5. Kumar S, Maurya VK, Prasad AK, Bhatt MLB, Saxena SK. Structural, glycosylation and antigenic variation between 2019 novel coronavirus (2019-nCoV) and SARS coronavirus (SARS-CoV). *Virusdisease*. 2020;**31**(1):13–21.
6. Ellinghaus D, Degenhardt F, Bujanda L, Buti M, Albillos A, Invernizzi P, et al. The ABO blood group locus and a chromosome 3 gene cluster associate with SARS-CoV-2 respiratory failure in an Italian-Spanish genome-wide association. *Analysis*. 2020;**2020**(05):pp. 31.20114991.
7. Terao C, Bayoumi N, McKenzie CA, Zelenika D, Muro S, Mishima M, et al. Quantitative variation in plasma angiotensin-I converting enzyme activity shows allelic heterogeneity in the ABO blood group locus. *Ann Hum Genet*. 2013;**77**(6):465–71.
8. Luo JQ, He FZ, Luo ZY, Wen JG, Wang LY, Sun NL, et al. Rs495828 polymorphism of the ABO gene is a predictor of enalapril-induced cough in Chinese patients with essential hypertension. *Pharmacogenet Genomics*. 2014;**24**(6):306–13.
9. Pleiotropic effect of common variants at ABO Glycosyltranferase locus in 9q32 on plasma levels of pancreatic lipase and angiotensin converting enzyme. *PLoS One*. 2014;**9**(2):e55903.
10. Wu O, Bayoumi N, Vickers MA, Clark P. ABO(H) blood groups and vascular disease: a systematic review and meta-analysis. *Journal of thrombosis and haemostasis : JTH*. 2008;**6**(1):62–9.
11. Paré G, Chasman DI, Kellogg M, Zee RY, Rifai N, Badola S, et al. Novel association of ABO histo-blood group antigen with soluble ICAM-1: results of a genome-wide association study of 6,578 women. *PLoS Genet*. 2008;**4**(7):e1000118.
12. Delanghe JR, De Buyzere ML, Speeckaert MM. C3 and ACE1 polymorphisms are more important confounders in the spread and outcome of COVID-19 in comparison with ABO polymorphism. *Eur J Prev Cardiol*. 2020;2047487320931305.
13. Breiman A, Ruvën-Clouet N, Le Pendu J. Harnessing the natural anti-glycan immune response to limit the transmission of enveloped viruses such as SARS-CoV-2. *PLoS Pathog*. 2020;**16**(5):e1008556.
14. Tendulkar AA, Jain PA, Velaye S. Antibody titers in Group O platelet donors. *Asian journal of transfusion science*. 2017;**11**(1):22–7.
15. Gérard C, Maggipinto G, Minon JM. COVID-19 & ABO blood group: another viewpoint. *Br J Haematol*. 2020.
16. Shaikh S, Sloan SR. Clearance of maternal isohemagglutinins from infant circulation (CME). *Transfusion*. 2011;**51**(5):938–42.
17. Liu YJ, Chen W, Wu KW, Broadberry RE, Lin M. The development of ABO isohemagglutinins in Taiwanese. *Hum Hered*. 1996;**46**(4):181–4.
18. Grifoni A, Weiskopf D, Ramirez S, Smith D, Crotty S. Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. *Cell*. 2020:S0092-8674(20)30610-3
19. Larsen MD, de Graaf EL, Sonneveld ME, Plomp HR, Linty F, Visser R, et al. Afucosylated immunoglobulin G responses are a hallmark of enveloped virus infections and show an exacerbated phenotype in COVID-19. 2020:2020.05.18.099507.
20. Zhao J, Yang Y, Huang H, Li D, Gu D, Lu X, et al. Relationship between the ABO Blood Group and the COVID-19 Susceptibility. *medRxiv*. 2020;**2020**(03):11.20031096.
21. Zeng X, Fan H, Lu D, Huang F, Meng X, Li Z, et al. Association between ABO blood groups and clinical outcome of coronavirus disease 2019: Evidence from two cohorts. 2020;2020(04):15.20063107.
22. Zietz M, Tatonetti NP. Testing the association between blood type and COVID-19 infection, intubation, and death. 2020;2020(04):pp. 08.20058073.
23. Göker H, Aladağ Karakulak E, Demiroğlu H, Ayaz Ceylan ÇM, Büyükaşik Y, Inkaya A, et al. The effects of blood group types on the risk of COVID-19 infection and its clinical outcome. *Turkish journal of medical sciences*. 2020.


# Amelioration of COVID-19-related cytokine storm syndrome: parallels to chimeric antigen receptor-T cell cytokine release syndrome

## Case series

Coronavirus disease 2019 (COVID-19) severity appears to parallel the host immune response, with a subset of patients developing COVID-19 cytokine storm syndrome (CSS).[1] Serum inflammatory cytokines are elevated in COVID-19[2–5] and interleukin 6 (IL-6) appears to play a central role in COVID-19-related CSS.[6–8] Based on the success of IL-6-receptor blockade for chimeric antigen receptor T-cell therapy associated cytokine release syndrome (CAR-T cell CRS), similar strategies using tocilizumab are being investigated in COVID-19. However, early reports described only modest elevations of IL-6 of approximately 50 pg/ml (reference range <7 pg/ml) in severe COVID-19[2–4,9] compared to IL-6 levels often >10 000 pg/l in CAR-T cell CRS,[10] leading authors to conclude that COVID-19 pathophysiology is attributable to alternate mechanisms apart from CSS.[11]

Two central mechanistic considerations may help resolve this controversy. First, determining if COVID-19 is associated with markedly elevated IL-6, in the range seen in CAR-T cell CRS, is crucial. Second, current trials are focussing on mortality and ventilation endpoints, but data pertaining to the effect of IL-receptor blockade on inflammatory cytokine levels and cardiorespiratory outcomes are needed to established biological efficacy. We therefore conducted a preliminary evaluation of tocilizumab on inflammatory cytokines including IL-1β, IL-6, IL-10 and tumour necrosis factor alpha (TNF-α), and physiological parameters in five consecutive patients with severe COVID-19 CSS. Study approval was obtained from the institutional research ethics board.

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection was confirmed by real-time reverse transcription polymerase chain reaction from a tracheal aspirate. All patients underwent invasive mechanical ventilation and were diagnosed with acute respiratory distress syndrome (ARDS).[12] Two patients required veno–venous extracorporeal membrane oxygenation (VV-ECMO) for refractory hypoxaemia. Tocilizumab was administered (single 400 mg dose)[13]

as part of clinical care, based on objective manifestations of COVID-19 CSS: (i) COVID-19 pneumonia requiring mechanical ventilation; (ii) fever ($T_{max}$ >38°C); (iii) C-reactive protein (CRP) >100 mg/l; and (iv) peak ferritin >1000 μg/l.

Serum was analysed for IL-1β, IL-6, IL-10 and TNF-α using the Quanterix® Single Molecule Array (Simoa®) HD-1 analytical platform daily between 08:00 and 09:00 hours.[14] Routine clinical laboratory data including full blood count, D-dimer (μg/l), CRP (mg/l), ferritin (μg/l), CD4/CD8 ratio, CD4 (%) and CD8 (%) were collected. Changes in IL-1β, IL-6, IL-10, TNF-α, and CRP were assessed using the Friedman's test, with a Wilcoxon signed-ranks test for *post hoc* comparisons ($\alpha = 0{\cdot}05$).

Four patients were male and one was female. The median (range) age was 61 (32–73) years and body mass was 70 (67–75) kg. Four patients presented to hospital with symptoms of fever, all five with cough, and two with headache. All five of the patients had lymphopenia [median (range) 0·6 (0·3–0·8)× $10^9$ cells] and elevated D-dimer [median (range) 2640 (630–7000) μg/l], ferritin [median (range) 2932 (1051–5638) μg/l] and CRP [median (range) 225 (126–374) mg/l]. The admission median (range) CD4 and CD8 percentages were 42 (35–53)% and 25 (16–33)% respectively, while the CD4/CD8 ratio was 1·46 (1·15–2·69).

All patients had markedly increased peak serum IL-6 levels during the course of observation [median (range) 2023 (360–13140) pg/ml] (Fig 1), commensurate in magnitude to that observed with CAR-T cell CRS.[10] Tocilizumab was administered at a median (range) of 2 (1–7) days following mechanical ventilation and was associated with significant reductions in IL-6 ($P = 0{\cdot}027$), IL-10 ($P = 0{\cdot}009$), TNF-α ($P = 0{\cdot}012$), as well as CRP ($P < 0{\cdot}001$) (Fig 1). The lymphocyte count increased following tocilizumab administration, but was not statistically significant (+1·0 × $10^9$ cells, 95% confidence interval −0·3 to 1·3; $P = 0{\cdot}063$; Fig 1). Statistical analyses of pre- and post-tocilizumab administration D-dimer and ferritin were not conducted due to missing daily values. The ratio of arterial oxygen tension to fraction of inspired oxygen percentage ($PaO_2/FiO_2$), an index of pulmonary gas exchange efficiency, increased from a pre-treatment value of 123 (range 60–140) to 251 (range 147–310; $P = 0{\cdot}043$) on day 6 following tocilizumab administration (Fig 2). Further, mean arterial pressure improved following tocilizumab on days 3 ($P = 0{\cdot}042$), 4 ($P = 0{\cdot}042$) and 6 ($P = 0{\cdot}041$) (Fig 2), which was reflected in markedly decreased intravenous noradrenaline dose requirements. As of 2 June 2020, four of the patients have been discharged home and one died in the intensive care unit.

Our case series provides evidence that patients with severe COVID-19 may exhibit hypercytokinaemia, in keeping with ranges described in CAR-T CRS. We further demonstrate reductions in serum inflammatory markers following tocilizumab administration that correlate with improved clinical

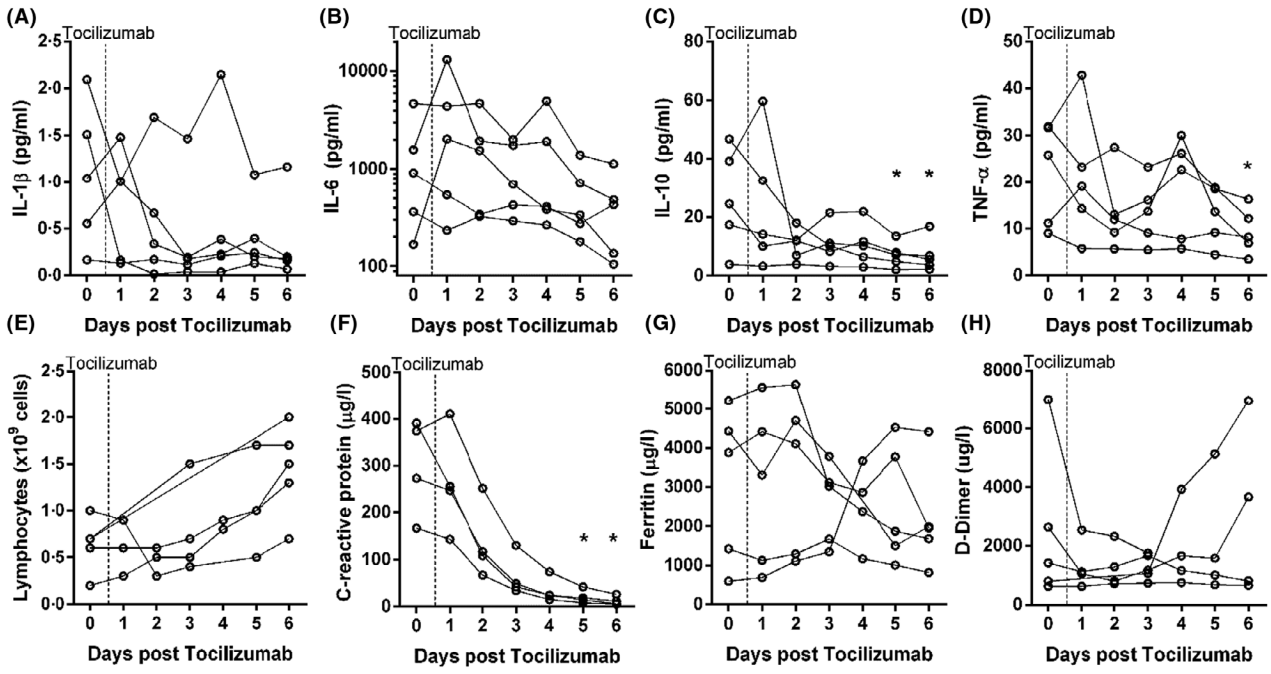


**Fig 1.** Tocilizumab reduces the concentration of circulating serum inflammatory cytokines and clinical laboratory variables. Top panel (A–D): levels of serum interleukin (IL)-1β, IL-6, IL-10 and tumour necrosis factor alpha (TNF-α) for each patient. Vertical dashed lines represent the timing of tocilizumab administration relative to daily measurements of cytokines. Bottom Panel (E–H): levels for lymphocyte count, C-reactive protein, ferritin and D-dimer for each patient. Vertical dashed lines represent the timing of tocilizumab administration relative to daily measurements of clinical laboratory value. *denotes a significant difference from Day 0, $P < 0{\cdot}05$.

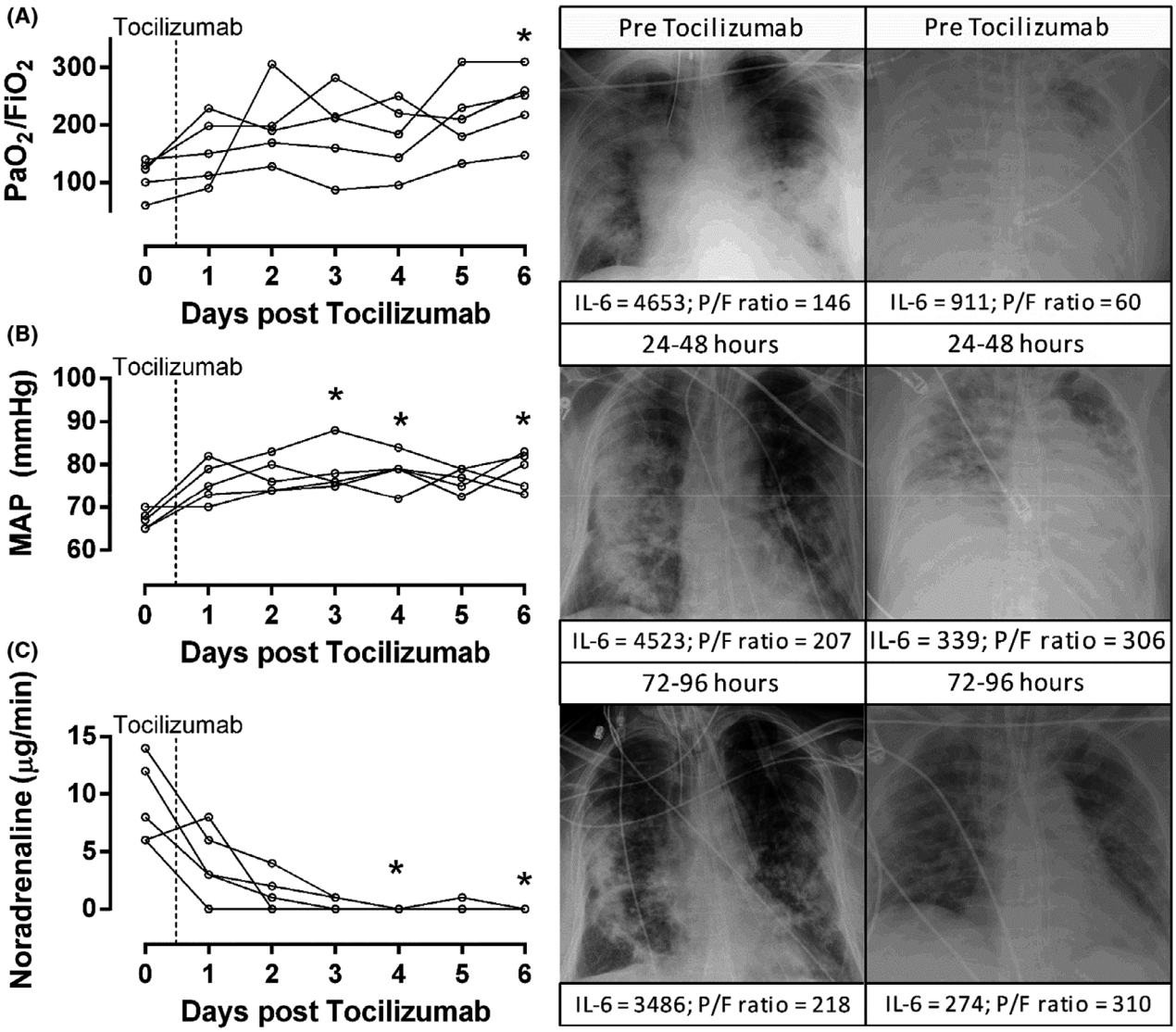


**Fig 2.** Changes in cardiorespiratory function following tocilizumab. The $PaO_2/FiO_2$ ratio, mean arterial pressure (MAP), and noradrenaline dose prior to and following tocilizumab administration. For panels A–C, the physiological variable is displayed on the *y*-axis with corresponding units, while days after tocilizumab administration are on the *x*-axis. Chest X-rays are presented for two patients within 24-h prior to drug administration and then 24–48 h and 72–96 h following drug administration. Individual IL-6 and $PaO_2/FiO_2$ (P/F) ratio data are displayed. For the patient on the left, note that tocilizumab treatment lead to radiographic improvement, despite persistently high inflammation (IL-6 >3400 pg/ml for all time points). This patient has been discharged home. The patient on the right required VV-ECMO for refractory hypoxaemia. Tocilizumab led to radiographic improvement in this patient, who has been discharged home. $PaO_2$, partial pressure of arterial oxygen; $F_IO_2$, fraction of inspired oxygen. *denotes a significant difference from Day 0, $P < 0{\cdot}05$.

parameters. Overall, these preliminary findings provide context for the biological plausibility of IL-6-receptor blockade in COVID-19 CSS. While tocilizumab has been used in IL-6-mediated inflammatory conditions, such as the CAR-T CRS,[10] investigation of the potential efficacy of tocilizumab for treating COVID-19 to date has been limited to cohorts that are not critically ill (e.g. requiring mechanical ventilation[13]). To the best of our knowledge, our present case series is the first to demonstrate an association between tocilizumab administration and reductions in multiple key inflammatory cytokines and laboratory variables in the severe spectrum of critically ill patients with COVID-19 (requiring invasive mechanical ventilation and VV-ECMO). Importantly, all patients deemed to have COVID-19 CSS had markedly elevated IL-6 levels, as high as 13 140 pg/ml, which to our knowledge is one of the highest serum IL-6 values reported in a patient with COVID-19 (Fig 1).[15] A recent case study on a single patient with COVID-19 with ARDS recorded extreme elevations of IL-6 in peripheral blood (*c.* 34 000 pg/ml) and pleural fluid (*c.* 24 000 pg/ml),[15] whereas the majority of studies report median IL-6 values of approximately 50 pg/ml in cohorts of patients with severe COVID-

19.[9] In fact, Chen *et al*.,[3] reported a median (interquartile range) IL-6 value of 41.5 (24·8–114.2) pg/ml in an observational study of 'severe' COVID-19, the median of which is >20-fold lower than that of our present patients.

The results of our present case series should not be used to unequivocally advocate for the use of tocilizumab in COVID-19, but instead provide context to upcoming and emerging trials of IL-6-receptor blockade. Attention should be given to defining COVID-19 CSS and elucidating the phenotype of patients most likely to benefit from IL-6-receptor blockade.

## Funding

Dr Sekhon is supported by the Vancouver Coastal Health Research Institute Clinician Scientist Award. This work was funded in part by the Vancouver General Hospital Foundation.

## Author contributions

Designed Research: Ryan L. Hoiland, Sophie Stukas, Jennifer Cooper, Cheryl L. Wellington, Mypinder S. Sekhon; Performed Research: Ryan L. Hoiland, Sophie Stukas, Jennifer Cooper, Sonny Thiara, Luke Y. C. Chen, Catherine M. Biggs, Kevin Hay, Agnes Y. Y. Lee, Kamran Shojania, Alym Abdulla, Cheryl L. Wellington, Mypinder S. Sekhon; Wrote the paper: Ryan L. Hoiland, Sophie Stukas, Jennifer Cooper, Luke Y. C. Chen, Cheryl L. Wellington, Mypinder S. Sekhon; Revision for critical intellectual content: Sonny Thiara, Catherine M. Biggs, Kevin Hay, Agnes Y. Y. Lee, Kamran Shojania, Alym Abdulla; Approval of final version: Ryan L. Hoiland, Sophie Stukas, Jennifer Cooper, Sonny Thiara, Luke Y. C. Chen, Catherine M. Biggs, Kevin Hay, Agnes Y. Y. Lee, Kamran Shojania, Alym Abdulla, Cheryl L. Wellington, Mypinder S. Sekhon.

## Conflicts of interest

The authors declare no conflicts financial or otherwise.

**Ryan L. Hoiland**[1,2,†]
**Sophie Stukas**[3,†]
**Jennifer Cooper**[3,†]
**Sonny Thiara**[4]
**Luke Y. C. Chen**[5]
**Catherine M. Biggs**[6]
**Kevin Hay**[5]
**Agnes Y. Y. Lee**[5]
**Kamran Shojania**[7]
**Alym Abdulla**[5]
**Cheryl L. Wellington**[3,‡]
**Mypinder S. Sekhon**[4,‡]

*[1]Department of Anesthesiology, Pharmacology and Therapeutics, University of British Columbia, Vancouver, [2]Centre for Heart, Lung, and Vascular Health, School of Health and Exercise Sciences, University of British Columbia – Okanagan, Kelowna, [3]Department of Pathology and Laboratory Medicine, International Collaboration on Repair Discoveries, School of Biomedical Engineering, Djavad Mowafaghian Centre for Brain Health, Faculty of Medicine, University of British Columbia, Vancouver, [4]Division of Critical Care Medicine, Department of Medicine, University of British Columbia, Vancouver, [5]Division of Hematology, Department of Medicine, University of British Columbia, Vancouver, [6]Division of Allergy and Immunology, Department of Medicine, University of British Columbia, Vancouver and [7]Division of Rheumatology, Department of Medicine, University of British Columbia, Vancouver, BC, Canada.*
*E-mail: mypindersekhon@gmail.com*

[†]Authors are denoted as co-first authors.
[‡]Authors are denoted as co-senior authors.

First published online 16 July 2020
doi: 10.1111/bjh.16961

## References

1. England JT, Abdulla A, Biggs CM, Lee AY, Hay KA, Hoiland RL, et al. Weathering the COVID-19 storm: lessons from hematologic cytokine syndromes. *Blood Rev*. 2020;**15**:100707.
2. Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y, et al. Dysregulation of immune response in patients with COVID-19 in Wuhan, China. *Clin Infect Dis*. 2020 [Epub ahead of print]. https://doi.org/10.1093/cid/ciaa248
3. Chen G, Wu D, Guo W, Cao Y, Huang D, Wang H, et al. Clinical and immunological features in severe and moderate Coronavirus Disease 2019. *J Clin Invest*. 2020;**130**:2620–9.
4. Wang Z, Yang B, Li Q, Wen L, Zhang R. Clinical Features of 69 cases with coronavirus disease 2019 in Wuhan, China. *Clin Infect Dis*. 2020 [Epub ahead of print]. https://doi.org/10.1093/cid/ciaa272
5. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020;**395**:497–506.
6. Wu Z, McGoogan JM. Characteristics of and important lessons from the Coronavirus Disease 2019 (COVID-19) outbreak in china summary of a report of 72314 cases from the Chinese center for disease control and prevention. *JAMA*. 2020;**323**:1239–42.
7. Chen T, Wu D, Chen H, Yan W, Yang D, Chen G, et al. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. *BMJ*. 2020;**368**:m1091.
8. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet*. 2020;**395**:1054–62.
9. Aziz M, Fatima R, Assaly R. Elevated Interleukin-6 and Severe COVID-19: a meta-analysis. *J Med Virol*. 2020 [Epub ahead of print]. https://doi.org/10.1002/jmv.25948
10. Maude SL, Frey N, Shaw PA, Aplenc R, Barrett DM, Bunin NJ, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. *N Engl J Med*. 2014;**371**:1507–17.
11. Leisman DE, Deutschman CS, Legrand M. Facing COVID-19 in the ICU: vascular dysfunction, thrombosis, and dysregulated inflammation. *Intensive Care Med*. 2020;**46**:1105–8.
12. Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, Fan E, et al. Acute respiratory distress syndrome: the Berlin definition. *J Am Med Assoc*. 2012;**307**:2526–33.
13. Xu X, Han M, Li T, Sun W, Wang D, Fu B, et al. Effective treatment of severe COVID-19 patients with tocilizumab. *Proc Natl Acad Sci*. 2020;**117**:10970–5.

14. Wilson DH, Rissin DM, Kan CW, Fournier DR, Piech T, Campbell TG, et al. The Simoa HD-1 analyzer: a novel fully automated digital immunoassay analyzer with single-molecule sensitivity and multiplexing. *J Lab Autom*. 2016;**21**:533–47.

15. Wang C, Kang K, Gao Y, Ye M, Lan X, Li X, et al. Cytokine levels in the body fluids of a patient with COVID-19 and acute respiratory distress syndrome: a case report. *Ann Intern Med*. 2020 [Epub ahead of print]. https://doi.org/10.7326/L20-0354

# Convalescent plasma for persisting COVID-19 following therapeutic lymphocyte depletion: a report of rapid recovery

We read with deep interest the report by Tepasse *et al.*[1] concerning two cases of persisting viraemia in coronavirus disease 2019 (COVID-19) with fatal outcome. Whilst severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection in the early stages of infection has been well described, less is known about the development of antibodies to SARS-CoV-2, clearance of RNA shedding and clinical outcome of COVID-19. In addition, the impact of immunosuppressive treatments on disease severity is not yet established, but several reports suggest a more prolonged disease in patients under rituximab, a B-cell depleting drug.[2–4] Here we report a case of persisting COVID-19, following combined treatment with rituximab and bendamustine for lymphoma, which immediately recovered after convalescent plasma transfusion. We think that this case raises promising perspectives for immunocompromised patients with persisting COVID-19.

A 76-year-old woman was diagnosed in 2019 with orbital and meningeal marginal zone lymphoma in the context of probable unrecognised Sjögren's syndrome with positive salivary gland biopsy (focus score = 2). Bendamustine and rituximab were administrated on 12 February (70 mg/m$^2$ of bendamustine) and 16 March 2020 (90 mg/m$^2$), inducing a decay of lymphocyte count from 1410/μl on 11 February to 160/μl on 17 March 2020. Granulocyte-colony stimulating factor prophylaxis was started thereafter.

She consulted her general practitioner on 26 March 2020 (Day 1) with fever, diarrhoea and deep fatigue. Home surveillance was initially decided. On 1 April (Day 7), she was referred to our hospital for severe pneumonia (tachypnoea, fever and desaturation requiring oxygen). Blood testing revealed lymphopenia (550/μl) in all lymphocytic subtypes: 88 lymphocyte T CD4 cells/μl (16·0%), 385 lymphocyte T CD8 cells/μl (69·6%), 3 lymphocyte B cells/μl (0·6%), 59 natural killer cells/μl (10·7%), associated with thrombocytopenia ($65 \times 10^9$/l) and an inflammatory syndrome [neutrophilic leucocytosis of $25{\cdot}11 \times 10^3$/μl and a C-reactive protein (CRP) of 24 mg/l]. Ground-glass bilateral opacities and consolidations were observed on chest computed tomography (CT). SARS-CoV-2 infection was confirmed by RNA reverse transcriptase-polymerase chain reaction (RT-PCR) on a nasopharyngeal swab.

A combination of lopinavir/ritonavir was given between days 9 and 24. Faced with worsening of clinical symptoms (confusion and increased oxygen requirement) and extension of the opacities on chest CT, a treatment with prednisone (50 mg/day) for 7 days was introduced on day 27. Apyrexia and oxygen withdrawal ensued. However, symptoms relapsed within 48 h of prednisone withdrawal, and persisted during the sixth week of admission, requiring oxygen administration due to desaturation, relapse of fever.

Follow-up chest CT on day 36 and day 44 showed an increase in ground-glass and consolidation opacities. SARS-CoV-2 RNA remained positive on 10 repeated nasopharyngeal swab tests (Fig 1). By contrast, SARS-CoV-2 antibodies remained undetectable at Day 47. Intravenous convalescent plasma obtained from SARS-CoV-2 survivors was administered starting at day 50 over 2 days, after obtaining the patient's informed consent, (2 units of 200 ml/day). No adverse events occurred. The patient tested positive for SARS-CoV-2 anti-nucleocapsid and anti-Spike immunoglobulin G (IgG) after the two first plasma units. Her health condition quickly improved, allowing definitively withdrawing oxygen, apyrexia ensued, and a decrease in CRP level within 24 h was objectified. SARS-CoV-2 RNA became undetectable on Day 57 and remained negative on Day 62. She returned home on Day 69 and completely recovered after 17 additional days of follow-up.

## Discussion

To date, treatment of COVID-19 is still challenging and there is no specific recommended therapy. Despite the sequential introduction of different treatments, our patient experienced an unusual delayed clinical worsening, a persisting clinical infection and a prolonged viral shedding. Such a course is unusual, as the median time to clinical worsening is approximately 8–10 days. Furthermore, the median time until viral RNA clearance attested by PCR on a nasopharyngeal swab, is estimated around 17–24 days in hospitalised patients.[5] Prolonged viral RNA shedding over 15 days is not infrequent, especially in elderly and severe COVID-19 cases.[6] In patients with prolonged viral shedding, the symptoms had retrieved whilst SARS-COV-2 RNA remained detectable in pharyngeal swabs at