Letter to the Editor

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Does COVID-19 alter the oxyhemoglobin dissociation curve? – An observational cohort study using a mixed-effect modelling

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To the Editor,

Mechanisms leading to hypoxemia in severe acute respiratory syndrome coronavirus-2 (SARS-CoV2) infection are multifactorial and not fully understood [1]. Coronavirus disease-2019 (COVID-19) is characterized by atypical acute respiratory distress syndrome (ARDS) with severe hypoxemia despite initially preserved lung compliance, usually reduced in typical ARDS [2]. Potential mechanisms have been investigated including diffuse pulmonary microembolism [3], deregulated hypoxemic reflex vasodilatation [4], and altered hemoglobin-mediated oxygen transport [5]. *In silico* models developed in a non-peer-reviewed work predicted that SARS-CoV2 would exhibit surface proteins able to bind to hemoglobin and alter its affinity to oxygen [5].

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However, such interaction has not been demonstrated experimentally or clinically. No significant differences in hemoglobin affinity for oxygen were found when comparing oxyhemoglobin dissociation curves in 14 COVID-19 patients to those in 11 healthy volunteers [6]. Yet, the limited sample size requiring data extrapolation in the low oxygen saturation (SO₂) area of the partial oxygen pressure (PO₂) curve, the extremely different physiological conditions in the two groups and the exclusion of PCO₂ and pH from the PO₂/SO₂ curve modeling prevented definitive conclusions. Here, we assessed the impact of COVID-19 on hemoglobin affinity to oxygen by modeling the PO₂/SO₂ relationships in COVID-19 and non-COVID-19 ARDS patients.

We collected blood gas analyses (ABL90 Flex, Radiometer Medical ApS, Denmark; operating temperature, 37 °C) in all consecutive COVID-19 and non-COVID-19 ARDS patients admitted to our intensive care unit in March–December 2020 and January 2018–December 2019, respectively. COVID-19 was diagnosed using the standard RT-PCR technique in swabs performed in upper respiratory airways with Cobas-SARS-CoV-2 kits® (Roche, France; sensitivity limit, 40 cycles). SO₂ data were fitted using the sigmoidal

Hill model: $SO_2 = \frac{PO_2^\gamma}{(P_{50,0} + \alpha(P_{CO_2} - 38) + \beta(pH - 7.4))^\gamma + PO_2^\gamma} + \varepsilon$, where ε is a random variable assumed Gaussian with mean zero and variance σ^2 ; $P_{50,0}$, the PO₂ value when hemoglobin is 50%-saturated with oxygen (P_{50}) at PCO₂ = 38 mmHg and pH = 7.40; α and β , the parameters quantifying PCO₂- and pH-related effects on P_{50} , respectively. The four model parameters ($P_{50,0}$, α , β , and γ) were assumed different in each patient, using a random effect approach. The parameter θ was modeled as a Gaussian random variable of mean μ_θ and variance σ_θ^2 . All random variables were assumed independent of each other, between patients and of ε . To allow for any effect of COVID-19, the mean value for each parameter was modeled as $\mu_\theta = \mu_{\theta,ref} + \delta_\theta \cdot 1_{COVID}$, where $1_{COVID} = 1$ for COVID-19 and =0 for non-COVID-19 patients. In each group, the “average patient” was defined as the patient with the median values for each $P_{50,0}$, α , β , and γ parameter.

This approach is similar to mixed-effect models used in population pharmacokinetics [7]. COVID-19-related effects on the oxyhemoglobin dissociation were considered significant if the 95%-confidence interval (95%CI) of at least one of $\delta P_{50,0}$, $\delta\alpha$, $\delta\beta$, or $\delta\gamma$ did not include zero. The model was fitted in R version-3.6.3 [8] using the nlme function (nlme package, version-3.1.151) [9] based on the maximum likelihood method. The 95%CI were determined using the Wald method. Model assumptions were checked graphically using standard validation methods including visual predictive checks and normal distribution checks by quantile-quantile plots. The results are expressed as median (25th–75th percentiles) or percentages. Comparisons were performed using Mann–Whitney and Chi-square tests as appropriate.

Overall, 5,291 and 3,449 blood gas measurements obtained from 124 COVID-19 (age, 59 years [50–71]; M/F sex ratio, 2.54) and 144 non-COVID-19 patients (age, 61 years [49–73]; M/F sex ratio, 2.87), respectively, were included ($p=0.41$ for age, $p=0.77$ for gender). ARDS etiologies in non-COVID-19 patients included bacterial pneumonia ($n=80$, 56%), influenza ($n=32$, 22%), and non-infectious

injuries ($n=44$, 31%). Invasive mechanical ventilation was used in 74 (60%) vs. 76 patients (53%) ($p=0.34$) and extracorporeal membrane oxygenation (ECMO) in six (5%) vs. eight patients (6%) ($p=0.99$). The number of blood gas measurements per patient was 18 [4–75] and 13 [4–26] in the COVID-19 and non-COVID-19 patients, respectively ($p=0.01$). Average hemoglobin was 11.1 g/dL [9.6–13.2] vs. 10.8 g/dL [9.4–12.7] ($p=0.4$). Average SO_2 was 95.7% [93.9–96.8] vs. 95.1% [91.7–96.3] ($p=0.03$), average PO_2 107 mmHg [91–128] vs. 95 mmHg [77–115] ($p=0.002$), average PCO_2 43 mmHg [38–48] vs. 42 mmHg [37–49] ($p=0.87$) and average pH 7.41 [7.38–7.45] vs. 7.40 [7.34–7.44] ($p=0.04$).

Our model well fitted the observed data showing, as expected, that PCO_2 increases P_{50} (α estimate >0 , 95%CI excluding zero) and pH decreases P_{50} (β estimate <0 , 95%CI excluding zero; Figure 1). The model-calculated $P_{50,0}$, α , β , and γ for each patient are given in Figure 2. $P_{50,0}$, β , and γ did not significantly differ between COVID-19 and non-COVID-19 patients (95%CI of $\delta P_{50,0}$, $\delta\gamma$, and $\delta\beta$ all included zero) whereas α did ($\delta\alpha = -0.025$, 95%CI $[-0.051; -1.7 \times 10^{-4}]$). This decrease in α , representing the

Figure 1: The oxyhemoglobin dissociation curve in COVID-19 and non-COVID-19 patients.

Single measurements are displayed as dots (red, 5,291 measurements in 124 COVID-19 patients; black, 3,449 measurements in 144 non-COVID-19 patients). SO_2 data were fitted using the sigmoidal Hill model: $SO_2 = \frac{PO_2^\gamma}{(P_{50,0} + \alpha(PCO_2 - 38) + \beta(pH - 7.4))^\gamma + PO_2^\gamma} + \epsilon$, where $P_{50,0}$, the PO_2 value when hemoglobin is 50%-saturated with oxygen (P_{50}) at $PCO_2 = 38$ mmHg and $pH = 7.40$; α and β , the parameters quantifying PCO_2 - and pH-related effects on P_{50} , respectively; and ϵ is a random variable assumed Gaussian with mean zero and variance σ^2 . Inter-patients variability was estimated at 1.5, 0.16, 0.06, and 9.79 mmHg for $P_{50,0}$, γ , α , and β , respectively (standard deviation of the random effect). The theoretical PO_2/SO_2 curves for the average patient in each group (continuous green line, COVID-19 patients; dashed green line, non-COVID-19 patients) are superimposable. The 95%-confidence intervals of the difference (δ) between COVID-19 and non-COVID-19 patients regarding $P_{50,0}$, β , and γ but not α included 0.

Figure 2: Individual parameters of the oxyhemoglobin dissociation curve in COVID-19 and non-COVID-19 patients.

Values of P_{50} (A), γ (Hill coefficient), (B), α (parameter quantifying PCO_2 -related effects on P_{50}), (C), and β (parameter quantifying pH-related effects on P_{50}), (D) are the fit result, obtained as the sum of the average value, corrected for COVID-19 when needed, and the individual random effect value. Hence, these values cannot be used for statistical inference; only the confidence interval of the model parameters can be interpreted.

slope of the effect of PCO_2 on P_{50} , from 0.049 to 0.025 in COVID-19 patients suggested a decreased sensitivity of P_{50} to PCO_2 . However, the 95%CI was large and its upper bound almost zero. Otherwise, when including mechanical ventilation and ECMO as covariates, decrease of P_{50} sensitivity to PCO_2 in COVID-19 patients was still present ($\delta\alpha = -0.06914$, 95%CI $[-0.102; -0.036]$ in non-ECMO-treated and non-mechanically ventilated patients). The theoretical PO_2/SO_2 curves for an average patient ($PCO_2 = 38$ mmHg, pH = 7.4) in each group were superimposable.

Although applied for the first time to characterize the oxyhemoglobin dissociation curve, our mixed-effect model including pH and PCO_2 as covariates showed only a slightly significant SARS-CoV2-induced effect on the

PO_2/SO_2 relationships that appeared less sensitive to PCO_2 in COVID-19 patients. Similar results were found if limiting the analysis to blood gas samples during the time of positive RT-PCR. However, since the Wald method is known to give too narrow 95%CI and correction for multiplicity resulted here in non-significant comparisons, this finding should be considered cautiously. In any case, the clinical relevance of such SARS-CoV2-related effects seems limited. Interestingly, a significant ~ 0.8 mmHg decrease in P_{50} once corrected for pH and PCO_2 was similarly reported in COVID-19 patients, with persistent left shift when only one value per patient was considered ($n=43$) [10]. Therefore, an overcorrection for PCO_2 when using the classical formula in COVID-19 patients may be hypothesized, artificially decreasing the apparent P_{50} .

Only samples encompassing extreme PO_2 and SO_2 , as provided in our study, allow assessment of the model accuracy in the far-left part of the PO_2/SO_2 curve. Additionally, we compared two groups with similar physiological settings and our model included the effects of pH and PCO_2 . Finally, our findings were consistent with another work showing that hemoglobin-oxygen dissociation curves generated from 11 critically ill COVID-19 patients matched the idealized curve, similarly to eleven non-COVID-19 patients [11].

Our study has limitations. Our mixed-effect model was used without prior validation in an external population. However, given its proximity with the Hill model and based on good agreement as shown in Figure 1, our model appeared adequate to describe the PO_2/SO_2 relationships while allowing potential patient-related effects. We could not rule out the presence of COVID-19 patient subpopulations in which hemoglobin physiology is affected. Moreover, our *ex vivo* modeling of oxygen affinity to hemoglobin may not take into account the presence of *in vivo* modulators specifically associated with COVID-19, even though no evidence suggests it to date.

To conclude, our study provides no evidence for clinically relevant SARS-CoV2-related alterations in the oxyhemoglobin dissociation curve. However, despite limited expected clinical consequences, we found a decrease impact of PCO_2 on P_{50} in the COVID-19 patients.

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Informed consent: The Ethics Committee waived informed consent from individuals included in this study as no additional intervention related to the research was performed.

Ethical approval: Research involving human subjects complied with all relevant national regulations,

institutional policies and is in accordance with the tenets of the Helsinki Declaration (as revised in 2013). The study was part of the COVID-ICU and French COVID-19 cohort registries and approved by our institutional ethics committee (IDRCB, 2020-A00256-33; CPP, 11-2020.02.04.68737).

References

1. Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC. Pathophysiology, transmission, diagnosis, and treatment of coronavirus disease 2019 (COVID-19): a review. *J Am Med Assoc* 2020;324:782–93.
2. Gattinoni L, Chiumello D, Caironi P, Busana M, Romitti F, Brazzi L, et al. COVID-19 pneumonia: different respiratory treatments for different phenotypes? *Intensive Care Med* 2020;46:1099–102.
3. Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, et al. Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in Covid-19. *N Engl J Med* 2020;383:120–8.
4. Reynolds AS, Lee AG, Renz J, DeSantis K, Liang J, Powell CA, et al. Pulmonary vascular dilatation detected by automated transcranial Doppler in COVID-19 pneumonia. *Am J Respir Crit Care Med* 2020;202:1037–9.
5. Liu W, Li H. COVID-19: attacks the 1-beta chain of hemoglobin and captures the porphyrin to inhibit human heme metabolism. Available from: https://chemrxiv.org/articles/preprint/COVID-19_Disease_ORF8_and_Surface_Glycoprotein_Inhibit_Heme_Metabolism_by_Binding_to_Porphyrin/11938173 [Accessed 30 March 2021].
6. Daniel Y, Hunt BJ, Retter A, Henderson K, Wilson S, Sharpe CC, et al. Haemoglobin oxygen affinity in patients with severe COVID-19 infection. *Br J Haematol* 2020;190:e126–7.
7. Dartois C, Brendel K, Comets E, Laffont CM, Laveille C, Tranchand B, et al. Overview of model-building strategies in population PK/PD analyses: 2002-2004 literature survey. *Br J Clin Pharmacol* 2007;64:603–12.
8. The R Core Team. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2020.
9. Pinheiro J, Bates D, The R Core Team. nlme: linear and non-linear mixed effects model. Available from: <https://svn.r-project.org/R/packages/trunk/nlme/> [Accessed 30 March 2021].
10. Vogel DJ, Formenti F, Retter AJ, Vasques F, Camporota L. A left shift in the oxyhaemoglobin dissociation curve in patients with severe coronavirus disease 2019 (COVID-19). *Br J Haematol* 2020;191:390–3.
11. DeMartino AW, Rose JJ, Amdahl MB, Dent MR, Shah FA, Bain W, et al. No evidence of hemoglobin damage by SARS-CoV-2 infection. *Haematologica* 2020;105:2769–73.