The Affinity of Hemoglobin for Oxygen Is Not Altered During COVID-19

Thomas Gille1,2*, Lucile Sesé1,2†, Eric Aubourg3, Emmanuelle E. Fabre4,5, Florence Cymbalista5,6, Kayaththiry Caroline Ratnam6, Dominique Valeyre2,7, Hilario Nunes2,7, Jean-Paul Richalet2 and Carole Planès1,2

1 Service de Physiologie et Explorations Fonctionnelles, Hôpital Avicenne, GHUPSSD, Assistance Publique—Hôpitaux de Paris, Bobigny, France, 2 Inserm UMR 1272 “Hypoxie et Poumon,” UFR SMBH Léonard de Vinci, Université Sorbonne Paris Nord, Bobigny, France, 3 CNRS, CEA, Astroparticule et Cosmologie, Université de Paris, Paris, France, 4 CNRS, CEA, Astroparticule et Cosmologie, Université de Paris, Paris, France, 5 Laboratoire de Biochimie, Hôpital Avicenne, GHUPSSD, Assistance Publique—Hôpitaux de Paris, Bobigny, France, 6 Laboratoire de Biochimie, Hôpital Avicenne, GHUPSSD, Assistance Publique—Hôpitaux de Paris, Bobigny, France, 7 Service de Pneumologie, Centre de Référence Maladies Pulmonaires Rares, Hôpital Avicenne, GHUPSSD, Assistance Publique—Hôpitaux de Paris, Bobigny, France

Background: A computational proteomic analysis suggested that SARS-CoV-2 might bind to hemoglobin (Hb). The authors hypothesized that this phenomenon could result in a decreased oxygen (O2) binding and lead to hemolytic anemia as well. The aim of this work was to investigate whether the affinity of Hb for O2 was altered during COVID-19.

Methods: In this retrospective, observational, single-center study, the blood gas analyses of 100 COVID-19 patients were compared to those of 100 non-COVID-19 patients. Fifty-five patients with carboxyhemoglobin (HbCO) ≥8% and 30 with sickle cell disease (SCD) were also included (“positive controls” with abnormal Hb affinity). P50 was corrected for body temperature, pH, and PCO2.

Results: Patients did not differ statistically for age or sex ratio in COVID-19 and non-COVID-19 groups. Median P50 at baseline was 26 mmHg [25.2–26.8] vs. 25.9 mmHg [24–27.3], respectively (p = 0.42). As expected, P50 was 22.5 mmHg [21.6–23.8] in the high HbCO group and 29.3 mmHg [27–31.5] in the SCD group (p < 0.0001). Whatever the disease severity, samples from COVID-19 to non-COVID-19 groups were distributed on the standard O2–Hb dissociation curve. When considering the time-course of P50 between days 1 and 18 in both groups, no significant difference was observed. Median Hb concentration at baseline was 14 g.dl−1 [12.6–15.2] in the COVID-19 group vs. 13.2 g.dl−1 [11.4–14.7] in the non-COVID-19 group (p = 0.006). Among the 24 COVID-19 patients displaying anemia, none of them exhibited obvious biological hemolysis.

Conclusion: There was no biological argument to support the hypothesis that SARS-CoV-2 could alter O2 binding to Hb.

Keywords: COVID-19, SARS-CoV-2, hemoglobin-oxygen affinity, P50, gas exchange, gas transport, hemolysis, anemia
INTRODUCTION

In December 2019, a novel coronavirus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in the Chinese city of Wuhan. The related coronavirus disease (COVID-19) rapidly spread worldwide during the following months, straining healthcare resources in many countries (Yu et al., 2020; Zhu et al., 2020). This pandemic urged the scientific community to quickly uncover and deliver information about the disease. Therefore, a substantial number of preprint articles have been made available, sparking a debate on whether they constitute reliable sources of scientific data (Smyth et al., 2020). Among them, an in silico modeling of molecular docking suggested that some structural and non-structural viral proteins might bind to hemoglobin (Hb) in several spots (Wenzhong and Hualan, 2020). The authors hypothesized that SARS-CoV-2 could dissociate iron ions from porphyrin, resulting in a decreased affinity of Hb for oxygen (O₂) and a decrease in O₂ binding. They also speculated that this mechanism could lead to hemolytic anemia, and that some by-products could participate in the pathophysiology of the disease. Indeed, an excess in free heme has previously been shown to promote oxidative and inflammatory stress (Wagener et al., 2020).

Although they were not supported by any experimental validation, such as in vitro biochemical interaction, nor any clinical observation, these conclusions were largely relayed in the media and social networks. One response on the ChemRxiv platform, identifying presumed flaws in the computational analysis, did not get as much audience (Read, 2020). Several academics called for research investigating the interaction between Hb and SARS-CoV-2 (Chowdhury and Anwar, 2020; Wagener et al., 2020). The aim of this work was therefore to investigate whether the affinity of Hb for O₂ was altered in COVID-19.

MATERIALS AND METHODS

Patient Selection

This retrospective, observational, single-center study compared 100 patients with COVID-19 and 100 control patients. The COVID-19 group (group 1) included patients with positive SARS-CoV-2 polymerase chain reaction (PCR) and at least one blood gas analysis (BGA) collected in Avicenne University Hospital, Bobigny, France, between 2020/03/16 and 2020/04/12, either in emergency room (ER), general ward or intensive care unit (ICU). One hundred patients were randomly selected, with a 1:4 stratification on the number of collected BGAs (one BGA vs. ≥2 BGAs) in order to favor the inclusion of patients with ≥2 samples, so that the time-course of P₅₀ could be evaluated.

The non-COVID-19 group was a historical “negative control” (group 2), it included patients with at least one BGA collected between 2019/03/01 and 2019/04/30. One hundred unmatched patients were randomly selected, with the same stratification. For each patient in COVID-19 and non-COVID-19 groups, 1–5 BGAs were selected (see below).

Sample Selection

BGAs were made using an ABL90 FLEX or an ABL800 FLEX analyzer (Radiometer, Bronshoj, Denmark). Samples with fetal Hb (HbF) >20%, sickle Hb (HbS), or any technical problem (air bubbles, sample insufficiently shaken…) were discarded. To avoid a disproportion in the weight of each patient in the analysis, and to obtain samples collected at different levels of oxygen therapy, the number of samples was limited to 5 per patient in COVID-19 and non-COVID-19 groups, which were selected as follows: (a) first BGA in ER (if applicable); (b) first BGA in ward (if applicable); (c) first BGA in ICU (if applicable); (d) BGA after 8 ± 3 days of hospitalization or last BGA before death if the patient died before D₅; (e) BGA after 15 ± 3 days of hospitalization or last BGA before death if the patient died before D₁₅.

Assessment of Hb Affinity

P₅₀ is the oxygen partial pressure when Hb is 50% saturated with O₂. It is negatively correlated with Hb affinity. For one BGA, a reliable value of P₅₀ can be calculated when Hb saturation is <97% (BGAs with saturation ≥97% were not used for these analyses). To allow comparisons between samples, all P₅₀ values were standardized for normal conditions (body temperature = 37°C; pH = 7.4; PCO₂ = 40 mmHg). The normal value of P₅₀ in these conditions is 26.8 mmHg (West and Luks, 2016). The oxyhemoglobin (HbO₂) dissociation model was computed taking into account carboxyhemoglobin (HbCO) and methemoglobin (MetHb), using Hill’s model corrected by Dash in the (pₐ) space of Roughton (Hill, 1921; Roughton and Darling, 1944; Dash et al., 2016): s is the combined O₂ + carbon monoxide (CO) saturation (fHbO₂ + fHbCO)/(1-fMetHb), and p = PO₂ + M PCO where M is the Haldane ratio of affinities (Douglas et al., 1912). The curve was first displaced by all known effects (temperature, pH, PCO₂), and the extra p scaling to match the BGA was measured. The same scaling was applied to the model P₅₀ (computed for O₂ saturation = 50%). This measured P₅₀ was then scaled back to standard conditions using Dash’s model.

A raise in 2,3-diphosphoglycerate (2,3-DPG) can induce a decrease in Hb affinity for O₂ (hence an increase in P₅₀). 2,3-DPG concentration ([2,3-DPG]) was not routinely measured in our patients, however factors modulating [2,3-DPG] were assessed, such as Hb concentration ([Hb]), age, phosphatemia, and history of heart failure (de Verdier and Garby, 1972; Purcell and Brozović, 1974). As hydroxychloroquine is known to provoke methemoglobinemia (Hall et al., 1986), the relation between hydroxychloroquine and MetHb level in the COVID-19 group was also assessed.

Model Validation

Two “positive control” groups for abnormal affinity were also used, to test if our model was able to detect clinically significant changes in Hb affinity in various conditions. All consecutive patients with HbCO ≥8%, starting from 2016/01/01, were included in the high HbCO group (group 3). One BGA per patient was selected. In this group, Hb was supposed to be
normal, but the presence of an unusual amount of CO was expected to stabilize Hb in its relaxed R-state and to provoke an increase in Hb affinity for O2 (West and Luks, 2016).

Finally, the last group comprised 30 patients with homozygous HbSS sickle cell disease: SCD group (group 4). Data from all available BGAs with Hb saturation <97% were collected (from 2015/07/27 to 2020/12/08). The SCD group was used to assess if our model using Dash’s equations was still valid with abnormal Hb, as HbS affinity for O2 is decreased (Huynh-Moynot et al., 2011; Ribeil et al., 2017).

Assessment of Hemolysis in Anemic Patients
Among COVID-19 patients, if at least one BGA showed a [Hb] ≤ 11 g.dl⁻¹, blood smears and patient files were reviewed with a hemobiologist and the following data throughout the study period were gathered and analyzed: [Hb] on complete blood count (CBC), mean corpuscular volume (MCV), reticulocyte count, presence or absence of schistocytes, plasmatic concentrations of total and unconjugated bilirubin, lactate dehydrogenase (LDH), haptoglobin, ferritin, and C-reactive protein (CRP).

Statistical Analysis
Demographic and blood gas characteristics were compared between COVID-19 and non-COVID-19 groups using χ² test (qualitative variables), Mann-Whitney or unpaired t-test (quantitative variables, according to distribution). Differences between measured HbO₂ and predicted HbO₂ were assessed by unpaired t test. Before/after comparisons in the COVID-19 group (mechanical ventilation, [Hb]) were performed with paired t-test. Comparisons between ≥3 groups were assessed with Kruskal-Wallis test and Dunn’s multiple comparison test. Spearman correlation coefficient (r) was employed to examine the relation between P₅₀ and [Hb], age or phosphatemia. For P₅₀ time-course, two-way ANOVA was performed. A p < 0.05 was considered significant. Prism® software was used (GraphPad Software Inc., San Diego, CA, United States).

RESULTS

Study Population
All 100 COVID-19 patients being hospitalized or at least seen in ER, none of them was asymptomatic. Fever, dyspnea, cough and other classical COVID-19 symptoms were common. In the non-COVID-19 group, the most frequent diagnoses were infection, airway disease (chronic obstructive pulmonary disease, asthma, bronchiectasis…), interstitial lung disease or heart failure (Supplementary Table 1). Patients in both COVID-19 and non-COVID-19 groups did not differ statistically for age or sex ratio. COVID-19 patients were significantly heavier and more frequently non-smokers. They required higher O₂ delivery at baseline (Table 1), and 80 finally necessitated O₂ therapy at some point.

Fifty-five patients had displayed a HbCO ≥ 8% since 2016 and were included in the high HbCO group (median HbCO level: 9.4% [8.6–12.6]). The reason for HbCO elevation was tobacco consumption in 26 (47.3%), CO poisoning in 15 (27.3%), and undetermined in the 14 others (25.4%). Thirty patients were included in the SCD group. One hundred and twenty-one BGAs were analyzed in the present study, among which 106 were collected in a context of vaso-occlusive crisis (VOC) and/or acute chest syndrome (ACS). Other indications were: respiratory infection without ACS (n = 7), scheduled health check (n = 6), thoracic pain without VOC (n = 2). Demographic characteristics are presented in Supplementary Table 2.

### TABLE 1 | Demographic and blood gas characteristics at baseline.

|                | COVID-19 (n = 100) | Non-COVID-19 (n = 100) | p   |
|----------------|--------------------|------------------------|-----|
| Age (years)    | 62 [48–72]         | 66.5 [52–76]           | NS  |
| Sex            |                    |                        |     |
| Male           | 70                 | 69                     | NS  |
| Female         | 30                 | 31                     |     |
| Body mass index* (kg.m⁻²) | 29.5 [28.1–31.3] | 25.4 [21.9–29.9]        | 0.0002 |
| Smoking history|                    |                        |     |
| Never smoker   | 51                 | 39                     |     |
| Former smoker  | 28                 | 36                     | 0.009 |
| Current smoker | 4                  | 16                     |     |
| Not available  | 17                 | 9                      |     |
| Pack-years*    | 20 [11–50]         | 30 [16–50]             |     |
| Place of first sample |            |                        |     |
| Emergency room | 84                 | 67                     |     |
| Ward           | 12                 | 22                     | 0.017 |
| Intensive care unit | 4            | 11                     |     |
| Severity       |                    |                        |     |
| Low dose O₂ (1-6 l.min⁻¹) | 35            | 21                     | 0.023 |
| High dose O₂ (≥7 l.min⁻¹ or ventilation) | 14 | 9 |     |
| Blood gas variables |            |                        |     |
| Temperature (°C) | 37.9 [37–38.7]     | 37 [36.5–37.1]         | <0.0001 |
| PO₂ (mmHg)     | 75.8 [65–83]       | 72.6 [60.2–84]         |     |
| PCO₂ (mmHg)    | 35.7 [32–39.5]     | 38 [31.8–43.2]         |     |
| pH             | 7.44 [7.41–7.47]   | 7.42 [7.38–7.46]       |     |
| Hemoglobin (g.dl⁻¹) | 14            | 13.2 [11.4–14.7]       | 0.006 |
| Oxyhemoglobin (%) | 93.2 [90.4–96.5]   | 92.6 [88.6–94.4]       |     |
| Oxygen content (ml.100 ml⁻¹) | 18.2 | 16.9 [14.1–19]        |     |
| Carboxyhemoglobin (%) | 0.9 [0.7–1.1]   | 1.3 [0.8–1.7]          |     |
| Methemoglobin (%) | 1.1 [1–1.2]      | 0.8 [0.6–1.1]          | <0.0001 |

*BMI: the number of available values was 70 and 76 for each group, respectively.

Data are presented as numbers, or medians and interquartile ranges between square brackets. Some blood gas variables were not statistically compared because of the patients receiving oxygen therapy. NS, non-significant.

**: the number of exploitable values (saturation < 97%) was 69 and 79 for each group, respectively.

### Supplementary Table 1

| Place of first sample | COVID-19 (n = 2) | Non-COVID-19 (n = 100) | p   |
|-----------------------|------------------|------------------------|-----|
| Emergency room        | 35               | 21                     |     |
| Ward                  | 14               | 9                      |     |
| Intensive care unit   | 14               | 9                      |     |

Bold values are significant p-values (i.e., under 0.05).
Blood Gas Characteristics
Among COVID-19 patients, 51 were on ambient air at baseline. Blood gases were analyzed from arterial sample for 48 of them and venous sample for the other 3. In the non-COVID-19 group, 70 patients were on ambient air at baseline, with 59 arterial and 11 venous samples (Table 2). Despite a trend for lower PO$_2$ in the COVID-19 group, no statistical difference was seen for PO$_2$, PCO$_2$ or pH between COVID-19 and non-COVID-19 patients in ambient air. Median HbCO level was slightly, but significantly, lower in COVID-19 patients. On the contrary, median MetHb level was slightly, but significantly, higher in COVID-19 patients.

Hb Affinity in COVID-19 and Non-COVID-19 Groups
A total number of 253 samples were selected for the 100 COVID-19 patients throughout the study period, and 271 in the non-COVID-19 group. Twenty-three COVID-19 patients and 27 non-COVID-19 patients had only 1 BGA. Raw HbO$_2$ values (without standardization) in relation to PO$_2$ in both groups are presented in Figure 1A, while HbO$_2$ values standardized for normal conditions (Std-HbO$_2$) in COVID-19 and non-COVID-19 groups are presented in Figures 1B,C, respectively. In both groups, mean difference between measured Std-HbO$_2$ value and predicted HbO$_2$ given by the standard O$_2$-Hb dissociation curve was very low: $-0.3 \pm 0.7\%$ ($p = 0.73$) and $-1.1 \pm 0.9\%$ ($p = 0.21$), respectively. This low dispersion was observed at any given PO$_2$ and whatever the level of oxygen therapy. Importantly, median P$_{50}$ at baseline was not different between COVID-19 group (26 mmHg [25.2–26.8]) and non-COVID-19 group (25.9 mmHg [24–27.3]; $p = 0.42$) (Table 1). As expected, it was significantly lower in the high HbCO group (22.5 mmHg [21.6–23.8]) and significantly higher in the SCD group (29.3 mmHg [27–31.5] ($p < 0.0001$ for all comparisons). No correlation was found between P$_{50}$ and age or phosphatemia (all correlation coefficients $r < 0.15$) or history of heart failure ($p = 0.28$) in both COVID-19 and non-COVID-19 groups. In the COVID-19 group, median MetHb level was significantly higher in the subgroup of samples collected in patients having received hydroxychloroquine ($n = 74$): 1.5% [1.2–1.8] vs. 1.1% [1–1.3] in the absence of hydroxychloroquine ($n = 177$) ($p < 0.0001$). Median P$_{50}$ in these two subgroups was 25.5 mmHg [24.9–26.5] vs. 26.1 mmHg [24.6–27.3], respectively ($p = 0.07$).

When considering P$_{50}$ time-course between days 1 and 18, no significant difference was observed between COVID-19 and non-COVID-19 patients: no group effect nor time effect ($p = 0.72$) (Figure 2). Global P$_{50}$ stability over time was similarly observed when considering only the most severe patients: eighteen COVID-19 patients necessitated mechanical ventilation, their median P$_{50}$ was 25.7 mmHg [25.1–26] with mechanical ventilation and 26 mmHg [25.2–26.9] without ($p = 0.19$) (Supplementary Figure 1).

Hb Affinity in the High HbCO Group
The graphical representations of raw HbO$_2$ and Std-HbO$_2$ in relation to PO$_2$ show that Std-HbO$_2$ reached an upper limit of 92.6% because of the competitive binding of CO to Hb (Supplementary Figures 2A,B). Contrary to COVID-19 and non-COVID-19 groups, mean difference between measured Std-HbO$_2$ and predicted HbO$_2$ was important in the high HbCO group: $-8.6 \pm 2.1\%$ ($p = 0.0001$). Taking into account the combined saturation of Hb with O$_2$ and CO (Std-SatO$_2$ + CO), compared to the standard O$_2$-Hb dissociation curve, a shift in the relation between combined saturation and PO$_2$ was observed to the left, indicating a lower P$_{50}$ and a greater Hb affinity for O$_2$ (Supplementary Figure 2C). At last, when the partial pressure in CO (PCO$_2$) was also taken into account, the median difference between measured combined saturation and predicted combined saturation was reduced to $-0.16 \pm 1.65\%$ ($p = 0.92$), indicating that our model was able to explain every kind of variation (Supplementary Figure 2D).

Hb Affinity in the SCD Group
In the same line, mean difference between measured Std-HbO$_2$ and predicted HbO$_2$ was important in the SCD group: $-5.3 \pm 4.1\%$ ($p < 0.0001$). As expected, a shift was observed to the right in the relation between Std-HbO$_2$ and PO$_2$, as compared to the standard O$_2$-Hb dissociation curve (Supplementary Figure 3), indicating that our model was able to detect clinically significant changes in Hb affinity even in a group of patients with abnormal Hb (here, a rise in P$_{50}$ with decreased Hb affinity). Taking into account combined Hb saturation (Std-SatO$_2$ + CO) and PCO$_2$ did not change those results. Of note, median P$_{50}$ while taking hydroxycarbamide was significantly less elevated than without treatment: 28.2 mmHg [27–31.2] vs. 30 mmHg [26.9–31.9], respectively ($p = 0.014$). However, all samples were retained for analysis in the present study. On the other hand, the effect of transfusion on P$_{50}$ could be assessed in a subgroup of 11 SCD patients: P$_{50}$ decreased in only 7 of them after transfusion, and the mean difference in P$_{50}$ before/after transfusion in the whole subgroup was $-0.5 \pm 1.7$ mmHg ($p = 0.38$).

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**Table 2** Characteristics of the arterial blood gas analyses collected in ambient air at baseline.

|                     | COVID-19 | Non-COVID-19 | $p$   |
|---------------------|----------|--------------|-------|
| **Temperature (°C)**| 37.8 [36.9–38.3] | 37 [36.5–37] | $<0.0001$ |
| **PO$_2$ (mmHg)**   | 71.5 [62.6–78.9] | 76.1 [65.8–89.5] | NS    |
| **PCO$_2$ (mmHg)**  | 35.7 [32.1–38.4] | 35.7 [30.2–38.7] | NS    |
| **pH**              | 7.44 [7.42–7.48] | 7.43 [7.41–7.48] | NS    |
| **Hemoglobin (g.dl$^{-1}$)** | 14.5 [13.3–15.6] | 13.4 [12.4–15] | 0.026 |
| **Oxyhemoglobin (%)** | 91.9 [89.8–93.9] | 93.3 [91–94.6] | NS    |
| **Oxygen content (ml.100 ml$^{-1}$)** | 18.9 [16.9–20.6] | 17.5 [15.9–19.2] | NS    |
| **Carboxyhemoglobin (%)** | 0.9 [0.7–1.2] | 1.3 [0.8–2] | 0.002 |
| **Methemoglobin (%)** | 1 [0.9–1.2] | 0.7 [0.6–1] | <0.0001 |
| **P$_{50}$ (mmHg)**  | 26.1 [25.4–26.7] | 26 [24.6–27.3] | NS    |

*Data are presented as medians and interquartile ranges between square brackets. NS, non-significant.

*P$_{50}$: the number of exploitable values (saturation < 97%) was 41 and 56 for each group, respectively.

*Bold values are significant p-values (i.e., under 0.05).
Anemia and Hemolysis

Median [Hb] at baseline was significantly higher in the COVID-19 group than in the non-COVID-19 group (Table 1). Twenty-four COVID-19 patients displayed [Hb] ≤ 11 g.dl⁻¹ at some point. Among them, 17 exhibited no biological sign of hemolysis, and the cause of anemia was undetermined for the other 7 (due to limited retrospective data). Among biological variables related to anemia, inflammation markers (ferritin, CRP) were the only significant differences between anemic patients from COVID-19 to non-COVID-19 groups (Supplementary Table 3).

Among the 24 anemic COVID-19 patients, before/after comparison of $P_{50}$ between highest and lowest [Hb] was possible in 16 of them. At highest [Hb] (mean: 12.9 ± 1.4 g.dl⁻¹), mean $P_{50}$ value was 25.6 ± 0.9 mmHg; whereas it was 25.5 ± 1.2 mmHg at lowest [Hb] (mean: 9.3 ± 1.2 g.dl⁻¹) ($p = 0.6$) (Supplementary Figure 4). Taking the whole COVID-19 population, no significant correlation was found between $P_{50}$ and [Hb] ($r = 0.19; p = 0.07$). Similarly, no correlation between these two variables was found in the SCD group either ($r = -0.06; p = 0.52$).

**DISCUSSION**

Comparing $P_{50}$ values and the distribution of HbO₂/PO₂ in relation to the standard O₂-Hb dissociation curve in 100 COVID-19 patients and 100 non-COVID-19 patients, we found no argument to support the hypothesis that SARS-CoV-2 can be responsible for a clinically significant alteration of O₂ binding to Hb, neither at baseline nor later in the disease course. In contrast, we were able to identify a shift in the relation between PO₂ and HbO₂, and variations in $P_{50}$ value, in 55 patients with HbCO ≥8% (increased Hb affinity) and 30 patients with sickle cell disease (decreased Hb affinity, even more when they were not treated by hydroxychloroquine). Moreover, no COVID-19 patients displayed hemolysis stigma.

Due to the huge number of BGA samples collected in our institution during the study period (3,706 in March-April 2019 and 5,346 between 2020/03/16 and 2020/04/12, regardless of the diagnosis), a random draw of patients had to be performed. Despite our 1:4 stratification on the number of collected samples, the COVID-19 group finally comprised 23 patients with only 1 BGA, whereas they were 27 in the non-COVID-19 group. This was due to two facts: for some patients with multiple BGAs, only one sample finally met our selection criteria; conversely, for some patients that had only one BGA during the predefined period, we could analyze other samples collected before or after. Eventually, we were able to compare 253 BGAs from 100 COVID-19 patients with positive SARS-CoV-2 PCR, to 221 samples from 100 non-COVID-19 controls, providing extensive information about blood gases and Hb affinity for O₂ in COVID-19.

Median $P_{50}$ corrected for body temperature, pH and PCO₂ at baseline was 26 mmHg [25.2–26.8] in the COVID-19 group vs. 25.9 mmHg [24–27.3] in the non-COVID-19 group. These values are slightly lower than the normal theoretical value of 26.8 mmHg, which is, however, calculated for normal HbCO and MetHb levels (West and Luks, 2016). In our study, non-COVID-19 patients displayed a slightly, but significantly, higher median HbCO level, which was consistent with the greater proportion of smokers in this group. On the contrary, COVID-19 patients displayed a slightly, but significantly, higher median MetHb level, probably secondary to the use of hydroxychloroquine in some of their patients.

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**FIGURE 1** | (A) Raw oxyhemoglobin (HbO₂) in relation to PO₂ in the COVID-19 group (red dots, 100 patients, 253 samples) and the non-COVID-19 group (blue dots, 100 patients, 221 samples). (B) Standardized HbO₂ (Std-HbO₂) in relation to PO₂ in the COVID-19 group, according to the level of oxygen therapy (light red triangles: ambient air; medium red dots: $O_2$ between 1 and 6 l.min⁻¹; dark red triangles: $O_2$ ≥ 7 l.min⁻¹ or ventilation). Measured HbO₂ was standardized for normal conditions (temperature = 37°C; pH = 7.4; PCO₂ = 40 mmHg) in order to compare it to the predicted HbO₂ given by the standard $O_2$-Hb dissociation curve, represented in black. (C) Std-HbO₂ for normal conditions in relation to $O_2$ in the non-COVID-19 group, according to the level of oxygen therapy (light blue triangles: ambient air; medium blue dots: $O_2$ between 1 and 6 l.min⁻¹; dark blue triangles: $O_2$ ≥ 7 l.min⁻¹ or ventilation).

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**Table 1**

| COVID-19 group | Non-COVID-19 group |
|----------------|--------------------|
| Median [Hb]    | 12.9 ± 1.4 g.dl⁻¹  | 10.1 ± 1.3 g.dl⁻¹ |
| $P_{50}$ value | 25.6 ± 0.9 mmHg    | 26.0 ± 1.0 mmHg   |

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**Supplementary Figure 4**

Comparing P₅₀ values and the distribution of HbO₂/PO₂ in relation to the standard O₂-Hb dissociation curve in 100 COVID-19 patients and 100 non-COVID-19 patients, we found no argument to support the hypothesis that SARS-CoV-2 can be responsible for a clinically significant alteration of O₂ binding to Hb, neither at baseline nor later in the disease course. In contrast, we were able to identify a shift in the relation between PO₂ and HbO₂, and variations in P₅₀ value, in 55 patients with HbCO ≥8% (increased Hb affinity) and 30 patients with sickle cell disease (decreased Hb affinity, even more when they were not treated by hydroxychloroquine). Moreover, no COVID-19 patients displayed hemolysis stigma.

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Median P₅₀ corrected for body temperature, pH and PCO₂ at baseline was 26 mmHg [25.2–26.8] in the COVID-19 group vs. 25.9 mmHg [24–27.3] in the non-COVID-19 group. These values are slightly lower than the normal theoretical value of 26.8 mmHg, which is, however, calculated for normal HbCO and MetHb levels (West and Luks, 2016). In our study, non-COVID-19 patients displayed a slightly, but significantly, higher median HbCO level, which was consistent with the greater proportion of smokers in this group. On the contrary, COVID-19 patients displayed a slightly, but significantly, higher median MetHb level, probably secondary to the use of hydroxychloroquine in some of their patients.
FIGURE 2 | Time-course of mean $P_{50}$ in the COVID-19 group (red circles) and the non-COVID-19 group (blue squares). Some days were regrouped to have sufficient number of samples ($D_{8-10}$: $n = 15$ in the COVID-19 group and $n = 10$ in the non-COVID-19 group; $D_{11-15}$: $n = 11$ in both groups; $D_{16-18}$: $n = 4$ and $n = 6$, respectively). Data are presented as means and SE.

them, a drug which can potentially raise MetHb level (Hall et al., 1986). Both HbCO and MetHb are well known to increase Hb affinity for O$_2$ and decrease $P_{50}$ (Douglas et al., 1912; Darling and Roughton, 1942; Roughton and Darling, 1944). Moreover, [2,3-DPG] can be modified in diverse conditions: chronic hypoxia, alkalosis, heart failure and anemia can increase [2,3-DPG]; while acidosis, blood transfusion, polycythemia, hypophosphatemia and greater age can decrease it (de Verdier and Garby, 1972; Purcell and Brozović, 1974). Because [2,3-DPG] was not routinely measured in our institution, and although the main confounding factors were assessed in the present work (pH, history of heart failure, phosphatemia, age), it is possible that some of our patients displayed a decreased [2,3-DPG]. This could be another possible explanation for $P_{50}$ values lower than 26.8 mmHg in our cohort. Anyway, the clinical significance of the effects of 2,3-DPG variation on oxygen affinity is thought to be minimal (Macdonald, 1977). Another limit of our study is that $P_{50}$ was calculated in a blood gas analyzer, i.e., not measured. The technique to directly measure $P_{50}$ is longer and not routinely performed: it consists in the exposure of a blood sample to an increasing partial pressure of oxygen and subsequent deoxygenation with nitrogen gas in a Hemox-Analyzer. However, as stated in the manufacturer reference manual, ABL FLEX analyzers can estimate a reliable value of $P_{50}$ from a blood sample with saturation <97%. We proceeded as would do the blood gas analyzer, but we calculated standardized $P_{50}$ using the equations validated by Dash (Dash et al., 2016). Indeed, our model was able to identify pathological $P_{50}$ values in our “positive control” groups, even in the presence of abnormal Hb: most $P_{50}$ values were lower than normal in the HbCO group, with a median $P_{50}$ of 22.5 mmHg [21.6–23.8]; on the contrary most $P_{50}$ values were higher than normal in the SCD group, with a median $P_{50}$ of 30 mmHg [26.9–31.9] in untreated patients and 28.2 mmHg [27–31.2] in the ones receiving hydroxycarbamide. Moreover, $P_{50}$ calculation by blood gas analyzers is, under certain conditions, routinely used by some referral centers in the diagnostic approach of hemoglobins with high O$_2$ affinity (Orvain et al., 2017). Anyway, in the present study, the sample distribution of high HbCO and SCD groups was shifted from the standard O$_2$-Hb dissociation curve, indicating a modified Hb affinity, whatever the potential lack of precision of $P_{50}$ calculation in our model compared to the gold-standard. On the contrary, no clinically significant change of Hb affinity could be observed in COVID-19 patients, whose samples were clearly distributed on the standard dissociation curve, as for the non-COVID-19 control group.

Our findings are in line with the results of a British study conducted in only 14 critically ill COVID-19 patients, and 11 age- and sex-matched controls (Daniel et al., 2020). Mean $P_{50}$ measured by Hemox-Analyzer, was not statistically different between both groups: 29 ± 2.3 vs. 28.5 ± 1.8 mmHg, respectively. The reasons why $P_{50}$ values were higher than the normal theoretical value were not discussed. In another British study, mean $P_{50}$ of 43 intubated and ventilated COVID-19 patients, retrospectively calculated from blood gas analyzer results, was 23.4 ± 3.13 mmHg, even significantly lower than a historical cohort of unmatched critically ill controls (24.6 ± 5.42 mmHg).
6-phosphate dehydrogenase (G6PD) deficiency uncovered in Pike et al., 2020) and 9 with previously undiagnosed glucose-paroxysmal nocturnal hemoglobinuria (Kulasekararaj et al., 2020; 2021). Fourteen additional patients were described: five with the subgroup of anemic COVID-19 patients (Algassim et al., 2020). Later, it was stated that AIHA could concern 12% et al., 2020) and 2 had Evans syndrome (Li et al., 2020; Wahlster et al., 2020). The draft of Wenzhong and Hualan (2020) hypothesizing that SARS-CoV-2 could “attack” hemoglobin received quite a wide coverage and drew the public’s attention, as well as some academics’. However, the present study found no biological argument to think that Hb affinity for O2 is significantly altered during COVID-19, nor that COVID-19 can directly induce significant hemolytic anemia.

Another claim in the preprint article of Wenzhong and Hualan (2020) was that SARS-CoV-2 could be responsible for hemolytic anemia. Indeed, potential causes of anemia are numerous and often intertwined in critically ill patients (hemodilution, iron deficiency by repeated blood sampling, surgical site bleeding or other invasive procedures, inflammation…) (Heming et al., 2011; Spinelli and Bartlett, 2016), particularly in such an inflammatory condition as COVID-19. In the present work, fever and dehydration could explain, at least in part, the higher median [Hb] in COVID-19 patients at baseline, compared to non-COVID-19 patients. Anyway, median [Hb] was normal at baseline, and although 24 COVID-19 patients later displayed anemia in the course of their disease, none of them exhibited obvious hemolysis. In a Chinese study comparing hematologic variables between critically ill COVID-19 and other COVID-19 patients not having required ICU, median [Hb] was normal at baseline in both groups, but the median [Hb] nadir was then lower in critically ill patients (11.1 g.dl$^{-1}$ [10.2–11.9] vs. 13.6 g.dl$^{-1}$ [12.7–15.1]) (Fan et al., 2020). In a literature review mostly analyzing data from Chinese centers, the authors stated that anemia was not a common laboratory finding in COVID-19. However, it cannot be excluded that occult intravascular hemolysis might occur at some level which could not be detected with classical biological signs, requiring more sensitive techniques such as detecting red blood cell microvesicles (Camus et al., 2015).

Several reports of acute hemolysis after SARS-CoV-2 infection were published, but not from direct viral action on Hb. Twelve patients presented with autoimmune hemolytic anemia (AIHA), among them 4 had B lymphoid malignancy, one had monoclonal gammopathy (Jensen et al., 2020; Lazarian et al., 2020; Lopez et al., 2020) and 2 had Evans syndrome (Li et al., 2020; Wahlster et al., 2020). Later, it was stated that AIHA could concern 12% of the subgroup of anemic COVID-19 patients (Algassim et al., 2021). Fourteen additional patients were described: five with paroxysmal nocturnal hemoglobinuria (Kulasekararaja et al., 2020; Pike et al., 2020) and with previously undiagnosed glucose-6-phosphate dehydrogenase (G6PD) deficiency uncovered in context of acute hemolysis (Afra et al., 2020a,b).

In conclusion, the COVID-19 pandemic has greatly promoted preprint servers, with no less than 12,194 preliminary reports about COVID-19 hosted on arXiv platforms at the time of writing (MedRxiv, 2021). While it is a thrilling way to rapidly share information about the disease, the absence of conventional peer-review is at risk of spreading erroneous conclusions, sometimes amplified by the media and/or social networks (Smyth et al., 2020). The studies involving human participants were reviewed and approved by the Comité Local d’Éthique pour la Recherche Clinique Avicenne-Jean Verdier-René Muret (CLEA), Hôpitaux Universitaires de Paris-Seine-Saint-Denis (HUPSSD), Assistance Publique—Hôpitaux de Paris (AP-HP), Bobigny, France (#CLEA-2020-129). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

**DATA AVAILABILITY STATEMENT**

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

**ETHICS STATEMENT**

The studies involving human participants were reviewed and approved by the Comité Local d’Éthique pour la Recherche Clinique Avicenne-Jean Verdier-René Muret (CLEA), Hôpitaux Universitaires de Paris-Seine-Saint-Denis (HUPSSD), Assistance Publique—Hôpitaux de Paris (AP-HP), Bobigny, France (#CLEA-2020-129). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

**AUTHOR CONTRIBUTIONS**

TG, LS, and EA wrote the manuscript. TG, J-PR, and CP conceived and planned the study. EA processed the data (consistency checks, oxyhemoglobin dissociation model, P_{50} measurement, data standardization). EF extracted laboratory data. FC and KR reviewed the blood smears and files of anemic patients. TG performed the statistical analyses. All authors discussed the results and contributed to the final manuscript.

**ACKNOWLEDGMENTS**

We would like to deeply thank Prof. Ranjan K. Dash, for fruitful discussions and sharing his latest matlab model. Many thanks also to Rémi Letestu, Sylvain Le Jeune, and Amélie Beaugrand for their precious help and advice.
**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2021.578708/full#supplementary-material

**Supplementary Figure 1** | $P_O_2$ with and without mechanical ventilation in the COVID-19 group. $P_O_2$ values were standardized for normal conditions (temperature=37°C; pH=7.4; $P_CO_2$=40 mmHg). Before/after comparison was possible in 13 out of 18 COVID-19 patients having required mechanical ventilation, no significant difference was found (paired t test, $p=0.38$).

**Supplementary Figure 2** | (A) Raw oxyhemoglobin ($HbO_2$) in relation to $P_O_2$ in the high $HbCO$ group (SS patients, 55 samples). (B) Standardized oxyhemoglobin ($Std-HbO_2$) in relation to $P_O_2$. Measured $HbO_2$ was standardized for normal conditions (temperature=37°C; pH=7.4; $P_CO_2$=40 mmHg) in order to compare it to the predicted $HbO_2$ given by the standard $O_2$-Hb dissociation curve, represented in black. (C) Standardized combined saturation for oxygen and carbon monoxide ($Std-SatO_2$, $CO$) in relation to $P_O_2$. (D) $Std-SatO_2$, $CO$ in relation to combined partial pressure ($P_O_2+M~P_CO_2$).

**Supplementary Figure 3** | (A) Raw oxyhemoglobin ($HbO_2$) in relation to $P_O_2$ in the SCD group (30 patients, 121 samples). (B) Standardized oxyhemoglobin ($Std-HbO_2$) in relation to $P_O_2$. Measured $HbO_2$ was standardized for normal conditions (temperature=37°C; pH=7.4; $P_CO_2$=40 mmHg) in order to compare it to the predicted $HbO_2$ given by the standard $O_2$-Hb dissociation curve, represented in black.

**Supplementary Figure 4** | Evolution of $P_O_2$ in 16 anemic COVID-19 patients between highest and lowest hemoglobin concentration ($Hb$). $P_O_2$ values were standardized for normal conditions (temperature=37°C; pH=7.4; $P_CO_2$=40 mmHg).

**Supplementary Table 1** | Main diagnosis in the non-COVID-19 group.

**Supplementary Table 2** | Demographic characteristics in high $HbCO$ and SCD groups.

**Supplementary Table 3** | Biological data related to anemia in patients from all groups with hemoglobin concentration ≤ 11 g.dl$^{-1}$.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.