVID-19
48 hospitalized patients (31 in intensive care units, 73 in medical wards, n=149 samples). Plasma HCQ
49 concentration were measured using high-performance liquid chromatography with fluorometric
50 detection. Modelling used Monolix-2019R2. Results: HCQ doses ranged from 200 to 800 mg/day
51 administered for 1 to 11 days and median HCQ plasma concentration was 151 ng/mL. Among the
52 tested covariates, only bodyweight influenced elimination oral clearance (CL) and apparent
53 volume of distribution (Vd). CL/F (F for unknown bioavailability) and Vd/F (relative
54 standard-error, %) estimates were 45.9L/h (21.2) and 6690L (16.1). The derived elimination half-life
55 (t1/2) was 102h. These parameters in COVID-19 differed from those reported in patients with
56 lupus, where CL/F, Vd/F and t1/2 are reported to be 68L/h, 2440L and 19.5h, respectively. Within
57 72h of HCQ initiation, only 16/104 (15.4%) COVID-19 patients had HCQ plasma levels above the
58 in-vitro half maximal effective concentration of HCQ against SARS-CoV-2 (240ng/mL).. HCQ did
59 not influence inflammation status (assessed by C-reactive protein) or SARS-CoV-2 viral clearance
60 (assessed by real-time reverse transcription-PCR nasopharyngeal swabs). Conclusion: The
61 inter-individual variability of HCQ pharmacokinetic parameters in severe COVID-19 patients was
62 important and differed from that previously reported in non-COVID-19 patients. Loading doses of
63 1600mg HCQ followed by 600mg daily doses are needed to reach concentrations relevant to
64 SARS-CoV-2 inhibition within 72 hours in $\geq 60\%$ (95% confidence-interval: 49.5-69.0%) of COVID-19
65 patients

66 **Keywords:** Hydroxychloroquine; Covid-19; Pharmacokinetics; Pharmacodynamics

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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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93 1. Introduction

94 A new human respiratory-tropic coronavirus, SARS-CoV-2, has spread rapidly worldwide.
95 COVID-19, the disease caused by this virus, has a very variable clinical presentation, ranging from
96 pauci-symptomatic to acute respiratory distress syndrome. Several drugs are being evaluated for the
97 treatment of covid-19 including hydroxychloroquine (C₁₈H₂₆ClN₃O) (HCQ). Some observational,
98 non-randomized studies have suggested the possible efficacy of HCQ associated or not with
99 azithromycin in COVID 19 patients contrasting with other studies [1-5]. Recent randomized
100 controlled trials showed that HCQ was not effective in hospitalized or non-hospitalized patients
101 with Covid-19 [6-9]. The results of over 120 randomized controlled trials for the treatment and
102 prevention of COVID-19 are pending. Doses of HCQ tested were highly variable, ranging from
103 400mg/day for few weeks up to 2.4g on day 1 as a loading dose followed by 400 mg/day for few
104 days, based on extrapolation from pharmacokinetics properties of HCQ derived from its approved
105 indications (malaria, auto-immune diseases) [10]. Yao et al. reported that HCQ possesses anti-viral
106 activity, against SARS-CoV2 in vitro [11] with an EC₅₀ (half maximal effective concentration) of
107 0.72μM (240ng/mL) of HCQ on Vero-cells. The antiviral effect of HCQ, has been suggested to result
108 from increasing intracellular pH leading to decreased phago-lysosome fusion, and impaired viral
109 receptor glycosylation. Moreover, HCQ has immune-modulating effect by inhibiting toll-like
110 receptor signaling, decreasing production of cytokines, especially IL-1 and IL-6, potentially
111 mitigating the cytokine release syndrome induced by SARS-CoV-2 infection [12-14].

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

122 2. Materials and Methods

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

132 2.1. Pharmacokinetic-dynamic modelling

133 Hydroxychloroquine time-courses were analyzed using the nonlinear mixed effect modelling
134 software program Monolix 2019R2 (www.lixoft.eu). To ensure full convergence of the program, the
135 iteration number was fixed to 1000 with 50 Markov Chain Monte Carlo. The effect of the
136 demographic and clinical characteristics which were thought to influence pharmacokinetics were
137 evaluated for the following covariates: bodyweight (BW), height, age, sex, hepatic function using
138 ALAT (alanine aminotransferase), creatinine clearance using MDRD (modification of diet in renal
139 disease equation), CRP (c-reactive protein) level, serum albumin, co-prescription with
140 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:

$$143 \quad P_i = P_{STD} \times (COV_i/COV_{STD})^{PWR}$$

144 where P_{STD} is the standard value of parameter and P_i and COV_i are the parameter and covariate
 145 values of the i^{th} 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, K_a (with F , as the
 154 unknown bioavailability). Given the lack of data on the absorption phase, K_a 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 negatvation 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-Meier curves were generated for each splitting factor. The time to
 164 negatvation 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 (C_p) as:

$$168 \quad CRP = CRP_0 \cdot \{ 1 - f_{HCQ} \cdot C_p / (C_{p50} + C_p) - (1 - f_{HCQ}) \cdot t / (t + t_{50}) \}$$

169 where CRP_0 , f_{HCQ} , C_{p50} 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 C_{p50}) 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
t _{1/2} , 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
t _{1/2} , 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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Table 2. Demographic and biological characteristics of 104 patients.

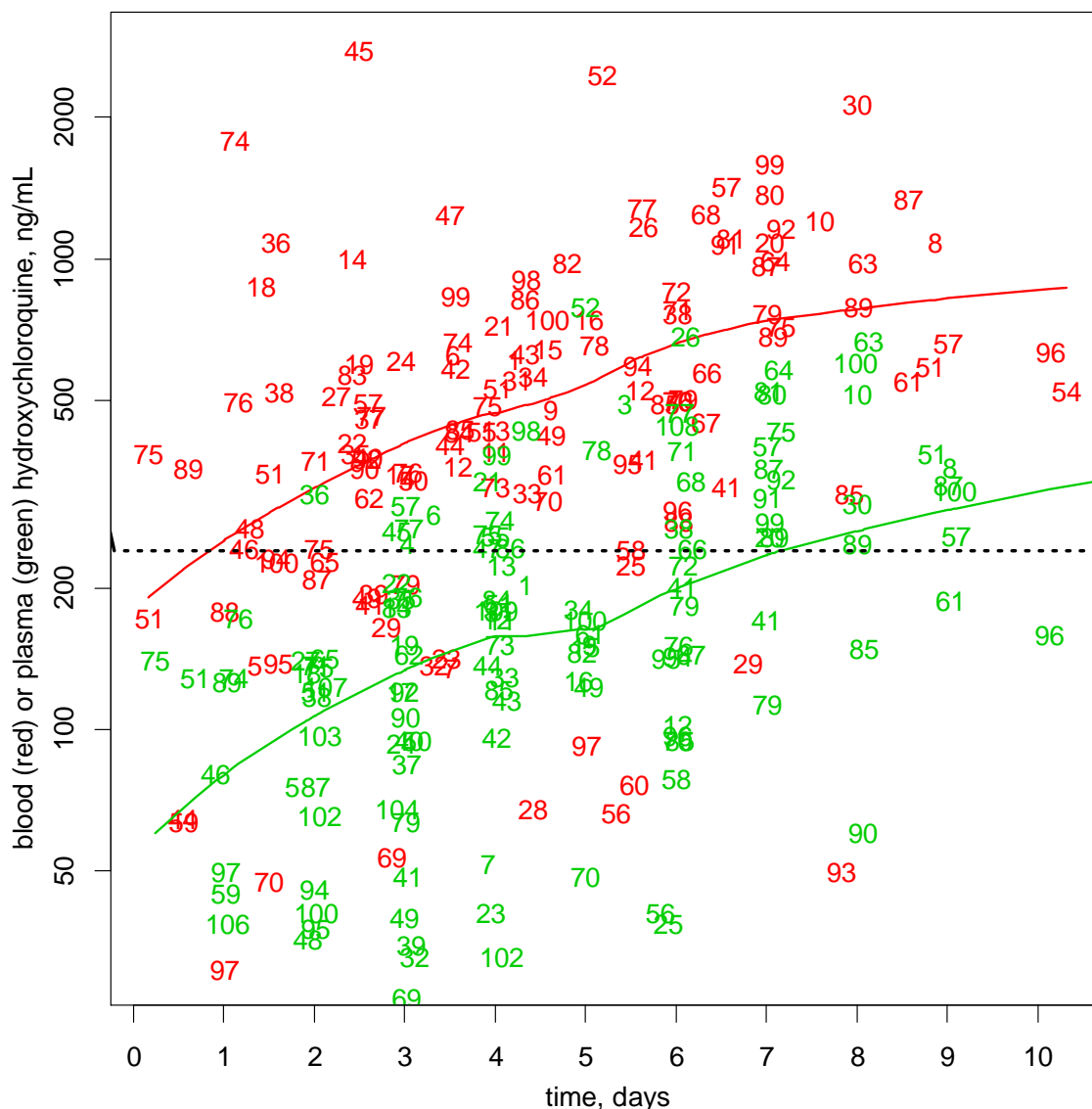
	Mean ± standard deviation	Minimum	Maximum
Age, years	63.0 ± 14.4	25	99
Weight, kg	79 ± 16	40	150
Height, cm	169 ± 11	146	192
BMI, kg/m ²	27.3 ± 5.0	17.8	51.9
Sex, (Female) %	32	NA	NA
Patient in intensive care, %	23	NA	NA
MDRD, mL/min/1.73m ² .	86.0 ± 33.6	5	194
Creatinine, µmol/L	98 ± 82	34	808
Albumin, 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
<hr/>			
Dose HCQ, mg/day	563 ± 99	200	800
Observation duration, Days	5.3 ± 2.3	1	12
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Blood concentration			
HCQ, ng/mL	586 ± 457	50	2792
<hr/>			
Plasma concentration			
HCQ, ng/mL	193 ± 152	12	795
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HCQBlood/HCQPlasma	4.0 ± 2.3	1	15

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BMI: Body Mass Index, NA: non-applicable, MDRD: (modification of diet in renal disease equation), HT: hematocrit, ALAT: alanine aminotransferase, ASAT: aspartate aminotransferase, HCQ: hydroxychloroquine. All data were collected at the time of HCQ sampling.



213

214 **Figure 1.** Observed blood (red) and plasma (green) hydroxychloroquine concentrations. Numbers
 215 stand for the patient identity and lines for the corresponding spline describing the overall trend for
 216 each matrix.

217 3.2. Pharmacokinetic modelling

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

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

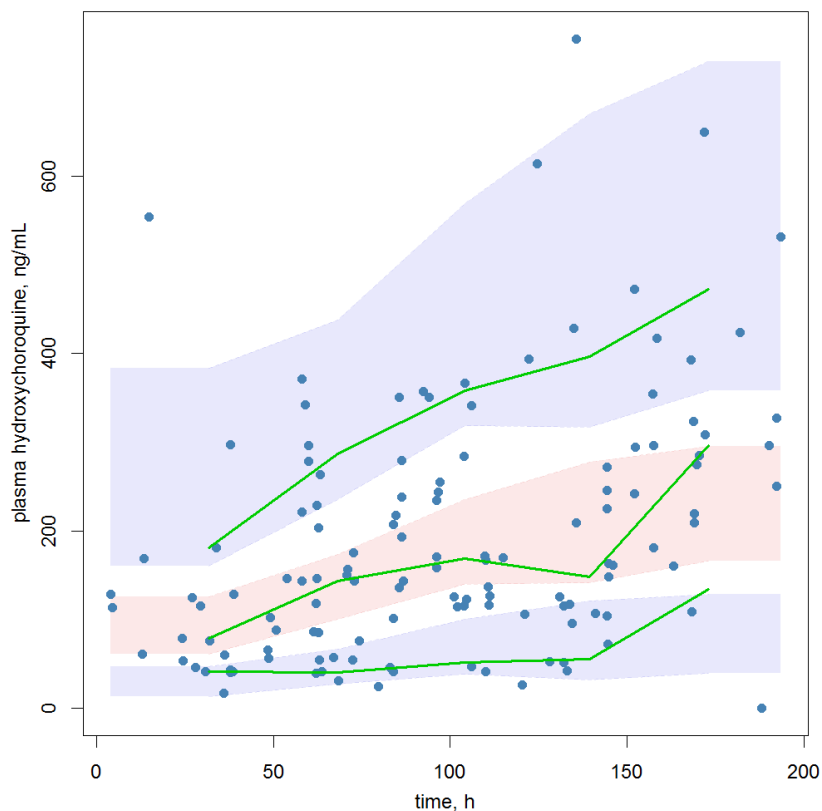
Parameter	Estimate	%res
ka, h-1	1.15	fixed
V/F, L	6690	16.1
β , V/F*(BW/70) ^{β}	1	fixed
CL/F	45.9	21.2
β , CL/F*(BW/70) ^{β}	0.75	fixed
η V/F	0.61	18.9
η CL/F	0.69	25.1
σ , ng/mL	64.1	9.76

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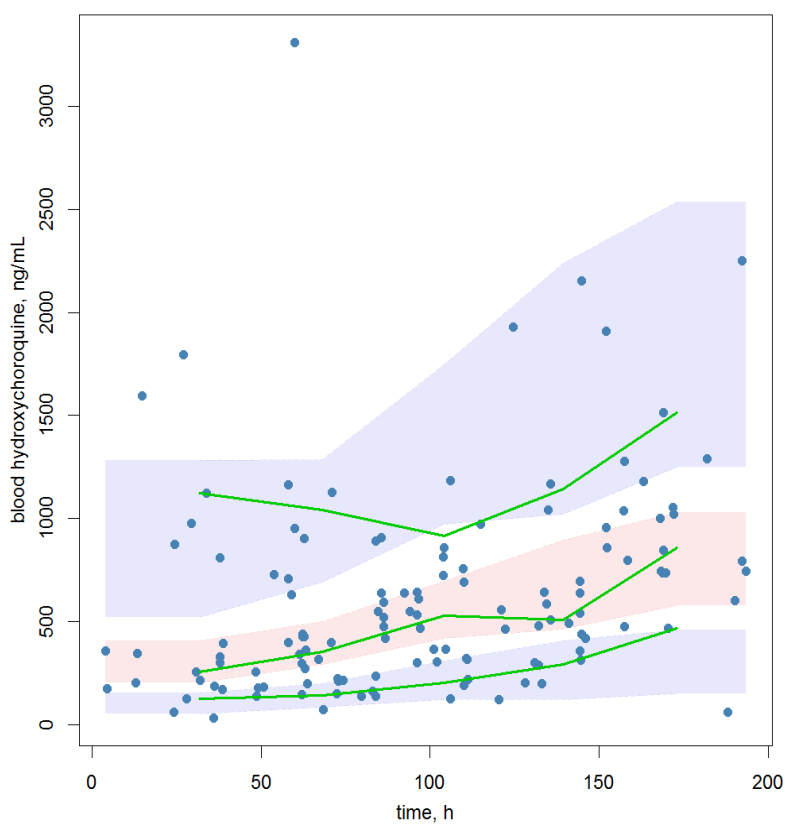
CL/F, apparent elimination clearance; V/F, apparent volume of distribution; Ka, absorption rate constant; F, unknown bioavailability; β , covariate effect parameter; η , between-subject variability; σ , proportional residual variability; BW, bodyweight (CL/F and V/F estimates are normalized to a 70 kg BW, i.e., for the i^{th} 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.

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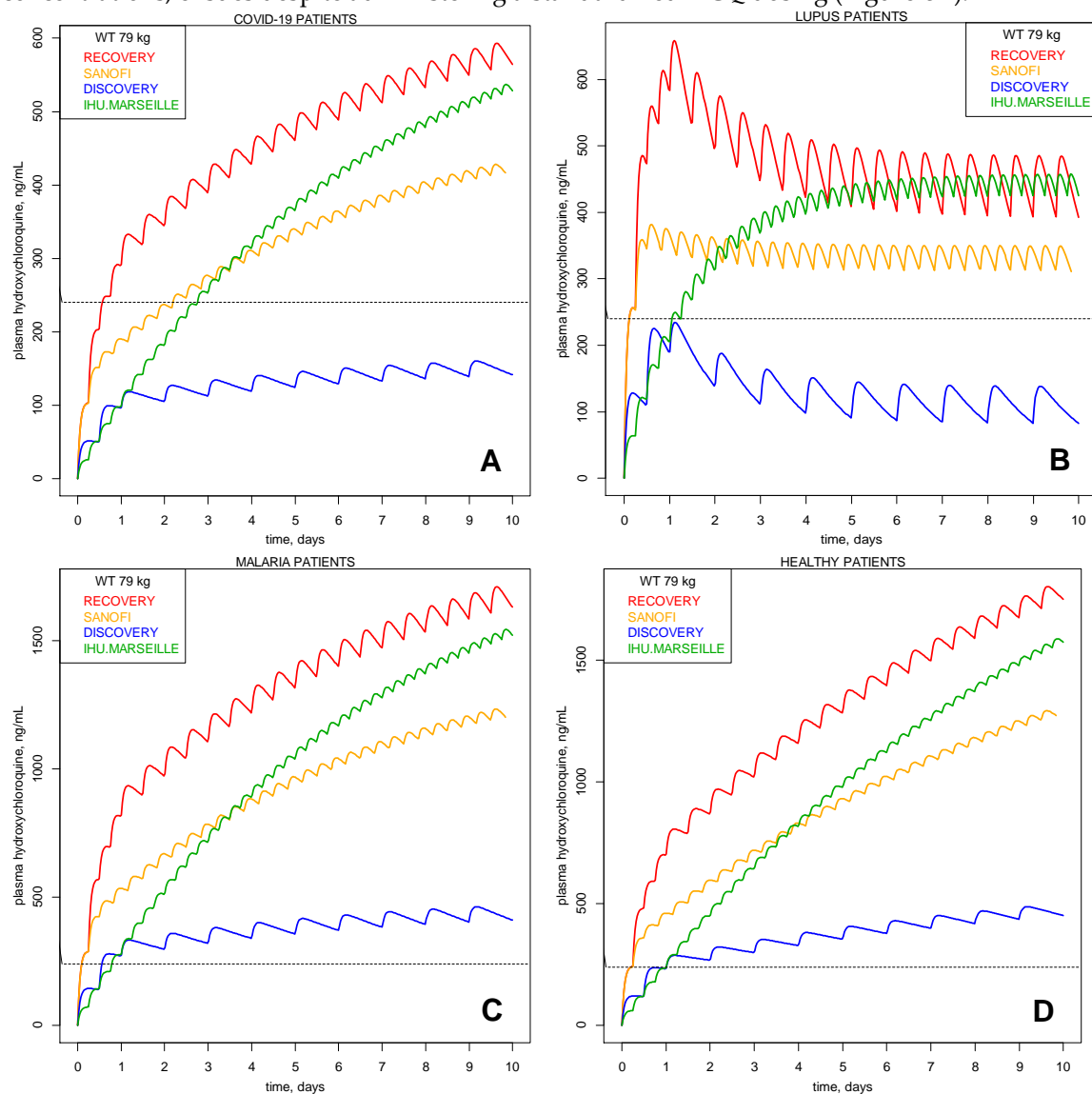
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Relying on our final pharmacokinetics parameters modelling, we generated representative plasma HCQ concentrations-time courses using various dosing regimens of major COVID-19 prospective trials testing HCQ (Figure-3A). Concentration vs. time profiles 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 ≥ 1600 mg followed by daily dose ≥ 600 mg reached theoretical concentrations in $\geq 40\%$ (95% confidence interval 30-50%) and $\geq 60\%$ (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).

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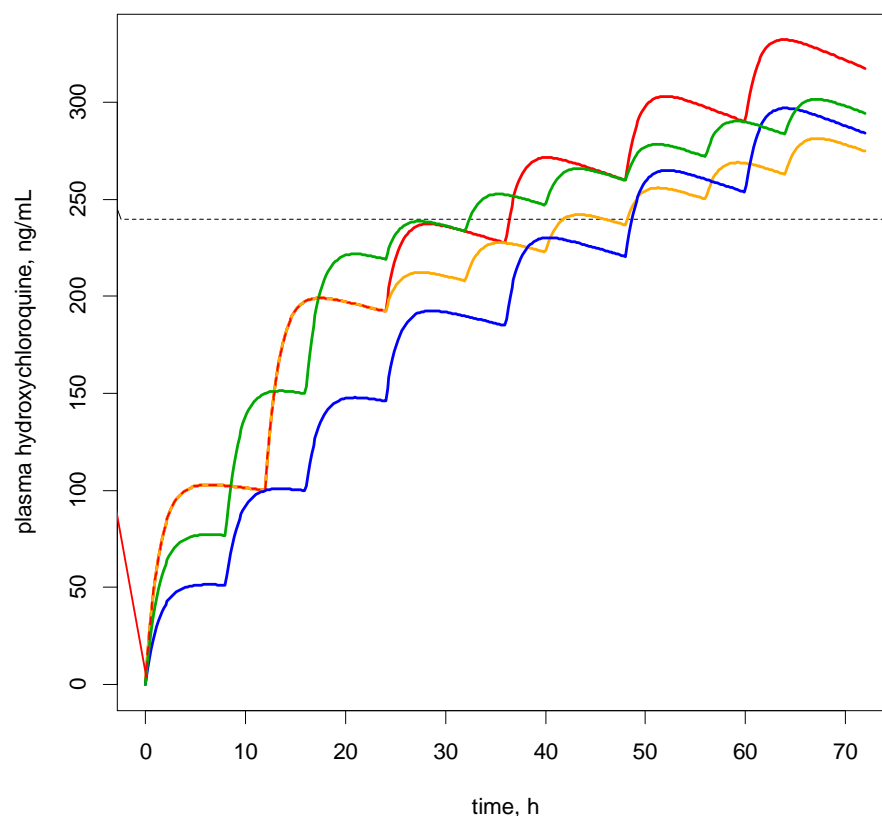
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Figure 3. Representative predicted plasma HCQ concentrations-time courses as a function of the dosing regimen evaluated in major prospective trials testing HCQ for COVID-19. Curves are drawn according to our final parameters for a typical patient weight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

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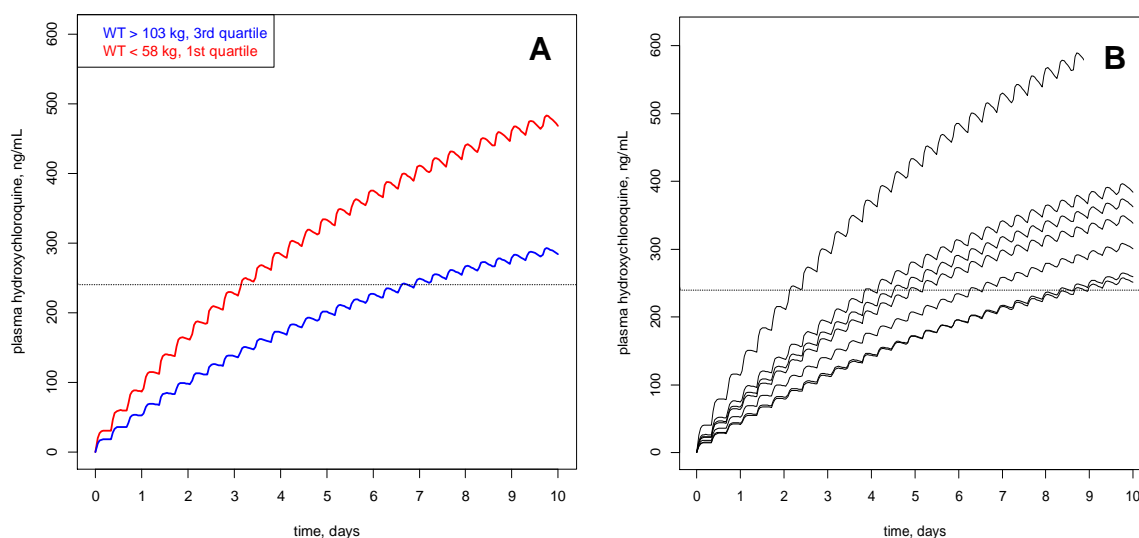
274 Dosing schedules are 2.4 g loading dose then 400 mg/12h (RECOVERY), 1.2 g loading dose then 200
 275 mg/8h (SANOFI), 200mg/8h (IHU.Marseille) and 800 mg loading dose then 400 mg/24h
 276 (DISCOVERY). Curves shown are using Covid-19 patients (A), lupus patients (B), malaria patients
 277 (C), and healthy subjects (D) parameters.

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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).



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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.

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292 3.3. Pharmacodynamic effects of HCQ in COVID-19

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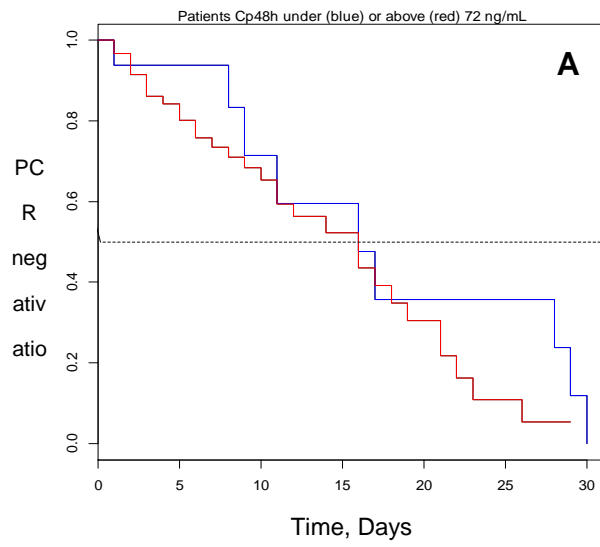
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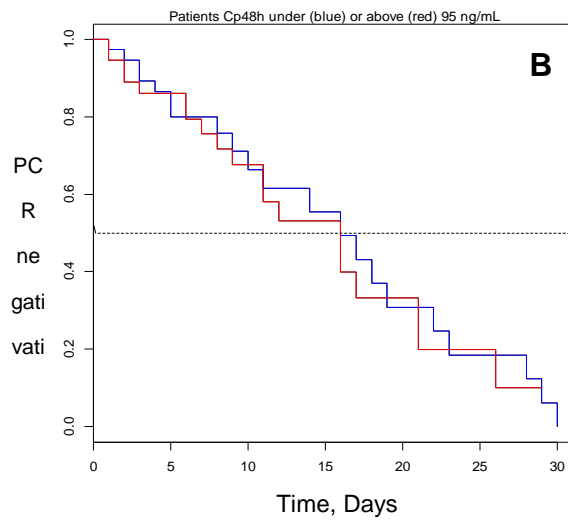
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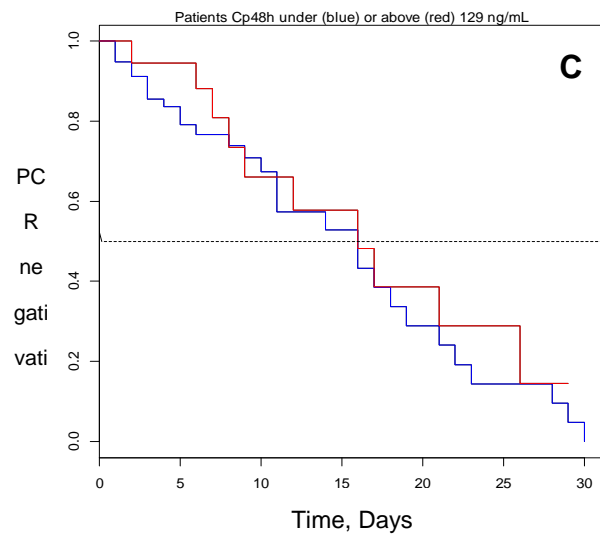
A total of 75 patients were available for a SARS-CoV-2 viral status analysis using nasopharyngeal swab. PCR follow-up was negative in 40 (53%). To assess the effect of plasma HCQ concentration on time-to-PCR negativation, patients were grouped as follows: individual predicted plasma HCQ concentration 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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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 unknown 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 $\geq 60\%$ (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
 412

Parameter	Estimate	%res
ka, h-1	0.75	fixed
V/F, L	1,990	15.9
β , V/F*(BW/70) ^{β}	1	fixed
β , V/F*(PLAT/300,000) ^{β}	-0.726	37
CL/F	14.7	13.5
β , CL/F*(BW/70) ^{β}	0.75	fixed
η V/F		
η CL/F		
σ , proportional	0.272	12.2

413
 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 i^{th} 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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