

## **A left shift in oxyhaemoglobin dissociation curve in patients with severe COVID-19**

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**Author Contributions:** DV designed the study, collected the data, analyzed the data, interpreted the data and drafted the first version of the manuscript. FF interpreted the data. AR interpreted the data. FV interpreted the data. LC designed the study and interpreted the data. All authors read and approved the final manuscript.

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## **Abstract**

Critically ill COVID-19 patients present with hypoxaemia and are mechanically ventilated to support gas exchange. We performed a retrospective, observational study of blood gas analyses (n=3,518) obtained from COVID-19 patients to investigate changes in haemoglobin oxygen affinity. Calculated oxygen tension at half-saturation ( $p_{50}$ ) was on average ( $\pm$  SD) 3.3 mmHg ( $\pm 3.13$ ) lower than the normal  $p_{50}$  value (23.4 vs 26.7 mmHg;  $p < 0.0001$ ). Compared to an unmatched historic control of patients with other causes of severe respiratory failure, patients with COVID-19 had a significantly higher Hb-O<sub>2</sub> affinity ( $p_{50}$  23.4 [SD  $\pm 3.13$ ] vs 24.6 [SD  $\pm 5.4$ ] mmHg;  $p < 0.0001$ ). We hypothesize that, due to the long disease process, acclimatisation to hypoxaemia could play a role.

## Introduction

Coronavirus-19 (COVID-19) is characterised by hypoxaemia that can precede radiological changes or other clinical symptoms including dyspnoea. (1) Given that the SARS-CoV-2 virus has a vascular tropism, (2) the physiological manifestation of the altered pulmonary perfusion, hypoxaemia is to a degree disproportionate to the severity of parenchymal lung disease. In addition, direct (midbrain (3)) or indirect (via metabolism of angiotensin II on the carotid bodies (4)) viral actions can affect respiratory drive and response to hypoxaemia. The duration of the disease is generally more prolonged compared to acute respiratory distress syndrome (ARDS) from other aetiology. (1) As reported by Daniel et al. in this Journal, (5) haemoglobin (Hb) oxygen ( $O_2$ ) affinity in 14 patients with COVID-19 was not different from 11 control participants, when affinity was measured *in vitro*, with a Hemox analyser, standardised pH of 7.4 and temperature. These  $p_{50}$  values were obtained directly from the blood gas analyser, without adjustments for physiological changes in  $CO_2$  or pH *in vivo*, which could be important in COVID-19. We hypothesised that *in vivo* Hb- $O_2$  affinity could be affected by other factors in COVID-19.

## Methods

To assess alterations in *in vivo* Hb- $O_2$  affinity, we performed a retrospective, observational analysis of all arterial and venous blood gases ( $n=3,518$ ) obtained from all intubated and ventilated patients ( $n=43$ ), with severe COVID-19 in one intensive care unit at Guy's and St Thomas' Hospital (London, United Kingdom) between 15<sup>th</sup> April and 15<sup>th</sup> May 2020. Institutional approval was gained from the local audit committee (project reference: 11013). The need for individual informed consent was waived for this retrospective analysis of data collected prospectively for routine care, with no breach of privacy or anonymity. The study qualified as a service evaluation as defined by the UK NHS Health Research Authority (NHS HRA) and therefore did not require review by a research ethics committee.

Measured values of  $pO_2$  and  $SO_2$  were compared to the standard oxyhaemoglobin dissociation curve for normal Hb- $O_2$  affinity. (6)  $P_{50}$  values were calculated using the Hill equation (7, 8) (after correcting for pH, temperature and base excess (9)) (Eq.1) and derived from Roche blood gas analyser (Cobas system, F. Hoffmann-La Roche Ltd, (10) Eq.2), and compared to the normal value (for pH 7.4, 37.0°C and  $pCO_2$  40mmHg) of 26.7 mmHg respectively. (11-13)

Results were compared to a historic, un-matched control cohort with an overall total of 15,945 arterial and venous blood gas samples obtained from 828 critically ill patients with acute respiratory failure (pneumonia/pneumonitis, or secondary ARDS, but presumed COVID-19 negative – as these samples were obtained in 2017 and earlier). One-sample t test was used for comparison between actual means (Eq.1 and Eq. 2) and normal value. Two-tailed, unpaired t test was used for comparison between means of COVID-19 and control samples. Statistical analysis was performed using Prism (GraphPad Software).

We calculated the  $p_{50}$  using two methods:

$$\text{Hill (Eq.1)} \quad p_{50} = pO_{2(corr)} \times \left( \frac{1-SO_2}{SO_2} \right)^{\frac{1}{2.711}}$$

$$\text{with } pO_{2(corr)} = pO_2 \times 10^{[0.48(pH-7.4)-0.024(T-37)-0.0013 \times \text{Base Excess}]}$$

$$\text{Roche (Eq.2)} \quad p_{50} = 26.7 \times 10^{(lg pO_2 - lg pO_2^k)}$$

$$\text{with } lg pO_2^k = \frac{lg Q + 4.172}{2.9} \text{ and } Q = \frac{SO_2}{1-SO_2}$$

## Results

3,518 blood gas analyses of 43 patients (34 [79%] male; mean age 53 years [range 26-77]) were obtained (*Table 1*). *eFigure 1 (supplement)* presents  $pO_2$  and  $SO_2$  values. *Figure 1* shows the distribution of  $p_{50}$  values derived by Hill equation (Eq.1). Compared to the standard  $p_{50}$  value of 26.7 mmHg, Eq.1 presented a difference of 3.3 mmHg (mean  $p_{50}$  23.4 mmHg [99%CI 23.23 - 23.50;  $p < 0.0001$ ]) and Eq.2 a difference of 1.9 mmHg (mean  $p_{50}$  24.8 mmHg [99%CI 24.68 – 25.00;  $p < 0.0001$ ]). *Table 1* shows the comparison to the control group data. Data on intra and inter subject variability, as well as data on temporal trends is shown in the *supplement*.

## Discussion

When compared to the control group, patients with COVID-19 had lower pH and higher  $pCO_2$ , but not significantly different temperature. Henceforth, a lower Hb- $O_2$  affinity (i.e. a right-shift) would have been expected. However, we demonstrated a lower  $p_{50}$  (i.e. a left shift) in the

COVID-19 group (for both equations). We included two different equations in order to strengthen the methodology, and the results obtained from these equations were in good agreement (Pearson's  $R^2$  0.65). The left shift is reflected in the opposing alterations in  $pO_2$  and  $SO_2$ . Patients with COVID-19 had a significantly lower  $pO_2$ , while showing a higher  $SO_2$ . In contrast with typical ARDS, changes in Hb- $O_2$  affinity could reflect the severity and the duration of hypoxaemia prior to presentation to critical care.

Hb- $O_2$  affinity is an important link between alveolar  $O_2$  tension and tissue oxygen supply. Hb- $O_2$  affinity is characterized in terms of  $p_{50}$  (the  $O_2$  tension where 50% of the Hb is oxygenated), and is the defining factor for binding the  $O_2$  that diffuses from the pulmonary alveoli into the blood and its release in peripheral tissues. Siggaard-Andersen et al have shown agreement between the arterial and the venous values for standard  $p_{50}$  based on widely different oxygen saturation levels.(14) Changes in Hb- $O_2$  affinity are crucial ways of adjusting both arterial  $O_2$  loading and peripheral  $O_2$  unloading in order to ensure aerobic metabolism when inspired  $pO_2$  decreases and/or  $O_2$  demand increases. The functional properties of Hb may improve tissue  $O_2$  supply with ODC shifts in either directions - increased Hb- $O_2$  affinity increases  $O_2$ -loading under conditions such as severe hypoxia (15, 16), while decreased Hb- $O_2$  affinity favours the release of bound oxygen from the Hb molecule.

Hb- $O_2$  binding is considered cooperative, that is, binding of the first molecule of  $O_2$  to Hb causes an increase in the  $O_2$  affinity of the remaining Hb-subunits. According to mathematical modelling, (17) an increase in Hb- $O_2$  affinity resulting from a  $p_{50}$  change of -3 mmHg (as seen in our data using Eq.1) only slightly increases  $SO_2$  (by 1%) in arterial blood in normoxia ( $PaO_2$  90 mmHg). However, in hypoxia ( $PaO_2$  45 mmHg), the increased Hb- $O_2$  affinity increases arterial  $SO_2$  by approximately 4.5%. While being at a disadvantage under normoxaemia, humans with a high Hb- $O_2$  affinity (adolescents from a family with Hb Andrew-Minneapolis, a stable beta-chain mutant with whole blood  $p_{50}$  ~17 mmHg) respond more appropriately to altitude induced hypoxia. (16) Increased Hb- $O_2$  affinity results in oxygenation benefits during severe hypoxia and increases survival during acute hypoxia in several animal models. (15, 18) Thus, a high Hb- $O_2$  affinity may be of particular importance for  $O_2$  loading in hypoxic conditions. (19) There are well described existing strategies of shifting the ODC to the left and increasing  $SO_2$  at a given  $pO_2$ . A fast increase in Hb- $O_2$  affinity is mediated by a reduction of  $CO_2$  and increase of pH via hyperventilation under environmental hypoxia. This reversible

alteration can occur rapidly within seconds to minutes. A slower mechanism is a decrease in 2,3-Diphosphoglycerate (DPG) or other organic phosphates. (17) Reduced 2,3-DPG levels were observed in critically ill normoxaemic patients, however, the effect on  $p_{50}$  was diminished potentially due to acidaemia in this cohort. (20)

A hypothetical explanation for our findings for patients with COVID-19 could be the response to prolonged periods of hypoxia. Patients with COVID-19 often present to hospital after a period lasting on average 15 days – during which patients may suffer from “happy hypoxia”, a term coined for the phenomenon that profoundly low oxygen saturation levels are found in individuals with relatively little subjective sensation of dyspnoea. (21) It has been hypothesized that SARS-CoV-2 may exert an idiosyncratic effect on the respiratory system via angiotensin-converting-enzyme 2 receptors in the carotid body and the midbrain, and this may lead to attenuation of the perceived dyspnoea. (3, 4, 22) The patients in our COVID-19 group might have had unrecognised hypoxia for a significant period prior to their hospital admission. Furthermore, even after hospital admission, many patients with COVID-19 remain relatively stable for a few days before they deteriorate and are admitted to the intensive care unit. (1) Thus, when compared to a general critical care population (e. g. patients with ARDS in whom hypoxia has to develop within 7 days – as per the Berlin definition), patients with respiratory failure secondary to COVID-19 may have a much longer time to “acclimatise” to hypoxemia. These changes in  $p_{50}$  continued to be present during the length of stay in intensive care, therefore we suspect a sustained response, which could be explained by reduced 2,3-DPG levels. (23) The mechanisms and the importance of this phenomenon requires further studies.

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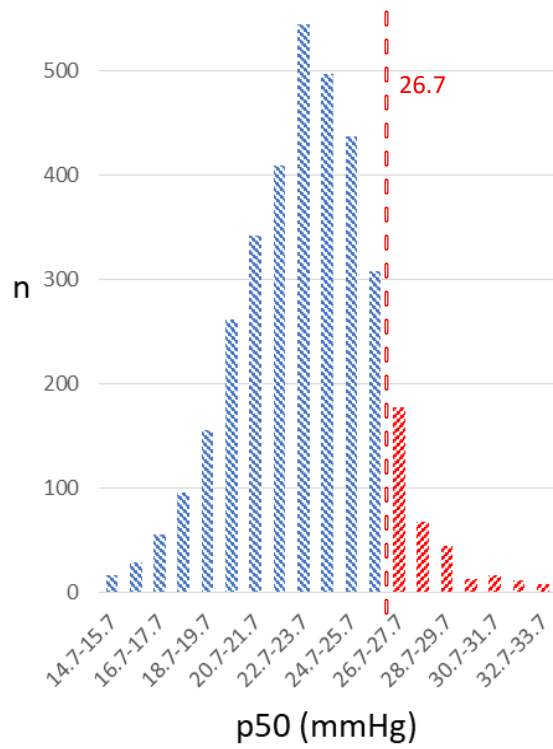
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|  |        | COVID-19 |       |       |        |       | CONTROL |       |       |        |       |  |                                      |
|--|--------|----------|-------|-------|--------|-------|---------|-------|-------|--------|-------|--|--------------------------------------|
|  |        | n        | Mean  | SD    | CI 99% |       | n       | Mean  | SD    | CI 99% |       | Group with COVID-19 showed               | unpaired t test (two-tailed p-value) |
| <b>p<sub>50</sub></b><br><b>(Eq.1)</b> | mmHg   | 3,518    | 23.37 | 3.13  | 23.23  | 23.50 | 15,945  | 24.59 | 5.42  | 24.48  | 24.70 | lower p <sub>50</sub>                    | p<0.0001                             |
| <b>p<sub>50</sub></b><br><b>(Eq.2)</b> | mmHg   | 3,518    | 24.8  | 3.7   | 24.7   | 25.0  | 15,945  | 25.7  | 6.0   | 25.6   | 25.8  | lower p <sub>50</sub>                    | p<0.0001                             |
| <b>pH</b>                              |        | 3,518    | 7.382 | 0.077 | 7.379  | 7.386 | 15,932  | 7.397 | 0.072 | 7.395  | 7.398 | lower pH                                 | p<0.0001                             |
| <b>pO<sub>2</sub></b>                  | mmHg   | 3,518    | 77.9  | 28.0  | 76.7   | 79.1  | 15,945  | 86.6  | 62.5  | 85.3   | 87.8  | lower pO <sub>2</sub>                    | p<0.0001                             |
| <b>SO<sub>2</sub></b>                  | %      | 3,518    | 94.2  | 7.9   | 93.9   | 94.6  | 15,945  | 93.1  | 10.4  | 92.9   | 93.3  | higher SO <sub>2</sub>                   | p<0.0001                             |
| <b>pCO<sub>2</sub></b>                 | mmHg   | 3,518    | 46.1  | 11.8  | 45.5   | 46.6  | 15,936  | 43.1  | 10.1  | 42.9   | 43.4  | higher pCO <sub>2</sub>                  | p<0.0001                             |
| <b>BE</b>                              | mmol/L | 3,518    | 1.2   | 4.3   | 1.0    | 1.3   | 15,925  | 0.7   | 4.2   | 0.6    | 0.8   | higher BE                                | p<0.0001                             |
| <b>Hct</b>                             | %      | 3,483    | 26.5  | 4.0   | 26.4   | 26.7  | 15,703  | 29.7  | 6.7   | 29.6   | 29.8  | lower Hct                                | p<0.0001                             |
| <b>Hb</b>                              | g/L    | 3,518    | 81.2  | 12.4  | 80.6   | 81.7  | 15,941  | 93.8  | 20.2  | 93.4   | 94.2  | lower Hb                                 | p<0.0001                             |
| <b>PaO<sub>2</sub>/FiO<sub>2</sub></b> | mmHg   | 2,627    | 215.7 | 111.5 | 210.1  | 221.3 | 13,941  | 299.4 | 236.5 | 294.2  | 304.6 | lower PaO <sub>2</sub> /FiO <sub>2</sub> | p<0.0001                             |
| <b>Temp</b>                            | °C     | 3,518    | 36.8  | 0.8   | 36.8   | 36.8  | 15,945  | 36.8  | 0.8   | 36.8   | 36.8  | NS                                       | p=0.057                              |

**Table 1** Summary of results from patients with COVID-19 and un-matched control group from critically ill patients without COVID-19. PaO<sub>2</sub>/FiO<sub>2</sub> ratio was calculated for arterial samples only. Values are reported as mean, standard deviation (SD) and 99% confidence interval (CI 99%). BE, base excess; Hct, haematocrit; Temp, temperature; NS, no significant difference.



**Figure 1.** Distribution of  $p_{50}$  values calculated using Hill equation (Eq.1) from measured  $pO_2$  and  $SO_2$  ( $n=3,518$ ). Blue indicates left shift and red indicates right shift of oxyhaemoglobin affinity from the standard  $p_{50}$  value. Dashed line at 26.7 mmHg indicates standard value for  $p_{50}$ .

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## **Supplementary information**

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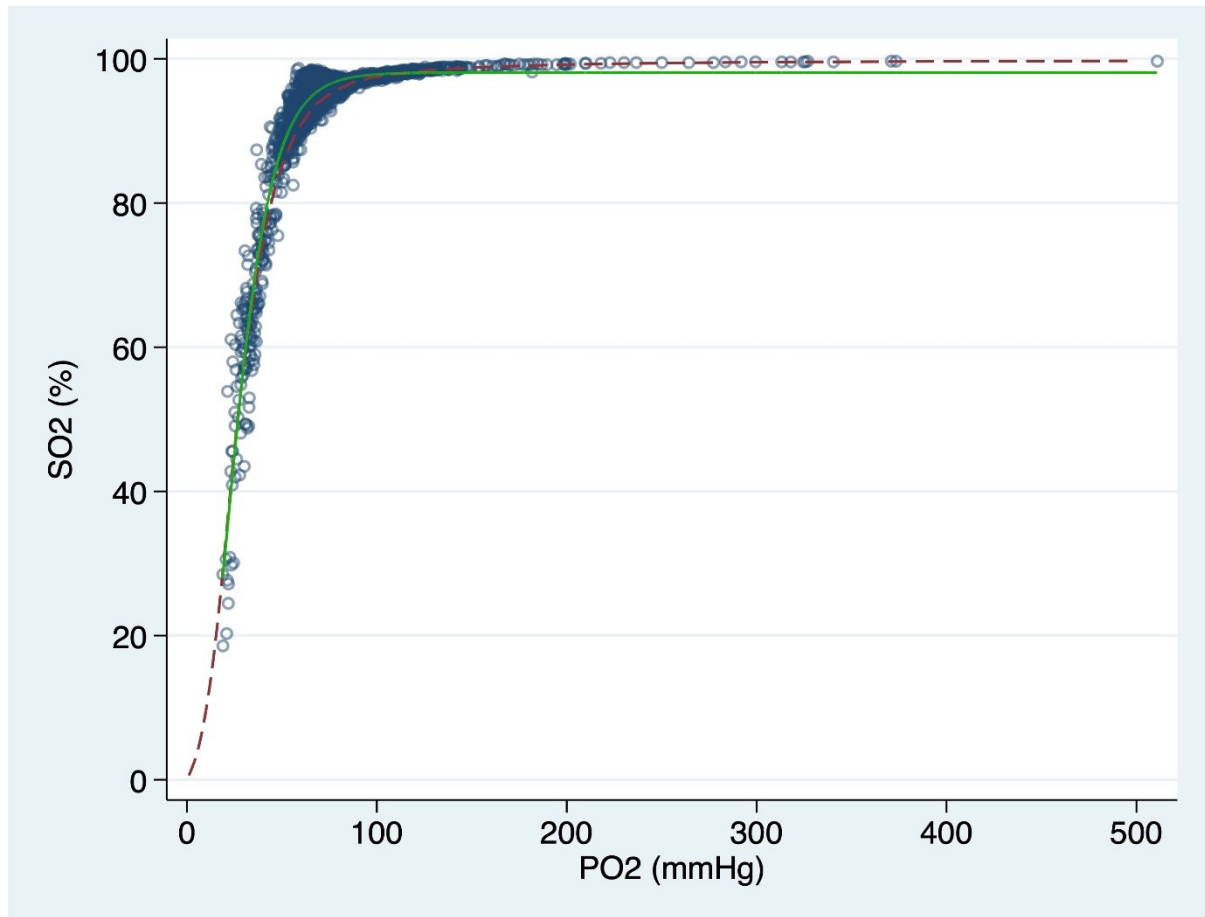
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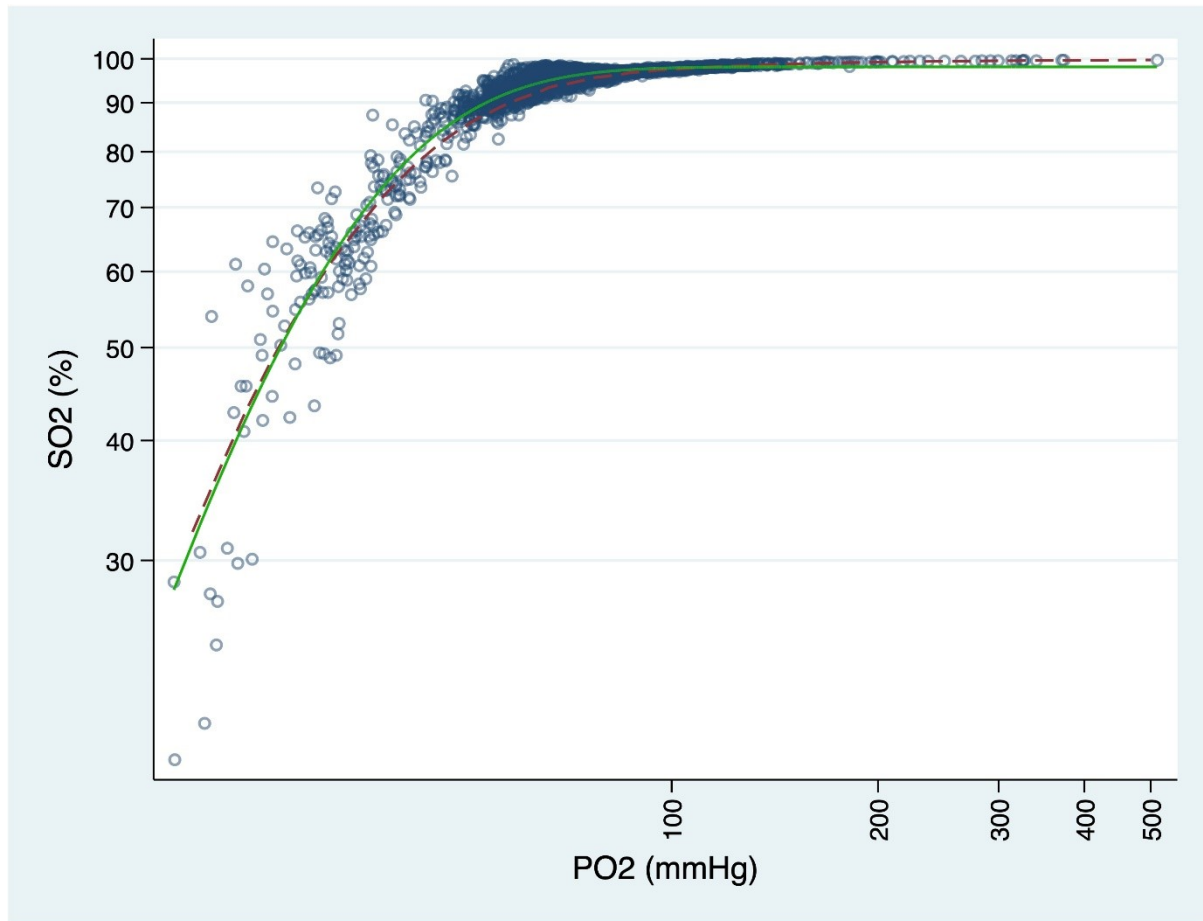
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## Supplemental results

### Oxyhaemoglobin dissociation curve

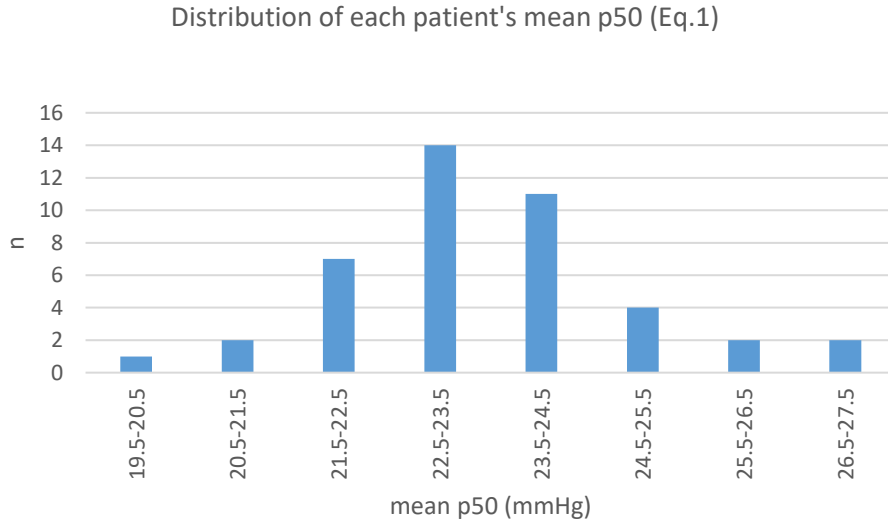


**eFigure 1.**  $\text{SO}_2$  with respective  $\text{pO}_2$  for 3,518 blood gas analyses (circles) and the sigmoid fitting line (green line). For comparison standard oxyhaemoglobin dissociation curve is shown (dashed red line). (6)



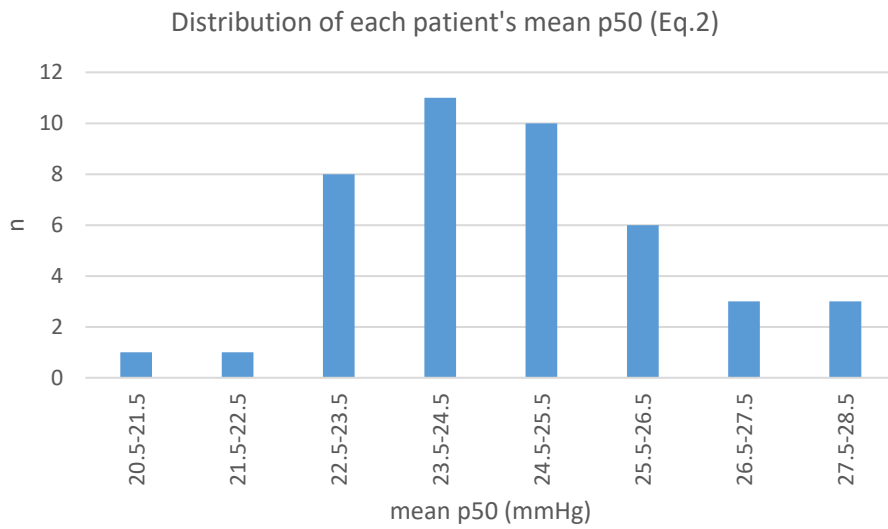
**eFigure 2.** Hill plot showing SO<sub>2</sub> with respective pO<sub>2</sub> for 3,518 blood gas analyses (circles) and the sigmoid fitting line (green line). For comparison standard oxyhaemoglobin dissociation curve is shown (dashed red line). (6)

## Inter subject variability



**eFigure 2** Distribution of the mean  $p_{50}$  calculated for each subject according to the following equation (see Eq. 1 in the main text):  $p_{50} = pO_{2(corr)} \times \left( \frac{1-SO_2}{SO_2} \right)^{\frac{1}{2.711}}$ ,

$$\text{where } pO_{2(corr)} = pO_2 \times 10^{[0.48(pH-7.4)-0.024(T-37)-0.0013 \times \text{Base Excess}]}$$



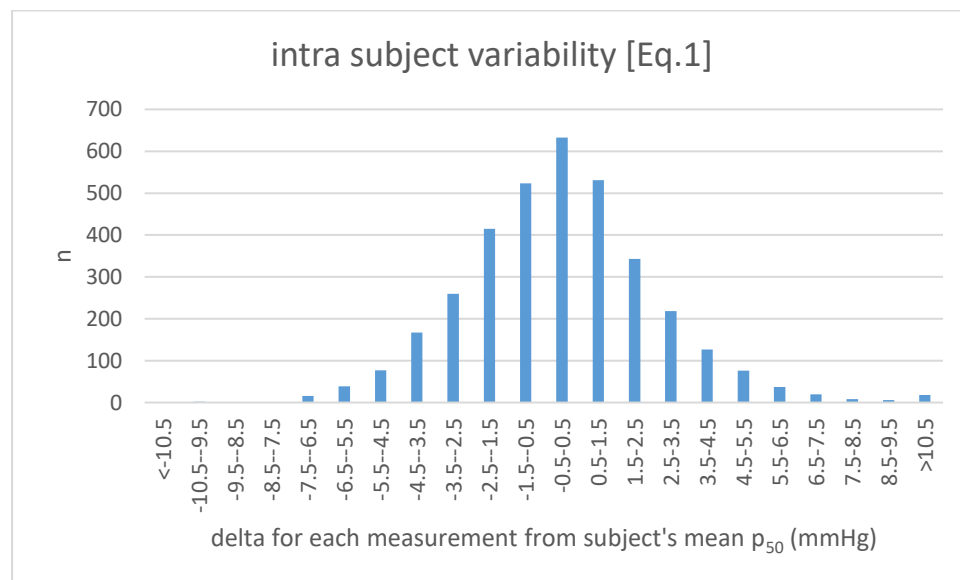
**eFigure 3** Distribution of the mean  $p_{50}$  calculated for each subject according to the following equation (see Eq. 2 in the main text):  $p_{50} = 26.7 \times 10^{(\lg pO_2 - \lg pO_2^k)}$ ,

$$\text{where } \lg pO_2^k = \frac{\lg Q + 4.172}{2.9} \text{ and } Q = \frac{SO_2}{100\% - SO_2}.$$

### Individual's mean p<sub>50</sub>

The mean (SD) of each individual's mean p<sub>50</sub> for Eq.1 was 23.46 mmHg (1.39) and Eq. 2 was 24.74 mmHg (1.58). Thus, the left shift is still present when only using one value per patient (n=43). Comparing this to normal p<sub>50</sub> (26.7 mmHg) with one sample t test showed a statistically significant difference (p<0.0001 with df=42 respectively; t = 15.2850 for Eq.1 and t = 9.673 for Eq.2)

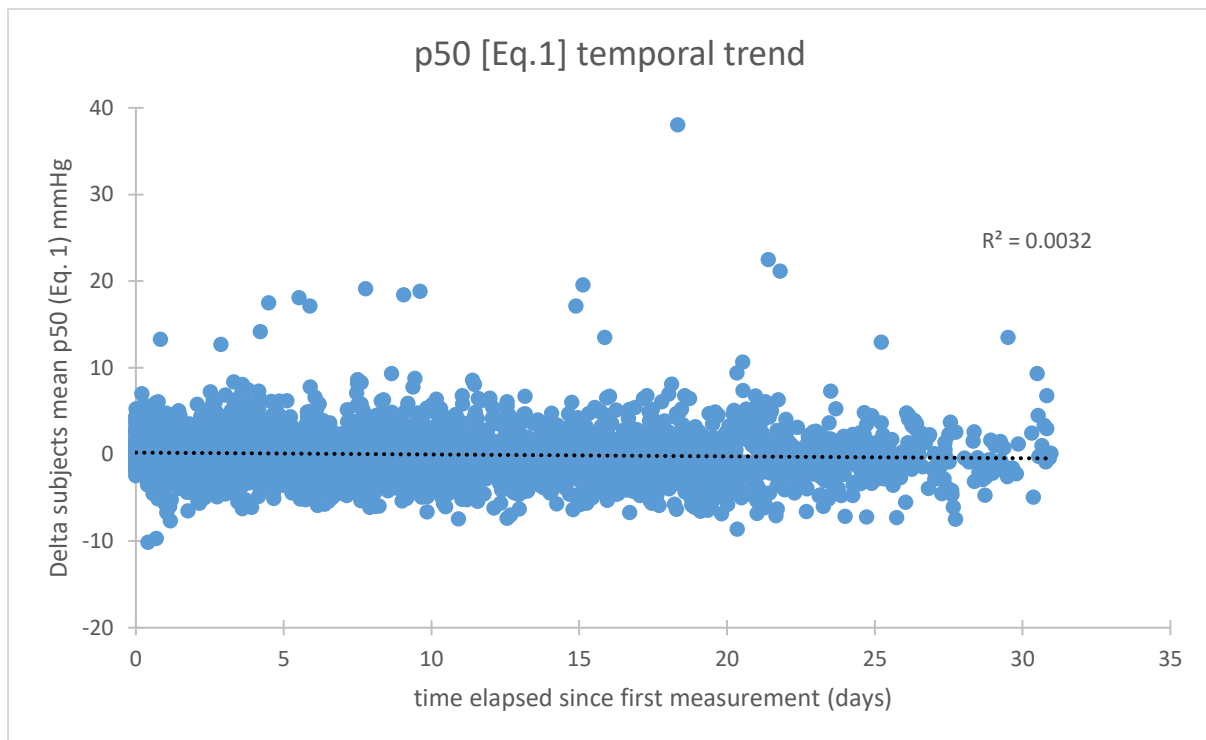
### Intra subject variability



**eFigure 4** Distribution of absolute difference between each measurement and the respective individual's mean p<sub>50</sub> calculated with the Eq.1.

## Time

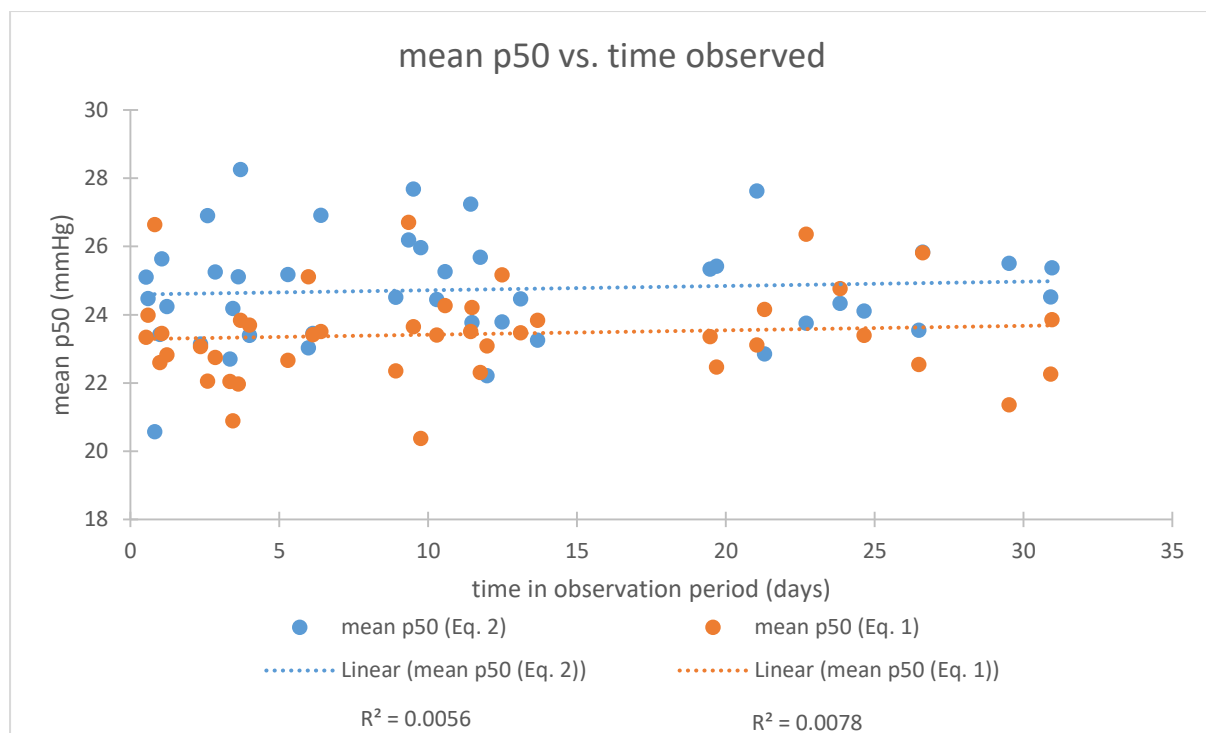
Patients spent a mean of 11.5 days in ICU during the observation period. Each individual's  $p_{50}$  varied during their time in ICU. However, plotting each measurement's deviation from the individual's mean  $p_{50}$  against elapsed time from first measurement did not show a relevant unidirectional change (eFig. 5).



**eFigure 5** Delta between each individual measurement from the mean  $p_{50}$  of that subject plotted over time starting from the first measurement.

Furthermore, individuals who spent a longer period in ICU during the observation period, did not show a different mean  $p_{50}$  and there was no specific correlation between mean  $p_{50}$  and time spent in ICU during the observation period (eFig. 6).





**eFigure 6** Distribution of the individual patients' mean p50 calculated with Eq.1 and Eq.2 plotted according to their length of stay during the observation period.