5. Yang M, Ng MH, Li CK. Thrombocytopenia in patients with severe acute respiratory syndrome (review). *Hematology*. 2005;**10**:101–5.
6. Assinger A. Platelets and infection – an emerging role of platelets in viral infection. *Front Immunol*. 2014;**5**:649. https://doi.org/10.3389/fimmu.2014.00649
7. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet*. 2020;**395**:507–13.
8. Xu P, Zhou Q, Xu J. Mechanism of thrombocytopenia in COVID-19 patients. *Ann Hematol*. 2020;1–4.
9. Zulfiqar AA, Lorenzo-Villalba N, Hassler P, Andrès E. Immune thrombocytopenic Purpura in a patient with covid-19. *N Engl J Med*. 2020;**382**:e43.
10. Nham E, Ko JH, Jeong BH, Huh K, Cho SY, Kang CI, et al. Severe thrombocytopenia in a patient with COVID-19. *Infect Chemother*. 2020 [published online ahead of print, 2020 Apr 23].
11. Ahmed MZ, Khakwani M, Venkatadasari I, Horgan C, Giles H, Jobanputra S, et al. Thrombocytopenia as an initial manifestation of Covid-19; Case Series and Literature review. *Br J Haematol*. 2020;**189**(6):1057–8.
12. Rodeghiero F, Stasi R, Gernsheimer T, Michel M, Provan D, Arnold DM, et al. Standardization of terminology, definitions and outcome criteria in immune thrombocytopenic purpura of adults and children: report from an international working group. *Blood*. 2009;**113**:2386–93.
13. Venkata C, Kashyap R, Farmer JC, Afessa B. Thrombocytopenia in adult patients with sepsis: incidence, risk factors, and its association with clinical outcome. *J Intensive Care*. 2013;**1**:9. https://doi.org/10.1186/2052-0492-1-9.
14. Chan KH, Lee P, Chan CY, Lam KBH, Ho P. Monitoring respiratory infections in covid-19 epidemics. *BMJ*. 2020;**369**:m1628.
15. Woodruff M, Ramonell R, Cashman K, Nguyen D, Ley A, Kyu S, et al. Critically ill SARS-CoV-2 patients display lupus-like hallmarks of extrafollicular B cell activation. *medRxiv*. 2020 Preprint. https://doi.org/10.1101/2020.04.29.20083717.
16. Hadjadj J, Yatim N, Barnabei L, Corneau A, Boussier J, Pere H, et al. Impaired type I interferon activity and exacerbated inflammatory responses in severe Covid-19 patients. *medRxiv*, 2020 Preprint. 1101/2020.04.19.20068015.
17. Jenks SA, Cashman KS, Woodruff MC, Lee FE, Sanz I. Extrafollicular responses in humans and SLE. *Immunol Rev*. 2019;**288**:136–48.
18. Thachil J, Tang N, Gando S, Falanga A, Cattaneo M, Levi M, et al. ISTH interim guidance on recognition and management of coagulopathy in COVID-19. *J Thrombosis Haemostasis: JTH*. 2020;**18**:1023–6.
19. Pavord S, Thachil J, Hunt B, Murphy M, Lowe G, Laffan M, et al. Practical guidance for the management of adults with Immune Thrombocytopenia during the COVID-19 pandemic. *Br J Haematol*. 2020 [Epub ahead of print]. https://doi.org/10.1111/bjh.16775
20. Lan L, Xu D, Ye G, Xia C, Wang S, Li Y, et al. Positive RT-PCR test results in patients recovered from COVID-19. *JAMA*. 2020;**323**:1502–3
21. Salje H, Tran Kiem C, Lefrancq N, Courtejoie N, Bosetti P, Paireau J, et al. Estimating the burden of SARS-CoV-2 in France. *Science* 2020;**369**:208–11.

Single-cell oxygen saturation imaging shows that gas exchange by red blood cells is not impaired in COVID-19 patients

SARS-CoV-2 coronavirus infection is characterised by a marked inflammatory state and viral pneumonitis. A striking clinical feature is severe hypoxaemia, often in the presence of near-normal lung mechanics. Several hypotheses have been put forward to explain these findings,[1,2] including pulmonary microvascular thrombosis, dysregulated hypoxic pulmonary vasoconstriction and dysfunctional gas transport by red blood cells (RBCs). Derangement in convective O_2 transport is an attractive hypothesis as this would explain why COVID-19 hypoxaemia is often refractory to supplemental oxygen. A controversial *in silico* prediction postulated that the virus attacks haemoglobin (Hb),[3] and despite subsequent criticism,[4] a number of hypotheses have emerged linking Hb with COVID-19, such as the association between thalassaemias or fetal Hb with disease severity.[5–7] Notwithstanding these opinions, studies in China have confirmed modestly lower Hb levels in severe COVID-19[8,9] and greater heterogeneity in terms of RBC volume, quantified as RBC Distribution Width-Standard Deviation (RDW-SD).[10]

In a recent letter to this Journal, Hb oxygen affinity was shown to be unaltered in a cohort of 14 patients infected with SARS-CoV-2.[11] However, steady-state measurements of affinity cannot predict the kinetics of gas exchange by RBCs, which may become rate-limiting in COVID-19 due to impaired perfusion of the injured lung and inflammation-triggered RBC deformations that expand intracellular diffusion path length. Moreover, measurements on whole blood report an ensemble population average, which cannot resolve the presence of small subpopulations of dysfunctional RBCs, if these emerge in COVID-19. Indeed, given that RDW-SD increases in COVID-19, O_2 handling must be interrogated with cellular resolution.

We recently designed single-cell oxygen saturation imaging to assess O_2 unloading kinetics and O_2 storage capacity on a cell-by-cell basis.[12] We now applied this technique to study blood from COVID-19 patients at the John Radcliffe Hospital, Oxford, UK. Ten SARS-CoV-2-positive patients [9/10 confirmed by polymerase chain reaction (PCR) result, remaining patient diagnosed clinically] were recruited to this study through the Oxford GI Biobank (ethics 16/YH/0247). In half of the patients, blood was sampled within the first two weeks of diagnosis, and for the other half, sampling was in the subsequent fortnight. Three patients were asymptomatic healthcare workers, identified by voluntary PCR testing, and the

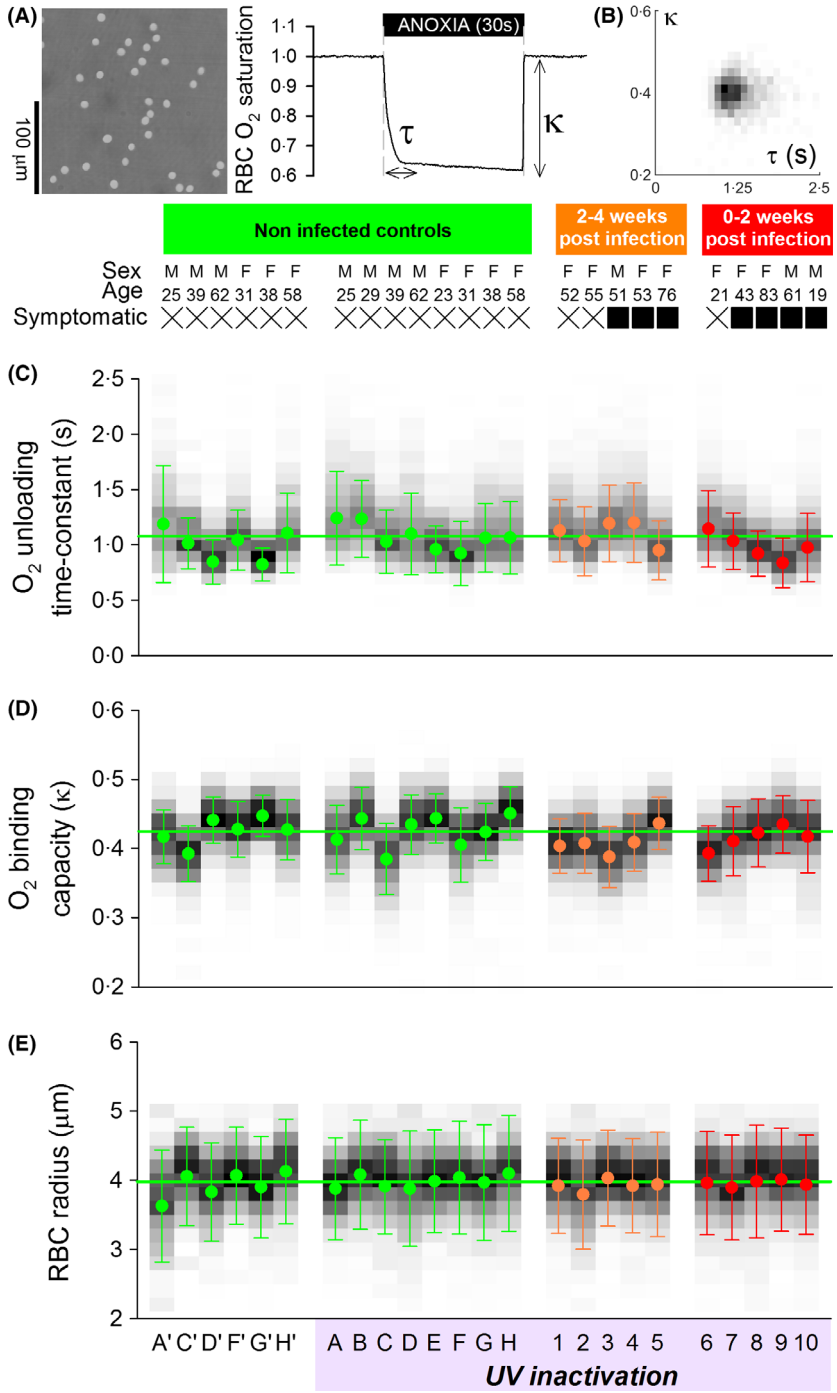

Fig 1. Single-cell characterisation of O_2 handling in red blood cells (RBCs) from patients with SARS-CoV-2 infection. (A) RBCs were loaded with CytoTracker Green and DeepRed to measure O_2 saturation during rapid and transient deoxygenation (N_2-bubbled solution, with 1 mmol/l dithionite). Protocol quantifies O_2 handling in terms of unloading time-constant (τ) and binding capacity (κ) in fresh venous blood sample from COVID-19 patients. (B) Cell-by-cell frequency distribution of τ and κ shows single population. Quantification of (C) τ, (D) κ and (E) radius in non-infected controls (A–H), and patients (1–10) tested positive for SARS-CoV-2 at two time points relative to infection. Viral inactivation by UV light in the presence of 10 mmol/l ascorbate was performed; based on findings from control blood, this treatment had no effect on measured parameters. Greyscale shading illustrates histogram distribution of each parameter. Data presented as mean, and error bars denote width of distribution at half maximal height. >1000 cells per sample.

remaining seven presented with COVID-19 symptoms. None of the patients had a history of RBC disorders or haemoglobinopathy. For reference measurements, healthy donors were recruited. Venous blood samples were spun down (1200 RCF for 3 min at room temperature) to collect 10 µl of red cells, which were re-suspended in 4 ml of HEPES-buffered

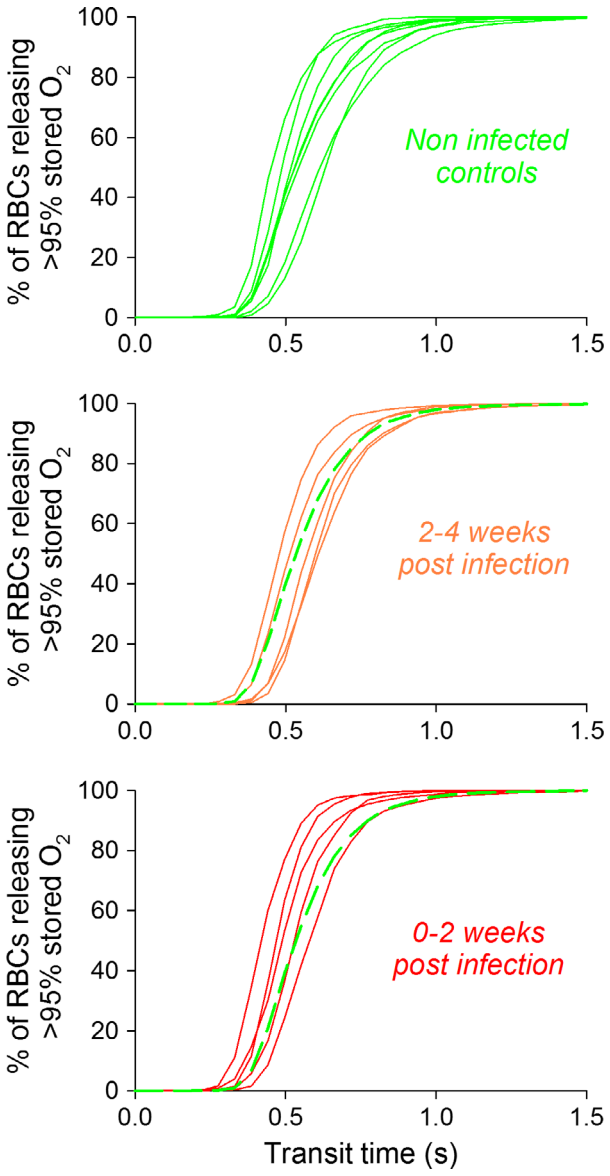

Fig 2. Fraction of red blood cells (RBCs) that deoxygenate by >95% during given transit time. SARS-CoV-2 infection does not affect O_2 release kinetics. Analysis of histogram distributions shown in Fig 1 using equations derived previously in Richardson *et al.*[12]

Tyrode solution containing 10 mmol/l ascorbate and then treated with UV-C light (4000 μW/cm^2 at a distance of 6 cm for 15 min, UVS-18 EL Series UV lamp, Analytik-Jena, Germany) to inactivate the virus. Ascorbate was included to protect Hb from oxidative damage by UV light. After resuspending in fresh Tyrode solution (containing no ascorbate), cells were loaded with CellTracker DeepRed and Green (ThermoFisher Scientific, Waltham, MA, USA) to produce HbO_2-sensitive fluorescence on a confocal imaging system.[12] Rapid solution switching between an oxygenated and deoxygenated microstream triggered O_2 exchange, which was imaged at high temporal resolution for each RBC individually (Fig 1A). Repeating this several times yielded a measure of O_2 unloading kinetics (time constant τ), normalised O_2 binding capacity (κ) and cell radius in the horizontal plane. Data were analysed in terms of their frequency distribution (Fig 1B).

RBC τ, κ and radius were no different in COVID-19 patients, compared to controls (Fig 1C–E). There was also no evidence for a change in the distribution of these functional variables, arguing against the emergence of dysfunctional subpopulations over the course of infection. When expressed in terms of the fraction of cells that release >95% of stored oxygen in a given time (equivalent to capillary transit), there was no effect of SARS-CoV-2 infection on RBC O_2 handling (Fig 2).

Our findings add new evidence that SARS-CoV-2 infection does not lead to dysfunctional convective transport. The cause of hypoxaemia in COVID-19 patients is therefore unlikely to relate to impaired O_2 handling by RBCs, either as a result of direct coronavirus infection or a consequence of the inflammatory state. Our findings argue against a mechanistic link between Hb variants and disease outcomes.

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Author contributions

KCP, NR and PS performed the research. KD, SM and PS designed the research study. NR and PK contributed essential reagents or tools. KCP, KD and PS analysed the data. PS wrote the paper. The authors thank Dr Barbara Kronsteiner and Dr Emily Adland for coordinating blood sample supply.

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References

1. Gattinoni L, Chiumello D, Rossi S. COVID-19 pneumonia: ARDS or not? *Crit Care*. 2020a;**24**:154.
2. Gattinoni L, Coppola S, Cressoni M, Busana M, Rossi S, Chiumello D. COVID-19 does not lead to a "Typical" acute respiratory distress syndrome. *Am J Respir Crit Care Med*. 2020b;**201**:1299–300.
3. Liu W, Li H. COVID-19: attacks the 1-beta chain of hemoglobin and captures the porphyrin to inhibit human heme metabolism. *ChemRxiv*. 2020. https://doi.org/10.26434/chemrxiv.11938173.v11938178.
4. Read RJ. Flawed methods in "COVID-19: Attacks the 1-beta chain of hemoglobin and captures the porphyrin to inhibit human heme metabolism". *ChemRxiv*. 2020. https://doi.org/10.26434/chemrxiv.12120912.v12120912.
5. Cavezzi A, Troiani E, Corrao S. COVID-19: hemoglobin, iron, and hypoxia beyond inflammation. A narrative review. *Clin Pract*. 2020;**10**:1271.
6. Lansiaux E, Pebay PP, Picard JL, Son-Forget J. COVID-19: beta-thalassemia subjects immunised? *Med Hypotheses*. 2020;**142**:109827.
7. Sotoudeh E, Sotoudeh H. A hypothesis about the role of fetal hemoglobin in COVID-19. *Med Hypotheses*. 2020;**144**:109994.
8. Bao J, Li C, Zhang K, Kang H, Chen W, Gu B. Comparative analysis of laboratory indexes of severe and non-severe patients infected with COVID-19. *Clin Chim Acta*. 2020;**509**:180–94.
9. Liu X, Zhang R, He G. Hematological findings in coronavirus disease 2019: indications of progression of disease. *Ann Hematol*. 2020;**99**:1421–8.
10. Wang C, Deng R, Gou L, Fu Z, Zhang X, Shao F, et al. Preliminary study to identify severe from moderate cases of COVID-19 using combined hematology parameters. *Ann Transl Med*. 2020;**8**:593.
11. 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. https://doi.org/10.1111/bjh.16888
12. Richardson SL, Hulikova A, Proven M, Hipkiss R, Akanni M, Roy NBA, et al. Single-cell O_2 exchange imaging shows that cytoplasmic diffusion is a dominant barrier to efficient gas transport in red blood cells. *Proc Natl Acad Sci USA*. 2020;**117**:10067–78.