RESEARCH ARTICLE

Tocilizumab in hospitalized patients with COVID-19: Clinical outcomes, inflammatory marker kinetics, and safety

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Abstract

Coronavirus disease 2019 (COVID-19) due to infection with severe acute respiratory syndrome coronavirus 2 causes substantial morbidity. Tocilizumab, an interleukin-6 receptor antagonist, might improve outcomes by mitigating inflammation. We conducted a retrospective study of patients admitted to the University of Washington Hospital system with COVID-19 and requiring supplemental oxygen. Outcomes included clinical improvement, defined as a two-point reduction in severity on a six-point ordinal scale or discharge, and mortality within 28 days. We used Cox proportional-hazards models with propensity score inverse probability weighting to compare outcomes in patients who did and did not receive tocilizumab. We evaluated 43 patients who received tocilizumab and 45 who did not. Patients receiving tocilizumab were younger with fewer comorbidities but higher baseline oxygen requirements. Tocilizumab treatment was associated with reduced C-reactive protein, fibrinogen, and temperature, but there were no meaningful differences in time to clinical improvement (adjusted hazard ratio [aHR], 0.92; 95% confidence interval [CI], 0.38–2.22) or mortality (aHR, 0.57; 95% CI, 0.21–1.52). A numerically higher proportion of tocilizumab-treated patients had subsequent infections, transaminitis, and cytopenias. Tocilizumab did not improve outcomes in hospitalized patients with COVID-19. However, this study was not powered to detect small differences, and there remains the possibility for a survival benefit.

KEYWORDS
coronavirus, COVID-19, immunomodulatory, SARS-CoV-2, tocilizumab

1 | INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel human pathogen responsible for the largest global challenge to public health and humanity in over a century. Due to the high pathogenicity of this novel virus and a lack of pre-existing immunity, millions of individuals have been infected worldwide.[1] Among those infected, approximately 14% develop progressive pulmonary disease (coronavirus disease 2019 [COVID-19]) that results in critical illness in 5% of individuals.[2] Given high mortality rates in older patients and those with underlying comorbidities, there is an urgent need to identify effective therapies.

A profound inflammatory response to viral infection appears to be responsible for severe disease manifestations in patients with COVID-19.

High serum levels of C-reactive protein (CRP), ferritin, and interleukin 6 (IL-6) are observed in patients requiring oxygen support and who progress to respiratory failure, cardiovascular collapse, and death and are consistent with a hyperinflammatory cytokine storm.[3] These laboratory and clinical findings are similar to the cytokine release syndrome (CRS) that may develop in cancer patients receiving chimeric antigen receptor immunotherapy (CAR-T cell therapy),[4] which has overlapping characteristics with macrophage activation syndrome (MAS).[5] Cytokine storm, and the resulting immunopathology, has also been identified as a cause of morbidity associated with related coronaviruses (SARS-CoV-1, Middle East respiratory syndrome-CoV) and other respiratory viruses.[6–9]

Treatment with corticosteroids, which suppress immune responses nonspecifically, has not been shown to improve outcomes with other severe respiratory viral infections or acute respiratory distress syndrome in the general population.[10] However, recent clinical trial data demonstrate a mortality benefit of low dose dexamethasone for COVID-19 in hospitalized patients requiring supplemental oxygen or mechanical ventilation,[11] supporting the notion that hyperinflammation contributes to disease pathogenesis among patients with severe COVID-19 disease. The increasing use of cytokine-specific immunomodulatory agents in other conditions has led to intense interest in the use of these agents for COVID-19. The rationale for use of immunomodulatory therapies in patients with COVID-19 is extrapolated from the experience in CAR-T cell therapy recipients, in whom IL-6 receptor blockade with tocilizumab (Actemra) can rapidly attenuate symptoms of CRS.[4] In this context, tocilizumab does not appear to independently increase risk for infection,[12,13] although infectious complications are reported with chronic use in rheumatoid arthritis,[14] particularly with concurrent immunosuppressants. Cytokine blockade for hyperinflammatory syndromes is supported by additional lines of evidence, including the finding that treatment with recombinant IL-1 receptor antagonist, anakinra (Kineret), may improve outcomes in patients with MAS-like features in the setting of sepsis.[15]

Since the beginning of the pandemic, multiple observational reports have suggested that tocilizumab improves respiratory, radiological, and inflammatory parameters associated with COVID-19, although there are conflicting results with regard to clinical outcomes.[16–29] Observational studies using either another anti-IL-6 receptor antagonist, sarilumab (Kevzara), or anakinra have shown similar findings.[30–33] The safety of immunomodulatory therapy in the context of an active infection remains incompletely understood; IL-6 is an essential aspect of immune control for many viral infections, but overproduction of IL-6 may be detrimental to viral clearance and survival.[8,34] Results from a randomized controlled trial indicate that tocilizumab did not result in reduced mortality or need for mechanical ventilation,[35] although there was evidence for shorter duration of hospitalization and intensive care unit stays in preliminary results from another trial,[36] and the incidence of adverse events were similar to the placebo groups. There remains a critical need to understand the efficacy and safety of tocilizumab in patients with COVID-19. The aims of this observational study were to comprehensively describe the clinical course and outcomes of hospitalized patients with COVID-19 who were treated with tocilizumab and compare to patients from the same time period who were not treated with tocilizumab, describe

adverse effects, and to evaluate the kinetics of inflammatory markers after tocilizumab therapy.

2 | METHODS

2.1 | Patients

We used the University of Washington (UW) Enterprise Data Warehouse to retrospectively identify all individuals admitted to three UW Hospitals across the Seattle metropolitan region between March 19th (the first date of tocilizumab administration) and April 24th, 2020 with SARS-CoV-2 detection in a respiratory sample. Patients were excluded if they did not require supplemental oxygen, died or were placed on comfort measures within 2 days of admission (in the no tocilizumab cohort only), or received an alternative anti-cytokine therapy as part of clinical care or a trial. This study was approved by the UW Institutional Review Board.

2.2 | Treatment, supportive care, and monitoring

Hospital system-wide guidelines pertaining to the use of COVID-19-specific therapies were formulated using the limited available data by an interdisciplinary committee. During the study period, this guidance recommended use of open label hydroxychloroquine or investigational remdesivir in patients requiring supplemental oxygen and not participating on a corresponding clinical trial. The guidelines recommended consideration of a single dose of tocilizumab 400 mg intravenously in patients who had persistent fever and either impending or current respiratory failure, hemodynamic instability requiring vasopressor or ionotropic support, or a serum IL-6 level greater than five times the upper limit of normal (range, 0–6 pg/ml). Tocilizumab was contraindicated in patients with sepsis due to another infection, transaminases > 5 times the upper limit of normal, severe neutropenia (neutrophil count < 500 cells/mm^3), or severe thrombocytopenia (platelet count <50,000 cells/mm^3). Corticosteroids and other antivirals were not recommended at that time. Patients received prophylactic heparin or enoxaparin for prevention of deep vein thrombosis at the discretion of the treating physician. Laboratory testing for inflammatory markers (IL-6, CRP, ferritin, fibrinogen, lactate dehydrogenase [LDH], and D-dimer) were recommended at the time of admission, every 3 days, with clinical deterioration, and before discharge.

2.3 | Outcomes

The primary outcome was sustained (≥3 days) clinical improvement within 28 days after baseline. For patients treated with tocilizumab, baseline was considered to be at the time of tocilizumab administration. For patients not treated with tocilizumab, baseline was considered to be 2 days after hospitalization to align with the median number of days between hospitalization and tocilizumab administration in the

tocilizumab cohort (median, 1.8; interquartile range [IQR], 0.8–3.8). Clinical improvement was defined as a two-point reduction in patients' baseline status on a six-point ordinal scale (modified from the WHO guidelines), or live discharge from the hospital, whichever came first. The ordinal scale consisted of 1, discharged; 2, hospitalized but not requiring oxygen; 3, hospitalized and requiring any supplemental oxygen; 4, hospitalized and requiring high-flow oxygen devices or noninvasive ventilation; 5, hospitalized and requiring invasive ventilation; and 6, death.[37] We also analyzed mortality within 28 days.

We described additional clinical outcomes of sepsis (defined as requiring hemodynamic support with vasopressors, with resolution at the time of vasopressor discontinuation), thrombotic events, bacteremia, and microbiologically confirmed pneumonia with another pathogen prompting antimicrobial therapy. We also described the kinetics of inflammatory laboratory markers, temperature, and heart rate.

2.4 | Data collection and statistical considerations

We abstracted data from medical records and electronic databases from the time of index admission for COVID-19 through 28 days after baseline as defined above. We described the proportion of patients with clinical improvement (as defined above) overall and stratified by baseline severity categories. We estimated the cumulative incidence of clinical improvement, overall and within strata of baseline oxygen requirements, treating death as a competing risk. To mitigate differences in baseline characteristics between those who did and did not receive tocilizumab, we modeled the probability of treatment using baseline characteristics and calculated weights for inverse probability of treatment weighting (IPTW) analyses to weight the distribution of covariates to that of the overall population. Weights were standardized using the marginal probability of treatment and truncated at the 98th percentile (values >4) to reduce variability.[38] We carried out multivariable Cox proportional-hazards modeling to evaluate the association of receipt of tocilizumab with (1) clinical improvement and with (2) mortality using IPTW models. Robust variances were utilized in IPTW models to account for inclusion of the weights.[38] We also computed the restricted mean survival time (RMST) difference for time to clinical improvement and adjusted these models using IPTW. This analysis provides an intuitively appealing estimate of the difference in the mean number of days to clinical improvement between treatment groups.[39] Variables with a $p < .2$ in univariable analyses were candidates for inclusion in the multivariable model; tocilizumab was included in all models, irrespective of univariate results. Variables were retained in the multivariable model if their inclusion modified the tocilizumab hazard ratio by more than 10% or if they were significantly associated with the outcome. In Cox models and RMST calculations for time to clinical improvement, patients who died before Day 28 without clinical improvement were assigned a censored follow-up time at Day 28, essentially giving them the worst possible "infinite" outcome. Additionally, we described the kinetics of changes in vital signs and

laboratory results, as well as the occurrence of other clinical events including thrombosis and infections. SAS version 9.4 (SAS Institute) and RStudio were used for analyses.

3 | RESULTS

3.1 | Patient and treatment characteristics

Between March 19th and April 24th, 2020, 44 patients were treated with tocilizumab within our hospital system. During this time period, an additional 120 patients were hospitalized with COVID-19 but did not receive tocilizumab. After excluding patients who did not require supplemental oxygen, who died or were placed on comfort measures within 2 days of admission, or who were enrolled in a placebo-controlled trial of IL-6 receptor blockade with sarilumab, there were 43 patients in the tocilizumab cohort and 45 patients in the non-tocilizumab cohort (Figure S1). Demographic and clinical characteristics of each cohort are shown in Table 1 and stratified by baseline severity in Table S1. Compared to patients who did not receive tocilizumab, patients treated with tocilizumab were more likely to be younger, of white Hispanic race and ethnicity, without chronic lung disease, full code status, and receiving invasive ventilation (42% vs. 20%) or high-flow oxygen (47% vs. 22%). The invasive ventilation groups were similar overall. Most patients in both groups were treated with hydroxychloroquine, a minority (30%) were treated with remdesivir or placebo as part of a double-blind randomized clinical trial,[40] and one patient received open-label compassionate use remdesivir. No patients received corticosteroids for the treatment of COVID-19. The majority of patients received a single 400 mg intravenous dose of tocilizumab (median, 4.7 mg/kg; Table S2); 3 patients received a second dose within 24 h after the first.

3.2 | Clinical improvement

All patients were followed for up to 28 days after the baseline date or the time of death, whichever occurred first. In the tocilizumab cohort, 26 (60%) patients were discharged from the hospital, 9 (21%) died, and 8 (19%) remained hospitalized. In the no tocilizumab cohort, 27 (60%) patients were discharged from the hospital, 15 (33%) died, and 3 (7%) remained hospitalized. Among patients not requiring invasive ventilation at baseline and who did not have a "do not intubate" code status, 5 of 15 (33%) and 0 of 3 (0%) patients progressed to invasive ventilation in the tocilizumab and no tocilizumab cohorts, respectively. The proportion of patients in each baseline severity category who met the clinical improvement endpoint are depicted in Figure 1, and longitudinal patient outcomes are depicted in Figure S2.

There were no differences in the cumulative incidence of clinical improvement among patients who did and did not receive tocilizumab overall or stratified by baseline severity categories (Figure 2). Among patients who improved, the median times to clinical improvement in the tocilizumab versus no tocilizumab cohorts, respectively, were 8 versus 6 days in the low-flow subgroup, 11 versus 9 days in the

TABLE 1 Baseline demographics and clinical characteristics

	No tocilizumab (N = 45)	Tocilizumab (N = 43)	Total (N = 88)	p[a]
Age, year				<.001
<50	2 (4)	16 (37)	18 (20)	
50–70	17 (38)	20 (47)	37 (42)	
≥70	26 (58)	7 (16)	33 (38)	
Male sex	31 (69)	30 (70)	61 (69)	.93
Race and ethnicity				<.001
Non-Hispanic white	30 (67)	8 (19)	38 (43)	
Non-Hispanic black	3 (7)	4 (9)	7 (8)	
Hispanic white	7 (16)	18 (42)	25 (28)	
Other	5 (11)	11 (26)	16 (18)	
Unknown	0 (0)	2 (5)	2 (2)	
Body-mass index				.14
<29.9	23 (51)	17 (40)	40 (45)	
30–39.9	14 (31)	22 (51)	36 (41)	
≥40.0	8 (18)	4 (9)	12 (14)	
Past diagnoses[b]				
Any diagnosis	40 (89)	31 (72)	71 (81)	.05
Chronic lung disease	18 (40)	3 (7)	21 (24)	<.001
Diabetes	16 (36)	22 (51)	38 (43)	.14
Cardiovascular disease	32 (71)	26 (60)	58 (66)	.29
Chronic kidney disease	13 (29)	7 (16)	20 (23)	.16
Immunocompromised	10 (22)	4 (9)	14 (16)	.10
Autoimmune disease	1 (2)	1 (2)	2 (2)	1
Code status "do not intubate"	17 (38)	5 (12)	22 (25)	.005
Hospital location				.05
1	11 (24)	20 (47)	31 (35)	
2	12 (27)	5 (12)	17 (19)	
3	22 (49)	18 (42)	40 (45)	
COVID-19 specific therapies[c]				
Hydroxychloroquine, open label	25 (56)	35 (81)	60 (68)	.009
Hydroxychloroquine or placebo, blinded trial	0 (0)	1 (2)	1 (1)	.008
Remdesivir or placebo, blinded trial	15 (33)	11 (26)	26 (30)	.57
Remdesivir, open label	0 (0)	1 (2)	1 (1)	1.0
Oxygen support category				<.001[d]
Low-flow oxygen therapy	26 (58)	5 (12)	31 (35)	
Noninvasive ventilation or high-flow oxygen therapy	10 (22)	20 (47)	30 (34)	
Invasive ventilation	9 (20)	18 (42)	27 (31)	

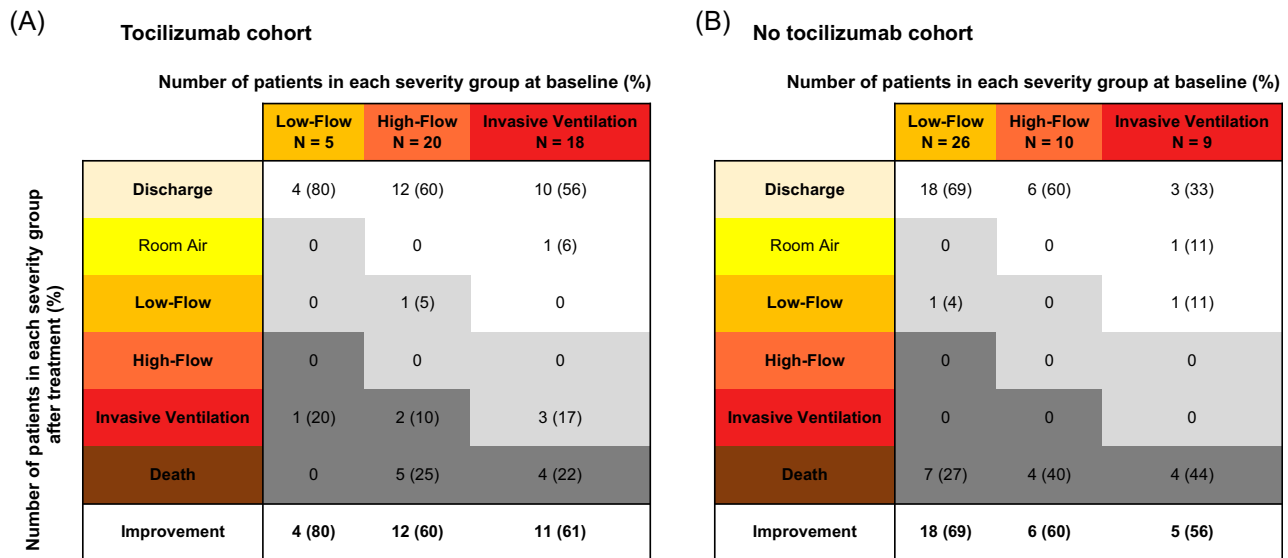*Note:* Data are presented as no. (%), unless otherwise indicated.

Abbreviation: COVID-19, coronavirus disease 2019.

[a]χ^2/Fisher's exact test for categorical variables, as appropriate.

[b]Each category was considered independently for % and p value calculations. Chronic lung disease included chronic obstructive pulmonary disease, asthma, and obstructive sleep apnea. Cardiovascular disease included hypertension, cardiovascular or cerebrovascular disease, and heart failure. Immunocompromised included diagnoses of cancer, human immunodeficiency virus (HIV), solid organ transplant, and bone marrow transplant.

[c]Each category was considered independently for % and p value calculations. At any time during the index hospitalization.

[d]Across all three oxygen support categories.

(A)

Tocilizumab cohort

Number of patients in each severity group at baseline (%)

		Low-Flow N = 5	High-Flow N = 20	Invasive Ventilation N = 18
Number of patients in each severity group after treatment (%)	Discharge	4 (80)	12 (60)	10 (56)
	Room Air	0	0	1 (6)
	Low-Flow	0	1 (5)	0
	High-Flow	0	0	0
	Invasive Ventilation	1 (20)	2 (10)	3 (17)
	Death	0	5 (25)	4 (22)
	Improvement	**4 (80)**	**12 (60)**	**11 (61)**

(B)

No tocilizumab cohort

Number of patients in each severity group at baseline (%)

	Low-Flow N = 26	High-Flow N = 10	Invasive Ventilation N = 9
Discharge	18 (69)	6 (60)	3 (33)
Room Air	0	0	1 (11)
Low-Flow	1 (4)	0	1 (11)
High-Flow	0	0	0
Invasive Ventilation	0	0	0
Death	7 (27)	4 (40)	4 (44)
Improvement	**18 (69)**	**6 (60)**	**5 (56)**

FIGURE 1 The proportion of patients in each baseline severity category who had clinical improvement by 28 days among patients in the tocilizumab and no tocilizumab groups. White boxes indicate clinical improvement for the indicated baseline severity category, light gray indicate neither improvement nor worsening, and dark gray indicate worsening. Percentages are column percentages based on the baseline severity category

high-flow subgroup, and 16 versus 13 days in the invasive ventilation subgroup (Table S3).

To mitigate differences in baseline characteristics between tocilizumab treated and non-tocilizumab treated patients, we generated standardized IPTW weights from propensity scores (Figure S3A,B). After truncating the two highest values, the IPT weighted population showed improvement in the balance of baseline characteristics between patients who did and did not receive tocilizumab (Figures S3C,D; Table S4). In an adjusted Cox model using the truncated IPTW weights, receipt of tocilizumab was not associated with a difference in clinical improvement (adjusted hazard ratio [aHR], 0.92; 95% confidence interval [CI], 0.38–2.22; Table 2). Exploratory models within baseline oxygen categories had similar findings but were limited by small numbers within stratum (data not shown). There was also no evidence for a significant difference in the time to clinical improvement in an IPTW adjusted RMST analysis, which demonstrated that patients receiving tocilizumab improved an average of 1.08 days faster (RMST, −1.08; 95% CI, −5.59 to 8.63 days; $p = .61$). A cumulative incidence plot of time to clinical improvement using the IPT weighted population is shown in Figure S4.

3.3 | Mortality

An adjusted Cox model using the truncated IPTW weights demonstrated a lower observed risk of mortality within 28 days among patients treated with tocilizumab compared to those not treated with tocilizumab (aHR, 0.57; 95% CI, 0.21–1.52; Table 2). However, this estimate was highly variable and was not significant; as such, we cannot infer any difference in mortality between the treatment groups. A Kaplan–Meier survival curve using the IPT weighted population is shown in Figure S5.

3.4 | Other clinical outcomes

Sepsis within 1 day of tocilizumab or baseline date in the non-tocilizumab group occurred in nine and seven patients, respectively. Of the 9 tocilizumab patients, 7 survived (78%) with a median time to sepsis resolution of 3.7 days (IQR, 1.5–7.3); 2 patients died with ongoing sepsis 5 and 14 days after tocilizumab. Of the 7 non-tocilizumab treated patients, 3 survived (43%) with a median time to resolution of 13 days (IQR, 13–15); 4 patients died with ongoing sepsis at 1, 5, 12, and 14 days after baseline.

We also explored the potential benefit of tocilizumab for reducing thrombotic events. A numerically higher proportion of patients developed thrombotic events in the tocilizumab cohort ($n = 4$, 9%) than in the non-tocilizumab cohort ($n = 2$, 4%; Table S5). In addition, we evaluated potential adverse events associated with tocilizumab treatment. We found that a numerically higher proportion of patients in the tocilizumab cohort had bacteremia, secondary pneumonia, transaminitis, neutropenia, or thrombocytopenia within 14 days after baseline in the tocilizumab cohort (Table 3).

3.5 | Effect of tocilizumab on clinical and laboratory markers of inflammation

Laboratory values were similar at baseline for the tocilizumab and no tocilizumab cohorts (Figure 3). Among most patients who received tocilizumab, there was a rapid and sustained decrease in temperature (Figure S6), CRP, and fibrinogen. There was an initial increase followed by a decrease in IL-6 levels after administration of tocilizumab as expected given the mechanism of action of blocking the IL-6 receptor. Levels of LDH declined over days, and there was minimal change in ferritin and D-dimer levels. Heart rates rapidly declined but changes
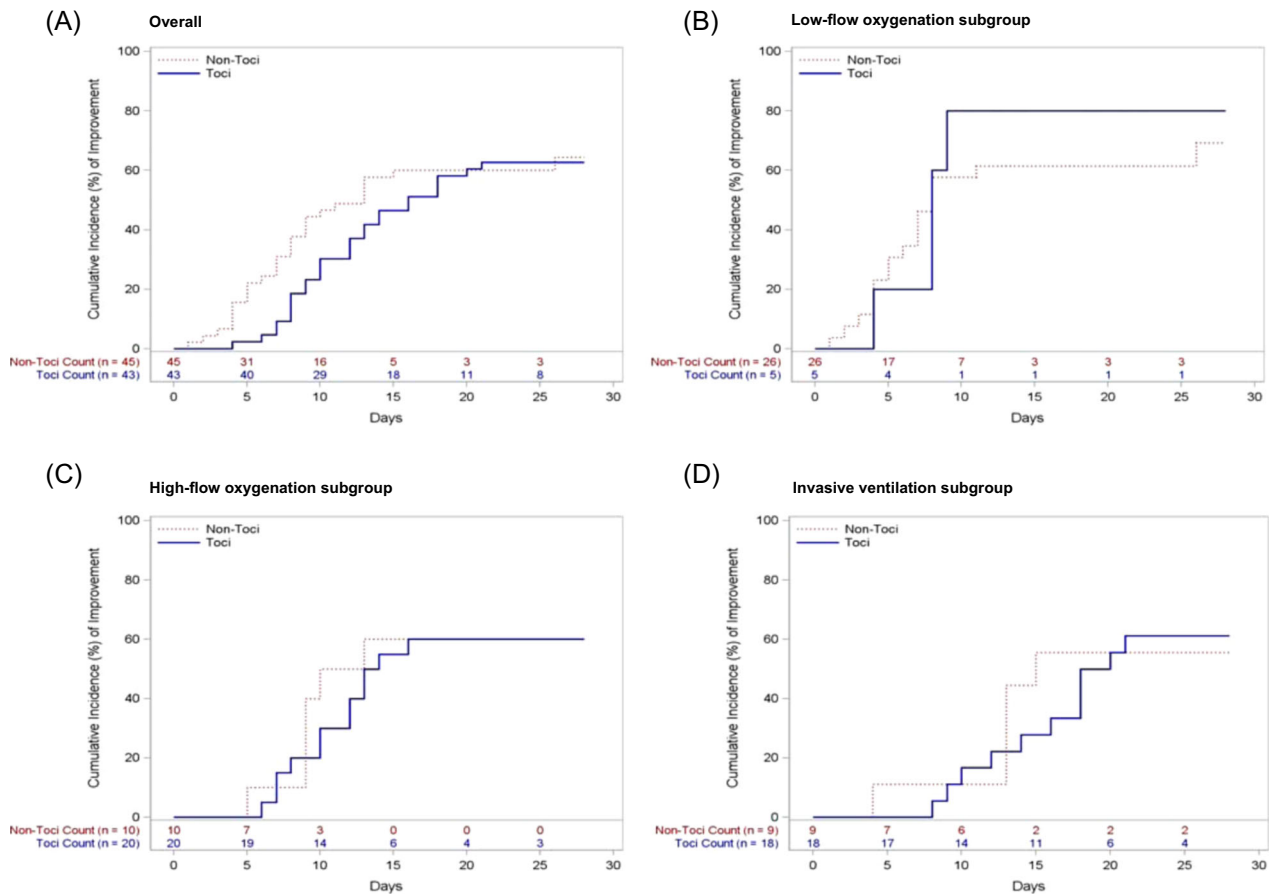
FIGURE 2 Cumulative incidence of sustained (≥3 days) clinical improvement within 28 days among patients who did and did not receive tocilizumab using primary, unweighted data. Death was treated as a competing risk event

were not sustained over time (Figure S6). In contrast, levels of all biomarkers were relatively stable in the no tocilizumab cohort.

4 | DISCUSSION

Morbidity and mortality remain unacceptably high in patients with COVID-19. The clinical utility of directed immunomodulatory agents to preempt or reverse the cytokine-driven inflammation responsible for much of this morbidity has biological plausibility,[41] but there are limited data to guide treatment strategies. Although tocilizumab resulted in normalization of multiple inflammatory biomarkers, we did not find clear evidence that tocilizumab improved clinical outcomes, although we were not powered to detect small effects, and we cannot rule out a survival benefit. There were numerically more infections, transaminitis, and cytopenias in individuals treated with tocilizumab.

Published observational studies have provided mixed results in terms of the associated benefits of tocilizumab treatment in hospitalized patients with COVID-19. Studies including a comparator arm of patients with COVID-19 who did not receive tocilizumab have substantial heterogeneity in patients, their treatments, definitions of outcomes, and analytic approaches.[17–21,24–29] One of the largest and most rigorous studies in mechanically ventilated patients indicated a significant survival

benefit, despite a higher incidence of infections in the tocilizumab group.[26] However, these studies are limited by residual indication bias among those receiving tocilizumab. Rapidly evolving improvements in supportive care strategies may also confound findings of better outcomes with tocilizumab over time. The first available data from randomized controlled trials of tocilizumab in moderately ill hospitalized patients with COVID-19 in North America did not meet their primary endpoints for a reduction in intubation or death among patients randomized to tocilizumab.[35] In a larger trial, there was evidence for a shorter duration of hospitalization and intensive care unit stays.[36] Patients receiving tocilizumab did not have an increased incidence of adverse events in either trial.

In our study, we did not demonstrate an apparent clinical benefit of treatment with tocilizumab, contrary to what has been published in many observational studies. Although patients receiving tocilizumab in our study were younger and sicker on presentation, no clear benefits were seen in comparisons stratified by baseline disease severity, IPTW models that established more balance between the tocilizumab and no tocilizumab groups, or adjusted models. Our study was not confounded by the use of corticosteroids, unlike most other observational studies. This is relevant given emerging data of the benefit of corticosteroids in patients with severe disease.[42] The possibility still remains that there is a clinical scenario in which tocilizumab is beneficial, but this will be best

TABLE 2 Multivariable Cox models of time to clinical improvement or death within 28 days using IPTW weights (truncated to account for outliers) to weight the distribution of covariates to that of the overall population

Variable	Adjusted hazard ratio[a]	p Value	95% confidence interval
Clinical improvement			
Tocilizumab			
No	1.00		
Yes	0.92	.85	0.38–2.22
Age group, year			
<50	1.00		
50-70	1.81	.18	0.76–4.27
≥70	1.35	.67	0.33–5.44
Sex			
Male	1.00		
Female	0.95	.92	0.37–2.44
Race and ethnicity			
Not non-Hispanic White	1.00		
Non-Hispanic White	0.58	.29	0.21–1.59
Cardiovascular disease			
No	1.00		
Yes	0.46	.08	0.19–1.11
Hospital			
1 or 2	1.00		
3	3.35	.002	1.57–7.15
Code status "do not intubate"			
No	1.00		
Yes	0.11	<.001	0.04–0.29
Oxygen support category			
Low-flow oxygen therapy	1.00		
Noninvasive ventilation or high-flow oxygen therapy	0.51	.25	0.16–1.59
Invasive ventilation	0.15	.002	0.05–0.49
Mortality			
Tocilizumab			
No	1.00		
Yes	0.57	.26	0.21–1.52
Age group, year			
<70	1.00		
≥70	3.99	.006	1.48–10.77
Race and ethnicity			
Not non-Hispanic White	1.00		
Non-Hispanic White	0.38	.08	0.13–1.12
Hospital			
1 or 3	1.00		
2	2.44	.11	0.82–7.29
Code status "do not intubate"			
No	1.00		
Yes	10.61	.003	2.18–51.71
Oxygen support category			
Low-flow oxygen therapy	1.00		
Noninvasive ventilation or high-flow oxygen therapy	2.65	.09	0.87–8.0
Invasive ventilation	12.08	.001	2.71–53.81

Abbreviation: IPTW, inverse probability of treatment weighting.

[a]A hazard ratio (HR) less than 1 indicates a lower likelihood of clinical improvement or mortality, as relevant to the model.

	Tocilizumab cohort (N = 43)	No tocilizumab cohort (N = 45)
Bacteremia[a]	4 (9)[b]	2 (4)[c]
Ventilator-associated pneumonia[d]	9 (21)[e]	5 (11)[f]
AST or ALT >5x baseline[g]	4 (9)	2 (4)
Absolute neutrophil count <1000 cells/mm[3h]	0 (0)	0 (0)
Platelet count <100,000 cells/mm[3i]	5 (12)	2 (4)

Note: Data are presented as number of individuals (%) and represent new events that occurred within 14 days of the baseline date.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate transaminase.

[a]Defined as positive blood cultures that were treated with antibiotics.

[b]Pathogens consisted of coagulase-negative Staphylococci (CONS), *Serratia marscecens*, and *Enterococcus faecalis*.

[c]Pathogens consisted of methicillin-sensitive *Staphylococcus aureus* (MSSA), *Granulicatella adiacen*, and CONS.

[d]Defined as microbiologic documentation in addition to antimicrobial therapy. All events occurred in patients who were mechanically ventilated in both cohorts.

[e]Pathogens consisted of MSSA, Haemophilus influenzae, Escherichia coli, Serratia marscecens, methicillin-resistant Staphylococcus aureus (MRSA), Klebsiella pneumoniae, and Klebsiella variicola.

[f]Pathogens consisted of Haemophilus influenzae, Sterptococcus pneumoniae, MSSA, Enterobacter aerogenes, and Serratia marcescens.

[g]7 patients did not have any results, and 11 patients did not have any results after baseline.

[h]1 patient did not have any results, and 7 patients did not have any results after baseline.

[i]5 patients had an absolute neutrophil count < 1,000 cells/mm^3 at baseline; 3 patients did not have any results after baseline.

demonstrated through randomized clinical trials. Some studies report higher rates of infections in patients receiving tocilizumab,[25–27] although this was not observed in the randomized trials[35,36] In our study, a numerically higher proportion of patients in the tocilizumab cohort had a microbiologically confirmed secondary infection, although this was likely related to more patients requiring invasive ventilation in the tocilizumab group, resulting in more diagnoses of ventilator-associated pneumonias. We found numerically higher rates of transaminitis and cytopenias in patients receiving tocilizumab, but these comparisons were limited by a small number of events and may also reflect differences in the distribution of disease severity between the two groups. Similar to the preliminary data from the randomized trials, our findings do not suggest obvious safety concerns related to tocilizumab use for COVID-19.

We also tested the novel hypothesis that thrombotic events may be reduced in tocilizumab-treated patients. This could be an additional benefit of tocilizumab based on the high frequency of venous thrombosis in patients with COVID-19.[43] It is biologically plausible that tocilizumab could reduce risk for thrombosis given studies demonstrating reduced plasma levels of the procoagulant Factor XIII (fibrin-stabilizing factor), D-dimer levels, and other inflammatory markers in patients receiving tocilizumab.[44–47] Although we demonstrate a clear anti-inflammatory effect with rapid and marked reductions in serum levels of CRP and fibrinogen, a higher proportion of patients in the tocilizumab group in our study had thrombotic events, which may be due to the higher baseline

disease severity in the tocilizumab group. It is possible that thromboembolic events were underdiagnosed overall during the study period.[43,48] The low number of events and differences in characteristics between the patient subgroups limit conclusions, but the role of tocilizumab for reducing thrombosis may warrant additional study in randomized trials.

Finally, we evaluated the effect of tocilizumab on clinical and laboratory markers of inflammation. Similar to other studies of tocilizumab in patients with CRS and COVID-19,[16,49] we demonstrated a rapid and sustained effect on lowering body temperature, CRP, and fibrinogen. This may play a role in the higher rates of resolution of sepsis physiology that we describe in our cohort, as reported in another study,[20] and as suggested by the shorter duration of intensive care unit stays in a randomized trial.[36] The anti-inflammatory effects also suggest that the approximately 4 mg/kg dose used in our patients was sufficiently bioactive. Studies using subcutaneous tocilizumab demonstrate similar anti-inflammatory effects, and this mode of administration could be further studied for its clinical utility.[50] Whether the kinetics of inflammatory biomarkers correlate with response to tocilizumab warrants further study.

The primary limitations of our study include those inherent to a retrospective, non-randomized design. Higher baseline severity status in the tocilizumab cohort, as well as other differences in patient characteristics, resulted in imbalances in the patient cohorts. To control for
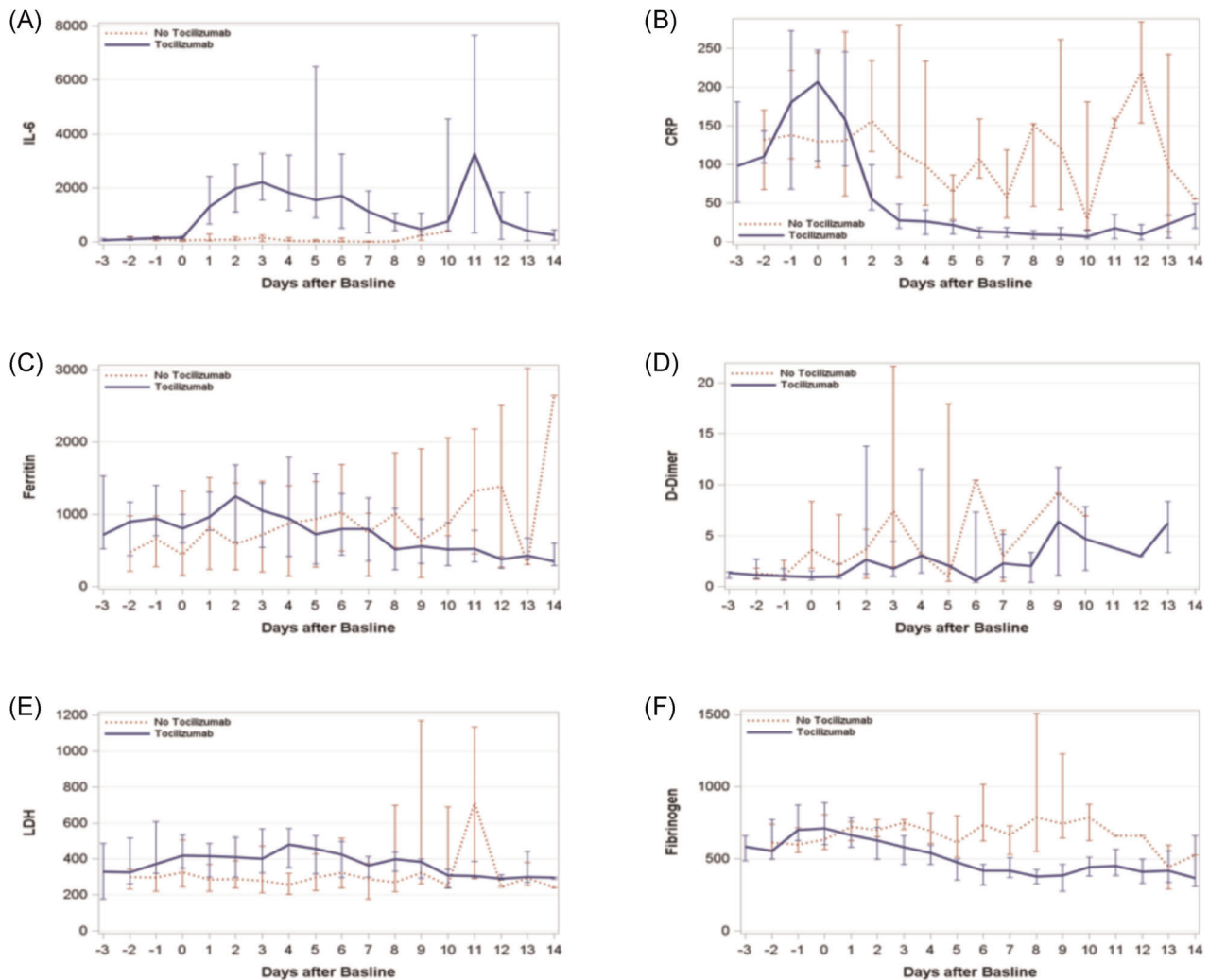
FIGURE 3 Kinetics of inflammatory laboratory markers. Units for other laboratory values are ng/ml for ferritin, mcg/ml for D-dimer, mg/dl for fibrinogen. The presented data indicate medians and the associated interquartile ranges. If multiple values were available for the same day, the maximum value was used. The number of patients contributing results to each panel are in Table S6. In Panel A, the late secondary increase in IL-6 levels was driven by two individuals who had progressive cardiopulmonary disease with concurrent bloodstream infections and ventilator-associated pneumonias at the time of secondary IL-6 increase. CRP, C-reactive protein (mg/L); IL-6, interleukin 6 (pg/ml); LDH, lactate dehydrogenase (U/L)

some of these differences, we compared outcomes within baseline severity categories and performed IPTW adjusted analyses. For most comparisons, we were limited to detecting relatively large differences. To avoid instability in the models, we did not include chronic lung disease, which only affected three individuals in the tocilizumab group, or hydroxychloroquine, which does not affect outcomes of hospitalized patients with COVID-19. Although the use of remdesivir may have affected our findings, it was used in a minority of participants, and blinded receipt of remdesivir versus placebo was balanced across the two cohorts. The observation that tocilizumab was used in sicker patients indicates opportunities for larger studies of earlier administration. Given that obesity is a risk factor for worse outcomes with COVID-19 and that more than half of our participants had a body mass index greater than 30, weight-based dosing could be considered to attain more standardized drug exposure across patients. Strengths of this study include the relatively large

cohort of patients requiring noninvasive and invasive oxygenation, along with careful adjustment for differences in patient characteristics and other confounders. We performed an RMST analysis for time to clinical improvement that is novel to studies of tocilizumab for COVID-19 and provide a tangible measure of treatment effect.[39] We performed comprehensive chart review and data abstraction to describe and analyze a variety of clinical and laboratory outcomes to evaluate the potential benefits and side effects of tocilizumab therapy, including novel comparisons of thrombotic events and resolution of sepsis physiology. Our data were not confounded by steroids, and most patients did not receive remdesivir.

In conclusion, there was no clear evidence of improved clinical outcomes among hospitalized patients with COVID-19 who received one dose of 400 mg of tocilizumab without concurrent steroids or effective antivirals. Tocilizumab was associated with a robust reduction in

inflammatory biomarkers; further study in patients with sepsis physiology may be warranted. Randomized controlled trials using tocilizumab in the context of concurrent effective antiviral and steroid therapy will be particularly important to guide evolving treatment strategies.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Joshua A. Hill, M.M., H. Nina Kim, and Guang-Shing Cheng designed the study and interpreted the data. Joshua A. Hil, M.M., Shireesha Dhanireddy, Margaret Green, Rupali Jain, Christine Johnston, Joanne Huang, and Guang-Shing Cheng contributed to data collection. Joshua A. Hil, H. Nina Kim, Hu Xie, and Wendy M Ieisenring analyzed the data and created the figures. Joshua A. Hil drafted the manuscript. All authors contributed to the writing and revision of the manuscript and approved the final version.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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