Case report

Cytokine profiles in severe SARS-CoV-2 infection requiring extracorporeal membrane oxygenation support

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A R T I C L E   I N F O

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A B S T R A C T

It has been postulated that the underlying pathophysiology of COVID-19 is mediated by cytokine storm resulting in a hyperinflammatory state. A similar kind of cytokine-storm has been described in individuals undergoing veno-venous extracorporeal membrane oxygenation (VV ECMO) support. There is therefore concern that initiation of VV ECMO support among COVID19 patients could further exacerbate this dysregulated inflammatory response. In this prospective cohort study, we describe the clinical course and cytokine fluctuations in eight subjects treated with VV ECMO for management of refractory respiratory failure from COVID19. Among all eight patients, cytokine elevations were noted among Interleukin 6 (IL-6), Interleukin 10 (IL-10), and Interleukin 2 Receptor (CD25) soluble (sIL2R). Although further research is necessary, among our cohort of patients it did not appear that initiation of VV ECMO worsened cytokine storm.

The highest cause of morbidity and mortality in COVID-19 is acute respiratory failure that may require mechanical ventilation [1]. A small number of these cases progress to refractory hypoxemic respiratory failure, prompting initiation of veno-venous extracorporeal membrane oxygenation (VV-ECMO) support. It has been postulated that a substantial portion of the pathophysiology in SARS-CoV-2 infection is mediated by host immune activation [2], also referred to as cytokine storm. Although cytokines have an important role in defense against pathogens, dysregulated response can result in endothelial dysfunction, impaired oxygen extraction and loss of vascular tone [3]. Reports from Wuhan, China found critically ill COVID-19 patients to have significant elevations particularly in IL-6, but additionally IL-2, IL-7, IL-10, and TNF alpha in critically ill patients with COVID19. [4,5].

A similar kind of cytokine storm has previously been described in individuals undergoing ECMO support. Studies have shown that among patients who require ECMO support, IL-6 is persistently elevated. Additionally, animal studies have shown that this increase in IL-6 during ECMO contributes to lung parenchymal damage [6]. Given that cytokine storm has been implicated as a major contributor to acute lung injury, shock and mortality among COVID19 patients, there is concern that initiation of VV ECMO support could further exacerbate this dysregulated inflammatory response. We describe the clinical course and cytokine fluctuations in eight subjects treated with VV ECMO for management of refractory respiratory failure from COVID-19 infection.

This study comprised a cohort of the first eight COVID-19 patients admitted to Yale New Haven Hospital who required VV ECMO support during March–April 2020. Hospital guidelines included treatment with hydroxychloroquine for moderately ill patients as defined by any oxygen requirement, and tocilizumab, an IL-6 receptor monoclonal antibody for severely ill patients as defined by high sensitivity C-reactive protein (hsCRP) greater than 70 and oxygen requirement of 3L nasal cannula or greater.

All eight subjects were male; an average age of 45.5 years (range 32–56); most common ethnicity Hispanic (87.5%) and most prevalent risk factors were obesity and hypertension (Table 1). The most common presenting symptoms were fever, cough and shortness of breath. Initial bloodwork revealed elevated inflammatory biomarkers with an average hsCRP of 146ng/mL, ferritin 4724ng/mL, and procalcitonin of 1.2ng/mL, along with absolute lymphocyte count 1.1k/ul. Initial chest x-ray demonstrated bilateral airspace opacities in all subjects.

All patients were treated with hydroxychloroquine on the day of presentation. Seven patients received tocilizumab within 48 hours of admission, and the remaining patient on day five of hospitalization. All patients were treated for ARDS with lung protective ventilation, prone-ventilation and neuro-muscular blockade. The mean time from admission to initiation of mechanical ventilation was 12 days and the mean...
and the mean time from mechanical ventilation to ECMO was 19 days from the start of symptoms. Of the 8 patients in this study, 4 were successfully decannulated, 1 remained on ECMO, and 3 died at the time of this report.

Throughout hospitalization, cytokine levels were measured every 48 hours during the hospitalization. The most significant cytokine elevations were among Interleukin 6 (IL-6), Interleukin 10 (IL-10), and Interleukin 2 Receptor (CD25) soluble (sIL2R). One patient had a transient elevation in Interferon gamma and Interleukin 17, however these normalized early in hospitalization. Importantly, Interleukin 1 beta, Interleukin 2, Interleukin 4, Interleukin 5, Interleukin 8, Interleukin 12, Interleukin 13 and Tumor Necrosis Factor-alpha remained within normal range throughout their hospitalization.

IL-6 levels were increased in all patients early in their course, with peak levels following administration of tocilizumab (Fig. 1). Among the five patients who remained alive at the conclusion of the study (ECMO survivors), IL-6 levels normalized an average of 11 days after tocilizumab and remained normal in four of these patients, even after initiating ECMO support. The fifth patient began to have an increase in IL-6 on day 24 of illness, the day before starting ECMO and continued to remain elevated. Among the three patients who died, two had improved but persistently elevated IL-6 levels. The third patient’s level normalized 12 days after tocilizumab yet began to increase five days after ECMO initiation. All three had elevated IL-6 levels at time of death.

Additionally, IL-10 was more than 7-fold greater than normal in two of three patients who died. Of the five patients who remained alive at the time of this report, two had normal IL-10 levels throughout their course, and the other three had self-limiting elevations, no more than 3 times normal, prior to ECMO.

Seven patients had sIL2R more than 1.5-fold greater than normal within 48 hours of hospitalization. Among the five patients who remained alive, two patients’ levels normalized prior to ECMO support. Two patients’ levels normalized while on ECMO support, however one patient’s level began to increase after day 18, five days after ECMO initiation. The fifth patient had persistently elevated sIL2R levels. Among the three patients who died, two patients experienced fluctuations of their sIL2R, yet they remained elevated. The third patient’s level was normal around the time of starting ECMO and it remitted normal when he died.

Studies have hypothesized that IL-6 may act as a marker of COVID-19 severity [4], which is supported by the initial IL-6 elevation seen in this cohort of critically ill patients. Furthermore, prior studies examining cytokines in ECMO demonstrate that consistently elevated IL-6 levels are correlated with mortality risk [2]. For this reason, there has been a theoretical concern regarding the utilization of VV-ECMO support in ARDS from COVID-19. Despite this, only one of our patients had an increase in IL-6 within 48 hours of ECMO initiation. Additionally, there were no consistent cytokine changes found with administration of steroids. Our limited sample size and differences in therapeutic approach among participants limits our ability to draw conclusions regarding the impact of different therapies on the development of cytokine storm upon ECMO initiation. With regard to mortality, among our patients who remained alive, four continued to have normal IL-6 levels and of those who died, two had elevated IL-6 levels at the time of death. Among these eight patients, four had normal cytokine levels 8 days after ECMO initiation. We propose that by this stage of illness, even after inflammation has improved, the prolonged effects of cytokine storm leave behind extensive damage.

*** Cytokine panel tests were performed via a quantitative multiplex bead assay by a CLIA-certified diagnostic lab in Salt Lake City, Utah. Lower limit of detection was 5pg/mL.

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### Table 1

**Patient clinical course.**

| Patient | Past medical history | Day of presentation (symptom onset – day 0) | Day of tocilizumab administration | Day of Intubation | Day of ECMO cannulation | Other Medications | Other Interventions | Outcome |
|---------|----------------------|-------------------------------------------|----------------------------------|------------------|------------------------|------------------|-------------------|--------|
| #1      | Hypertension, remote lupus, BMI 41.6 | 7                                         | 7                                | 9                | 21                     | methylprednisolone D9-12; D15-17; D25-28 ruxolitinib D29-39 methylprednisolone D10-16 | Tracheostomy D38 | Remains on ECMO, guarded prognosis (D45) | Died D36 |
| #2      | Type 2 diabetes, hypothyroidism, BMI 34.7 | 9                                         | 9                                | 9                | 10                     | remdesivir D11-20 methylprednisolone D25-28 ruxolitinib D29-37 methylprednisolone D15-18 remdesivir D28-38 | Tracheostomy D26 | Decannulated Day 35, remains mechanically ventilated (D39) | Died D36 |
| #3      | BMI 31.4              | 8                                         | 8                                | 16               | 23                     | remdesivir D11-20 methylprednisolone D25-28 ruxolitinib D29-37 methylprednisolone D15-18 remdesivir D28-38 | Tracheostomy D39 | Decannulated D47, remains mechanically ventilated (D54) | Remains on ECMO (D49) |
| #4      | Hypertension, hypothyroidism, BMI 34.7 | 14                                        | 16                               | 14               | 26                     | methylprednisolone D15-18 | Convalescent Serum D18 Tracheostomy D28 Cytokine Filter D31-36 CVVH D17-36 | Died D36 |
| #5      | Healthy, BMI 29.3     | 7                                         | 8                                | 7                | 20                     | methylprednisolone D15-18 | Convalescent Serum D18 Tracheostomy D28 Cytokine Filter D31-36 CVVH D17-36 | Died D36 |
| #6      | Healthy, BMI 28.4     | 10                                        | 12                               | 15               | 17                     | darunavir/ritonavir D10-17 methylprednisolone D17-22; D31-34 atazanavir D6-16 methylprednisolone D14-20; D35-41 | CVVH D18-44 | Died D44 |
| #7      | Hypertension, BMI 27.0 | 5                                         | 6                                | 12               | 18                     | methylprednisolone D12-15; D33-35 | None | Decannulated D23, remains mechanically ventilated (D35) | Died D44 |
| #8      | Healthy, BMI 35.1     | 3                                         | 8                                | 13               | 13                     | methylprednisolone D12-15; D33-35 | None | Decannulated D23, remains mechanically ventilated (D35) | Died D44 |
Declaration of competing interest

The authors declare that they have no conflicts of interest.

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Fig. 1. Cytokine trends among survivors vs. non-survivors.