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Modelling the distribution of the oxygen-haemoglobin dissociation curve in vivo: An observational study

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ABSTRACT

Few studies have explored the variability of the oxygen-haemoglobin dissociation curve *in vivo*.

96,428 blood gas measurements were obtained (80,376 arterial, 6959 venous) from a cohort of 7656 patients who were admitted to a large UK teaching hospital between 1 February 2020 and 31 December 2021 for a Covid-19 related admission with a positive PCR. There was consistent variation of the distribution of the oxygen-haemoglobin curve across most oxygen saturation strata with typical values at 91–92 % saturation (mean 8.1kPa, standard deviation sd 0.6 kPa or 60.8 mmHg sd 4.5 mmHg), with the exception of the highest strata of oxygen saturation of 99–100 % (mean 17.7 kPa, sd 8.1kPa or 132 mmHg sd 60.8).

The higher oxygen partial pressures at higher oxygen saturations are a concern in view of the increased mortality observed in RCTs of higher oxygen saturation targets. However, the observational study design precludes any attribution of causality.

1. Introduction

The relationship between blood oxygen saturation and the partial pressure of plasma oxygen dissolved called the oxygen-haemoglobin dissociation curve, and the evidence-base that underpins this was initially developed in the first half of the 20th Century in a series of controlled laboratory experiments (Antonini, 1979). These *in vitro* observations were translated into clinical medicine with limited validation of the assumptions *in vivo* environment, (Antonini, 1979), and existing equations used to model the oxygen-haemoglobin dissociation curve may be based on as few as ten individuals (Collins et al., 2015).

One *in vivo* study replicated the oxygen-haemoglobin dissociation curve which had been generated *in vitro* (Antonini, 1979) using data from 3524 arterial blood samples (Collins et al., 2015), and this represents the only representation of the association between oxygen saturation and dissolved blood oxygen concentrations from a large real-world clinical dataset. Another study described the variance in oxygen affinity to haemoglobin as defined as the partial pressure of oxygen at 50 % oxygen saturation in 60 healthy young volunteers under experimental conditions (Balcerek et al., 2020). However, there are no descriptions from large real-world populations of the variability of the oxygen-haemoglobin dissociation curve that use both venous and arterial blood samples to cover the complete range of oxygen saturations.

Understanding the association between blood oxygen saturation and blood oxygen concentration in clinical patients with a wide spectrum of respiratory failure severity is important as it has implications for the ongoing debate about the optimal targets for arterial oxygen during the treatment of patients, with randomised-controlled trials suggesting that high-dose supplementary oxygen may paradoxically increase mortality in both children (Peters et al., 2024) and adults (Chu et al., 2018; Ni et al., 2019; Nielsen et al., 2024). The Covid-19 pandemic resulted in a large number of patients admitted to hospitals over a relatively short

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period of time, many of whom had blood gas measurements taken. This thus provided the opportunity to examine the oxygen-haemoglobin dissociation curve in patients with a range of severity of respiratory failure to help understand what these relationships are in real-world clinical patients.

Hence, we used a database of patients who were admitted with Covid-19 infection to a large teaching hospital to explore the variability of the association between blood oxygen saturation and partial pressure of oxygen in patients.

2. Methods

2.1. Study design and patient population

We conducted a cohort study in all patients admitted to Nottingham University Hospitals NHS trust between 1 February 2020 and 31 December 2021 for a Covid-19 related admission with a recent positive PCR.

2.1.1. Blood gas measurements

Blood gas measurements were obtained throughout the patients' time in Nottingham University Hospitals NHS Trust, which uses the Radiometer ABL90Flex and Radiometer ABL90FlexPlus for point of care blood gas measurements. Both of these devices perform their own internal calibration and internal quality control. In addition, every three months a manual calibration is performed. All arterial and venous blood gas measurements were electronically stored. The venous samples may have been taken from any venous source, as the precise point of sampling for each blood specimen is not recorded.

2.2. Data modelling

The blood gas results were used to construct oxygen dissociation curves by plotting the oxygen saturation (sO_2) against the partial pressure of oxygen from the same sample. A smooth curve of predicted sO_2 over measured blood partial pressure of oxygen was fitted with cubic regression splines in a general additive mixed model including a patient level random intercept using R (gamm function (Wood, 2011) from the mgcv package (Wood, 2004)). The patient level random intercept modelled the correlation between samples taken from the same patient at different time points. Default options were used to select the k value and checked to ensure it was greater than the effective degrees of freedom. Different basis types of thin plate splines and cubic splines were compared with Akaike Information Criterion (AIC), selecting the basis with the lowest AIC.

2.3. Clinical governance approvals

Approval for this work was granted via an NUH Clinical Effectiveness Team audit (reference: reference: 21–294 C) and IRAS (REC: 20/WM/0142, project ID: 282490, amendment No. SA02 20/07/21).

3. Results

96,428 consecutive blood gas results were available from 7656 individual eligible patients (Table 1). 4928 patients contributed more than one test to the analysis, with 1102 contributing more than 10 tests (see supplementary table 1).There was relatively consistent variation of the distribution of the partial pressures of blood oxygen that constitute the oxygen-haemoglobin curve across most oxygen saturation strata from 85 % to 96 % (Fig. 1) with typical values at 91–92 % saturation (mean 8.1kPa, standard deviation sd 0.6 kPa, or 60.8 mmHg sd 4.5 mmHg).

However, much higher mean oxygen partial pressures were observed in the maximal stratum of oxygen saturation of 99–100 % (17.7 kPa or 133 mmHg) along with substantially more dispersion of these values (sd 8.1kPa or 60.8 mmHg). As these higher oxygen partial pressures were skewed with a long tail, we have shown box plots in the supplementary Figure. The supplementary table examines the most extreme values from the box plot where oxygen saturation was 100 % and oxygen partial pressure was above 40 KPa. This shows they were sicker patients (with lower bicarbonate and haemoglobin), more likely to be current smokers, and many were being given high flow oxygen.

Table 1
Description of study population.

Characteristic	Arterial (median, IQR)	Venous (median, IQR)
No of individual patients	3300	6959
No of measurements	80376	16052
No of males (%)	54622 (68 %)	8750 (55 %)
Age, years	58 (48,66)	69 (54,80)
Height cm (median, IQR)	170 (163,178)	168 (160,175)
Weight kg (median, IQR)	87.1 (75.3100.0)	77.0 (64.8,92.8)
BMI (median, IQR)	30 (25,35)	27 (24,32)
Ethnicity other than White n %	32164 (42 %)	4146 (27 %)
Current smokers, N (%)	8632 (10.7 %)	2992 (18.6 %)
Temperature, °C	36.9 (36.5,37.4)	36.7 (36.4,37.1)
pH*	7.43 (7.37,7.47)	7.407 (7.36,7.44)
Oxygen partial pressure* , kPa	9.0 (8.0,10.5)	4.5 (3.4,6.1)
Oxygen partial pressure* , mmHg	67.3 (59.9,78.8)	34.0 (25.6,45.4)
Carbon dioxide partial pressure* , kPa	5.8 (4.8,7.1)	5.7 (4.9,6.4)
Carbon dioxide partial pressure* , mmHg	43.1 (36.3,53.0)	42.5 (37.0,48.3)
Oxygen saturation, %	93.7 (91.0,96.2)	63.3 (42.8,80.2)
Haemoglobin, g/L	101 (85,122)	122 (99,141)
Carboxyhaemoglobin, %	1.2 (0.9,1.6)	1.3 (1,1.6)
Methaemoglobin %	0.7 (0.5,0.9)	0.8 (0.5,1.2)
Oxygen haemoglobin %	91.8 (89.2,94.3)	61.8 (41.8,78.2)
Bicarbonate mEq/L	27.5 (24.9,30.6)	25.4 (22.8,27.6)

IQR = interquartile range
***Value uncorrected for temperature from point of care blood gas machine** (Nottingham University Hospitals NHS Trust uses the Radiometer ABL90Flex and Radiometer ABL90FlexPlus)

The modelled oxygen haemoglobin dissociation curve is presented in Fig. 2, with a cubic spline (k = 10, effective degrees of freedom = 8.97, AIC = 407525). Cubic splines were selected with a better model fit than thin plate splines (AIC = 440992). Scaled residuals were plotted against fitted values, estimated from the general additive mixed model fitting the smoothed oxygen dissociation curve (supplementary Figure 1). This showed 813 (0.8 %) measurements had an absolute scaled residual greater than 5. Excluding these measurements from the models and tables did not alter the estimates for the pO2 for the 50 % or 92 % oxygen saturation points on the curve (supplementary Figure 2), and there were no clinically meaningful differences in the stratified distributions and population characteristics (Supplementary Figure 1 and Supplementary Table 2).

4. Discussion

This *in vivo* modelling of the oxygen-haemoglobin dissociation curve in a large number of patients across a wide range of oxygen saturations demonstrates that there is a generally constant variation in the association between blood oxygen saturation and partial pressure across blood oxygen saturations of 85–96 %, but that this variance increases over tenfold for blood oxygen saturations of 99 % or more.

The strengths of this analysis include the use of a complete population of patients with a single disease process, minimising the risk of recruitment bias. The blood gas physiological data were taken from the same sample, ensuring that they were concurrent, avoiding issues of measurement error that may occur is saturations were derived from pulse oximetry (Crooks et al., 2022, 2023).

One limitation of these data are that they are 'real-world' clinical data, as opposed to data generated in controlled experimental conditions. This provides a degree of heterogeneity in the population which

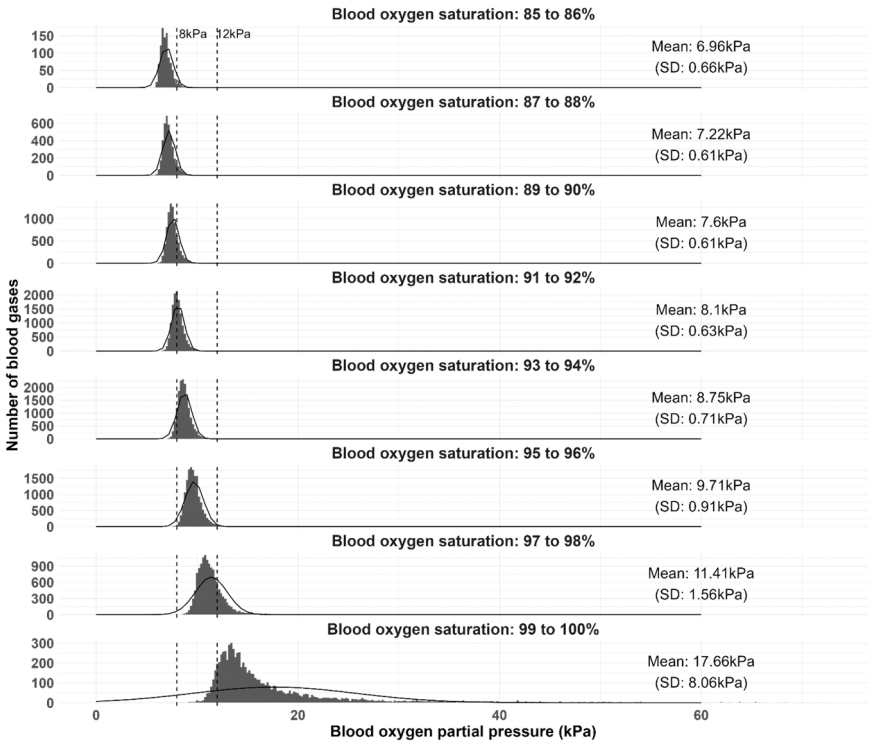

Fig. 1. Histograms demonstrating the association between oxygen saturation and blood partial pressure oxygen levels stratified by 2 % oxygen saturation increments from 85 % and above. Note: The Y axes vary for presentational purposes.

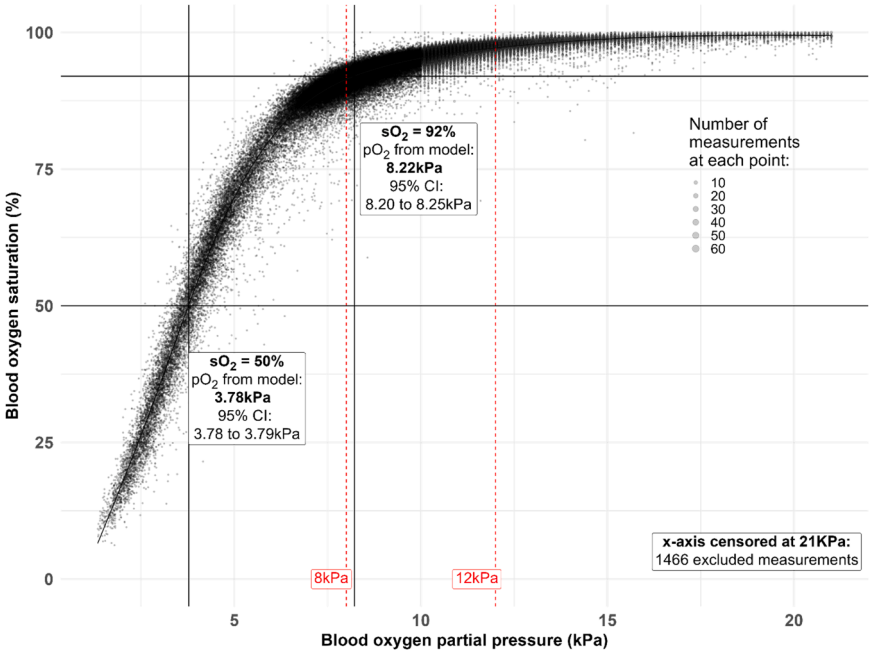

Fig. 2. Oxygen dissociation curve from general additive mixed model predicting sO2 from pO2 (not temperature corrected). Points indicate measurements and grey ribbon indicates fitted curve with 95 % CI from model prediction. Horizontal solid black lines indicate sO2 values of 50 % and 92 % with corresponding pO2 values indicated by vertical solid black lines.

will include a range of potentially important factors such as genetic variation, disease severity which may lead to acidosis, disease co-morbidities and medications, all of which may impact on the oxygen-haemoglobin dissociation curve and hence have the potential to represent confounding factors. Being an observational epidemiological study, there were no control groups and so the analysis is limited to showing how the oxygen haemoglobin dissociation curve varies in real-world conditions with particular interest in the higher oxygen saturation strata in patients with Covid-19 infection. Generalisability of these observations to populations that differ to ours or with disease processes other than Covid-19 infection is not possible in the absence of further independent studies that explore these associations in these individuals. In particular, there may be factors that modify the oxygen-haemoglobin dissociation curve that are as yet unknown, that may be important

modifying factors.

The use of real-world data is both a strength and a limitation. While we have large numbers of data measurements from a well-defined population with no excluded individuals which reduces the risk of bias, we also have no information on the quality of the process of implementing the blood gas measurement which could vary according to ease of sample collection, duration of distance from the patient to the arterial blood gas analyser and also if the sample was transported on ice or not in a patient population that were treated in two different hospital campuses. All of these factors have the potential to introduce variation into the dataset. However, this is more likely to be non-systemic error, and hence unlikely to erroneously change the overall analysis. Patients with Covid-19 often received supplementary oxygen due to respiratory failure, and again this will be another factor that can modify the physiological processes of oxygen transport, although as our data used blood gas analysis output this should not introduce any bias to the analysis.

The observation of interest in this analysis is the variance of the oxygen-haemoglobin dissociation model across the different strata of blood oxygen saturations. For blood oxygen saturation levels of above 96 %, there is a marked increase in the dispersion of the partial pressure of oxygen with the standard deviation increasing to approximately 1.6kPa from a baseline of 0.7kPa to 0.9kPa for lower oxygen saturations. This then increases substantially again to a standard deviation of 8.1kPa for oxygen saturations of 99–100 %.

These histograms and summary statistics demonstrate that while oxygen saturation is capped at 100 % due to the finite capacity of haemoglobin to carry oxygen, plasma can be exposed to and absorb very high levels of oxygen. These levels are not physiological, as they are not observed in nature but in essence represent the consequence of exposing patients to high-flow supplementary oxygen within a medical environment. The high blood oxygen concentrations observed are probably the end consequence of the complex interactions between supplementary oxygen concentrations, the degree of lung disease and respiratory rate.

The debate as to the optimal blood oxygen levels required to provide supportive care during illness is an active one, and is likely to depend on the individual patient and their clinical scenario. The national guidance for the care of Covid-19 patients in the United Kingdom advised target saturations of 92–96 % (National Institute of Clinical Excellence, 2020). However, there have been a number of randomised controlled trials in both children (Peters et al., 2024) and adults (Chu et al., 2018; Ni et al., 2019; Nielsen et al., 2024) suggesting that lower target blood oxygen levels are associated with better mortality outcomes. These data demonstrate how high-flow supplementary oxygen may result in supra-physiological partial pressures of blood oxygen. The observational nature of the data presented mean that they provide no clear causal link between exposure to high levels of oxygen in the blood and clinical outcomes such as mortality, but simply describe what is occurring in a large population of patients. These data thus complement the existing animal (Hochberg et al., 2021) and clinical studies (Peters et al., 2024; Chu et al., 2018; Ni et al., 2019; Nielsen et al., 2024) which collectively contribute to the understanding of oxygen physiology and transport both healthy individuals and sick patients.

In summary, these observational data provide a unique insight into the oxygen-haemoglobin dissociation curve *in vivo* in a large number of patients with a wide spectrum of severity of respiratory failure. As such, it sets the scene and highlights the need for further work to corroborate our findings in other large data sets in patients with other diseases because these findings could have particular implications for understanding the optimal titration of supplementary oxygen in sick patients to avoid inadvertent harm.

Ethics committee approval

Approval for this work was granted via an NUH Clinical Effectiveness Team audit (reference: 21–294 C). The analysis used anonymised patient data, and no individual patient consent was required.

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CRediT authorship contribution statement

Fogarty Andrew: Writing – original draft, Methodology, Conceptualization. **West Joe:** Writing – review & editing, Project administration, Data curation. **Crooks Colin:** Writing – review & editing, Writing – original draft, Methodology, Data curation. **Simmonds Mark:** Writing – review & editing, Resources, Data curation. **Morling Jo:** Writing – review & editing, Methodology. **Briggs Steve:** Writing – review & editing, Data curation, Conceptualization. **Juurlink Irene:** Writing – review & editing, Methodology, Data curation. **Hammond-Pears Susan:** Writing – review & editing, Data curation. **Cruickshank Simon:** Writing – review & editing, Data curation. **Card Tim:** Writing – review & editing, Data curation, Conceptualization. **Shaw Dominick:** Writing – review & editing, Conceptualization.

Conflict of Interest

None of the authors have any conflict of interest with the publication of these data.

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Author contributions

The database was established by JW, TC, CC, MS, SB, JM, IJ, SC and S H-P. The hypothesis was generated by AF and DS. The statistical analysis was by CC. AF drafted the manuscript. All authors contributed to editing and approved the final version of the manuscript. CC and SB had full access to the data.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.resp.2025.104400.

Data availability

The authors do not have permission to share data.

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