Hydroxychloroquine in COVID-19 patients: what still needs to be known about the kinetics

G. MARTIN-BLONDEL[1]*, S. RUIZ[2]*, M MURRIS[3], S FAGUER[4], V DUHALDE[5] , F EYVRARD[5], J IZOPET[6], JM MANSUY[6], Y ROLLAND[7], K DELAVIGNE[8], R GUIMBAUD[9], G PUGNET[10], JM CONIL[2], B GEORGES[2], P DELOBEL[1], V MINVILLE[2], S. SILVA SIFONTES[11], D. CONCORDET[12], P. GANDIA[12,13]**

1 CHU de Toulouse, Service des Maladies Infectieuses, 330 avenue de Grande-Bretagne, 31059, Toulouse cedex 9, France.
2 CHU de Toulouse, Pôle d'Anesthésie-Réanimation, 1 avenue du Professeur Jean Poulhès - TSA 50032 - 31059 Toulouse cedex 9, France
3 CRCM, CHU Toulouse, Hôpital Larrey, Service de Pneumologie, 20 chemin de Pourvourville, TSA 30030, 31059 Toulouse Cedex 9, France.
4 CHU de Toulouse, Pôle de Néphrologie et Transplantation d'Organes, 1 avenue du Professeur Jean Poulhès - TSA 50032 - 31059 Toulouse cedex 9
5 CHU de Toulouse, Pôle Pharmacie, 1 avenue du Professeur Jean Poulhès - TSA 50032 - 31059 Toulouse cedex 9, France
6 CHU de Toulouse, Laboratoire de Virologie, Institut Fédératif de Biologie, 330 avenue de Grande-Bretagne, 31059, Toulouse cedex 9, France.
7 CHU de Toulouse, Pôle de Gériatrie, 330 avenue de Grande-Bretagne, 31059, Toulouse cedex 9, France.
8 IUCT-Oncopole, Service d'Hématologie, avenue Irene Joliot-Curie – 31059 Toulouse Cedex 9, France
9 CHU de Toulouse, Pôle des Maladies de l'Appareil Digestif, 1 avenue du Professeur Jean Poulhès - TSA 50032 - 31059 Toulouse cedex 9, France
10 CHU de Toulouse, Service de Médecine Interne, 330 avenue de Grande-Bretagne, 31059, Toulouse cedex 9, France.
11 CHU de Toulouse, Pôle d'Anesthésie-Réanimation, 330 avenue de Grande-Bretagne, 31059, Toulouse cedex 9, France.
12 INTHERES, Université de Toulouse, INRA, ENVT, 23 Chemin des Capelles, BP 87614, 31 076, Toulouse Cedex 3, France.
13 CHU de Toulouse, Laboratoire de Pharmacocinétique et Toxicologie Clinique, Institut Fédératif de Biologie, 330 avenue de Grande-Bretagne, 31059, Toulouse cedex 9, France.

First co-authors:*
Prof. Guillaume MARTIN-BLONDEL and Dr Stéphanie RUIZ

Corresponding author: **
Prof. Peggy GANDIA, CHU de Toulouse, Laboratoire de Pharmacocinétique et Toxicologie Clinique, Institut Fédératif de Biologie, 330, avenue de Grande-Bretagne, 31059, Toulouse cedex 9, France.
Telephone: +335 67 69 03 82; Fax: +335 67 69 03 84
E-mail: gandia.p@chu-toulouse.fr

ABSTRACT

Different dosage regimens of hydroxychloroquine are used to manage COVID-19 patients, without information on the pharmacokinetics in this population.

Blood samples (n=101) were collected from 57 COVID-19 patients for 7 days and concentrations were compared with simulated kinetic profiles.

Hydroxychloroquine exposure is low and cannot be predicted by other populations.

KEY WORDS: COVID-19, hydroxychloroquine, drug monitoring, population pharmacokinetic modelling, dosage regimens

Introduction

Based on the *in vitro* activity against SARS-CoV-2 and preliminary clinical data, hydroxychloroquine is currently used in the management of COVID-19 patients [1–3]. Results of ongoing clinical trials are eagerly awaited. In the meantime, the efficacy as well as the dosage of hydroxychloroquine is highly debated. In their pilot study, Gautret et al. [2] administered the standard dosage used for treating systemic lupus erythematosus (SLE), which is 200 mg x 3 / day, as hydroxychloroquine is mainly prescribed for this indication [4]. Because of the severity of COVID-19 and the pharmacokinetics of hydroxychloroquine in SLE patients [5], a loading dose was rapidly included in the new hospital regimens to optimize drug distribution in tissues and more precisely in the lungs.

Due to the lack of information on the plasma/blood concentrations required in COVID-19 patients to induce a virological/clinical effect, in many European countries these concentrations are monitored in patients whether or not they are included in clinical research protocols.

Our aim was to determine whether or not the pharmacokinetics in SLE patients as described by Morita et al. [6] can be applied to COVID-19 patients. This is essential information for further exploration of the pharmacokinetic-pharmacodynamic relationship.

Patients and methods

According to the guidelines established by the French National Team AC43-ANRS/STP-SFPT in March/April 2020, blood samples were collected at different time points (from 48h to 192h) during clinical management of COVID-19 patients after treatment initiation and within 30 minutes before drug administration. Plasma concentrations were determined using a validated chromatographic analytical method which presents a lower limit of quantification of 0.05 mg/l, and an intra- and inter-day variability < 4% and <10% respectively.

To ensure that these concentrations were in keeping with the expected values at least in SLE patients for the same dosage regimens, we used the population pharmacokinetic model published by Morita et al. [6] Hydroxychloroquine is mainly eliminated by hepatic metabolism (i.e. CYP3A4 and CYP2C8) with no reported difference between the Japanese and Caucasian populations concerning the prevalence of polymorphisms for these two cytochromes [7]. Therefore, the ethnicity of the patients in Morita's study [6] should not have a high impact on kinetics. We simulated the expected kinetic profiles in plasma (n=10,000) taking into account the significant covariate (i.e. body weight) which mainly explains the inter-individual variability of total clearance.

This study was entered in the Toulouse University Hospital register of retrospective studies (registration number: RnIPH 2020-33) and is covered by MR-004 (CNIL number: 2206723 v 0). It was approved by Toulouse University Hospital and ethical requirements were entirely respected.

Results

The average trough concentrations (+/- standard deviation) collected from day 3 to day 9 for 57 patients (101 blood samples) are shown in Table 1, whether or not they were treated in an intensive care unit. Currently, there are no clear guidelines for an optimal dosage regimen. Consequently, different dosage regimens were applied over the past weeks and were updated based on data that emerged: regimen 1 (200 mg x 3/day), regimen 2 (400 mg x 2 on day 1 followed by 200 mg x 3/day), regimen 3 (400 mg x 2 on day 1 followed by 400 mg x 1/day) and regimen 4 (600 mg x 2 followed by 400 mg x 1 /day). For regimen 4, which was exclusively used for patients in intensive care units, tablets were crushed and administered by feeding tube.

For each regimen applied at the Toulouse University Hospital, an example of the expected kinetic profiles for a body weight of 80 kg (the median weight in our population) are presented in Figure 1 with the corresponding serum concentration for each patient.

Co-prescriptions were checked for each patient included, in order to assess a possible influence on hydroxychloroquine pharmacokinetics. None of the patients had medications that could increase or decrease the oral bioavailability and / or clearance of hydroxychloroquine.

Discussion

The concentrations measured in COVID-19 patients show that hydroxychloroquine exposure tends to be low and in most instances lower than the values reported in SLE patients, in particular for the standard regimen of “200 mg x 3/day”. These preliminary results confirm that the pharmacokinetic behavior in COVID-19 patients cannot be predicted by the SLE population or by rheumatoid arthritis patients as recently reported by Perinel et al [8]. Furthermore, given the apparently large volume of distribution of hydroxychloroquine, Morita’s model [6], which is based on plasma concentrations, presents a major limitation for accurately predicting hydroxychloroquine accumulation in deep tissue. It is a major additional argument to urgently assess hydroxychloroquine pharmacokinetics in COVID-19 patients using both blood and plasma matrices. Moreover, monitoring hydroxychloroquine concentrations in bronchoalveolar fluids of COVID-19 patients could provide additional information on the degree of hydroxychloroquine lung impregnation [9,10] for a short period of treatment (i.e. few days). In fact, data exist from lung tissue [11] and BAL [12] for antibiotics such as macrolides that accumulate in deep tissues. Therefore, it would be logical to consider BAL as “quality control” that provides information on the degree of hydroxychloroquine exposure in the lung.

There is no established efficacy threshold for the moment. Therefore, the only relevant element to take into account when monitoring hydroxychloroquine concentrations seems to be toxicity and more precisely cardiotoxicity. A number of arguments suggest that a plasma concentration of 1 mg/l should not be exceeded, as initially suggested by the French National Team, AC43-ANRS/STP-SFPT. However, this concentration should be interpreted with caution because COVID-19 patients may (i) receive co-prescriptions which can worsen the cardiotoxicity of hydroxychloroquine (for example, azithromycin), (ii) have preexisting heart problems along with COVID-19 (EMA/202483/2020) and/or (iii) have hypokalemia.If this is the case, monitoring for heart problems by placing patients on telemetry to track EKG over the course of treatment, as is the current practice for ICU patients or at the University of Washington Medical Center [13], is not an option to be overlooked.

On April 2nd 2020, for an unknown reason, the French Ministry of Health imposed a dosage regimen identical to that used in SLE (no loading dose and 200 mg x 3/day) for patients treated outside the context of a clinical trial. Given (i) the variability of the physiopathological status of COVID-19 patients, (ii) the complex tissue distribution kinetics of hydroxychloroquine and (iii) the hydroxychloroquine concentration required to clear 100% of SARS-CoV-2 *in vitro* (≈ 7 mg/l) [13], it does not seem reasonable to impose the same dosing regimen on the entire COVID-19 population without any further pharmacokinetic-pharmacodynamic information.

In conclusion, a full hydroxychloroquine kinetic exploration is currently needed for COVID-19 patients.

Acknowledgements

We would like to thank Prof. Pierre-Louis Toutain for his expertise in the pharmacokinetic field, Dr. Michel Lavit, Dr. Thomas Lanot and Patrick Seraissol for their expertise in the analytical field, Eni Losha for his help in collecting data and the staff of technicians at the Pharmacokinetics and Toxicology Laboratory of Toulouse University Hospital.

Funding

This study was supported by internal funding.

Transparency declarations

None to declare

References

1. Molina JM, Delaugerre C, Goff JL, et al. No Evidence of Rapid Antiviral Clearance or Clinical Benefit with the Combination of Hydroxychloroquine and Azithromycin in Patients with Severe COVID-19 Infection. Médecine et Maladies Infectieuses **2020**; Available at: http://www.sciencedirect.com/science/article/pii/S0399077X20300858. Accessed 7 April 2020.

2. Gautret P, Lagier J-C, Parola P, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. Int J Antimicrob Agents **2020**; :105949.

3. CHEN J, LU H, LIU D. A pilot study of hydroxychloroquine in treatment of patients with common coronavirus disease-19 (COVID-19). Journal of Zhejiang University (Medical Science) **2020**; 49. Available at: http://www.zjujournals.com/med/EN/abstract/article_41137.shtml.

4. Rainsford KD, Parke AL, Clifford-Rashotte M, Kean WF. Therapy and pharmacological properties of hydroxychloroquine and chloroquine in treatment of systemic lupus erythematosus, rheumatoid arthritis and related diseases. Inflammopharmacology **2015**; 23:231–269.

5. Al-Rawi H, Meggitt SJ, Williams FM, Wahie S. Steady-state pharmacokinetics of hydroxychloroquine in patients with cutaneous lupus erythematosus. Lupus **2018**; 27:847–852.

6. Morita S, Takahashi T, Yoshida Y, Yokota N. Population Pharmacokinetics of Hydroxychloroquine in Japanese Patients With Cutaneous or Systemic Lupus Erythematosus. Ther Drug Monit **2016**; 38:259–267.

7. Škarić-Jurić T, Tomas Ž, Zajc Petranović M, et al. Characterization of ADME genes variation in Roma and 20 populations worldwide. PLoS ONE **2018**; 13:e0207671.

8. Perinel S, Launay M, Botelho-Nevers É, et al. Towards Optimization of Hydroxychloroquine Dosing in Intensive Care Unit COVID-19 Patients. Clin Infect Dis Available at: https://academic.oup.com/cid/advance-article/doi/10.1093/cid/ciaa394/5816960. Accessed 8 April 2020.

9. Popert AJ. Chloroquine: a review. Rheumatol Rehabil **1976**; 15:235–238.

10. McChesney EW. Animal toxicity and pharmacokinetics of hydroxychloroquine sulfate. The American Journal of Medicine **1983**; 75:11–18.

11. Kobuchi S, Fujita A, Kato A, Kobayashi H, Ito Y, Sakaeda T. Pharmacokinetics and lung distribution of macrolide antibiotics in sepsis model rats. Xenobiotica **2020**; 50:552–558.

12. Capitano B, Mattoes HM, Shore E, et al. Steady-state intrapulmonary concentrations of moxifloxacin, levofloxacin, and azithromycin in older adults. Chest **2004**; 125:965–973.

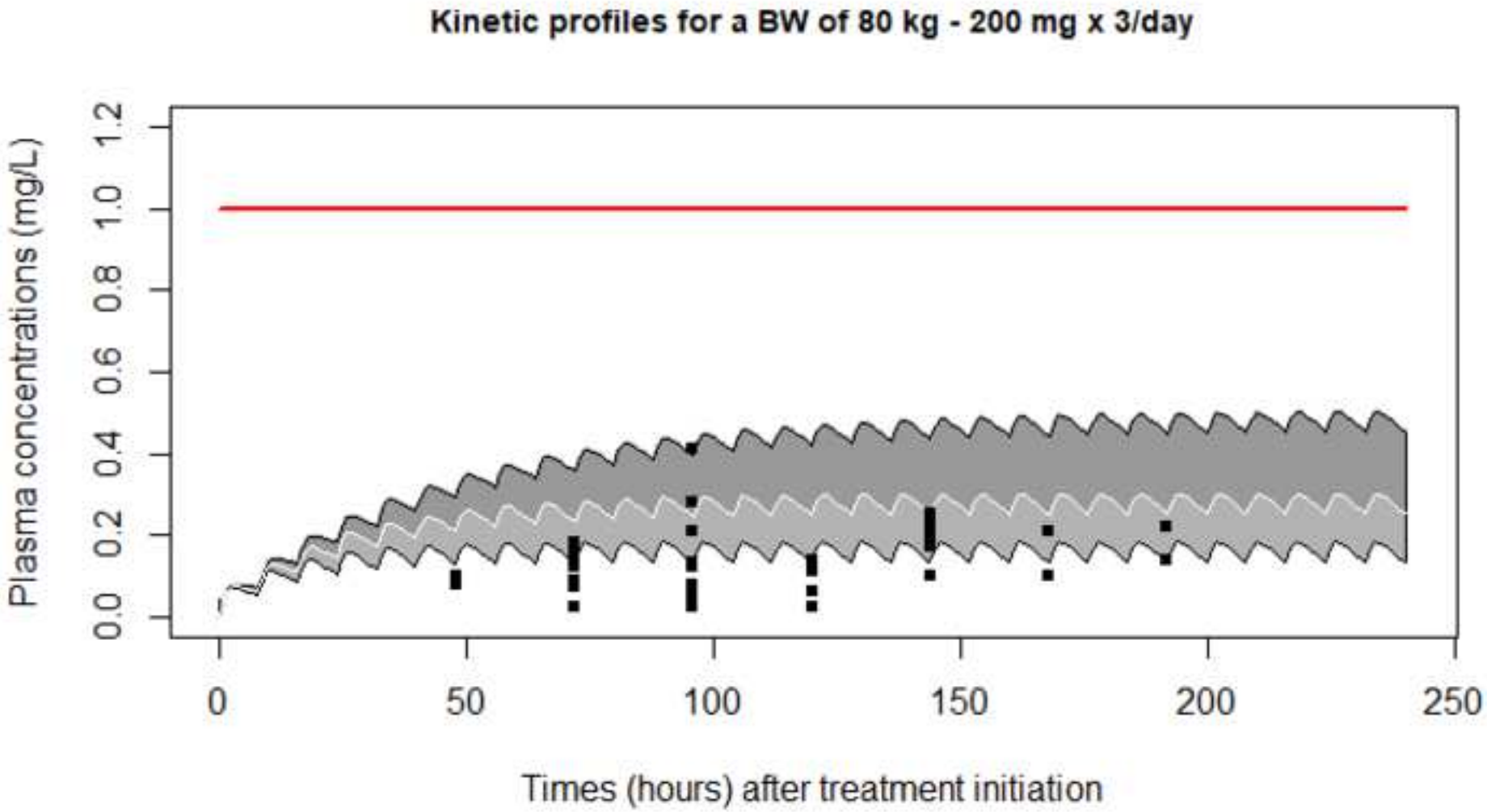
13. Arnold SL, Buckner F. Hydroxychloroquine for treatment of SARS-CoV-2 infection? Improving our confidence in a model-based approach to dose selection. Clin Transl Sci **2020**;

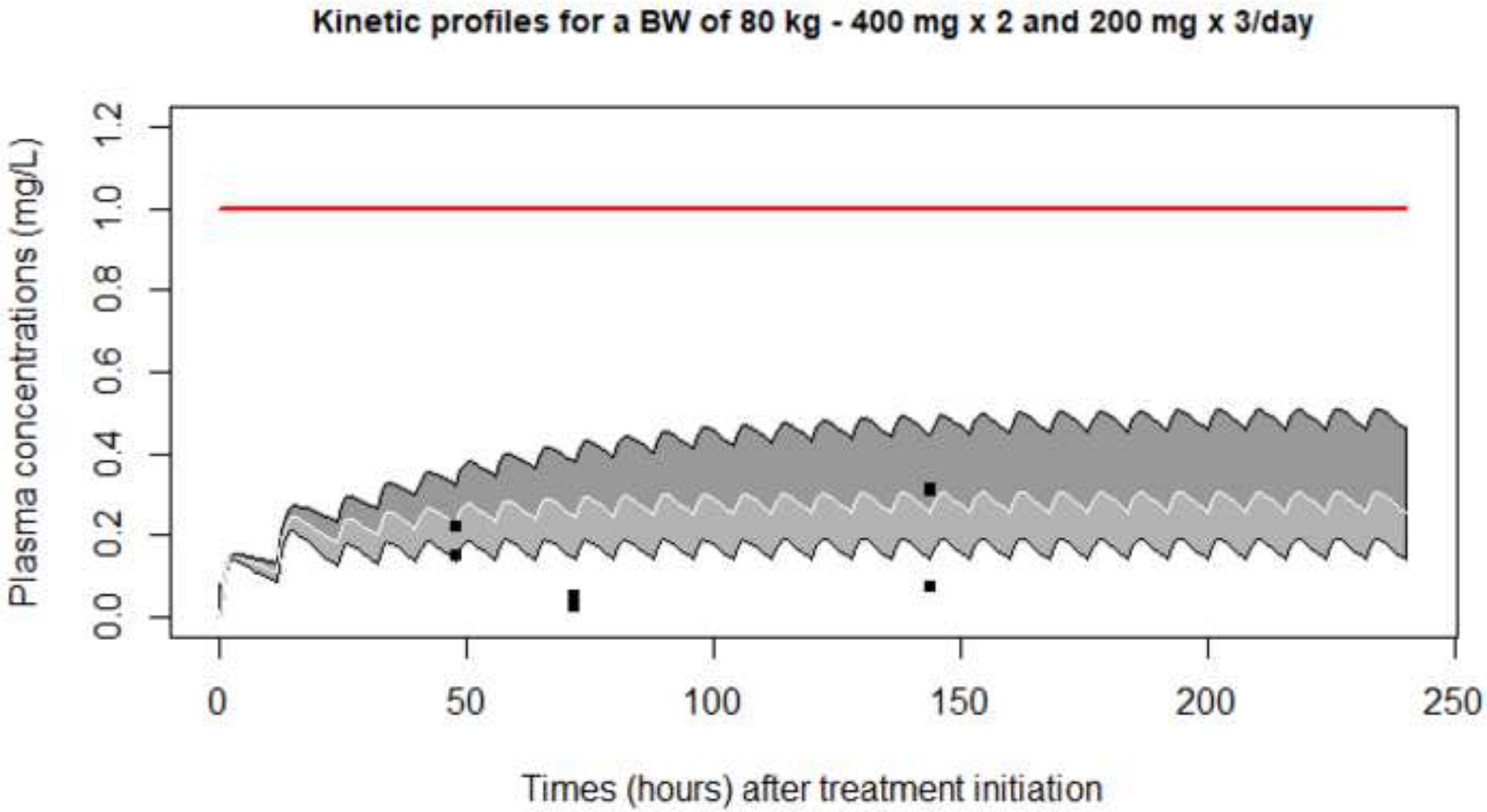
Table 1: Plasma trough hydroxychloroquine concentrations (i.e. sample collected within 30 minutes before drug administration) at different moments after treatment initiation in 57 COVID-19 patients (101 blood samples)

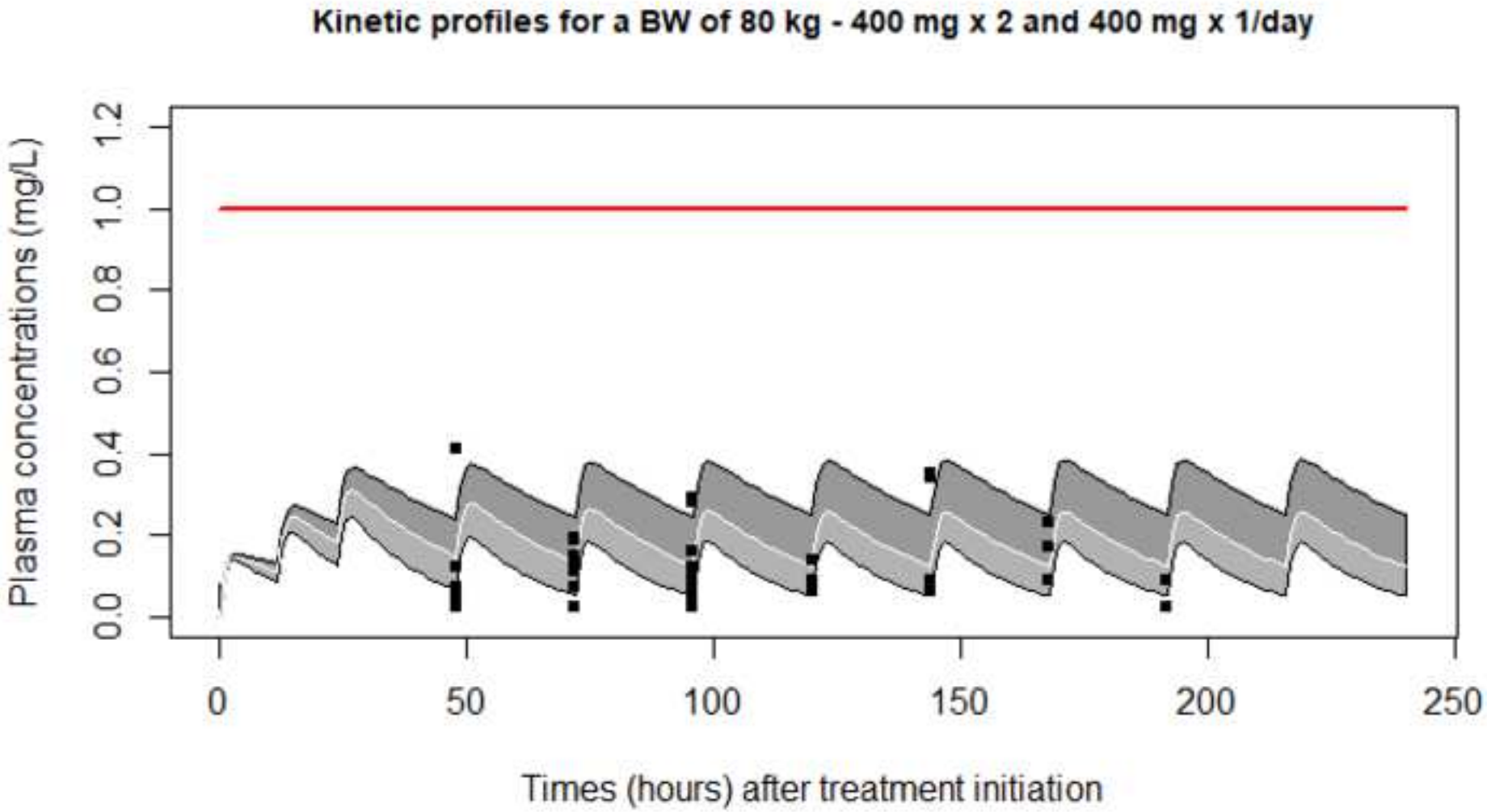
Dosage regimen	Time (hours) after treatment initiation							Body weight (kg) (min-max)
	48h	72h	96h	120h	144h	168h	192h	
	mean ± SD (mg/L) [number of patients]							[number of patients]
200 mg x 3 /day	0.09 ± 0.01 [3]	0.10 ± 0.04 [14]	0.14 ± 0.13 [10]	0.10 ± 0.05 [5]	0.19 ± 0.06 [5]	0.16 ± 0.08 [2]	0.18 ± 0.06 [2]	62.5-130 [25]
400 mg x 2 at day 1 200 mg x 3 /day	0.19 ± 0.05 [2]	0.04 ± 0.02 [2]	ND	ND	0.19 ± 0.17 [2]	ND	ND	85-125 [4]
400 mg x 2 at day 1 400 mg x 1 /day	0.09 ± 0.12 [9]	0.11 ± 0.06 [6]	0.13 ± 0.10 [9]	0.09 ± 0.03 [6]	0.13 ± 0.14 [6]	0.16 ± 0.07 [3]	0.07 ± 0.04 [3]	54-115 [22]
600 mg x 2 at day 1 400 mg x 1/day	0.22 ± 0.11 [6]	ND	0.24 ± 0.11 [4]	ND	0.2 [1]	ND	0.05 [1]	60-130 [6]

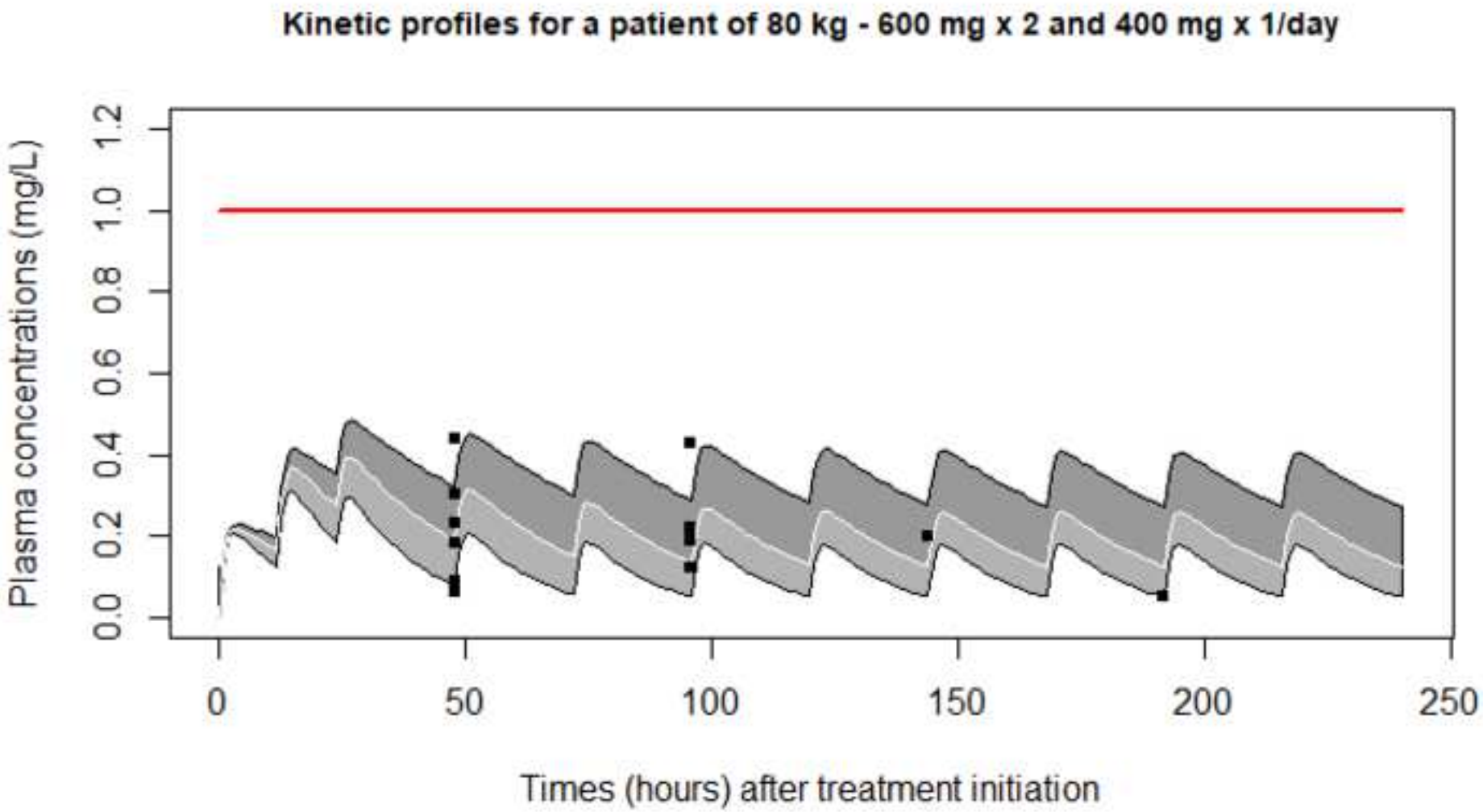
ND: no data. When a concentration was lower than the limit of quantification (0.05 mg/l), it was set at 0.025 mg/l.

Figure 1: Hydroxychloroquine plasma kinetic profiles (n=10000 *per* regimen) simulated with the population pharmacokinetic model published by Morita et al. [6] for a body weight of 80 kg and for different dosage regimens (200 mg x 3 /day ; 400 mg x 2 at day 1 followed by 200 mg x 3 /day ; 400 mg x 2 at day 1 followed by 400 mg x 1 /day; 600 mg x 2 at day 1 followed by 400 mg x 1/day). The median kinetic profile is indicated by the white curve. The "extreme" profiles found in less than 5% and 95% of the population are indicated by the black curves. The red line corresponds to the toxic plasma concentration. The black squares represent observed trough concentrations (i.e. within 30 minutes before drug administration) for patients who weigh approximately 80 kg (the median weight in our population). To facilitate the interpretation of the figure, all the trough concentrations that are presented are for the same moment before administration.


Kinetic profiles for a BW of 80 kg - 200 mg x 3/day
Plasma concentrations (mg/L)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
0
50
100
150
200
250
Times (hours) after treatment initiation


Kinetic profiles for a BW of 80 kg - 400 mg x 2 and 200 mg x 3/day
Plasma concentrations (mg/L)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
0
50
100
150
200
250
Times (hours) after treatment initiation


Kinetic profiles for a BW of 80 kg - 400 mg x 2 and 400 mg x 1/day
Plasma concentrations (mg/L)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
0
50
100
150
200
250
Times (hours) after treatment initiation


Kinetic profiles for a patient of 80 kg - 600 mg x 2 and 400 mg x 1/day
Plasma concentrations (mg/L)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
0
50
100
150
200
250
Times (hours) after treatment initiation