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LETTER TO THE EDITOR

Impaired respiratory function reduces haemoglobin oxygen affinity in COVID-19

Using homology modelling and molecular docking algorithms, Liu and Li predicted the existence of interactions between SARS-Cov-2 and haemoglobin (Hb) and suggested that these interactions may change haemoglobin-oxygen (Hb-O_2) affinity.[1] Hb-O_2 affinity has been extensively studied in intensive care unit (ICU) patients with COVID-19 (usually intubated and mechanically ventilated) showing normal to increased affinity.[2–4] Higher Hb-O_2 affinity facilitates Hb oxygenation in the lungs while reducing tissue O_2 unloading; a reduced Hb-O_2 affinity has the opposite effect. Only one study has investigated patients with COVID-19 outside the ICU.[5]

In a retrospective, cross-sectional study, we analysed Hb-O_2 affinity in COVID-19 patients hospitalised in general wards of our institution. The local ethics committee approved the study. Details on study protocol, methods and additional results are reported in Data S1. All patients had a laboratory-confirmed diagnosis of COVID-19 and an arterial blood gas analysis performed on admission, with a Hb-O_2 saturation (SO_2) of less than 97%. At the time of analysis, no patient was intubated, mechanically ventilated, or had received a COVID-19 vaccination. We determined oxygen tension at half-saturation of Hb (P_{50}), an index of Hb-O_2 affinity, using two equations for the determination of in vivo and standard P_{50} (Figure S1).[6] In vivo P_{50} reflects actual arterial blood P_{50}, as determined by Hb structure, red-cell 2,3-diphosphoglycerate (DPG), pH and arterial pressure of CO_2 ($PaCO_2$). Standard P_{50} is the P_{50} value measured at pH 7.4 and $PaCO_2$ 40 mm Hg; it is an index of Hb-O_2 affinity in which the influences of pH and $PaCO_2$ have been removed.[7]

General characteristics of study patients are shown in Table S1. Systemic inflammation was common (elevated C-reactive protein and serum ferritin), but only 12% of cases had procalcitonin concentrations greater than 2.00 ng/ml, suggestive of superimposed bacterial infection. Standard P_{50} was higher than in vivo P_{50} ($p < 0.001$), although median values of both parameters fell within the normal range. Table S2 shows correlations of in vivo and standard P_{50} with clinical and laboratory parameters. Multivariable analysis demonstrated that a compromised respiratory function, expressed by reduced SO_2 and a low ratio of arterial O_2 pressure to fraction of inspired oxygen (PaO_2/FiO_2) and high lactate and lactate dehydrogenase, were associated with higher P_{50} (Table 1). pH had a negative correlation with in vivo P_{50} and a positive correlation with standard P_{50}. Ninety-five patients (33%) died within 28 days from hospital admission. Patients

TABLE 1 Multiple regression analysis of factors related to P_{50}

	In vivo P_{50}		Standard P_{50}	
Variables	**PCor (95% CI)**	***p***	**PCor (95% CI)**	***p***
SO_2	−0.581 (−0.659 to −0.490)	<0.001	−0.563 (−0.645 to −0.469)	<0.001
pH	−0.675 (−0.739 to −0.599)	<0.001	0.399 (0.285 to 0.502)	<0.001
Lactate	0.287 (0.166 to 0.400)	<0.001	0.289 (0.167 to 0.402)	<0.001
Multiple regression r	0.769 (0.711 to 0.816)	<0.001	0.679 (0.603 to 0.742)	<0.001
The below analysis was performed excluding SO_2 from independent variables; pH and $PaCO_2$ were additionally excluded in standard P_{50} analysis				
PaO_2/FiO_2	−0.314 (−0.428 to −0.190)	<0.001	−0.146 (−0.278 to −0.009)	0.038
pH	−0.603 (−0.681 to −0.512)	<0.001	—	—
Lactate	0.324 (0.200 to 0.438)	<0.001	0.153 (0.016 to 0.284)	0.030
LDH	—	—	0.361 (0.235 to 0.475)	<0.001
OxyHb	−0.209 (−0.332 to −0.079)	0.002	−0.148 (−0.280 to −0.011)	0.036
Multiple regression *r*	0.501 (0.395 to 0.594)	<0.001	0.508 (0.399 to 0.603)	<0.001

Abbreviations: LDH, lactate dehydrogenase; OxyHb, arterial oxyhaemoglobin; PaO_2/FiO_2, PaO_2 to fraction of inspired oxygen; PCor, partial correlation coefficient.

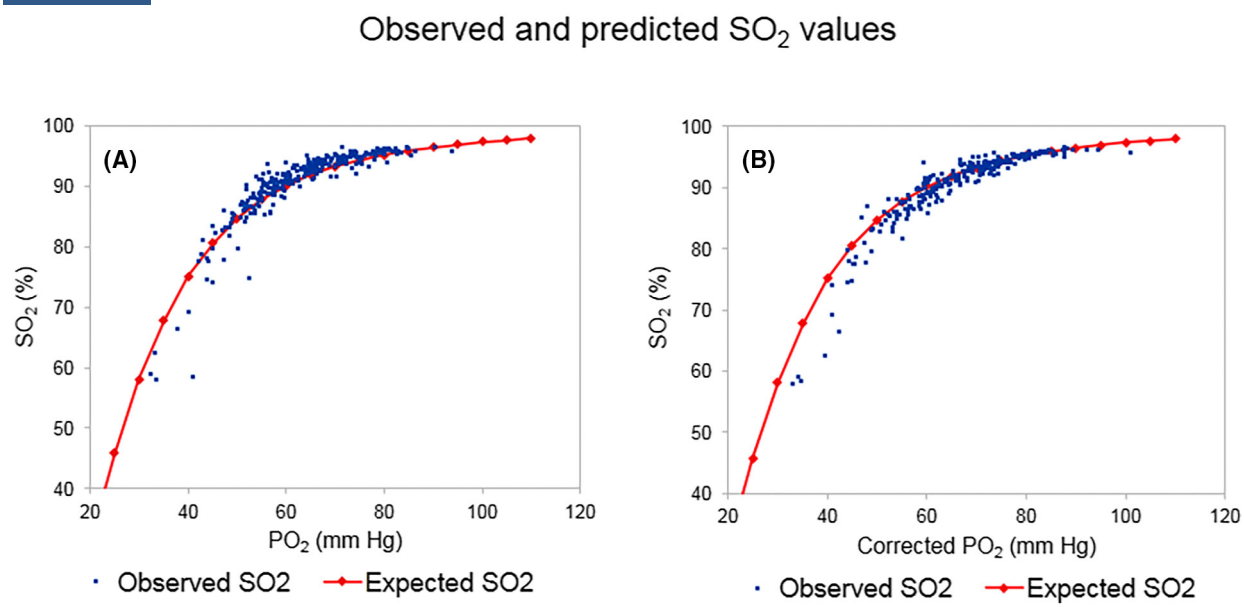

FIGURE 1 Distribution of observed and predicted SO_2 values in COVID-19 patients. (A) SO_2 is reported as a function of observed PaO_2. and (B) as a function of PaO_2 corrected for arterial blood pH and $PaCO_2$. Red marks represent the expected SO_2 value for any given PaO_2 according to Doyle equation; blue dots correspond to experimental data.

who died had more elevated in vivo P_{50} than those who survived, while no difference was observed for standard P_{50} (Table S3). However, on multivariable logistic regression, the effect of in vivo P_{50} disappeared and only advanced age, reduced PaO_2/FiO_2 ratio and increased lactate dehydrogenase were associated with mortality (Table S4).

Figure 1 shows SO_2 values as a function of measured PaO_2, or PaO_2 adjusted for pH and PaO_2; distribution of SO_2 was similar to the oxygen dissociation curve (ODC) obtained from Hill's equation as adapted by Doyle (Figure 1), in which normal P_{50}, measured at 37°C, pH 7.40 and $PaCO_2$ 40 mm Hg, is assumed to be 26.6 mm Hg,[8] showing an overall agreement between observed and expected SO_2. This curve almost perfectly overlapped the standard human Hb ODC reported by Severinghaus[9] (data not shown). Lower levels of SO_2 (<85%) were often shifted rightward with respect to the reference SO_2 curve expected for a given PaO_2, indicating that these patients had reduced Hb-O_2 affinity. Patients with SO_2 of less than 85% had a reduced PaO_2/FiO_2 ratio, high lactate (>1.7 mmol/L) and increased P_{50} (Table S5). In these patients, standard P_{50} was significantly higher than 28.0 mm Hg, the upper limit of normal ($p < 0.001$), and the elevation was not justified by pH or $PaCO_2$ changes. These results show that a severely compromised respiratory function is associated with reduced Hb-O_2 affinity.

Our data suggest a preeminent role of patients' respiratory status and acid-base balance in the regulation of both in vivo and standard P_{50}. pH was negatively related to in vivo P_{50}, since acidosis reduces Hb-O_2 affinity;[9] in contrast, pH had a positive correlation with standard P_{50}, as described in critically ill patients.[6] $PaCO_2$ behaved in the opposite way. The correlations with standard P_{50} are likely to represent the indirect effects of pH (and $PaCO_2$), possibly due to the upregulation of 2,3-DPG production by alkalemia.[10] Our results are partially at variance with previous reports showing increased or normal Hb-O_2 affinity in COVID-19.[2–6,11] Most of these studies were conducted in ICUs with determination of standard P_{50}; in these settings a permissive hypercapnia is often allowed when low tidal ventilation is adopted to reduce ventilator-associated lung injury[12] and may explain the reported reduction in standard P_{50}. Otherwise, reduction of standard P_{50} could result from 2,3-DPG downregulation in the presence of respiratory or metabolic acidosis. Compared with our data, ICU studies reported higher $PaCO_2$ and lower pH values,[3,4] which can cause standard P_{50} reduction, but also demonstrated that elevated P_{50} is usually associated with more severe disease and increased mortality.[4,5]

Some patients in our study had respiratory distress and were subsequently transferred to the ICU, but at the time of analysis they were spontaneously breathing, and most had normal blood pH with low $PaCO_2$. Median Hb was 129 g/L, confirming that COVID-19 patients in internal medicine units usually have only mild anaemia.[13] In addition, Hb was similar in patients with SO_2 of 85% or more and those with a SO_2 below 85%, indicating that anaemia was not involved in the upregulation of P_{50} associated with worsening respiratory function. Our data in patients with SO_2 below 85%, showing increased P_{50} and lactate concentration, confirm previous observations in acute myocardial infarction, suggesting that P_{50} elevation represents a compensatory change aimed at preventing tissue hypoxia when oxygen delivery is compromised,[14] whereas high plasma lactate is a marker of the O_2 debt associated with decreased oxygen transport and consumption.[15] Although

the reported results are preliminary and require confirmation, we document that a severely compromised respiratory function is accompanied by significant elevations of P_{50} in COVID-19 that is likely to be an expression of the organism's adaptation to hypoxaemia. This agrees with previous observations showing that a left-shifted ODC is associated with lower mortality in COVID-19 patients at hospital admission.[5]

KEYWORDS

COVID-19, haemoglobin-oxygen affinity, haemoglobin P50, oxyhaemoglobins, PaO2 to fraction of inspired oxygen ratio, SARS-CoV-2

AUTHOR CONTRIBUTIONS

Gaetano Bergamaschi designed the study, analysed data, performed statistical analysis and drafted the manuscript. Chiara Barteselli, Virginia Del Rio, Federica Borrelli de Andreis, Ivan Pellegrino, Caterina Mengoli, Emanuela Miceli, Marta Colaneri and Valentina Zuccaro set up the database, collected the data and critically reviewed the manuscript. Michele Di Stefano, Raffaele Bruno and Antonio Di Sabatino designed the study, analysed the data and critically reviewed the manuscript.

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CONFLICTS OF INTEREST

The authors declare no competing conflicts of interest.

DATA AVAILABILITY STATEMENT

The datasets used in this study are available from the corresponding author upon reasonable request. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

APPENDIX

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