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RESEARCH ARTICLE

Optimization of hydroxychloroquine dosing scheme based on COVID-19 patients' characteristics: a review of the literature and simulations

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ABSTRACT

1. During the recent COVID-19 outbreak hydroxychloroquine (HCQ) has been proposed as a safe and effective therapeutic option. However, a wide variety of dosing schemes has been applied in the clinical practice and tested in clinical studies.
2. An extended literature survey was performed investigating the pharmacokinetics, the efficacy and safety of HCQ in COVID-19 treatment. Population pharmacokinetic models were retrieved from the literature and after evaluation and assessment one was selected in order to perform simulations.
3. The most commonly applied dosing schemes were explored for patients with different weights and different levels of HCQ clearance impairment. Model-based simulations of HCQ concentrations revealed that high initial doses followed by low and sparse doses may offer significant benefits to patients by decreasing the viral load without reaching levels considered to produce adverse effects. For instance, the dosing scheme proposed for a 70 kg adult with moderate COVID-19 symptoms would be 600 mg upon diagnosis, 400 mg after 12 h, 300 mg after 24 h, 200 mg after 36 h, followed by 200 mg BID for 4 d, followed by 200 mg OD for 5 d.
4. Based on the results from simulations performed and the currently published knowledge regarding HCQ in COVID-19 treatment, this study provides evidence that a high loading dose followed by sparse doses could offer significant benefits to the patients.

Introduction

During the recently emerged pandemic of coronavirus disease 2019 (COVID-19) due to SARS-CoV-2, hydroxychloroquine (HCQ) has been proposed as a drug of choice (Alpern & Gertner, 2020). COVID-19 severity has been categorized based on symptoms by the WHO in mild, moderate and severe, while a life-threatening state of critical disease has been noted, as well (WHO, 2020). Most interestingly, it has been shown that mortality, as well as disease severity and immune response are closely related to the viral load of the patient (Casadevall et al., 2020; Liu et al., 2020a; Pujadas et al., 2020; Wang et al., 2020; Zheng et al., 2020).

Up to this point, HCQ has been used for the treatment of malaria or autoimmune diseases such as rheumatoid arthritis, discoid and systemic lupus erythematosus (Plaquenil Label, 2017; Plaquenil SmPC, 2020). However, HCQ seems to offer a dual beneficial action for COVID-19 patients, as it exerts both immunomodulatory and antiviral effects (Drożdżal et al., 2020). In fact, some recent studies have revealed the drug's *in vitro* antiviral activity (Liu et al., 2020b; PAHO/WHO, 2020; Yao et al., 2020). A wide variety of observational and randomized controlled trials (RCT) worldwide are currently

evaluating the efficacy of HCQ for the treatment of COVID-19, implementing many different dosing schemes and patients with different levels of disease severity according to the WHO, (2020) categorization (Alpern & Gertner, 2020; Cortegiani et al., 2020; PAHO/WHO, 2020). Several studies have yielded positive results, indicating that HCQ may accelerate the alleviation of symptoms and diminish the length of hospital stay (Arshad et al 2020; Cavalcanti et al., 2020; Chen et al., 2020a; Gautret et al. 2020a,b; Tang et al 2020; Yu et al., 2020), while others yielded negative results, indicating that HCQ does not show any clinical effects in improving symptoms or was associated with increased length of hospital stay or disease progression (Barbosa et al., 2020; Chen et al., 2020b; Horby et al., 2020; Molina et al 2020). It has to be noted, that in many cases these studies report adverse effects promoted by HCQ, such as prolongation of the QT-interval or diarrhea (Gautret et al 2020b; Horby et al. 2020; Tang et al., 2020).

In view of the high heterogeneity of the so far study outcomes, the WHO recommends not to use HCQ outside the context of clinical trials (WHO, 2020). In any case, due to the lack of a better option, HCQ is currently included in the

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therapeutic protocols designed by numerous hospitals and health systems worldwide, for the treatment of COVID-19 patients (Singh et al., 2020). In addition, the FDA that initially approved the use of HCQ for COVID-19 (Alpern & Gertner, 2020) has recently revoked the emergency off-label use of this drug (FDA, 2020). It should be noted that currently there is not substantial evidence to prove the safety and efficacy of HCQ in the treatment of COVID-19, and thus results from well-designed randomized trials are required for this drug's repurposing (Elavarasi et al., 2020; Singh-Uttam et al., 2020).

Indicatively, some dosing schemes that have been applied or evaluated in clinical studies are presented in Table 1.

Considering all the above and based on the reported antiviral activity of the compound (Drożdżal et al., 2020; Liu et al., 2020b; Yao et al., 2020), there is currently a need to rationalize the administered dosing schemes in order to maximize the efficacy and safety of HCQ in patients with COVID-19. Eventually, additional time is needed in order to retrieve concrete results from new in vitro and in vivo studies specifically designed for HCQ against COVID-19. However, in view of its long-standing clinical use a lot of data and experience has been accumulated for this drug. In this vein, modeling and simulation techniques have been proved very useful as they can combine all the available data from in vitro, pre-clinical and clinical studies (Lavé et al., 2007; Lowe et al., 2007). Thus, by implementing all the relevant knowledge that has been gathered so far and using modeling and simulation techniques it is possible to attain a better approximation to the optimum dosing scheme.

The aim of the present study was firstly, to review all the relevant to HCQ literature, focusing on COVID-19 treatment and secondly, through simulations, to investigate the efficacy and safety of the dosing schemes of HCQ for COVID-19 treatment currently applied and to propose optimized dosing schemes.

Materials and methods

Pharmacokinetics of HCQ

After oral administration HCQ is rapidly and almost completely absorbed with bioavailability ranging from 0.67 to 0.74. Mean peak plasma concentrations (Cmax) after a single dose of 400 mg was approximately 105 ng/ml, and mean time to peak plasma concentration (Tmax) was 1.83 h. HCQ was found to present linear pharmacokinetics in a therapeutic dose range (Furst, 1996; Plaquenil SmPC, 2020).

HCQ binds avidly to tissues, leading to large volumes of distribution, significant accumulation of the drug and low clearance. In fact, the increased amount of time needed for this accumulation to occur, accounts for the delayed appearance of its clinical effects but also of its adverse effects. Approximately 30-40% of the administered dose is bound to proteins, both albumin and alpha glycoprotein. HCQ is metabolized by the liver with its main metabolite (desethylhydroxychloroquine) presenting some immunomodulatory activity (Furst, 1996; Munster et al., 2002).

The drug is primarily eliminated via the urine, where about 3% of the administered dose is recovered in 24 h. Following a single 200 mg oral dose the half-life of HCQ was found to be 537 h (22.4 d) (Plaquenil Label, 2017; Plaquenil SmPC, 2020).

Blood levels were found to vary significantly among individuals, with a variation in mean maximum/minimum concentration reaching 40%. This variability has been attributed

Table 1. Dosing schemes of hydroxychloroquine applied for the treatment of COVID-19.

Dosing Schemes*	Applied by	Reference
200 mg TID for 10 d	Observational study	Gautret et al., 2020a,b
400 mg at diagnosis, 400 mg 12 h later, followed by 200 mg BID for 5 d	Massachusetts General Hospital (clinical practice)	Massachusetts General Hospital, 2020
200 mg per day for 10 d	Italian Society of Infectious and Tropical Diseases Lombardy Section (clinical practice)	Singh et al.,2020
400 mg orally per day for 7–10 d	Central Clinical Task Force, Korea (clinical practice)	Singh et al.,2020
400 mg BID × 2 doses then 12 h later start 400 mg OD for 5–10 d	Mount Sinai Health System, Canada (clinical practice)	Singh et al.,2020
400 mg BID × 1 day followed by 200 mg BID × 4 d	Clinical guidance for patients with suspected or confirmed COVID-19 in Belgium (clinical practice)	Arnold & Buckner, 2020; Singh et al., 2020; Yao et al., 2020
400 mg twice daily for 2 doses on day 1, followed by 200 mg BID on days 2–5	Multi-center retrospective observational study	Arshad et al., 2020
400 mg BID for 7 d	*Randomized controlled trial* Mild-to-moderate hospitalized patients	Cavalcanti et al., 2020
A loading dose of 1200 mg daily for 3 d followed by a maintenance dose of 800 mg daily for 2 or 3 weeks	*Randomized controlled trial* Mild-to-moderate (2 weeks) or severe (3 weeks) disease	Tang et al., 2020
800 mg upon diagnosis and at 6 h, followed by 400 mg starting at 12 after the initial dose and then every 12 h for the next 9 d or until discharge	*Randomized controlled trial* (RECOVERY)	Horby et al., 2020
200 mg BID for 5 d	*Randomized controlled trial*	Chen et al., 2020a
200 mg BID for 7–10 d	Observational (retrospective)	Yu et al., 2020
400 mg per day for 5 d	*Randomized controlled trial*	Chen et al., 2020b
200 mg TID for 10 d	Observational (narrative review)	Molina et al., 2020
400 mg BID for 1–2 d and 3–4 subsequent d of 200 mg to 400 mg OD	*Randomized controlled trial*	Barbosa et al., 2020

*Doses are expressed in mg of hydroxychloroquine sulfate (200 mg tablet contains 155 mg base equivalent).

to the absorption and distribution rather than clearance characteristics of the drug (Al-Rawi et al., 2018; Miller et al., 1991).

Numerous factors have been shown to affect blood levels of HCQ, such as genetic variants of the CYP enzyme family, age, gender, body weight, gastric emptying, and co-administration with immunosuppressants, such as corticosteroids (Al-Rawi et al., 2018; Lee et al., 2016, 2017). Even though it has been reported that renal clearance of unchanged drug was approximately 16 to 30% and did not correlate with creatinine clearance (Miller et al., 1991), renal insufficiency has been reported to increase the risk of toxicity (Abdulaziz et al., 2018).

Model selection for simulations

A total of eight population pharmacokinetic studies of HCQ were identified in the literature (Balevic et al., 2019; Carmichael et al., 2003; Haas et al., 2019; Lim et al 2009; Morita et al., 2016; Rangwala et al., 2014; Rosenfeld et al., 2014; Vogl et al., 2014). Population parameter estimates and characteristics of the studies are presented in Table 2.

In the majority of studies assessed, samples were retrieved from patients suffering from severe diseases and receiving co-medications especially immunosuppressants that have been reported to alter HCQ pharmacokinetics (Lee et al., 2017). In addition, given that HCQ binds avidly to tissues, a fact that results in high volumes of distribution and slow clearance (Furst, 1996), a model with two compartments, a central one for plasma and tissues with an instantaneous distribution and a peripheral one standing for tissues where the drug distribution is slower seemed to be more appropriate for the description of HCQ disposition. In this vein, the model of Lim et al. (2009) was developed with samples retrieved from 91 healthy volunteers and patients with vivax malaria that did not receive any other medications and resulted in a two compartmental model. Also, the study of Lim et al. (2009) that included the highest number of samples, i.e., a total of 431 concentration measurements, from all the studies identified resulting in a two compartmental model, a fact that increases the reliability of the parameter estimates.

In view of the above, the model developed by Lim et al., (2009) was selected in order to perform the simulations. Parameters estimated in this study, were similar to those obtained with other population pharmacokinetic models developed (Rangwala et al., 2014; Rosenfeld et al., 2014), as well as to those retrieved after non-compartmental pharmacokinetic analysis (Furst, 1996; Munster et al., 2002).

Body weight has been reported to affect significantly HCQ in vivo concentrations (Plaquenil SmPC, 2020; Lee et al., 2017) and several models have identified it as a statistically significant covariate affecting clearance allometrically (Balevic et al., 2019; Morita et al., 2016; Vogl et al., 2014). Thus, weight has been included in the model used for simulations with an allometric exponent of 0.8, as estimated in these studies.

Toxicity, adverse effects and upper limit of hydroxychloroquine levels

The maximum tolerated dose in adults with rheumatoid arthritis has been reported 1200 mg per day (Munster et al., 2002), while in patients with newly diagnosed glioblastoma multiforme the maximum tolerated dose reported was 600 mg (Rosenfeld et al., 2014).

In order to prevent retinopathy that HCQ induces after long term use, a concentration range of 500–2000 ng/ml has

Table 2. Population pharmacokinetic models of hydroxychloroquine identified in the literature.

Population	Balevic et al., 2019 Pregnant women with rheumatoid arthritis	Carmichael et al., 2003 Rheumatoid arthritis	Morita et al., 2016 Cutaneous or systemic lupus erythematosus
Co-medication	Yes	No	Yes
Compartments	1	1	1
tlag (h)	No lag time	0.654	0.389
ka (1/h)	1.15	0.91	1.15
V/F (L)	1850	820	2440
Cl/F (L/h)	51	9. 13.13	68.2
Covariate	Weight (allometric exponent = 1)	No covariate	Weight (allometric exponent = 0.844)

Population	Haas et al., 2019 Renal carcinoma	Rosenfeld et al., 2014 Glioblastoma multiforme	Rangwala et al., 2014 Advanced solid tumours and melanoma	Vogl et al., 2014 Relapsed/refractory myeloma	Lim et al., 2009 Healthy adults and patients with vivax malaria
Co-medication	Yes	Yes	Yes	Yes	No
Compartments	2	2	2	2	2
tlag (h)	No lag time	1.06	No lag time	1.74	0.389
ka (1/h)	0.93	0.49	0.998	1.27	1.15
V/F (L)	599.89	361.28	485.747	243.87	437
V2/F (L)	3604.83	947.26	1406.52	2537.68	1390
Cl/F (L/h)	7.98	11.44	9.97	3	10.9
Q/F (L/h)	14.98	103.9	49.043	15	45.1
Covariate	No	No	No	Weight allometric (value not reported)	No

Tlag: absorption lag time; ka: first order absorption rate; V/F: apparent volume of distribution in the central compartment; Cl/F: apparent first order clearance from the central compartment; V2/F: apparent volume of distribution in the peripheral compartment; Q/F: apparent intercompartmental clearance between the central and the peripheral compartment.

been proposed to be safe and effective in patients with systemic lupus erythematosus under chronic therapeutic treatment (Durcan et al., 2015).

Regarding its acute use, the most significant adverse effects to consider are cardiac, gastrointestinal, extrapyramidal and neuropsychiatric effects (Plaquenil SmPC, 2020; Juurlink, 2020). The cardiotoxicity of chloroquines has been shown to be dose-dependent, with mean increases in QTc of 6.1 ms after a chloroquine dose of 600 mg and 28 ms after a chloroquine dose of 1200 mg. It should be noted that many drugs co-administered with HCQ for the treatment of COVID-19 as azithromycin, ceftriaxone and fluoroquinoles have also been proved to promote prolongation of the QTc interval, increasing the risk of cardiotoxicity (Briasoulis et al., 2011; Juurlink, 2020; Teng et al., 2019).

Gastrointestinal (GI) toxicity of HCQ has been studied in relation to blood levels in 212 patients with rheumatoid arthritis followed for 24 weeks. It was revealed that blood levels of 2250 ng/ml promote GI adverse effects in 30% of patients, while 5250 ng/ml in 50% of patients. This is a rather significant observation in terms of HCQ treatment, as generally GI symptoms are the first and most frequent to occur constituting a warning, even for cardiotoxicity (Munster et al., 2002; Tang et al 2020).

Risk factors related to HCQ toxicity include old age, renal and liver disease, genetic variants, concomitant drug use, high body mass index and, obviously, high dose and long duration of treatment (Marmor et al., 2016).

Symptoms of HCQ overdose manifest rapidly within 30 minutes after administration. They include headache, visual disturbances, cardiovascular collapse, convulsions, and hypokalemia, cardiac rhythm and conduction disorders, including QT prolongation, Torsade de pointes, ventricular tachycardia and ventricular fibrillation, followed by sudden potentially fatal respiratory and cardiac arrest (Plaquenil Label, 2017; de Olano et al., 2019).

In view of the above, HCQ levels of 2250 ng/ml, even though rather conservatory, were used as an upper limit, considering also the cardiotoxic effects of the drug, possible co-administered medications and impaired clearance due to co-morbidities (Abdulaziz et al., 2018).

It should be noted that this concentration is far below the cytotoxic concentration of the drug (CC50 = 249.50 μM or 83799 ng/ml) in green monkey kidney VeroE6 cells (Liu et al., 2020b). Regarding cytotoxicity, Yang et al., 2020 tested cytotoxicity of HCQ in 8 cell lines retrieved from heart, liver, kidney, retina, intestine and lung. This study identified that the lowest cytotoxic level of HCQ was 15.26 μM or 5125.40 ng/ml, i.e., a concentration far below the safety threshold selected in the present study (Yang et al., 2020).

Efficacy and lower limit of hydroxychloroquine levels

In the study of Liu et al. (2020b) the 50% maximal effective concentration (EC50) of HCQ against SARS-CoV-2 was estimated using the same cell line for 4 different multiplicities of infections (MOIs) namely (0.01, 0.02, 0.2, and 0.8) after treatment with the drug for 1 h. The EC50s found were 4.51, 4.06, 17.31, 12.96 μM or 1514.46, 1363.6, 5813.9 and 4352.8 ng/ml,

Table 3. Population parameters used for simulations (Lim et al., 2009).

Parameters	Parameter estimates	Inter-individual variability
tlag (h)	0.389	0.0359
ka (1/h)	1.15	–
V/F (L)	437	0.232
V2/F (L)	1390	0.715
Cl/F (L/h)	10.9	0.161
Weight exponentially on Cl/F	0.8	–
Q/F (L/h)	45.1	–

tlag: absorption lag time; ka: first order absorption rate; V/F: apparent volume of distribution in the central compartment; Cl/F: apparent first order clearance from the central compartment; V2/F: apparent volume of distribution in the peripheral compartment; Q/F: apparent intercompartmental clearance between the central and the peripheral compartment; Weight exponentially on Cl/F: allometric exponent for weight scaling of apparent clearance.

respectively (Liu et al., 2020b). Most interestingly, in another study performed by Yao et al. (2020), also in green monkey kidney VeroE6 cells, a time – dependency of the EC50 was demonstrated, with EC50 values corresponding to 6.14 μM or 2062.25 ng/ml and 0.72 μM or 241.82 ng/ml after 24 and 48 h, respectively.

This phenomenon was attributed to the fact that HCQ is accumulated within the cells, and its actions present a delay to be manifested (Yao et al., 2020). Besides its antiviral activity, HCQ's therapeutic effects against COVID-19 reside also on its immunomodulatory effects which are manifested with blood concentrations above 500 ng/ml (Durcan et al., 2015).

Simulations

Simulations were performed using the R function 'Simulx' included in the 'mlxR 4.0' package (Lavielle, 2019). The model parameters used are summarized in Table 3. Inter-individual variability was also taken into account and thus a population of 500 patients was simulated. The dosing schemes explored were selected from published literature studies and simulations were performed assuming patients with weights of 50, 70 and 90 kg. Then, the dosing scheme providing the most favorable profile was sought.

HCQ is metabolized by the liver, while it is primarily excreted by the kidney. Both renal insufficiency and impaired hepatic clearance, can reduce HCQ's total clearance. It has been reported that renal clearance constitutes the 55% of total HCQ's clearance (White et al., 2020). COVID-19 patients have been proved to present increased risk of renal impairment (Cheng et al., 2020; Naicker et al., 2020; Ronco and Reis 2020), while extracorporeal membrane oxygenation (ECMO) has been showed to impair the drug's clearance (Tukacs, 2018). Therefore, during simulations an intermediate and a high level of renal impairment reducing the total clearance of HCQ by 30 and by 50%, i.e., a population with an apparent clearance of 7.63 and 5.45 L/h, were also considered.

Results and discussion

Simulations were performed in order to explore the expected blood levels of HCQ upon administration of various dosing

schemes currently used in clinical practice, as well as, in order to propose an optimal dosing scheme in relation to patients' body weight, clearance impairment and COVID-19 severity.

The lower and upper bounds, selected herein, are in line with other published studies (Arnold & Buckner, 2020; Perinel et al., 2020; Yao et al., 2020), while concentrations achieved with the simulations are in accordance with the dose-con-centrations results presented in previously pharmacokinetic analyses (Morita et al., 2016; Rangwala et al., 2014; Vogl et al., 2014) indicating the good predictability of our simulations.

As it may be noted in Figures 1 and 2, low frequent doses of HCQ result after a period of 5 d in toxic levels even in patients with normal body weight, while high initial bolus doses do not increase blood levels significantly. This phe-nomenon is due to HCQ pharmacokinetics that imply its accumulation into tissues leading to delayed clearance (Furst, 1996).

In terms of efficacy, three factors should be considered for the determination of the appropriate HCQ blood levels: (a) higher multiplicity of infection *in vitro*, i.e., higher viral load, results in higher EC50, (b) increasing the duration of exposure to HCQ results in lower EC50 that decreases from 2062.25 ng/ml to 241.82 ng/ml in 24 h, and (c) the immuno-modulatory effect of the drug is promoted at concentrations over 500 ng/ml. Therefore, blood levels should ideally reach 1500 ng/ml during the first days upon diagnosis, especially in severe cases with high viral load, and be kept above 500 ng/ml for the following days (Durcan et al., 2015; Liu et al., 2020b; Yao et al., 2020).

On the other hand, levels exceeding 2250 ng/ml are con-sidered more probable to promote gastrointestinal adverse effects, which seem to constitute a warning for cardiotoxicity as they occur first and are more common (Munster et al., 2002; Gautret et al. 2020a,b; Plaquenil SmPC, 2020). In add-ition, this concentration is far below the concentration found to be cardiotoxic *in vitro*, i.e., 5125.40 ng/ml. More interest-ingly, cardiotoxicity of chloroquines is known to be dose-dependent, with 600 mg of chloroquine, a far more toxic compound than HCQ (Liu et al., 2020b), promoting a pro-longation of 6.1 ms, when prolongations less than 10 ms are considered to be of low concern (US FDA E14, 2017). In gen-eral, significant prolongations of the QT interval with HCQ have been noted with either high doses or with long term-use and frequent doses (Gautret et al. 2020a,b; Jankelson et al., 2020; Juurlink, 2020; Saleh et al., 2020). As a result, in the present study the dosing schemes proposed, were designed in order to avoid unnecessarily high and fre-quent doses.

Based on simulations among the currently published dos-ing schemes, the most adequate dose for a patient of 50 or 70 kg, even with 30% impaired clearance, would be 800 mg once daily on day 1, followed by 200 mg twice daily for 7 d (Scheme_7) or 400 mg upon diagnosis, 400 mg 12 h later, fol-lowed by 200 mg BID for 5 d (Scheme_2). For a patient of 90 kg, 400 mg BID for 2 doses followed 12 h later by 400 mg OD for 5–10 d (Scheme_5) seems to be the most adequate

scheme. In this vein, for a patient of 70 kg with 50%, a dose of 200 mg per day for 10 d (Scheme_3) seems to constitute a safe option, even though this dosing scheme is not expected to be effective enough to significantly decrease the viral load, during the first days of the treatment.

Through model-based simulations, optimal dosing schemes were developed (Table 4 and Supplementary mater-ial) and the corresponding HCQ blood concentrations versus time were retrieved (Figures 3 and 4). Dosing schemes were designed in order to achieve a fast onset of "high" concen-trations during the initial phase of the disease, since an initial higher viral load is anticipated, especially for patients with severe COVID-19, and then keep HCQ blood levels below 2250 ng/ml and over 500 ng/ml, at all times. This can be noted in Figures 3 and 4 where the simulated HCQ concen-trations versus time profiles for 500 volunteers are presented as 90% prediction intervals. In Table 4 the dosing scheme designed for a patient of 70 kg with normal clearance is pre-sented, while Tables with the dosing schemes designed for patients of 30 kg, 50 kg, 90 kg, 110 kg, 70 kg with 30% impaired clearance and 70 kg with 50% impaired clearance are included in the Supplementary Material.

It should be noted that some of the dosing schemes applied currently in the clinical setting start with a loading dose of 400 mg. Despite that, the rest of the treatment in most cases differs significantly, as in most cases doses that lead to drug accumulation and thus increase the possibility of adverse effects are widely noted in the literature. In add-ition, only one dose scheme is used in all cases and all patients, irrespectively of their body weight, HCQ clearance or their condition.

In this study, the need to reach higher concentrations in patients whose immune system is not effectively reducing the viral load, leading to more intense symptoms is addressed. In fact, it has been shown that viral load relates significantly to disease severity and immune response. High viral loads were linked to intense immune response, even in peripheral tissues, complicating the patient's condition and increasing the risk of mortality (Casadevall et al., 2020; Liu et al., 2020a; Pujadas et al., 2020; Wang et al., 2020; Zheng et al., 2020). HCQ in an appropriate dosage may be of bene-fit thanks to its dual action, i.e., immunomodulatory and antiviral.

In addition, the possibility of impaired clearance is explored and its effect on the drug's levels estimated. Based on the pharmacokinetics of HCQ, the fact that the drug binds avidly to tissues and that patient's weight can signifi-cantly affect its clearance was taken under consideration. Thus, depending on the patient and his/her condition, the dosing schemes proposed in this study actually present some significant differences compared to those currently applied (e.g., Supplementary Table S4 and S6), improving the possibility of a safer use of HCQ, depending on patient's characteristics.

A possible disadvantage of the dosing schemes proposed is their complexity, as they involve different doses and vary-ing frequency of administration, especially during the first days upon diagnosis. However, based on the results from
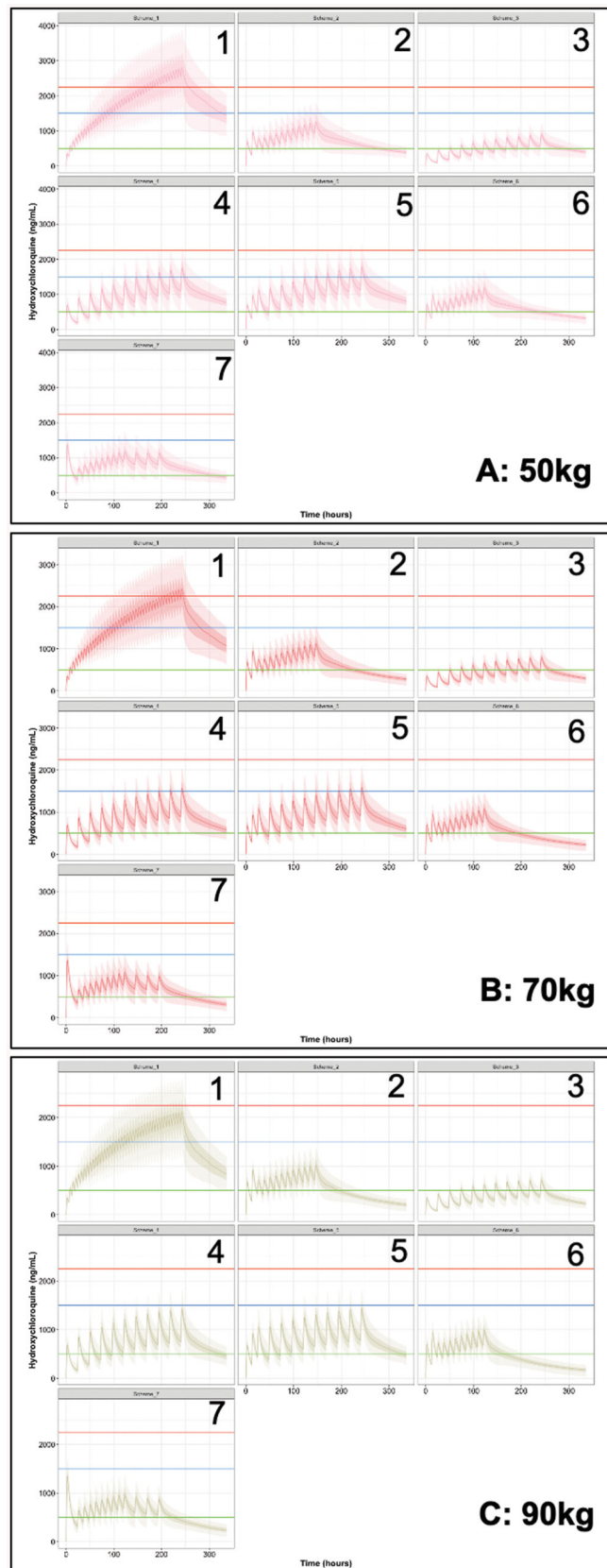
Figure 1. Simulated hydroxychloroquine concentrations (ng/ml) versus time (hours) in 500 patients receiving dosing schemes proposed in the literature for **A**: a population of 50 kg. **B:** a population of 70 kg, **C:** a population of 90 kg. **Scheme_1**: 200 mg TID for 10 d used by Gautret et al France (Gautret et al., 2020a,b). **Scheme_2:** 400 mg at diagnosis, 400 mg 12 h later, followed by 200 mg BID for 5 d used by the Massachusetts General Hospital (Massachusetts General Hospital, 2020). **Scheme_3:** 200 mg per day for 10 d used by the Italian Society of Infectious and Tropical Diseases Lombardy Section (Singh et al., 2020). **Scheme_4:** 400 mg orally per day for 7–10 d used by the Central Clinical Task Force, Korea. (Singh et al., 2020). **Scheme_5:** 400 mg BID × 2 doses then 12 h later start 400 mg OD for 5–10 d used by the Mount Sinai Health System, Canada (Singh et al., 2020). **Scheme_6:** 400 mg BID for day 1, followed by 200 mg BID for 4 d. (Singh et al., 2020). **Scheme_7:** 800 mg once daily on day 1, followed by 200 mg twice daily for 7 d (Perinel et al., 2020) Red line: 2235 ng/ml, known to promote adverse effects to 30% of patients Blue line: 1500 ng/ml, levels to achieve during the first doses. Green line: 500 ng/ml, minimum levels eliciting immunomodulatory effect.
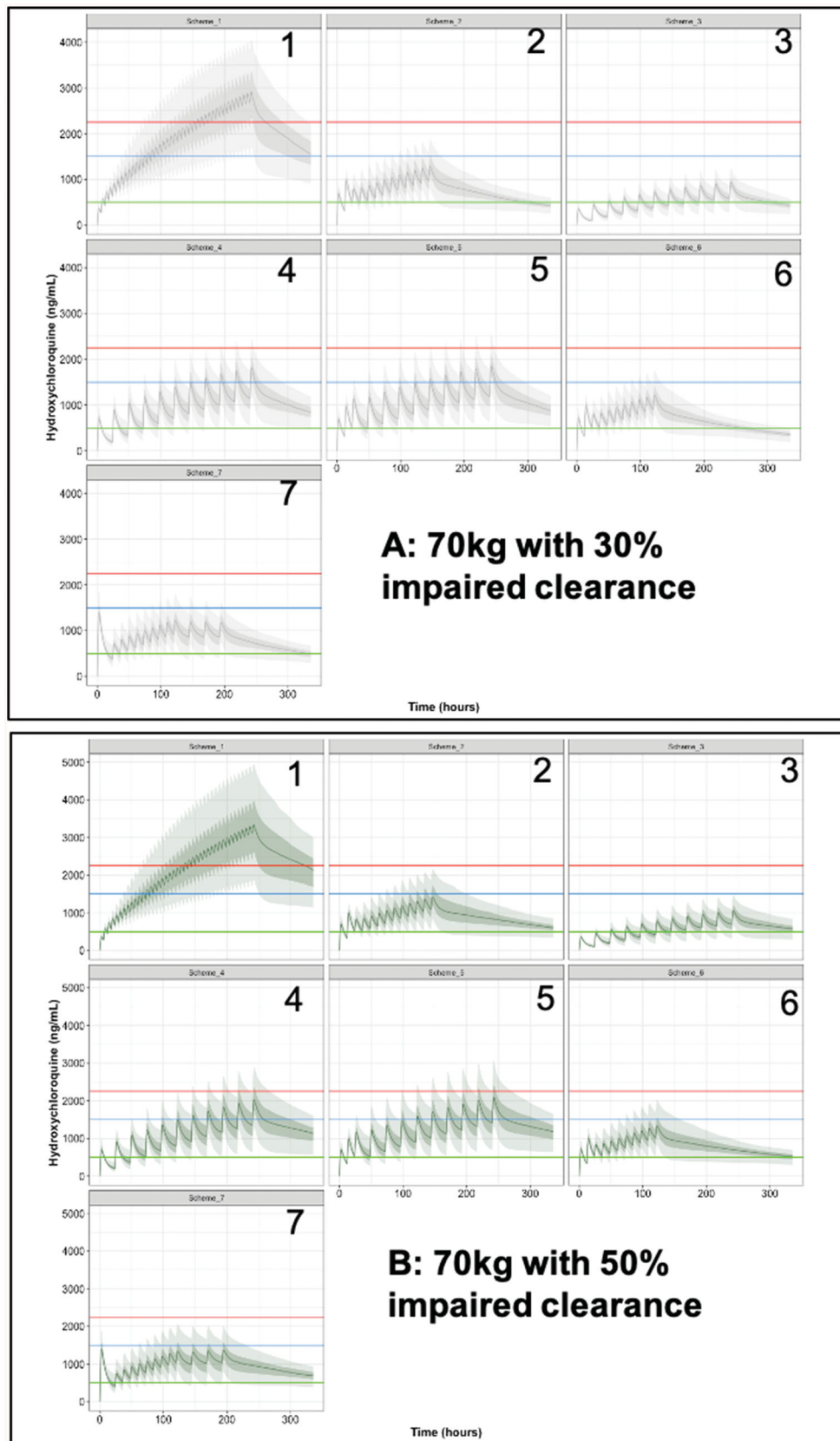
Figure 2. Simulated hydroxychloroquine concentrations (ng/ml) versus time (hours) in 500 patients receiving dosing schemes proposed in the literature for **A**: a population of 70 kg with 30% impaired clearance and **B**: a population of 70 kg with 50% impaired clearance. **Scheme_1**: 200 mg TID for 10 d used by Gautret et al. France (Gautret et al., 2020a,b). **Scheme_2:** 400 mg at diagnosis, 400 mg 12 h later, followed by 200 mg BID for 5 d used by the Massachusetts General Hospital (Massachusetts General Hospital, 2020). **Scheme_3:** 200 mg per day for 10 d used by the Italian Society of Infectious and Tropical Diseases Lombardy Section. (Singh et al., 2020). **Scheme_4:** 400 mg orally per day for 7–10 d used by the Central Clinical Task Force, Korea. (Singh et al., 2020). **Scheme_5:** 400 mg BID × 2 doses then 12 h later start 400 mg OD for 5–10 d used by the Mount Sinai Health System, Canada. (Singh et al., 2020). **Scheme_6:** 400 mg BID for day 1, followed by 200 mg BID for 4 d (Singh et al., 2020). **Scheme_7:** 800 mg once daily on day 1, followed by 200 mg twice daily for 7 d (Perinel et al., 2020) Red line: 2235 ng/ml, known to promote adverse effects to 30% of patients. Blue line: 1500 ng/ml, levels to achieve during the first doses. Green line: 500 ng/ml, minimum levels eliciting immunomodulatory effect.

Table 4. Proposed dosing scheme for treatment of COVID-19 with hydroxychloroquine for a 70 kg patient, depending on disease severity.

Patient of 70kg					
Mild		Moderate		Severe	
Time (h)	Doses (mg)	Time (h)	Doses (mg)	Time (h)	Doses (mg)
0	400	0	600	0	800
12	400	12	400	12	400
24	300	24	300	24	400
36	300	36	200	36	300
48	200	48	200	48	200
72	200	60	200	60	200
96	200	72	200	72	200
120	200	84	200	84	200
144	200	96	200	96	200
168	200	108	200	108	200
192	200	120	200	120	200
216	200	144	200	132	200
240	200	168	200	144	200
		192	200	156	200
		216	200	168	200
		240	200	192	200
				216	200
				240	200

*Doses are expressed in mg of hydroxychloroquine sulfate (a 200 mg tablet contains 155 mg base equivalent).

simulations performed and the currently published data regarding hydroxychloroquine in COVID-19 treatment, these schemes could offer significant benefits to the patients, while after the first 3 d, in most cases they require only a "low" dose once or twice daily.

A maximum of 10 d of treatment is proposed in view of the course of COVID-19 (Harapan et al., 2020). In fact, as it has been shown for the 90% of mild cases, viral clearance is achieved within 10 d post-onset (Liu et al., 2020a). However, clinical evaluation of patients has to be performed and decisions should be made on a case-by-case basis. Therefore, continuation of a daily dose may be required for a longer period, keeping HCQ concentrations steady. Despite the high doses proposed upon diagnosis, the MTD of HCQ is not exceeded. It should be noted that continuous ECG monitoring should be conducted, especially during the first-high dose phase, in patients with moderate and severe COVID-19.

HCQ concentrations in the lungs have been reported to be significantly higher than the corresponding ones in plasma (Yao et al., 2020). Therefore, reaching HCQ blood concentrations of 1500 ng/ml assures that the EC50 concentrations are achieved within the main organ affected by the disease (Harapan et al., 2020). In addition, there is some evidence that the virus attacks red blood cells, rendering them incapable of transporting oxygen (Liu & Li, 2020) and T cells, decreasing their number significantly (Qin et al., 2020). Thus, a high loading dose providing an initial phase of high blood levels, followed by sparse small doses is anticipated to be both more efficacious and safer in comparison to a frequent low dose scheme. This can also be supported by both the pharmacokinetics of HCQ and by its time-dependent EC50, which resulted in 9-fold lower values after an incubation of 48 h, as compared to an incubation period of 24 h (Yao et al., 2020). Consequently, in a dosage regimen with a high initial dose followed by small maintenance doses, the viral load is expected to decrease during the initial phase, while the virus becomes more susceptible to the drug's concentration, allowing, therefore, for a gradual decrease of the dose to be administered.

Regarding the immunomodulatory effect of the drug, it has been found to be exerted with relatively low HCQ blood-levels (range 500–2000 ng/ml) (Durcan et al., 2015).

It's worth mentioning that the aim of the present study was not to propose a cure for COVID-19. Instead, given that, HCQ is used both in the clinical setting and in clinical trials as an option for the management of COVID-19 with a 'one-dose fits all' approach, this study aimed to provide guidance on the dose to be selected depending on patient's characteristics.

Other studies, where modeling and simulation approaches have been used in order to identify an optimized dosing scheme for HCQ in COVID-19 treatment suggest, as well, that a higher dose upon diagnosis will significantly benefit the patients (Arnold & Buckner, 2020; Fan et al., 2020; Garcia-Cremades et al 2020; Perinel et al., 2020; White et al., 2020; Yao et al., 2020). However, in these studies the currently applied dosing schemes were not investigated, while they focused primarily on efficacy or on safety aspects. In addition, patients' characteristics were not taken into consideration and a specific dose was proposed for all cases. Only in the study of White et al., (2020) renal impairment and body weight were taken under consideration. The remarkable effect on HCQ's blood concentrations was made evident and the investigators addressed the importance of dose adjustment per weight (White et al., 2020). In the present study, an integrated approach was used taking into consideration all the main aspects of treatment with HCQ, while the proposed dosing schemes were designed by taking into consideration patient's weight, disease severity, and his/her HCQ clearance.

It is worth mentioning, that the significant advantage of a high loading dose has been demonstrated in an open-label, randomized, controlled trial including 150 patients with COVID-19 (Tang et al., 2020), while the safety of a sparse dosing strategy in order to avoid accumulation has also been indicated in a recently published study for chloroquine (Karalis et al., 2020), that exhibits similar chemical structure, mechanism of action and adverse effects with HCQ (Singh et al., 2020).

Conclusion

After an extensive literature survey and simulations performed, several dosing schemes of HCQ have been proposed for the treatment of COVID-19 in relation to patient's weight and disease severity that could indicate patient's viral load. A high initial dose followed by lower sparse doses seems to be the most appropriate approach to apply in this case, as it is postulated to be more effective and safer compared to small frequent doses. Indeed, these dosing schemes were designed aiming to lower the viral load both in blood and in the lungs, without allowing for HCQ accumulation that could lead to adverse effects. Despite their complexity in terms of clinical practice we believe that they may offer significant advantages to clinicians coping with COVID-19.
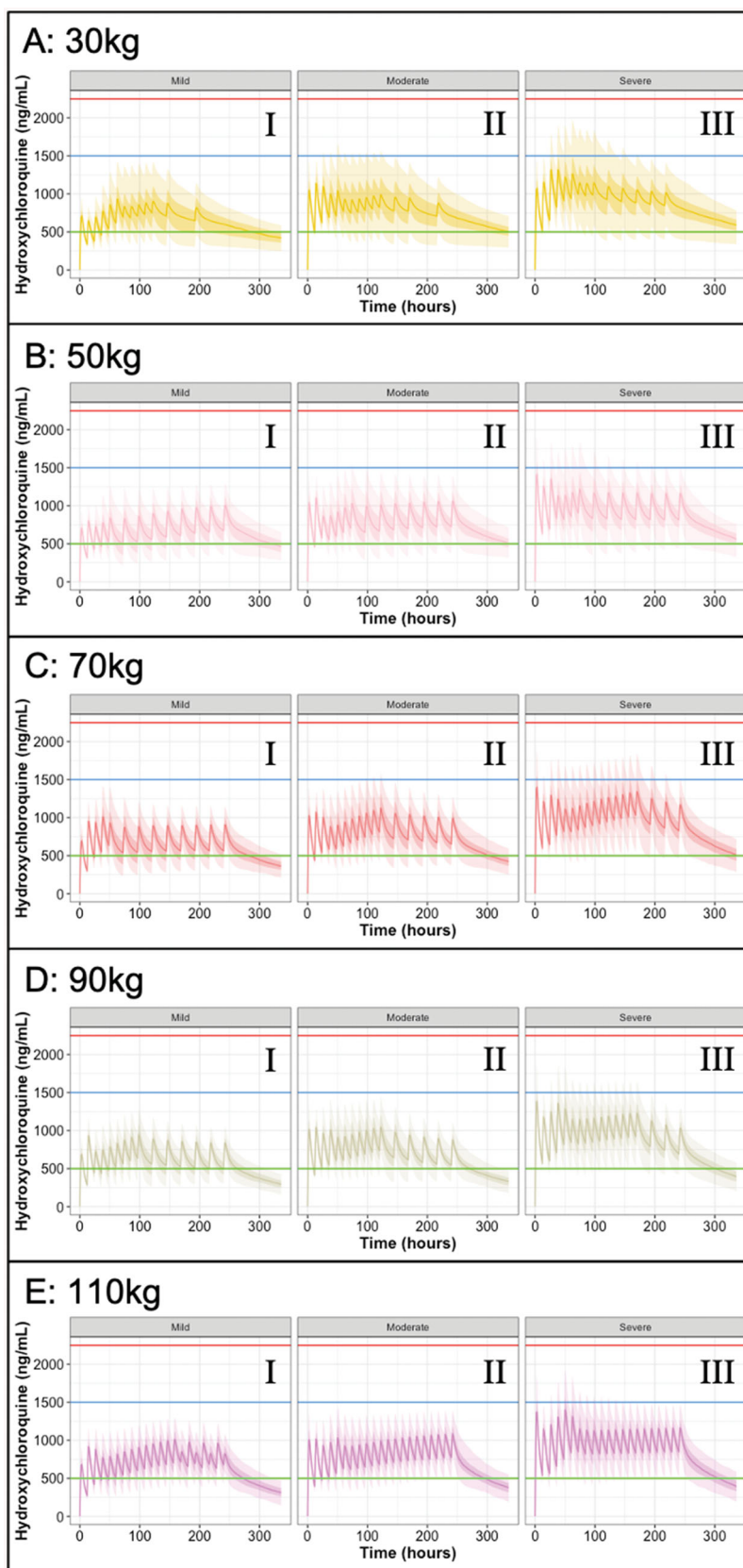
Figure 3. Simulated hydroxychloroquine concentrations (ng/ml) versus time (hours) in 500 patients receiving the proposed dosing schemes depending on disease severity, i.e., mild (I), moderate (II) or severe (III) for **A**: a patients' population of 30 kg, **B**: a patients' population of 50 kg, **C**: a patients' population of 70 kg, **D**: a patients' population of 90 kg, **E**: a patients' population of 110 kg. Red line: 2235 ng/ml, known to promote adverse effects to 30% of patients. Blue line: 1500 ng/ml, levels to achieve during the first doses. Green line: 500 ng/ml, minimum levels eliciting immunomodulatory effect.
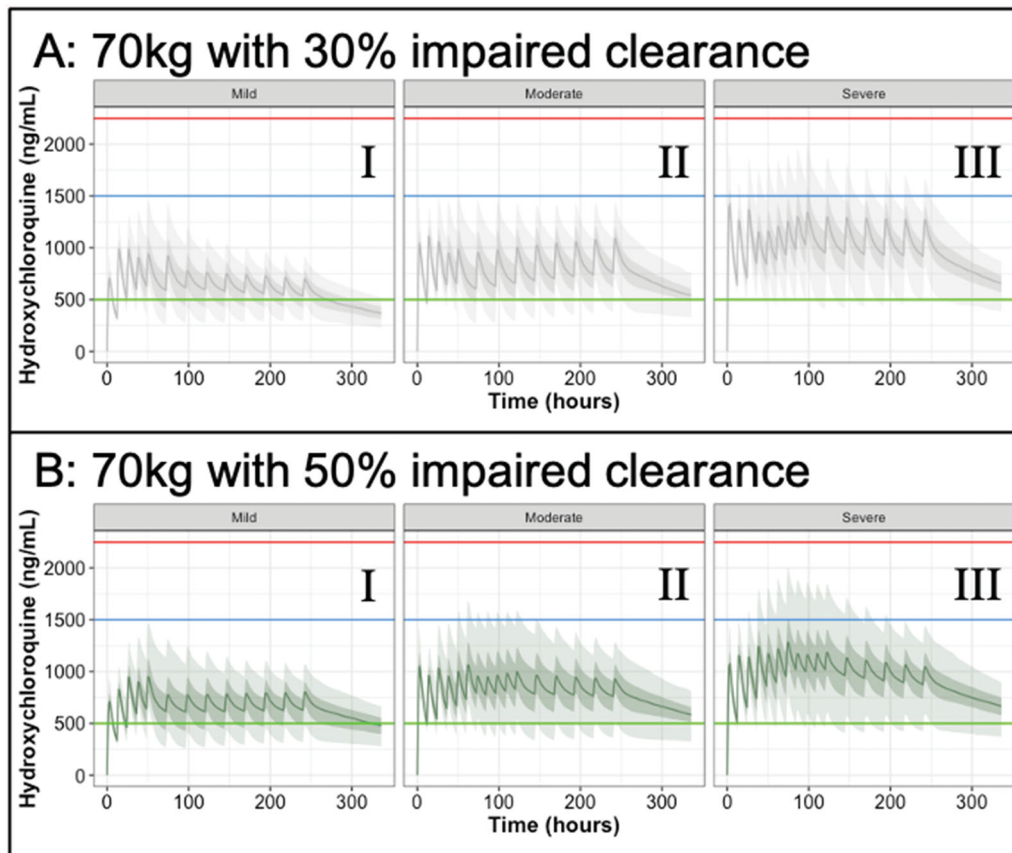
Figure 4. Simulated hydroxychloroquine concentrations (ng/ml) versus time (hours) in 500 patients receiving the proposed dosing schemes depending on disease severity, i.e., mild (I), moderate (II) or severe (III) for **A**: a patients' population of 70 kg with 30% impaired clearance, **B**: a patients' population of 70 kg with 50% impaired clearance. Red line: 2235 ng/ml, known to promote adverse effects to 30% of patients. Blue line: 1500 ng/ml, levels to achieve during the first doses. Green line: 500 ng/ml, minimum levels eliciting immunomodulatory effect.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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