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Thromboinflammation response to tocilizumab in COVID-19

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Abstract

Background: Coronavirus disease-19 (COVID-19) spans a wide spectrum of illness. Severe cases of COVID-19 can manifest inflammation in organs other than the lung, in tissues not known to support viral replication, and also in a hypercoagulable state. These observations have suggested that severe acute respiratory syndrome coronavirus 2 can provoke a hyperimmune response in some cases that could lead to secondary organ damage.

Methods: With evidence of elevated levels of interleukin-6 (IL-6) in patients with severe COVID-19, we conducted a small pilot off-label compassionate care study of the IL-6 receptor inhibitor tocilizumab in patients with severe COVID-19.

Results: A single infusion of tocilizumab in patients with severe COVID-19 manifested rapid declines in C-reactive protein and d-dimer and gradual rises in lymphocyte and platelet counts.

Conclusions: These findings suggest both pathophysiological mechanisms and clinical benefit that might be seen with IL-6 inhibition in severe COVID-19.

KEYWORDS
COVID-19, COVID-19 coagulopathy, interleukin-6, thromboinflammation, tocilizumab

1 INTRODUCTION

The pandemic coronavirus disease-19 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) manifests a highly variable course in different individuals. Whereas most patients experience few if any symptoms, a relevant proportion develop severe respiratory disease, inflammation in other organs, and a hypercoagulable state. An excessive immune response to various viral infections has been associated with hyperinflammation and multiorgan immune-mediated pathology. Elevated levels of various cytokines have been observed in severe cases of SARS-CoV and Middle East respiratory syndrome coronavirus as well as in murine...
models in the 1918 influenza. It is not clear whether these cytokines were involved with actual pathology and, if so, which cytokines and what organ injury.

SARS-CoV-2 suppresses the initial type I interferon response that is critical for control of viral infections. This presumably allows the virus to escape early immune suppression and to replicate more extensively. When the adaptive immune response is activated later, profound activation of viral-specific cytolytic T cells ensues in response to the high viral burden. The massive expansion of cytolytic T cells results in considerable tissue damage of virally infected cells. The release of large quantities of damage-associated molecular patterns from dying cells can trigger a secondary enhanced innate immune cytokine response. Thus, whereas it may be detrimental to suppress the initial innate immune response, it may be desirable to suppress the secondary adaptive and innate immune responses. Thus, the timing of cytokine suppression is critical.

A possible clue linking excessive cytokine release with immune-mediated pathology derives from studies with chimeric antigen receptor – T-cell (CAR-T) therapy of hematopoietic malignancies. Such therapy involves infusing patients with large numbers of autologous T cells expressing a chimeric receptor targeting the tumor. This results in massive activation of the infused T cells by the tumor antigens, tumor lysis, the release of large amounts of several innate cytokines, including interleukin (IL)-1, tumor necrosis factor (TNF), and IL-6, and a multiorgan inflammatory response, including coagulopathies and, in some cases, respiratory failure. Inhibition of the IL-6 receptor (IL-6R) with tocilizumab was shown to be effective in reducing the inflammatory response to CAR-T therapy and in 2017 was approved by the US Food and Drug Administration for treatment of CAR-T-induced inflammatory cytokine release syndrome. Given the likely profound activation of T cells in severe COVID-19 cases, we reasoned that IL-6 blockade might also be therapeutic for these patients.

IL-6 can induce a wide array of proinflammatory mediators. This in part relates to different types of cis and trans IL-6 signaling. In cis signaling, IL-6 binds to membrane-bound IL-6 receptor (mIL-6R) in a complex with glycoprotein 130 (gp130) and then signals through Janus kinases (JAKs) and signal transducer and activator of transcription 3. Although gp130 is ubiquitously expressed, mIL-6R is largely restricted to immune cells. On trans signaling, high circulating levels of IL-6 bind to the soluble form of IL-6R, which can form a complex with gp130 on nearly all cell surfaces. This results in IL-6 signaling of cells that lack mIL-6R, such as endothelial cells. These cells can then express several cell adhesion molecules and chemokines that can considerably amplify the inflammatory response. IL-6 is also a potent activator of the coagulation system during infection, increasing mononuclear cell expression of tissue factor, which complexes with factor Vlla leading to downstream thrombin activation and generation of fibrin clots. IL-6 inhibition can block tissue factor–induced thrombin generation. This crosstalk between the coagulation and the inflammatory system is essential to prevent microbial invasion, as it creates a localized fibrin network that can limit the spread of infection.

Given the likely profound activation of T cells in patients with severe COVID-19, coupled with the experience from CAR-T therapy, we elected to treat a small group of severe COVID-19 cases with tocilizumab on an off-label compassionate care basis. Careful attention was given to markers of inflammation, evidence of coagulopathy, and cytopenias.

## MATERIALS AND METHODS

### 2.1 Patients

Between March 10 and April 12, 2020, patients who were COVID-19-positive were considered for treatment with tocilizumab if they met the following criteria: intubated and a PaO2:FiO2 < 150, temperature > 38.3°C, ferritin > 1000 ng/mL, d-dimer > 800 ng/mL, lactate dehydrogenase (LDH) > 250 U/L, CRP > 70, and lymphocyte count < 0.6 x 10⁹/L. Age- and sex-matched patients who were COVID-19-positive and also intubated but did meet all of the laboratory inclusion criteria comprised a comparison group and received standard of care. All patients received hydroxychloroquine 400 mg twice daily for 1 day and then 200 mg twice daily for 4 days more. Tocilizumab was administered as a one-time infusion of 400 mg in eligible patients. The study was approved by the University of Vermont Committee for the Protection of Human Subjects.

### 2.2 Laboratory methods

We recorded laboratory results from routine patient care for CRP, d-dimer, ferritin, and platelet and lymphocyte counts. All patients tested positive for SARS-CoV-2.

### 2.3 Statistical methods

All observations were divided into early and late periods. The early period for treated patients ran from the date of the first available laboratory result through the day of their tocilizumab infusion. The late period ran from the day after infusion to the last available laboratory result. For the control group, the early period ended halfway through their entire observation time, and the late period included the remainder.

We performed five independent least-squares linear regressions for each of five laboratory analytes as the outcome variable. In each case, the predictors were treatment group (tocilizumab vs control), observation period, and their interaction. All regressions were adjusted for clustering within each patient.

Given the absence of randomization, the inherent differences in the two groups at baseline, the presence of multiple comparisons, and the fact that we did not specify an analytic plan beforehand, we
consider these analyses to be descriptive and hypothesis generating rather than valid tests of established hypotheses. Nonetheless, if the tocilizumab group had a change in the laboratory value that was different than the change in the control group, we would expect the coefficient on the interaction to be significantly different than zero. All statistical analyses were performed in Stata 15.1 (StataCorp, LLC, College Station, Texas, USA).

3 | RESULTS

During the 5-week period of this study, all six patients who met the criteria for tocilizumab treatment were men, between the ages of 39 and 79, and receiving mechanical ventilation (Table 1). From the remainder of intubated patients who did not meet all the criteria for treatment, six male individuals were chosen who closely matched the age of the treated patients (Table 1). Six patients met the criteria for treatment with tocilizumab including PaO2/FIO2 < 150 mm Hg, temperature > 38.3°C, ferritin > 1000 ng/mL, d-dimer > 800 ng/mL, LDH > 250 U/L, CRP > 70 mg/L, and lymphocyte count < 0.6 x 10^9/L. Two individuals had a body mass index > 50 kg/m^2, indicative of severe obesity. These six individuals received a single dose of tocilizumab (400 mg intravenously).

All observations were divided into early and late periods. The early period for treated patients ran from the date of the first available laboratory result through the day of their tocilizumab infusion. The day of infusion was arbitrarily set as day 0 so that results of treated patients could be aligned and thus more easily compared. The late period ran from the day after infusion to the last available laboratory result. For the control group, the early period ended halfway through their entire observation time, and the late period included the remainder.

Following tocilizumab infusion there was a very striking decline in elevated levels of CRP and d-dimer, within 24 hours in some cases (Figure 1). In parallel, there was a slower rise in the numbers of platelets and lymphocytes. In addition, there was considerable improvement in the PaO2/FIO2 for the four patients in whom it was tested (Table 1). There was little or no alteration in the ferritin levels following tocilizumab treatment. By contrast, in the patients not receiving tocilizumab, there was a variable and inconsistent pattern to change in CRP and d-dimer. Similarly, the levels of platelets and lymphocytes did not change, nor did the ferritin levels in patients not receiving tocilizumab. There were no cases of nosocomial infection in either group.

Table 2 reports the regression models. CRP fell in both groups over time. Although CRP was higher in the tocilizumab group during the early period, it fell much more and the interaction term was significant, suggesting a benefit of tocilizumab. A parallel pattern was observed for d-dimer, although the difference by treatment did not achieve significance given the limited number of patients. Platelet counts were similar in the two groups in the early period but rose significantly more in the tocilizumab group in the later period (P = .02). Lymphocyte counts also rose in several patients following tocilizumab,

| TABLE 1 | Patient demographics |
|-----------------|------------------|
| **Patients receiving tocilizumab** | |
| **Sex** | **Age** (y) | **BMI** (kg/m2) | **Comorbidities** | **Evidence of thrombosis** | **Anticoagulation** | **Hemodynamic shock** | **Survival (length of hospital stay)** | **PaO2/FIO2 (mm Hg/FractionO2) (last available)** |
| 1 | Male | 72 | 25.8 | Multiple sclerosis | PE | UFH | No | No | NA |
| 2 | Male | 47 | 34.0 | Graves’ disease | No | UFH | Yes | Yes (18 d) | NA |
| 3 | Male | 39 | 52.8 | Morbid obesity | No | UFH | No | No | 310 |
| 4 | Male | 50 | 50.8 | Morbid obesity | No | UFH | No | Yes (11 d) | 195 |
| 5 | Male | 76 | 21.1 | None known | PE | UFH | Yes | No | 180 |
| 6 | Male | 72 | 32.6 | Prostate cancer | No | UFH | No | No | 448 |
| **Patients not receiving tocilizumab** | |
| 7 | Male | 76 | 31.4 | Hypertension | No | LMWH | No | No | 187 |
| 8 | Male | 79 | 22.1 | Ulcerative colitis | No | LMWH | No | Yes (6 d) | 240 |
| 9 | Male | 46 | 21.8 | None known | No | UFH | No | Yes (9 d) | 243 |
| 10 | Male | 72 | 25.1 | Myeloma, hypertension, atrial fibrillation | No | LMWH | No | Yes (11 d) | 117 |
| 11 | Male | 67 | 29.1 | Chronic kidney disease, hypertension | No | UFH | Yes | Yes (20 d) | 223 |
| 12 | Male | 58 | 32.7 | Diabetes, obstructive sleep apnea | No | LMWH | Yes | No | 71 |

Abbreviations: BMI, body mass index; LMWH, low-molecular-weight heparin; NA, not applicable; UFH, unfractionated heparin.
but the difference from untreated patients did not achieve significance. There was no difference between the two groups for ferritin levels, which did not decline during the period of observation. Although there was no increased survival of the patients treated with tocilizumab (Table 1), they were also more severely ill than the control patients based on inclusion laboratory criteria. Nonetheless, the rapid resolution of many laboratory abnormalities suggests that cytokine inhibition can be effective if given earlier.

### DISCUSSION

Although this was a small nonrandomized preliminary study of patients with severe COVID-19, the findings were nonetheless striking for the rapid reversal of thromboinflammatory biomarkers following a single dose of tocilizumab. These included rapid reductions in CRP and D-dimer and gradual rises in platelet and lymphocyte counts. These findings underscore the possibility that many aspects
of severe COVID-19, especially coagulopathy, may be secondary to an excessively immune response to SARS-CoV-2. Particularly striking was the rapid decline in elevated d-dimer in patients with severe COVID-19 following a single dose of tocilizumab. Coagulopathy is now appreciated as a significant component of morbidity in COVID-19, and this may be due to pulmonary vascular endotheliopathy and deposition of fibrin thrombi in small and large vessels of the lung. There is also a high risk of venous thromboembolism, which can be predicted by admission elevation of d-dimer. IL-6–mediated activation of mononuclear cells to express tissue factor may lead to significant activation of the coagulation cascade and thrombin generation in these patients. Previous studies observed that IL-6 blockade may block tissue factor-induced thrombin generation and fibrin formation, which would be expected to lead to a decrease in fibrin degradation products such as d-dimer.

Because tocilizumab was considered as compassionate care for these patients with severe COVID-19, all patients were already on mechanical ventilation. Nonetheless, the rapid reversal of several laboratory abnormalities, particularly evidence of coagulopathy with resolution of d-dimer elevations in this setting of critical illness, suggests that this treatment deserves study earlier in the disease course, with a hypothesis that it could reduce macro- and micro-thrombi and subsequent pulmonary failure. Such studies should include careful translational biology studies to assess biomarker responses and, given the marked reduction of d-dimer (a venous thrombosis risk factor) with tocilizumab, evaluate thrombosis outcomes secondarily. The Global COVID-19 Thrombosis Collaborative Group suggested that treatment interventions be adapted across the course of severity of infection, and a recently proposed staging paradigm for coagulopathy in COVID-19 might provide a useful framework for patient classification for such trials. A parallel example to this is the rapid emergence of trials of full-intensity heparin treatment in medical ward patients to try to prevent pulmonary deterioration, after findings in critical illness suggested possible benefit (NCT04362085).

Severe COVID-19 has close parallels with a number of seemingly disparate syndromes that might all be classified as hyperinflammatory disorders. CAR-T therapy exposes patients to a large number of T cells that become activated upon contact with targeted tumor cells, often resulting in an inflammatory syndrome that includes hypercoagulation and even acute respiratory distress syndromes. A disorder possibly related mechanisms is toxic shock syndrome, a multiorgan inflammatory syndrome occasionally seen in young women. In these cases, tampons infected with Staphylococcus release an enterotoxin that acts as a superantigen by binding both the major histocompatibility complex class II molecule and the β-chain of several T-cell receptors. This activates a significant portion of the T-cell repertoire, similar to CAR-T therapy, resulting in injury to many organs, including skin, liver, and lung, also with coagulopathy and sometimes acute respiratory distress syndrome. How this might lead to elevation of cytokines, such as IL-6, is less clear. One possibility is that the cytolytic activity of the activated T cells results in lysis of tumors and normal tissues with the release of cellular components known as damage-associated molecular patterns that strongly activate the innate immune response, including macrophages, with release of IL-1, TNF, and IL-6, among other cytokines and chemokines. In addition, lung epithelium is a source of IL-6, which could be released during lung damage in all of these disorders. In this regard, it is of some interest to note that individuals with HIV and low T-cell counts have been noted to have fewer severe cases among those who contract COVID-19.

An additional parallel can be made between severe COVID-19 and hemophagocytic lymphohistiocytosis (HLH). HLH is a severe

### TABLE 2 Regression analyses

| Parameter               | Coefficient | 95% confidence interval | P value |
|-------------------------|-------------|-------------------------|---------|
| C-reactive protein (mg/L) |             |                         |         |
| Tocilizumab             | 169         | 93, 245                 | .001    |
| Period (late = 1)       | −32         | −54, −11                | .007    |
| Tocilizumab * period interaction | −155 | −206, −104              | .001    |
| Constant                | 108         | 66, 150                 | <.001   |
| D-dimer (ng/mL)         |             |                         |         |
| Tocilizumab             | 18 354      | −2984, 39 692           | .09     |
| Period (late = 1)       | −50         | −661, 562               | .86     |
| Tocilizumab * period interaction | −14 319 | −33 879, 5241           | .14     |
| Constant                | 1329        | 808, 1850               | <.001   |
| Ferritin (ng/mL)        |             |                         |         |
| Tocilizumab             | −9.5        | −1137, 1118             | .99     |
| Period (late = 1)       | −51         | −1558, 1456             | .94     |
| Tocilizumab * period interaction | −162 | −1748, 1424             | .83     |
| Constant                | 1803        | 747, 2860               | .003    |
| Platelets (1000/mm³)    |             |                         |         |
| Tocilizumab             | −43         | −195, 108               | .54     |
| Period (late = 1)       | −50         | −121, 21                | .15     |
| Tocilizumab * period interaction | 119 | 22, 216                 | .02     |
| Constant                | 244         | 112, 377                | .002    |
| Lymphocytes (1000/mm³)  |             |                         |         |
| Tocilizumab             | −0.07       | −0.40, 0.26             | .65     |
| Period (late = 1)       | 0.2         | −0.14, 0.53             | .22     |
| Tocilizumab * period interaction | 0.35 | −0.20, 0.91             | .19     |
| Constant                | 0.56        | 0.00, 0.12              | .001    |

Note: The effect of tocilizumab was modeled independently for each laboratory parameter using ordinary least-squares linear regression. The coefficient on tocilizumab estimates the differences between the treated and untreated patients at baseline. The coefficient on period estimates the effect of time across all patients. The coefficient on the interaction estimates the effect of treatment. Each row presents a coefficient along with its 95% confidence interval and associated P value.
Inflammatory syndrome characterized by fever, hepatitis, spleen and lymph node enlargement, and pancytopenia. It is often observed secondary to certain viral infections as well as autoimmune syndromes such as juvenile inflammatory arthritis. An additional laboratory characteristic is elevated ferritin, which we observed in our severe COVID-19 cases. HLH is likely the result of T-cell activation and JAK inhibitors. These agents are currently in clinical trials for patients with COVID-19 (NCT04377620).

In conclusion, compassionate use tocilizumab treatment in patients with severe COVID-19 reduced coagulation activation and inflammation, supporting the tight linkage between inflammation and thrombosis in these patients.

**RELATIONSHIP DISCLOSURE**

The authors declare no conflict of interest.

**AUTHOR CONTRIBUTIONS**

RCB was the main investigator selecting patients eligible for tocilizumab infusion and prescribed the medication after institutional review board approval. BL, MC, and MG assisted RCB in analyzing the laboratory data, reviewing the most updated literature on COVID-19 coagulopathy, and contributed to the writing of the article.

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