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). Serum inflammatory cytokines are elevated in COVID-19 and interleukin 6 (IL-6) appears to play a central role in COVID-19-related CSS. 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 1-7 pg/ml) in severe COVID-19 compared to IL-6 levels often >10 000 pg/l in CAR-T cell CRS, leading authors to conclude that COVID-19 pathophysiology is attributable to alternate mechanisms apart from CSS.

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 establish 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). Two patients required veno-venous extracorporeal membrane oxygenation (VV-ECMO) for refractory hypoxaemia. Tocilizumab was administered (single 400 mg dose).

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British Journal of Haematology, 2020, 190, e126-e156
as part of clinical care, based on objective manifestations of COVID-19 CSS: (i) COVID-19 pneumonia requiring mechanical ventilation; (ii) fever ($T_{\text{max}}>38^\circ\text{C}$); (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. 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 ($\alpha=0.05$).

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) 0.16 (0.13–0.18) x 10⁹ 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) 30023 (360–13140) pg/ml] (Fig 1), commensurate in magnitude to that observed with CAR-T cell CRS. Tocilizumab was administered at a median (range) of 2 (1–7) days following mechanical ventilation and was associated with significant reductions in IL-6 ($P=0.027$), IL-10 ($P=0.009$), TNF-α ($P=0.012$), as well as CRP ($P<0.001$) (Fig 1). The lymphocyte count increased following tocilizumab administration, but was not statistically significant (+1.0 x 10⁹ cells, 95% confidence interval −0.3 to 1.3; $P=0.063$; 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 (PaO₂/FiO₂), an index of pulmonary gas exchange efficiency, increased from a pre-treatment value of 123 (range 60–140) to 251 (range 147–310; $P=0.043$) on day 6 following tocilizumab administration (Fig 2). Further, mean arterial pressure improved following tocilizumab on days 3 ($P=0.042$), 4 ($P=0.042$) and 6 ($P=0.041$) (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, $P<0.05$.](https://example.com/fig1)
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, 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). 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). 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), whereas the majority of studies report median IL-6 values of approximately 50 pg/ml in cohorts of patients with severe COVID-
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\(^1\), 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\(^1\)*

\(^1\)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, Djavud Movafaghian 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.

\(^\dagger\)Authors are denoted as co-senior authors.

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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 in infection and a prolonged viral shedding. Such a course is unusual due to desaturation, relapse of fever.

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 administered 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/\(\mu\)l on 11 February to 160/\(\mu\)l 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/\(\mu\)l) in all lymphocytic subtypes: 88 lymphocyte T CD4 cells/\(\mu\)l (16-0%), 385 lymphocyte T CD8 cells/\(\mu\)l (69-6%), 3 lymphocyte B cells/\(\mu\)l (0-6%), 59 natural killer cells/\(\mu\)l (10-7%), associated with thrombocytopenia (65 x 10\(^9\)/l) and an inflammatory syndrome [neutrophilic leucocytosis of 25-11 x 10\(^9\)/l 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