

1 **Effect of Systemic Inflammatory Response to SARS-CoV-2 on Lopinavir**
2 **and Hydroxychloroquine Plasma Concentrations**

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45 **Abstract**

46 *Background:* Coronavirus disease 2019 (COVID-19) leads to inflammatory cytokine
47 release, which can downregulate the expression of metabolizing enzymes. This cascade
48 affects drug concentrations in the plasma. We investigated the association between
49 lopinavir (LPV) and hydroxychloroquine (HCQ) plasma concentrations and the values of
50 acute phase inflammation marker C-reactive protein (CRP).

51 *Methods:* LPV plasma concentrations were prospectively collected in 92 patients
52 hospitalized at our institution. Lopinavir/ritonavir was administered 12-hourly,
53 800/200 mg on day 1, and 400/100 mg on day 2 until day 5 or 7. HCQ was given at 800
54 mg, followed by 400 mg after 6, 24 and 48 hours. Hematological, liver, kidney, and
55 inflammation laboratory values were analyzed on the day of drug level determination.

56 *Results:* The median age of study participants was 59 (range 24–85) years, and 71%
57 were male. The median duration from symptom onset to hospitalization and treatment
58 initiation was 7 days (IQR 4–10) and 8 days (IQR 5–10), respectively. The median LPV
59 trough concentration on day 3 of treatment was 26.5 µg/mL (IQR 18.9–31.5). LPV
60 plasma concentrations positively correlated with CRP values ($r=0.37$, $p<0.001$), and
61 were significantly lower when tocilizumab was preadministered. No correlation was
62 found between HCQ concentrations and CRP values.

63 *Conclusions:* High LPV plasma concentrations were observed in COVID-19 patients. The
64 ratio of calculated unbound drug fraction to published SARS-CoV-2 EC₅₀ values
65 indicated insufficient LPV concentrations in the lung. CRP values significantly correlated
66 with LPV but not HCQ plasma concentrations, implying inhibition of cytochrome P450
67 3A4 (CYP3A4) metabolism by inflammation.

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69

70 Introduction

71 Clinical trials have been launched to find effective treatment against the novel
72 coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the cause
73 of the respiratory illness termed coronavirus disease 2019 (COVID-19) (1, 2).
74 Approximately 15% of COVID-19 patients develop severe pneumonia (3). Cytokine
75 release syndrome is an important factor for disease progression. Thus, treatment
76 rationales for COVID-19 have focused on both antiviral activity and control of the
77 infection-induced cytokine storm (4). Direct interaction between the 2 modalities must
78 be evaluated, however, because infectious and inflammatory diseases have an impact on
79 drug metabolism (5, 6).

80 The release of inflammatory cytokines such as interleukin-6 (IL-6) activates intracellular
81 signaling cascades, leading to the downregulation of cytochrome P450 enzymes (CYPs)
82 (7). The decrease in expression and activity of CYPs is explained by transcriptional
83 suppression of CYP mRNA, triggering a decrease in enzyme synthesis (5, 6). Systemic
84 inflammation affects CYPs differently with a more pronounced decrease in CYP3A4
85 expression followed by CYP2B6, CYP2C19, CYP2C9, CYP2D6 and CYP1A2 (5, 6, 8).
86 Correlations have been reported between elevated C-reactive protein (CRP) values and
87 high plasma levels of antipsychotic drugs (9) and voriconazole (10). CRP production is
88 triggered by IL-6, and conversely, IL-6 suppression can be monitored with plasma CRP
89 levels (11).

90 The HIV drug lopinavir/ritonavir (LPV/r) has been repurposed for the treatment of
91 SARS-CoV-2 (2). Recent brief reports of 8 (12), 12 (13), and 21 (14) COVID-19 patients,
92 noted considerably higher LPV plasma concentrations than those observed in HIV
93 patients (15). Considering the inhibition of drug metabolism by cytokine release, and the

administration of LPV/r (metabolized by CYP3A4), we had the rationale to prospectively monitor LPV plasma concentrations in our cohort of COVID-19 patients.

The objective of this study was to investigate the association between CRP values and LPV plasma concentrations in COVID-19 patients. With this approach, we aimed to underscore the hypothesis that high inflammatory markers in the blood correlate with high LVP plasma concentrations. For comparison, we measured hydroxychloroquine (HCQ) concentrations, because it is characterized by a different metabolism (16). We also performed an age-stratified analysis to explore the combined effect of aging and inflammation on drug plasma levels. Finally, we discuss our LPV plasma trough concentration results in the context of calculated unbound concentrations in the lung compartment and published 50% effective concentrations (EC50) values for SARS-CoV-2.

Methods

All adults (≥ 18 years) hospitalized at the University Hospital in Basel between 25 February and 30 April 2020 for a COVID-19 infection (confirmed by real-time reverse transcriptase-polymerase chain reaction [RT-PCR] from nasopharyngeal swab specimens) were screened for study eligibility. The study was part of a COVID-19 cohort consortium investigation and approved by the northwest/central Switzerland Ethics Committee (EKNZ 2020-00769).

Study population: COVID-19 patients were eligible, if they were treated with LPV/r. Patients were excluded if LPV drug concentrations were not measured. Prior to administration of LPV/r and HCQ, all concomitant drugs were reviewed for potential drug-drug interactions (DDI) via a website incorporated in our institutional treatment recommendations (17). Concomitant intake of CYP3A4 inhibitors or inducers was

119 stopped or switched to another compound with similar therapeutic effect.
120 Corticosteroids were not administered, except in 3 individuals in whom a low-dose long-
121 term treatment with prednisone was continued (5 mg/d in 2 patients, 10 mg/d in 1
122 patient). Other drugs affecting inflammation were not administered, with the exception
123 of tocilizumab (TCZ).

124 *Treatment concepts for COVID-19 and dosing rationale:* Our institutional treatment
125 recommendations include the administration of LPV/r and HCQ for hospitalized
126 patients. To achieve rapidly high LPV/r plasma concentrations, we administered a
127 double dose in the first 24 hours. This approach in the early treatment phase was
128 presumed necessary to suppress the high SARS-CoV-2 viral load in the early stage of
129 disease ("hit early and hit hard"). The LVP/r treatment schedule included 800/200 mg
130 twice daily on day 1, followed by a maintenance dose of 400/100 mg every 12 hours for
131 another 4 to 6 days. LVP/r treatment was combined with HCQ for 2 days (i.e., 800 mg
132 loading dose followed by 400 mg at 6 hours, 24 hours, and 48 hours). In patients with
133 clinical signs and findings suggestive for COVID-19-induced hyper-inflammation, the use
134 of TCZ was considered at the discretion of the treating COVID-19 care team. Parameters
135 for consideration were defined in the institutional diagnostic recommendations for
136 COVID-19. They included clinical signs (breathing frequency ≥ 30 per minute, O₂
137 saturation $< 93\%$), laboratory results (CRP ≥ 75 mg/L) and the extent of radiological
138 findings in the computed tomography scan of the lung (typical ground-glass opacities,
139 infiltrates in ≥ 4 lobes or considerable progression of infiltrates within 24 to 48 hours).
140 TCZ was administered intravenously at a dose of 8 mg/kg body weight, with single dose
141 or 2 doses within 24 hours.

142 *Quantification of LPV and HCQ plasma concentrations:* The institutional diagnostic
143 recommendations for COVID-19 suggest obtaining LVP plasma trough levels on day 2 or

144 3 of treatment. LPV levels were quantified by using commercial calibrators and controls
145 for liquid chromatography mass spectrometry methods (Recipe Chemicals +
146 Instruments, Munich, Germany). The lower limit of quantification was 0.1 µg/mL.
147 HCQ levels were measured from available plasma material obtained for LPV trough
148 determination. HCQ was quantified with a validated liquid chromatography mass
149 spectrometry method developed by the laboratory of clinical chemistry at the University
150 Hospital in Zurich, Switzerland. The lower limit of quantification was 10 ng/mL.

151 *Data management, variable categorization, and statistical analysis:* Patient
152 demographics, laboratory data, vital parameters, and medication records were extracted
153 from the electronic medical reports and the institutional Clinical Data Warehouse.

154 Information on the time interval between onset of symptoms consistent with COVID-19
155 and (i) hospitalization and (ii) initiation of antiviral treatment were investigated
156 prospectively. Laboratory results obtained at the day of drug level measurement were
157 used for this analysis.

158 Because age-related physiological changes can affect drug pharmacokinetics (18), we
159 categorized patients as <65 years or ≥ 65 years. As indicated earlier, we used a tentative
160 CRP cutoff value of 75 mg/L to aid decision making for the administration of TCZ. This
161 CRP level was used as marker for inflammation for the analysis in the study (i.e., <75 vs
162 ≥ 75 mg/L).

163 In patients receiving TCZ prior to the measurement of LPV or HCQ plasma
164 concentrations, a time interval cutoff of 12 hours for inflammation inhibition and
165 consecutive effect on drug metabolism was predefined. This value was chosen after
166 consideration of various parameters (i.e., presumed time to clinical resolution of
167 cytokine release syndrome after TCZ administration (19), dynamics of CRP levels in
168 infections (20), drug administration schedule). Hence, in the case of TCZ administration

169 at ≤ 12 hours prior to the measurement of LPV trough levels, the interval between the
170 two time points was considered to be too short for having an effect on LPV plasma
171 concentrations.

172 Absolute numbers, percentages, medians, and interquartile ranges (IQRs) were used to
173 report demographic characteristics and laboratory results. The Mann-Whitney U test
174 was used to compare continuous data, and the Spearman correlation coefficient to
175 explore associations of interest. All statistical analyses were performed with GraphPad
176 Prism and SPSS.

177

178 Results

179 Of 170 COVID-19 patients hospitalized in our institution within the study time frame, 92
180 RT-PCR confirmed positive cases with available LPV plasma concentrations were
181 included in the study. The median age of study participants was 59 (IQR 48–70; range
182 24–85) years, and the majority were males (71%). The median time from onset of
183 symptoms to hospitalization was 7 (IQR 4–10) days, and from onset of symptoms to
184 initiation of LPV/r and HCQ treatment, was 8 (IQR 5–10) days. Twenty-seven (29%)
185 individuals were transferred to the intensive care unit (ICU) during the hospitalization.
186 Overall, 35 (38%) patients received TCZ, 19 (54%) prior to LPV plasma concentration
187 measurement and 16 (46%) afterward. The median CRP values at the day of LPV plasma
188 measurements in these TCZ groups were 88.9 (IQR 48.2–153.2) mg/L, 79.9 (IQR 48.2–
189 129.6) mg/L and 105.4 (IQR 51.9–153.7) mg/L, respectively. In 3 individuals who
190 received TCZ before measurement of LPV plasma concentrations, the time interval
191 between the two time points was ≤ 12 hours. For analysis purposes, the LPV plasma
192 levels of these 3 patients were assigned to the group who received TCZ after drug level
193 measurement. The CRP values in these individuals were 44.2, 124.8 and 165.8 mg/L.

194 Patients admitted to the ICU tended to have a higher body weight, lower albumin and
195 hemoglobin levels, higher creatine kinase and CRP values than did patients who were
196 not treated in the ICU (**Table 1**). Twenty (22%) patients presented with moderate or
197 severe renal impairment. **Table 1** shows the demographic and clinical characteristics of
198 the patients.

199 *LPV levels and impact of inflammation:* LPV trough levels (12 h \pm 3 hours after the last
200 drug intake) ranged from 7.7 to 42.3 $\mu\text{g/mL}$ with a median value of 26.5 (IQR 18.9–31.5)
201 $\mu\text{g/L}$ (**Figure 1**). LPV plasma concentrations were measured after a median time of 3
202 (IQR 3–4) days and correlated positively with CRP values ($r=0.37$, $p<0.001$, 92
203 observations) and leukocytes ($r=0.32$, $p=0.002$, 91 observations). When stratifying
204 patients by predefined CRP level, we observed significantly higher LPV concentrations in
205 patients with CRP ≥ 75 mg/L than in those with <75 mg/L (median levels: 30.7 vs 20.9
206 $\mu\text{g/mL}$, $p < 0.001$) (**Figure 2A**). TCZ administration >12 hours prior to LPV
207 measurement demonstrated significantly lower LPV plasma concentrations (median
208 18.7 $\mu\text{g/mL}$) than did the comparison group (i.e., no TCZ administration or TCZ
209 administration ≤ 12 hours prior to LPV measurement) (median 28.8 $\mu\text{g/mL}$, $p<0.001$,
210 **Figure 3**). No other significant correlations were found with any other parameters listed
211 in **Table 1**.

212 *Combined effect of age and inflammation on LPV concentrations:* Median LPV plasma
213 trough levels were insignificantly higher in patients who were ≥ 65 years (26.9 $\mu\text{g/mL}$, $n =$
214 33) than in those who were <65 years (24.5 $\mu\text{g/mL}$, $n = 59$) (**Figure 2A**). Accordingly,
215 median LPV concentrations were not different in patients with CRP values ≥ 75 mg/L
216 and who were ≥ 65 vs <65 years (median levels: 31.0 vs 30.6 $\mu\text{g/mL}$, $p = 0.825$) or in
217 patients with CRP values <75 mg/L who were ≥ 65 vs <65 years (median levels: 24.7 vs
218 20.2 $\mu\text{g/mL}$, $p=0.362$) (**Figure 2A**).

219 *HCQ concentrations:* HCQ concentrations were measured in 59 patients from available
220 plasma samples, and ranged from 56 to 454 ng/mL with a median value of 171 (IQR
221 128–207) ng/mL (**Figure 1**). In 51 plasma samples, the median time interval since the
222 last drug intake was of 22 (range 12–31, IQR 14–23) hours, and the values showed no
223 correlation with CRP values ($r=0.044$, $p=0.76$) or any other laboratory parameter listed
224 in **Table 1**. HCQ plasma concentrations were not statistically different in patients with
225 CRP values <75 or ≥ 75 mg/L (median levels: 149 versus 148 ng/mL, $p=0.959$) (**Figure**
226 **2B**). There was no correlation between LPV and HCQ plasma concentrations ($r=0.197$,
227 $p=0.166$, $n=51$).

228

229 Discussion

230 Median LPV trough concentrations were an unexpected 3.5-fold higher in patients
231 infected with SARS-CoV-2 than in HIV-infected patients, as reported historically (i.e., 7.1
232 $\mu\text{g/mL}$) (15). Our prospective analysis on LPV plasma concentrations in 92 patients is in
233 line with recent observations in small series that reported LPV plasma concentrations
234 from 13 to 18 $\mu\text{g/mL}$ in COVID-19 patients (12–14). The even higher trough
235 concentrations in our study (i.e., median 26.5 $\mu\text{g/mL}$) might be explained by the double
236 dose LPV/r dose (800/200 mg) at day 1 and the differences in the severity of COVID-19
237 between the studies. The median CRP values available in two of the aforementioned
238 brief reports were 13.6 mg/L (12) and 48.9 mg/L (13) vs 65 mg/L in our study.

239 We investigated possible reasons for high LPV plasma concentrations in COVID-19
240 patients. HCQ-mediated inhibition of the hepatic organic anion transporting polypeptide
241 1A2 (OATP1A2) (21), and interference with liver entry and subsequent metabolic
242 elimination is, in our view, not plausible. OATP1A2 is expressed on the apical membrane
243 of cholangiocytes, where it reabsorbs drugs excreted into the bile (22). Inhibition of this

244 transporter would likely facilitate LPV/r biliary elimination. Viral-induced liver damage
245 may cause impaired drug metabolism and high LPV plasma concentrations. However,
246 the vast majority of individuals in our study population had only mildly elevated liver
247 enzymes (**Table 1**). The effect of the double dose within the first 24 hours on LPV
248 plasma trough concentrations measured after a median time of 3 (IQR 3-4) days is
249 difficult to assess. LPV/r 800/200 mg single dose pharmacokinetic studies reported
250 concentrations <12 µg/mL (23) or <14 µg/mL (24), 12 hours after intake. In a study
251 with healthy HIV-negative volunteers, LPV trough levels ranged from 8.3 to 13.8 µg/mL
252 at day 2 of treatment with 800/200 mg twice daily (25). In our study population, 81
253 (88%) samples had LPV plasma levels >14 µg/mL, 66 (72%) >20 µg/mL and 35 (38%) >
254 30 µg/mL. these data together with the LPV pharmacokinetics data in the literature (23-
255 25), noticeably suggests that the elevated LPV trough concentrations observed in
256 COVID-19 patients cannot be explained only by the effect of the initial double dose. Our
257 findings support the hypothesis that the systemic inflammatory response in COVID-19
258 patients inhibits drug metabolism, leading to elevated LPV plasma concentrations.
259 Conversely, blocking inflammation with TCZ was associated with lower LPV plasma
260 concentrations. This is possibly explained by the fact that TCZ inhibition of inflammatory
261 cytokines leads to a normalization of CYP metabolism.

262 Aging is associated with physiological changes and decline of the immune function,
263 which altogether can impact drug pharmacokinetics (18). However, LPV plasma trough
264 concentrations were not significantly different in patients who ≥65 than in those who
265 were <65 years in our study.

266 Inflammation has been shown to have the greatest impact on CYP3A4 expression (7).
267 This increase may, in turn, impact the magnitude of DDIs, because LPV/r inhibits
268 CYP3A4 in a concentration-dependent manner (26). Co-administered CYP3A4

269 substrates can – *per se* – be also affected by inflammation, and can further increase the
270 magnitude of DDIs. This interaction is illustrated by a case series of 12 patients who
271 were followed up for direct oral anticoagulant treatment (DOAC) before and after being
272 infected with SARS-CoV-2. LPV/r was started while DOAC was maintained at the same
273 dose. DOAC levels after initiation of LPV/r treatment showed an average 6-fold increase
274 (27). The co-administration of the strong CYP3A4 inhibitor ritonavir has been shown to
275 increase rivaroxaban levels by 2.5-fold in healthy volunteers (28), whereas rivaroxaban
276 plasma concentrations were increased by 7- up to 31-fold in COVID-19 patients treated
277 with LPV/r (27). Notably, the disappearance of the inhibitory effect on CYP3A4 may take
278 up to 5 days after stopping LPV/r (29).

279 The high LPV plasma concentrations observed in COVID-19 patients inevitably raise the
280 question about the LPV levels that can be achieved in the lung. LPV/r is thought to act by
281 inhibiting the enzyme 3-chymotrypsin-like protease (3CL^{pro}) of SARS-CoV-2, thereby
282 disrupting the cleavage of the viral protein and release from the host cell (30).
283 Coronavirus proteases, including 3CL^{pro}, do not contain a C2-symmetric pocket, resulting
284 in an unspecific inhibition (31). Recently, Choy et al. (32) investigated the EC₅₀ of LPV in
285 inhibiting SARS-CoV-2 replication in Vero E6 cells. The cells were treated with the
286 compound for 1 hour prior to the infection by the virus at a multiplicity of infection of
287 0.02. The authors reported EC₅₀ values of 26.63 and 26.10 μ M, measuring infectious
288 virus and viral RNA, respectively. These values correspond to *in-vitro* concentrations of
289 16.7 and 16.4 μ g/mL respectively (32). The antiviral activity *in vivo* is estimated by
290 calculating the ratio of unbound drug concentrations achieved in the lung at the
291 administered dose to the *in vitro* EC₅₀ value ($R_{L,TEC}$) (33). LPV plasma measurements in
292 12 COVID-19 patients showed median total and unbound trough concentrations of 18.0
293 μ g/mL and 0.16 μ g/mL, respectively, resulting in an unbound fraction of 0.88% (13).

294 This fraction is consistent with the results from a previous study (34). The simultaneous
295 determination of LPV in epithelial lining fluid (ELF) and plasma indicated an
296 ELF/plasma ratio of 1.77 (35). Considering our total observed LPV trough plasma
297 concentrations, the extrapolated unbound LPV trough level is 0.23 $\mu\text{g/mL}$. This value
298 corresponds to an unbound LPV level in ELF of 0.41 $\mu\text{g/mL}$, which gives a R_{LTEC} of 0.025.
299 Even though the majority of the observed total LPV plasma concentrations in COVID-19
300 patients were above the published EC_{50} values for SARS-CoV-2 (32), boosted LPV is
301 unlikely to attain sufficient effective levels in the lung to inhibit the virus. In line with
302 these arguments, current available clinical data do not demonstrate evidence for the
303 efficacy of LPV/r for COVID-19 (36, 37).

304 HCQ has been used historically for malaria and immune diseases. Its ability to inhibit
305 SARS-CoV-2 is thought to be due to an increase in endosomal pH, thereby impairing the
306 entry of the virus into the cell. HCQ also interferes with the glycosylation of cellular
307 receptors for SARS-CoV-2, resulting in reduced virus-cell binding. Finally, HCQ has
308 immunomodulatory activity that may suppress the cytokine storm (16).

309 The median HCQ concentrations observed in our study (i.e., 171 ng/mL, IQR 128–207) is
310 comparable to those reported in another study with COVID-19 patients (220 ± 110
311 ng/mL) (38), and to steady-state trough levels observed in patients with lupus
312 erythematosus (i.e., 103–130 ng/mL)(39). Thus, the HCQ plasma concentrations in
313 COVID-19 patients, in contrast to reported LPV plasma concentrations, were not higher
314 than those previously observed in studies with other indications. Furthermore, no
315 correlation was observed with CRP values. This difference may possibly be explained by
316 the different metabolic pathways of HCQ and LPV/r, as inflammation affects CYPs
317 differently (7). Furthermore, HCQ is known to have higher concentrations in tissue than
318 in plasma (approximately 200- to 700-fold higher), resulting in a large distribution

319 volume and a long half-life (33). Therefore, HCQ plasma concentrations from COVID-19
320 patients might not be suitable to reflect the effect of inflammation given that HCQ does
321 not achieve steady-state concentrations during the short treatment course. Similar to
322 LPV/r, HCQ was shown to have a low R_{LTC} (i.e., 0.11–0.34), indicating that HCQ levels
323 achieved *in vivo* do not result in adequate clinical activity against SARS-CoV-2 (33).
324 These calculations are supported by a studies failing to demonstrate a benefit of HCQ in
325 both hospitalized patients with COVID-19 (40), and as prophylaxis after SARS-CoV-2
326 exposure (41).

327 Some limitations of this study should be acknowledged. We did not consider IL-6
328 measurement as a routine diagnostic value within our COVID-19 cohort, and hence, in
329 study. IL-6 is a central mediator of the acute-phase response and a primary determinant
330 of hepatic production of CRP. IL-6 has many other pathophysiologic roles in humans
331 (42) and its diagnostic value for COVID-19, in particular for non-severe cases, is
332 unknown. The selection of cutoff of 12 hours in the case of TCZ administration prior to
333 measurement of LPV plasma concentrations was clinically reasonable but arbitrary.
334 However, this limitation applied to only 3 patients, and had no statistical influence on
335 the results.

336 In conclusion, high LPV trough plasma concentrations were observed in COVID-19
337 patients. However, the calculated unbound concentrations in the lung indicates
338 insufficient levels to inhibit SARS-CoV-2 replication. LPV levels correlated positively
339 with CRP values and negatively with the preadministration of TCZ, indicating that
340 COVID-19 related cytokine release significantly inhibits CYP3A4. Caution is advised
341 when prescribing CYP3A4 substrates with a narrow therapeutic index to COVID-19
342 patients because of the risk of elevated drug levels and related toxicities.

343

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355

356 **Conflict of Interest**

357 All other authors report no potential conflict of interest..

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360 **References**

- 361 1. Fajgenbaum DC, Khor JS, Gorzewski A, Tamakloe MA, Powers V, Kakkis JJ,
362 Repasky M, Taylor A, Beschloss A, Hernandez-Miyares L, Go B, Nimgaonkar V,
363 McCarthy MS, Kim CJ, Pai RL, Frankl S, Angelides P, Jiang J, Rasheed R, Napier E,
364 Mackay D, Pierson SK. 2020. Treatments Administered to the First 9152
365 Reported Cases of COVID-19: A Systematic Review. *Infect Dis Ther*
366 doi:10.1007/s40121-020-00303-8.
- 367 2. WHO. "Solidarity" clinical trial for COVID-19 treatments. Available at:
368 [https://www.who.int/emergencies/diseases/novel-coronavirus-2019/global-](https://www.who.int/emergencies/diseases/novel-coronavirus-2019/global-research-on-novel-coronavirus-2019-ncov/solidarity-clinical-trial-for-covid-19-treatments)
369 [research-on-novel-coronavirus-2019-ncov/solidarity-clinical-trial-for-covid-19-](https://www.who.int/emergencies/diseases/novel-coronavirus-2019-ncov/solidarity-clinical-trial-for-covid-19-treatments)
370 [treatments](https://www.who.int/emergencies/diseases/novel-coronavirus-2019-ncov/solidarity-clinical-trial-for-covid-19-treatments).
- 371 3. Wu Z, McGoogan JM. 2020. Characteristics of and Important Lessons From the
372 Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report
373 of 72 314 Cases From the Chinese Center for Disease Control and Prevention.
374 *JAMA* [epub ahead of print] doi:10.1001/jama.2020.2648.
- 375 4. Zhang S, Li L, Shen A, Chen Y, Qi Z. 2020. Rational Use of Tocilizumab in the
376 Treatment of Novel Coronavirus Pneumonia. *Clin Drug Investig* 40(6): 511-8.
- 377 5. Renton KW. 2005. Regulation of drug metabolism and disposition during
378 inflammation and infection. *Expert Opin Drug Metab Toxicol* 1(4): 629-40.
- 379 6. Morgan ET. 2009. Impact of infectious and inflammatory disease on cytochrome
380 P450-mediated drug metabolism and pharmacokinetics. *Clin Pharmacol Ther*
381 85(4): 434-8.
- 382 7. Dickmann LJ, Patel SK, Rock DA, Wienkers LC, Slatter JG. 2011. Effects of
383 interleukin-6 (IL-6) and an anti-IL-6 monoclonal antibody on drug-metabolizing
384 enzymes in human hepatocyte culture. *Drug Metab Dispos* 39(8): 1415-22.
- 385 8. Morgan ET, Goralski KB, Piquette-Miller M, et al. 2008. Regulation of drug-
386 metabolizing enzymes and transporters in infection, inflammation, and cancer.
387 *Drug Metab Dispos* 36(2): 205-16.
- 388 9. Hefner G, Shams ME, Unterecker S, Falter T, Hiemke C. 2016. Inflammation and
389 psychotropic drugs: the relationship between C-reactive protein and
390 antipsychotic drug levels. *Psychopharmacology* (Berl) 233(9): 1695-705.
- 391 10. Vreugdenhil B, van der Velden W, Feuth T, Kox M, Picckers P, van de Veerdonk FL,
392 Blijlevens NMA, Brüggemann RJM. 2018. Moderate correlation between systemic
393 IL-6 responses and CRP with trough concentrations of voriconazole. *Br J Clin*
394 *Pharmacol* 84(9): 1980-8.
- 395 11. Kojima T, Yabe Y, Kaneko A, Hirano Y, Ishikawa H, Hayashi M, Miyake H, Takagi H,
396 Kato T, Terabe K, Wanatabe T, Tsuchiya H, Kida D, Shioura T, Funahashi K, Kato
397 D, Matsubara H, Takahashi N, Hattori Y, Asai N, Ishiguro N. 2013. Monitoring C-
398 reactive protein levels to predict favourable clinical outcomes from tocilizumab
399 treatment in patients with rheumatoid arthritis. *Mod Rheumatol* 23(5): 977-85.
- 400 12. Schoergenhofer C, Jilma B, Stimpfl T, Karolyi M, Zoufaly A. 2020.
401 Pharmacokinetics of Lopinavir and Ritonavir in Patients Hospitalized With
402 Coronavirus Disease 2019 (COVID-19). *Ann Intern Med* [epub ahead of print]
403 doi:10.7326/m20-1550.
- 404 13. Gregoire M, Le Turnier P, Gaborit BJ, Veyrac G, Lecomte R, Boutoille D, Canet E,
405 Imbert BM, Bellouard R, Raffi F. 2020. Lopinavir pharmacokinetics in COVID-19
406 patients. *J Antimicrob Chemother* [epub ahead of print]
407 doi:10.1093/jac/dkaa195.

- 408 14. Baldelli S, Corbellino M, Clementi E, Cattaneo D, Gervasoni C. 2020.
409 Lopinavir/ritonavir in COVID-19 patients: maybe yes, but at what dose? *J*
410 *Antimicrob Chemother* [epub ahead of print] doi:10.1093/jac/dkaa190.
- 411 15. Croxtall JD, Perry CM. 2010. Lopinavir/Ritonavir: a review of its use in the
412 management of HIV-1 infection. *Drugs* 70(14): 1885-915.
- 413 16. Tripathy S, Dassarma B, Roy S, Chabalala H, Matsabisa MG. 2020. A review on
414 possible modes of actions of Chloroquine/ Hydroxychloroquine: Repurposing
415 against SAR-COV-2 (COVID 19) pandemic. *Int J Antimicrob Agents* [epub ahead of
416 print] doi:10.1016/j.ijantimicag.2020.106028.
- 417 17. University of Liverpool. Interaction Checker, COVID-19 Drug Interactions.
418 Available at: <https://www.covid19-druginteractions.org/>.
- 419 18. Stader F, Kinvig H, Penny MA, Battegay M, Siccardi M, Marzolini C. 2020.
420 Physiologically Based Pharmacokinetic Modelling to Identify Pharmacokinetic
421 Parameters Driving Drug Exposure Changes in the Elderly. *Clin Pharmacokinet*
422 59(3): 383-401.
- 423 19. Le RQ, Li L, Yuan W, Shord SS, Nie L, Habtemariam BA, Przepiorka D, Farrell AT,
424 Pazdur R. 2018. FDA Approval Summary: Tocilizumab for Treatment of Chimeric
425 Antigen Receptor T Cell-Induced Severe or Life-Threatening Cytokine Release
426 Syndrome. *Oncologist* 23(8): 943-7.
- 427 20. Markanday A. 2015. Acute Phase Reactants in Infections: Evidence-Based Review
428 and a Guide for Clinicians. *Open Forum Infect Dis* 2(3): ofv098.
- 429 21. Xu C, Zhu L, Chan T, Lu X, Shen W, Madigan MC, Gillies MC, Zhou F. 2016.
430 Chloroquine and Hydroxychloroquine Are Novel Inhibitors of Human Organic
431 Anion Transporting Polypeptide 1A2. *J Pharm Sci* 105(2): 884-90.
- 432 22. Lee W, Glaeser H, Smith LH, Roberts RL, Moeckel GW, Gervasini G, Leake BF, Kim
433 RB. 2005. Polymorphisms in human organic anion-transporting polypeptide 1A2
434 (OATP1A2): implications for altered drug disposition and central nervous system
435 drug entry. *J Biol Chem* 280(10): 9610-7.
- 436 23. Ofotokun I, Chuck SK, Binongo JN, Palau M, Lennox JL, Acosta EP. 2007.
437 Lopinavir/Ritonavir pharmacokinetic profile: impact of sex and other covariates
438 following a change from twice-daily to once-daily therapy. *J Clin Pharmacol*
439 47:970-7.
- 440 24. Eron JJ, Feinberg J, Kessler HA, Horowitz HW, Witt MD, Carpio FF, Wheeler DA,
441 Ruane P, Mildvan D, Yangco BG, Bertz R, Bernstein B, King MS, Sun E. 2004. Once-
442 daily versus twice-daily lopinavir/ritonavir in antiretroviral-naïve HIV-positive
443 patients: a 48-week randomized clinical trial. *J Infect Dis* 189:265-72.
- 444 25. Nijland HM, L'Homme R F, Rongen GA, van Uden P, van Crevel R, Boeree MJ,
445 Aarnoutse RE, Koopmans PP, Burger DM. 2008. High incidence of adverse events
446 in healthy volunteers receiving rifampicin and adjusted doses of
447 lopinavir/ritonavir tablets. *Aids* 22:931-5.
- 448 26. Eichbaum C, Cortese M, Blank A, Burhenne J, Mikus G. 2013. Concentration effect
449 relationship of CYP3A inhibition by ritonavir in humans. *Eur J Clin Pharmacol*
450 69(10): 1795-800.
- 451 27. Testa S, Prandoni P, Paoletti O, Morandini R, Tala M, Dellanoce C, Giorgi-
452 Pierfranceschi M, Betti M, Battista Danzi G, Pan A, Palareti G. 2020. Direct oral
453 anticoagulant plasma levels' striking increase in severe COVID-19 respiratory
454 syndrome patients treated with antiviral agents: The Cremona experience. *J*
455 *Thromb Haemost* 18(6): 1320-3.

- 456 28. Mueck W, Kubitz D, Becka M. 2013. Co-administration of rivaroxaban with drugs
457 that share its elimination pathways: pharmacokinetic effects in healthy subjects.
458 *Br J Clin Pharmacol* 76(3): 455-66.
- 459 29. Stader F, Khoo S, Stoeckle M, Back D, Hirsch HH, Battegay M, Marzolini C. 2020.
460 Stopping lopinavir/ritonavir in COVID-19 patients: duration of the drug
461 interaction effect. *J Antimicrob Chemother* [epub ahead of print] doi:
462 10.1093/jac/dkaa253.
- 463 30. Zhang L, Lin D, Sun X, Curth U, Drosten C, Sauerhering L, Becker S, Rox K,
464 Hilgenfeld R.. 2020. Crystal structure of SARS-CoV-2 main protease provides a
465 basis for design of improved α -ketoamide inhibitors. *Science* 368(6489): 409-12.
- 466 31. Sheahan TP, Sims AC, Leist SR, Schäfer A, Won J, Brown AJ, Montgomery SA, Hogg
467 A, Babusis D, Clarke MO, Spahn JE, Bauer L, Sellers S, Porter D, Feng JY, Cihlar T,
468 Jordan R, Denison MR, Baric RS. 2020. Comparative therapeutic efficacy of
469 remdesivir and combination lopinavir, ritonavir, and interferon beta against
470 MERS-CoV. *Nat Commun* 11(1): 222.
- 471 32. Choy KT, Wong AY, Kaewpreedee P, Sia SF, Chen D, Hui KPY, Chu DKW, Chan
472 MCW, Cheung PP, Huang X, Peiris M, Yen HL. 2020. Remdesivir, lopinavir,
473 emetine, and homoharringtonine inhibit SARS-CoV-2 replication in vitro.
474 *Antiviral Res* 178: 104786.
- 475 33. Fan J, Zhang X, Liu J, Yang Y, Zheng N, Liu Q, Bergman K, Reynolds K, Huang SM,
476 Zhu H, Wang Y. 2020. Connecting hydroxychloroquine in vitro antiviral activity to
477 in vivo concentration for prediction of antiviral effect: a critical step in treating
478 COVID-19 patients. *Clin Infect Dis* [epub ahead of print] doi:10.1093/cid/ciaa623.
- 479 34. Dumond JB, Rigdon J, Mollan K, Tierney C, Kashuba AD, Aweeka F, Collier AC.
480 2015. Brief Report: Significant Decreases in Both Total and Unbound Lopinavir
481 and Amprenavir Exposures During Coadministration: ACTG Protocol
482 A5143/A5147s Results. *J Acquir Immune Defic Syndr* 70(5): 510-4.
- 483 35. Atzori C, Villani P, Regazzi M, Maruzzi M, Cargnel A. 2003. Detection of
484 intrapulmonary concentration of lopinavir in an HIV-infected patient. *AIDS*
485 17(11): 1710-1.
- 486 36. Ford N, Vitoria M, Rangaraj A, Norris SL, Calmy A, Doherty M. 2020. Systematic
487 review of the efficacy and safety of antiretroviral drugs against SARS, MERS or
488 COVID-19: initial assessment. *J Int AIDS Soc* 23(4): e25489.
- 489 37. Cao B, Wang Y, Wen D, Liu W, Wang J, Fan G, Ruan L, Song B, Cai Y, Wei M, Li X, Xia
490 J, Chen N, Xiang J, Yu T, Bai T, Xie X, Zhang L, Li C, Yuan Y, Chen H, Li H, Huang H,
491 Tu S, Gong F, Liu Y, Wei Y, Dong C, Zhou F, Gu X, Xu J, Liu Z, Zhang Y, Li H, Shang L,
492 Wang K, Li K, Zhou X, Dong X, Qu Z, Lu S, Hu X, Ruan S, Luo S, Wu J, Peng L, Cheng
493 F, Pan L, Zou J, Jia C, et al. 2020. A Trial of Lopinavir-Ritonavir in Adults
494 Hospitalized with Severe Covid-19. *N Engl J Med* 382(19): 1787-99.
- 495 38. Martin-Blondel G, Ruiz S, Murris M, Faguer S, Duhalde V, Eyvrad F, Izopet J,
496 Mansuy JM, Rolland Y, Delavigne K, Guimbaud R, Pugnet G, Conil JM, Georges B,
497 Delobel P, Minville V, Silva Sifontes S, Concorde D, Gandia P. 2020.
498 Hydroxychloroquine in COVID-19 patients: what still needs to be known about
499 the kinetics. *Clin Infect Dis* [epub ahead of print] doi:10.1093/cid/ciaa558.
- 500 39. Morita S, Takahashi T, Yoshida Y, Yokota N. 2016. Population Pharmacokinetics of
501 Hydroxychloroquine in Japanese Patients With Cutaneous or Systemic Lupus
502 Erythematosus. *Ther Drug Monit* 38(2): 259-67.
- 503 40. Geleris J, Sun Y, Platt J, Zucker J, Baldwin M, Hripcsak G, Labella A, Manson D,
504 Kubin C, Barr RG, Sobieszczyk ME, Schluger NW. 2020. Observational Study of

- 505 Hydroxychloroquine in Hospitalized Patients with Covid-19. *N Engl J Med* 382
506 (25): 2411-18.
- 507 41. Boulware DR, Pullen MF, Bangdiwala AS, Pastick KA, Lofgren SM, Okafor EC,
508 Skipper CP, Nascene AA, Nicol MR, Abassi M, Engen NW, Cheng MP, LaBar D,
509 Lothar SA, MacKenzie LJ, Drobot G, Marten N, Zarychanski R, Kelly LE, Schwartz
510 IS, McDonald EG, Rajasingham R, Lee TC, Hulsiek KH. 2020. A Randomized Trial
511 of Hydroxychloroquine as Postexposure Prophylaxis for Covid-19. *N Engl J Med*
512 [epub ahead of print] doi:10.1056/NEJMoa2016638.
- 513 42. Papanicolaou DA. 2000. Interleukin-6: The Endocrine Cytokine. *The Journal of*
514 *Clinical Endocrinology & Metabolism* 85(3): 1331-3.

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Table 1. Demographic, clinical and laboratory characteristics of the study population on the day of LPV plasma concentration measurement.

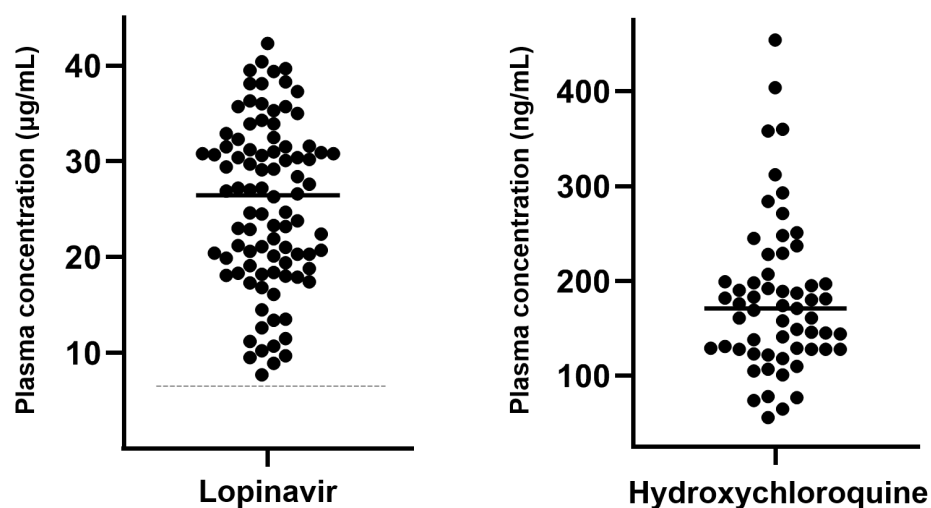
Parameters	All patients (N = 92)	No ICU (N = 65)	ICU (N = 27)
Male sex, n (%)	65 (71)	44 (68)	21 (78)
Age, years, median (range)	59 (24-85)	59 (24-85)	60 (32-85)
Weight, kg (IQR) (n = 83)	84 (70-94)	81 (70-92)	90 (84-100)
Time from symptom onset to hospitalization, day	7 (4-10)	7 (4-9)	8 (5-11)
Time from symptom onset to LPV/r + HCQ treatment, day	8 (5-10)	8 (5-10)	8 (6-11)
Time from hospitalization to LPV/r + HCQ treatment, day	0 (0-1)	1 (0-1)	0 (0-1)
Albumin, g/L (n = 84)	28 (24-31)	29 (26-32)	24 (21-28)
Hemoglobin, g/L (n = 90)	130 (116-142)	132 (120-146)	121 (111-133)
Leukocytes, 10 ⁹ /L (n = 91)	5.3 (4.3-7.2)	5.3 (4.3-7.2)	5.4 (4.2-7.6)
Thrombocytes, 10 ⁹ /L (n = 86)	238 (184-312)	238 (185-321)	238 (186-278)
ASAT, U/L (n = 90)	43 (28-57)	36 (26-54)	51 (43-69)
ALAT, U/L (n = 90)	37 (23-52)	37 (21-53)	37 (30-48)
Alkaline phosphatase, IU/L (n = 87)	61 (48-74)	62 (49-74)	57 (44-76)
GGT, U/L (n = 87)	55 (34-94)	54 (32-92)	63 (38-121)
Bilirubin, μ mol/L (n = 87)	17 (12-22)	17 (13-22)	16 (11-21)
Pancreatic amylase, U/L (n = 83)	38 (29-55)	35 (27-48)	45 (36-108)

eGFR, mL/min/1.73 m ² (n = 92)	81 (64-97)	81 (65-97)	84 (57-98)
Creatine kinase, U/L (n = 84)	106 (57-226)	88 (54-154)	209 (78-547)
CRP, mg/L (n = 92)	65 (36-113)	53 (28-102)	89 (57-139)
Systolic blood pressure, mmHg (n = 91)	112 (99-121)	114 (105-124)	93 (84-118)
Pulse, beats/min (n = 91)	66 (60-73)	67 (61-73)	62 (55-69)
Body temperature, Celsius (n = 76)	36.8 (36.5-37.1)	36.8 (36.5-37.0)	37.1 (36.5-37.5)
Pulse oximetry, % (n = 91)	92 (90-94)	92 (90-94)	90 (87-92)

519 Variables are median and interquartile range (IQR) unless stated otherwise. Laboratory
 520 values were not available for all patients. The number of patients with measurements is
 521 indicated for each separate laboratory parameter.

522 ASAT = aspartate aminotransferase, ALAT = alanine aminotransferase, CRP = C-reactive
 523 protein, eGFR = estimated glomerular filtration (using CKD-EPI formula), GGT = gamma-
 524 glutamyl transferase, HCQ = hydroxychloroquine, ICU = intensive care unit, LPV/r =
 525 lopinavir/ritonavir.

526 **Figure 1. Lopinavir (n = 92) and hydroxychloroquine (n = 59) plasma**
527 **concentrations in COVID-19 patients**



528

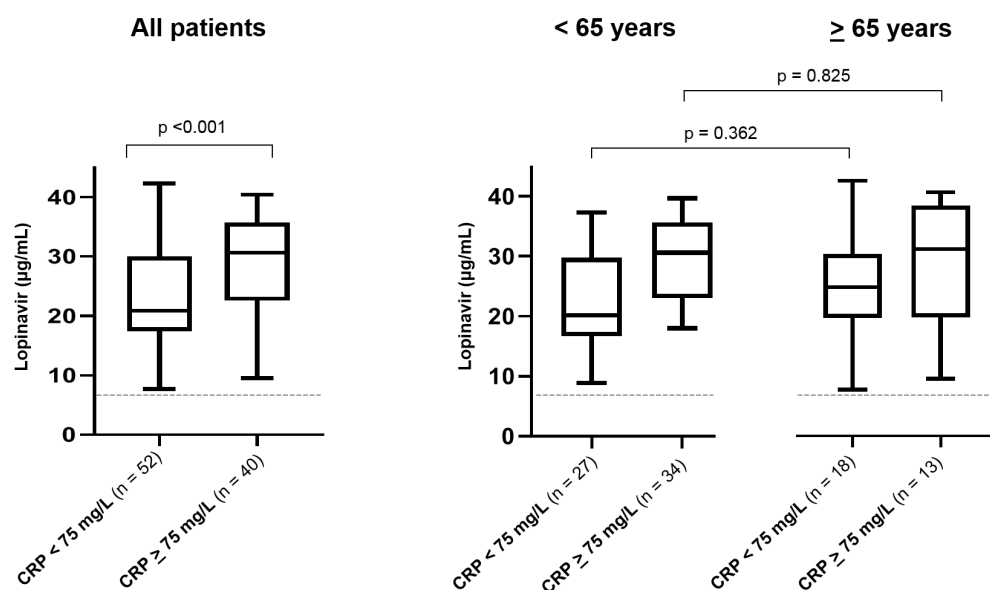
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530 The medial Lopinavir plasma concentration was 26.5 (IQR 18.9–31.5) µg/mL. The
531 median Hydroxychloroquine plasma concentration was 171 (IQR, 128–207) ng/mL. The
532 dashed line represents the historical lopinavir trough level observed in HIV-infected
533 individuals treated with lopinavir/ritonavir 400/100 mg twice daily (i.e., 7.1 µg/mL)
534 (15).

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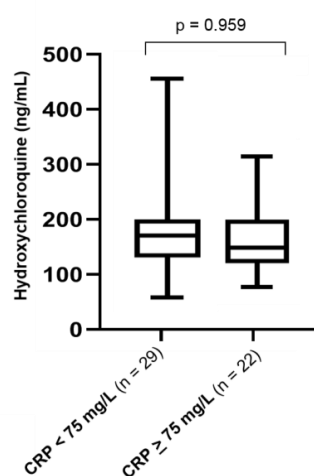
536 **Figure 2.** Box plots (showing the 5th, 25th, 50th, 75th and 95th percentiles) of lopinavir
537 trough concentrations by CRP values in all patients and by age group (A) and box plots
538 of hydroxychloroquine concentrations by CRP values for COVID-19 patients with trough
539 levels (B).

540 **A**



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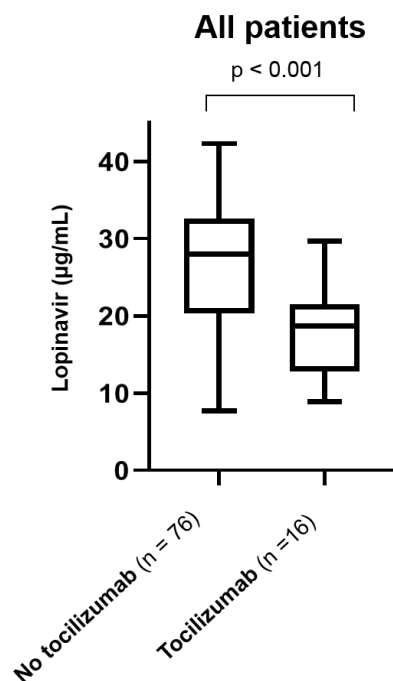
542 **B**



543

544 CRP = C-reactive protein. The dashed line represents the historical lopinavir trough level
545 observed in HIV-infected individuals treated with lopinavir/ritonavir 400/100 mg twice
546 daily (i.e., 7.1 $\mu\text{g/mL}$) (15).

547 **Figure 3.** Box plots (showing the 5th, 25th, 50th 75th and 95th percentiles) of lopinavir
548 plasma trough concentrations in COVID-19 patients by administration of tocilizumab.



549
550 The left bar includes LPV plasma levels from COVID-19 patients with no TCZ
551 administration (n = 57) or TCZ administration < 12 hours prior to LPV measurement (n
552 = 19), (median 28.8 µg/mL). The right bar represents LPV samples from COVID-19
553 patients with TCZ administration > 12 hours prior to LPV measurement (median 18.7
554 µg/mL).

555