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RESEARCH ARTICLE

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Tocilizumab for the treatment of adult patients with severe COVID-19 pneumonia: A single-center cohort study

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Abstract

Coronavirus disease 2019 (COVID-19) can lead to a massive cytokine release. The use of the anti-interleukin-6 receptor monoclonal antibody tocilizumab (TCZ) has been proposed in this hyperinflammatory phase, although supporting evidence is limited. We retrospectively analyzed 88 consecutive patients with COVID-19 pneumonia that received at least one dose of intravenous TCZ in our institution between 16 and 27 March 2020. Clinical status from day 0 (first TCZ dose) through day 14 was assessed by a 6-point ordinal scale. The primary outcome was clinical improvement (hospital discharge and/or a decrease of ≥2 points on the 6-point scale) by day 7. Secondary outcomes included clinical improvement by day 14 and dynamics of vital signs and laboratory values. Rates

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of clinical improvement by days 7 and 14 were 44.3% (39/88) and 73.9% (65/88). Previous or concomitant receipt of subcutaneous interferon-β (adjusted odds ratio [aOR]: 0.23; 95% confidence interval [CI]: 0.06-0.94; $P = .041$) and serum lactate dehydrogenase more than 450 U/L at day 0 (aOR: 0.25; 95% CI: 0.06-0.99; $P = .048$) were negatively associated with clinical improvement by day 7. All-cause mortality was 6.8% (6/88). Body temperature and respiratory and cardiac rates significantly decreased by day 1 compared to day 0. Lymphocyte count and pulse oximetry oxygen saturation/FiO_2 ratio increased by days 3 and 5, whereas C-reactive protein levels dropped by day 2. There were no TCZ-attributable adverse events. In this observational single-center study, TCZ appeared to be useful and safe as immunomodulatory therapy for severe COVID-19 pneumonia.

KEYWORDS

COVID-19, immunomodulation, pneumonia, SARS-CoV-2, tocilizumab

1 | INTRODUCTION

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome (SARS) coronavirus 2 (SARS-CoV-2), has spread to reach pandemic proportions.[1] Although most cases have a mild to moderate disease course, a significant proportion of patients require hospital admission due to the development of pneumonia, which may ultimately result in acute respiratory distress syndrome (ARDS).[2] The case fatality rate among critically ill COVID-19 patients has been reported to exceed 50%.[3,4]

Beyond the direct viral cytopathic effect, the pathogenesis of severe SARS-CoV-2 infection involves a hyperinflammatory state that resembles the cytokine release syndrome (CRS) observed in patients with immune-mediated conditions or receiving chimeric antigen receptor (CAR) T-cell therapy.[5] The use of immunomodulatory therapies (such as corticosteroids, Janus kinase inhibitors, or colchicine) has been accordingly proposed to abrogate this deleterious hyperinflammatory response, although available clinical experience is limited.

The humanized anti-interleukin (IL)-6 receptor monoclonal antibody tocilizumab (TCZ) is being used off-label in patients with severe COVID-19 on the basis of the previous experience with CAR T-cell therapy-induced CRS and known safety profile.[6-8] However, until the results of ongoing clinical trials become available, supporting evidence was initially restricted to case reports[9-11] and a small study with only 21 patients from China.[12] Whether the blockade of IL-6 signaling at earlier stages of infection may modify the course of COVID-19 and reduce the requirements for invasive respiratory support remains to be addressed. We described our experience with patients consecutively diagnosed with COVID-19 pneumonia treated with TCZ and analyzed baseline predictors of therapeutic response to this immunomodulatory approach.

2 | METHODS

2.1 | Study population and design

The present retrospective cohort study was performed at the University Hospital "12 de Octubre", a 1300-bed tertiary care center that serves a population of approximately 450 000 inhabitants in Madrid (Spain). Consecutive patients diagnosed with COVID-19 pneumonia that received at least one dose of TCZ from 16 to 27 March 2020, were included. The present study was conducted in accordance with the amended Declaration of Helsinki. The local Institutional Research Board (Drug Research Ethics Committee [CEIm] of the Hospital Universitario "12 de Octubre") approved the study protocol (CEIm no. 20/117) and granted a waiver of informed consent due to its observational design. The research was performed in accordance with the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines.

For analysis purposes, the date of administration of the first TCZ infusion was considered as day 0. Patients were followed up to hospital discharge or death (whichever occurred earlier), or 15 April 2020. Demographics and major comorbidities; clinical presentation; serial assessment of vital signs, respiratory status, laboratory values, and radiological features; antiviral and other immunomodulatory therapies; attributable adverse events; and outcomes were collected from electronic medical records using a standardized case report form. All laboratory and imaging investigations were performed as part of standard of care. Respiratory function was assessed by means of the pulse oximetry oxygen saturation/fraction of inspired oxygen (SpO_2/FiO_2) ratio, which shows a good correlation with the partial pressure of arterial oxygen (PaO_2)/FiO_2 ratio.[13] Variations (Δ) in vital signs and laboratory values between days 0 and 3 were calculated ($[X_{day\ 3} - X_{day\ 0}] \times 100/X_{day\ 0}$). To this end, the latest measurement available throughout this 3-day period was used.

A 6-point ordinal scale was applied to analyze clinical status on the basis of the following categories: 1 = not hospitalized; 2 = hospitalized, not requiring supplemental oxygen; 3 = hospitalized, requiring low-flow supplemental oxygen (FiO_2 <40%); 4 = hospitalized, requiring high-flow supplemental oxygen (FiO_2 ≥40%) or noninvasive mechanical ventilation; 5 = hospitalized, requiring invasive mechanical ventilation (IMV), extracorporeal membrane oxygenation (ECMO), or both; and 6 = death.

2.2 | Study outcomes

The *primary study outcome* was the proportion of patients achieving clinical improvement (defined by hospital discharge and/or a decrease of ≥2 points from baseline [day 0] on the 6-point ordinal scale) by day 7 after the first TCZ dose. *Secondary outcomes* included the proportion of patients with clinical improvement by day 14, as well as the dynamics of vital signs (axillary temperature, respiratory and heart rate, and SpO_2/FiO_2 ratio) and laboratory values (absolute lymphocyte count, and serum C-reactive protein [CRP], lactate dehydrogenase [LDH], and D-dimer levels) from days 0 to 14. Due to the exploratory nature of the research, no sample size estimation on the basis of the above-detailed outcomes was performed.

2.3 | Antiviral and immunomodulatory therapies

In keeping with clinical practice guidelines proposed by the Spanish Ministry of Health[14] and local protocols in effect at that time, coformulated lopinavir/ritonavir (200/100 mg twice daily orally for up to 14 days) and/or hydroxychloroquine (400 mg twice orally for the first 24 hours, followed by 200 mg twice daily for 5-10 days) were prescribed to patients with pneumonia. Subcutaneous (SC) interferon (IFN)-β (250 μg every 48 hours) was added according to the criteria of the treating physician. Since these drugs were used off-label, written or oral informed consent was obtained from the patient or relatives. Corticosteroid therapy could be used at different regimens (intravenous [IV] methylprednisolone 0.5-1 mg/kg daily for ≤5 days or as boluses of 100-250 mg daily for 3 days). Empirical antibiotics were associated if bacterial superinfection was suspected. All patients received thromboprophylaxis with low-molecular-weight heparin.

Due to initial uncertainties on TCZ effectiveness and safety and potential drug shortages, a multidisciplinary committee that included all the clinical specialties involved and the Department of Pharmacy was established to standardize therapeutic decisions. The off-label use of TCZ was considered in patients potentially eligible for IMV, with bilateral (or rapidly progressive) interstitial or alveolar infiltrates in chest X-ray or computerized tomography (CT) scan, and fulfilling at least one of the following criteria: (a) respiratory frequency more than 30 breaths per minute and/or SpO_2 less than 92% on room air; (b) CRP levels more than 10 mg/dL; (c) IL-6 levels more than 40 pg/mL; and/or (d) D-dimer level more than 1500 ng/mL. Exclusion criteria included the presence of alanine aminotransferase and/or aspartate aminotransferase levels more than five times the upper normal limit, uncontrolled systemic infection due to other pathogens, or complicated acute diverticulitis or bowel perforation. An initial IV 400 mg (if body weight <75 kg) or 600 mg (if body weight ≥75 kg) TCZ dose was administered as 1-hour infusion. A second 400 mg dose was administered 12 hours later, whereas a third dose could be given after 24 hours from the first infusion to selected patients that had achieved only a partial response.

2.4 | Microbiological methods

The diagnosis of COVID-19 was made by means of SARS-CoV-2 real-time reverse transcription-polymerase chain reaction in nasopharyngeal or oropharyngeal swabs or sputum samples, as detailed in Supporting Information Methods. The diagnosis was also assumed in patients with a suggestive clinical and radiological presentation and compatible epidemiological history but repeatedly negative testing.

2.5 | Statistical analysis

Quantitative data were shown as the mean ± standard deviation or the median with interquartile range (IQR), whereas qualitative variables were expressed as absolute and relative frequencies. Categorical variables were compared using the χ^2 test. The Student *t* test or the Mann-Whitney *U* test were applied for continuous variables, as appropriate. Repeated measurements were compared using paired parametric or nonparametric tests (the Student *t* test for paired samples, the Wilcoxon signed-rank test or Friedman test), as dictated by data distribution. Baseline factors predicting clinical improvement at days 7 and 14 were analyzed by logistic regression, with associations expressed as odds ratios (ORs) with 95% confidence intervals (95% CIs). The potential effect of unintended variations in patient selection across the study period was assessed according to the calendar date of the first TCZ dose (16 to 20 March [first 5-day period], 21 to 25 March [second 5-day period]). Collinearity among explanatory variables was assessed by means of the variance inflation factor (VIF). The Hosmer-Lemeshow test was used to assess the goodness-of-fit of the models. The threshold for significance was set at a *P* value of less than .05. Statistical analysis was performed with SPSS version 20.0 (IBM Corp, Armonk, NY).

3 | RESULTS

3.1 | Characteristics of the study cohort

We included 88 patients whose demographics and clinical characteristics are shown in Table 1. A SpO_2/FiO_2 ratio of less than 316 (equivalent to a PaO_2/FiO_2 ratio <300[13]) was present at day 0 in 70.5% (62/88) of patients, whereas only three of them (3.4%) were

TABLE 1 Demographics and clinical characteristics of the study cohort (n = 88)

Variable	
Age [mean ± SD], y	46.8 ± 10.7
Male gender [n (%)]	58 (65.9)
Ethnicity [n (%)]	
Hispanic	54 (61.4)
Caucasian	31 (35.2)
Other	3 (3.4)
Comorbidities [n (%)]	
Hypertension	19 (21.6)
Diabetes mellitus	5 (5.7)
Dyslipidemia	14 (15.9)
Obesity	11 (15.1)
Chronic lung disease	12 (13.6)
Thyroid disease	6 (6.8)
Pregnancy	4 (4.5)
Malignancy	2 (2.3)
Signs and symptoms at presentation [n (%)]	
Fever	82 (93.2)
Cough	70 (79.5)
Productive cough	13 (14.8)
Dyspnea	55 (62.5)
Myalgias	40 (45.5)
Diarrhea	22 (25.0)
Chest pain	12 (13.6)
Olfactory and taste disorders	8 (9.1)
Rhinorrhea	4 (4.5)
Rash	1 (1.1)
Duration of symptoms [median (IQR)][a], d	10 (8-12)
Duration of dyspnea [median (IQR)][a], d	4 (2-6)
Vital signs [mean ± SD][b]	
Axillary temperature, °C	37.6 ± 0.9
Respiratory rate, breaths per min	27.3 ± 7.9
Heart rate, beats per min	84.9 ± 14.3
SpO_2/FiO_2 ratio	252 ± 98
Laboratory values [mean ± SD][b]	
White blood cell count, $\times 10^9$ cells/L	7.2 ± 3.3
Neutrophil count, $\times 10^9$ cells/L	5.6 ± 3.3
Lymphocyte count, $\times 10^9$ cells/L	0.9 ± 0.4
Neutrophil-to-lymphocyte ratio	7.7 ± 6.1
Platelet count, $\times 10^9$ cells/L	263 ± 100
ALT, U/L	62.5 ± 51.9
AST, U/L	62.2 ± 41.7
LDH, U/L	463.2 ± 153.5
CRP, mg/dLc	15.6 ± 8.3
CRP >10 mg/dL [n (%)][c]	64 (74.4)
Procalcitonin, ng/mL[d]	0.4 ± 0.2
IL-6, pg/mL[e]	109.6 ± 296.1
IL-6 >40 mg/dL [n (%)][e]	32 (58.2)
Ferritin, ng/mL[f]	1860 ± 2493
D-dimer, ng/mL[g]	650 ± 459
Chest imaging features [n (%)]	
Bilateral interstitial infiltrates	59 (67.0)
Bilateral alveolar infiltrates	25 (28.4)
Unilateral alveolar infiltrate	4 (4.5)
Prior or concomitant treatments	
HCQ [n (%)]	86 (97.7)
Interval [median (IQR)][h], d	2 (1-3)
LPV/r [n (%)]	73 (83.0)
Interval [median (IQR)][h], d	1 (0-3)
Azithromycin [n (%)]	65 (73.9)
Interval [median (IQR)][h], d	2 (1-3)
IFN-β [n (%)]	41 (46.6)
Interval [median (IQR)][h], d	1 (0-2)
Corticosteroids [n (%)]	7 (8.0)
Interval [median (IQR)][h], d	0 (0-0)

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; HCQ, hydroxychloroquine; IFN-β, interferon-β; IL-6, interleukin-6; IQR, interquartile range; LDH, lactate dehydrogenase; LPV/r, lopinavir/ritonavir; SD, standard deviation; SpO2/FiO2, pulse oximetry oxygen saturation/fraction of inspired oxygen.
[a]To the first tocilizumab dose (day 0).
[b]At the date of the first tocilizumab dose (day 0).
[c]CRP levels available for 86 patients at day 0.
[d]Procalcitonin levels available for 28 patients at day 0.
[e]IL-6 levels available for 55 patients at day 0.
[f]Ferritin levels available for 43 patients at day 0.
[g]D-dimer levels available for 37 patients at day 0.
[h]From the initiation of therapy to the first tocilizumab dose (day 0).

receiving IMV. Most patients with available data had serum CPR levels more than 10 mg/dL (74.4% [64/86]) and IL-6 levels more than 40 pg/mL (58.2% [32/55]). Three, two, and one doses of TCZ were administered in 8.0% (7/88), 63.8% (56/88), and 28.4% (25/88) of patients, respectively. The median intervals from the initiation of symptoms and, specifically, dyspnoea onset to the first dose were 10 (IQR: 8-12) and 4 (IQR: 2-6) days. No attributable adverse events were observed. The median follow-up period was 11 (IQR: 7-25) days. Individual patient trajectories are shown in Figure S1 in Supporting Information. All-cause mortality was 6.8% (6/88), with a median interval from day 0 to death of 6 (IQR: 3-11.5) days.

3.2 | Primary study outcome

The change in patient status according to the 6-point ordinal scale is shown in Figure 1. The proportion of patients achieving clinical improvement at day 7 (primary outcome) was 44.3% (39/88). In detail, 40.9% (36/88) had been discharged home by that day, whereas 3.4% (3/88) inpatients experienced a decrease of ≥2 points in the ordinal scale.

A number of significant differences emerged when patients with or without clinical improvement at day 7 were compared (Table 2). Those experiencing improvement were more likely to be male and had a previous diagnosis of hypertension, had a longer duration of symptoms, higher SpO_2/FiO_2 ratio, lower white blood cell and neutrophil counts, lower serum CRP, LDH, and IL-6 levels, and were less likely to have been treated with SC IFN-β. To better delineate baseline predictors of response, quantitative variables were dichotomized according to clinically relevant thresholds (ie, SpO_2/FiO_2 ratio <316; CPR >10 mg/dL; LDH >450 U/L; IL-6 >40 pg/mL). Previous or concomitant use of IFN-β (OR: 0.23; 95% CI: 0.06-0.94; $P = .041$) and serum LDH more than 450 U/L (OR: 0.25; 95% CI: 0.06-0.99; $P = .048$) were negatively associated with clinical improvement in the logistic regression model. No significant collinearity was observed (VIF values <1.6). The model remained essentially unchanged when the SpO_2/FiO_2 ratio and laboratory parameters were entered as continuous rather than categorical variables, or after adjusting for the 5-day inclusion period (data not shown).

3.3 | Secondary study outcomes

The rate of clinical improvement at day 14 was 73.9% (65/88). In detail, 64.8% (57/88) had been discharged, with a further 9.1% (8/88) of patients achieving a decrease of ≥2 points in the 6-point ordinal scale. Median 6-point ordinal score decreased from 3 points (IQR: 3-4) at day 0 to 1 point (IQR: 1-3) at day 14 (*P* value for repeated measures <.0001). At the univariate analysis, patients with clinical improvement by day 14 were younger, less likely to be male, exhibited longer duration of symptoms, and had higher SpO_2/FiO_2 ratio and lower LDH and IL-6 levels at baseline as compared with those with no improvement. In addition, they were less likely to have been treated with IFN-β and more likely to have received azithromycin (Table S1). Due to the low number of patients not experiencing improvement by day 14, multivariate analysis could not be performed.

The serial assessment of vital signs revealed that axillary temperature and respiratory and cardiac rates significantly decreased with respect to baseline already by day 1, whereas the SpO_2/FiO_2 ratio showed a significant increase only by day 5 (Figure 2). Regarding laboratory parameters, absolute lymphocyte counts significantly increased by day 3, whereas CPR levels dropped by day 2. D-dimers increased through days 2 and 3, although this parameter was available for a low number of patients (Figure 3).

Serum IL-6 was measured before the administration of TCZ (day 0 or before) in 62.5% (55/88) of patients, whereas 18.2% (16/88) had posttreatment levels assessed after a median of 4 days (IQR: 3-6.5). Comparison of paired measurements in patients with available data (n = 10) showed a significant increase between pre- and posttreatment times (median: 37.5 vs 206 pg/mL; *P* value for repeated measures = .002) (Figure S2).

We also explored the potential value of variations (Δ) in vital signs and laboratory parameters during the first 3 days from the first TCZ dose to predict response to therapy. Patients with clinical improvement by day 7 had higher Δ SpO_2/FiO_2 (median: 16.7% vs −20.3%; $P < .0001$) and lower Δ LDH (median: −0.28% vs 13.9%; $P = .011$) as compared to those with no improvement (Figure 4).

The proportions of patients with chest imaging performed (X-ray or CT) that showed radiological improvement with respect to baseline at days 3, 5, 7, and 10 were 19.4% (7/36), 26.1% (6/23), 16.7% (4/24), and 55.0% (11/20), respectively (Table S2).

Finally, we analyzed the separate impact of pretreatment patient status on outcomes. Most patients that did not require supplemental oxygen (category 2) or were receiving low-flow oxygen (category 3) at day 0 had been discharged home (category 1) by day 7 (66.7% [4/6] and 55.0% [22/40], respectively). On the contrary, the proportion of patients on high-flow oxygen (category 4) at baseline that required IMV or ECMO (category 5) or were dead (category 6) by day 7 were 20.5% (8/39) and 7.7% (3/39) (Figure 5). Regarding secondary outcome, 100.0% (6/6) and 75.0% (30/40) of patients in categories 2 and 3 at day 0 had been discharged by day 14, whereas

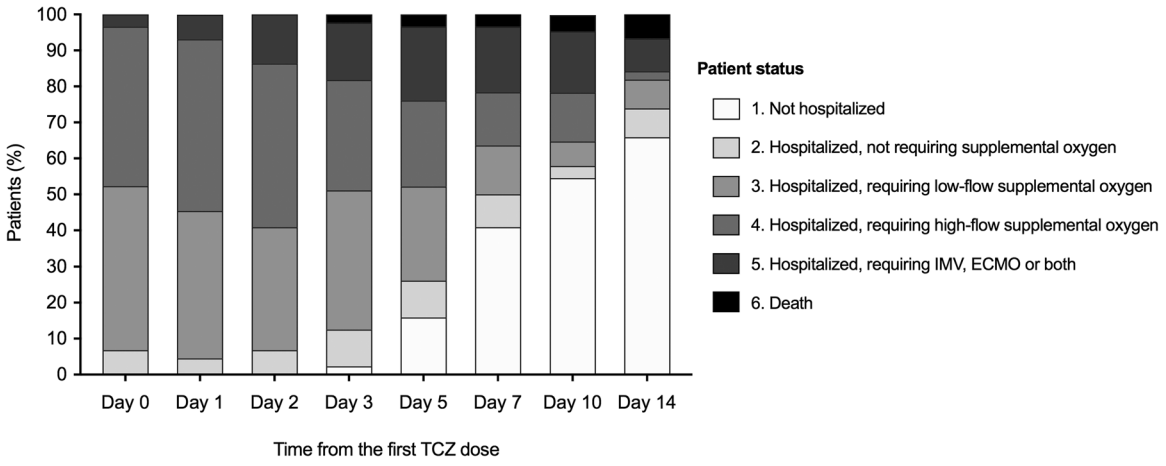

FIGURE 1 Patient status according to the 6-point ordinal scale at different times following the administration of the first dose of TCZ (day 0). ECMO, extracorporeal membrane oxygenation; IMV, invasive mechanical ventilation; TCZ, tocilizumab

TABLE 2 Univariate and multivariate analysis of factors present at the time of the first TCZ dose (day 0) predicting clinical improvement at day 7 (primary outcome)

Variable	Clinical improvement (n = 39)	No clinical improvement (n = 49)	*P* value	Univariate[a]			Multivariate[b]		
				OR	95% CI	*P* value	OR	95% CI	*P* value
Age, [mean ± SD], y	44.2 ± 12.1	48.9 ± 9.1	.039	**0.96**	**0.92-0.99**	**.043**	…	…	…
Male gender [n (%)]	21 (53.8)	37 (75.5)	.033	**0.38**	**0.15-0.94**	**.035**	…	…	…
Hispanic ethnicity [n (%)]	20 (51.3)	34 (69.4)	.083						
Hypertension [n (%)]	4 (10.3)	15 (30.6)	.021	**0.26**	**0.08-0.86**	**.027**	…	…	…
Diabetes mellitus [n (%)]	2 (5.1)	3 (6.1)	1.000						
Obesity [n (%)]	8 (20.5)	7 (14.3)	.440						
Chronic lung disease [n (%)]	3 (7.7)	9 (18.4)	.174						
Thyroid disease [n (%)]	2 (5.1)	4 (8.2)	.689						
Duration of symptoms [median (IQR)][c], d	11 (9.8-13)	10 (8-12)	.025	**1.19**	**1.02-1.41**	**.035**	…	…	…
Duration of dyspnea [median (IQR)][c], d	4 (2-6)	4 (2-7)	.599						
Three TCZ doses (vs one or two) [n (%)]	1 (2.6)	6 (12.2)	.127						
Respiratory rate, breaths per min [mean ± SD]	25.5 ± 8.0	28.8 ± 7.6	.080						
Heart rate, beats per min [mean ± SD]	83.3 ± 15.1	86.0 ± 13.7	.396						
SpO_2/FiO_2 ratio [mean ± SD]	282 ± 91	226 ± 97	.007	1.01	1.00-1.01	.010			
SpO_2/FiO_2 ratio <316 [n (%)]	23 (59.0)	39 (79.6)	.035	**0.37**	**0.14-0.95**	**.038**	…	…	…
Bilateral alveolar infiltrates [n (%)]	11 (28.2)	14 (28.6)	.970						
White blood cell count [mean ± SD], $\times 10^9$ cells/L	6.5 ± 2.4	7.8 ± 3.8	.074						
Neutrophil count [mean ± SD], $\times 10^9$ cells/L	4.9 ± 2.2	6.2 ± 3.8	.046	1.00	0.99-1.00	.065			
Lymphocyte count [mean ± SD], $\times 10^9$ cells/L	0.9 ± 0.4	0.8 ± 0.3	.390						
Neutrophil-to-lymphocyte ratio [mean ± SD]	6.6 ± 5.2	8.6 ± 6.6	.134						
CPR [mean ± SD][d], mg/dL	13.7 ± 7.8	17.1 ± 8.4	.054						
CPR level >10 mg/dL [n (%)][d]	24 (61.5)	40 (85.1)	.013	**0.28**	**0.10-0.78**	**.015**	…	…	…
LDH [mean ± SD], U/L	389.1 ± 110.8	521.2 ± 158.1	<.0001	0.99	0.98-0.99	.0003			
LDH >450 U/L [n (%)]	9 (25.0)	31 (67.4)	.0001	**0.16**	**0.06-0.43**	**.0002**	0.25	0.06-0.99	.048
IL-6 [mean ± SD][e], pg/mL	48.8 ± 74.6	168.1 ± 403.4	.135						
IL-6 level >40 pg/mL [n (%)][e]	11 (40.7)	21 (75.0)	.010	**0.23**	**0.07-0.72**	**.012**	…	…	…
Ferritin [mean ± SD][f], pg/mL	1182 ± 1003	2448 ± 3187	.097						

TABLE 2 (Continued)

Variable	Clinical improvement (n = 39)	No clinical improvement (n = 49)	*P* value	Univariate[a]			Multivariate[b]		
				OR	95% CI	*P* value	OR	95% CI	*P* value
Prior or concomitant treatments [n (%)]									
HCQ	38 (97.4)	48 (98.0)	1.000						
LPV/r	33 (84.6)	40 (81.6)	.712						
Azithromycin	29 (74.4)	36 (73.5)	.925						
IFN-β	13 (33.3)	28 (57.1)	.026	**0.38**	**0.16-0.89**	**.028**	0.23	0.06-0.94	.041
Corticosteroids	1 (2.6)	6 (12.2)	.127						

Abbreviations: CRP, C-reactive protein; HCQ, hydroxychloroquine; IFN-β, interferon-β; IL-6, interleukin-6; IQR, interquartile range; LDH, lactate dehydrogenase; LPV/r, lopinavir/ritonavir; OR, odds ratio; SD, standard deviation; SpO2/FiO2, pulse oximetry oxygen saturation/fraction of inspired oxygen; TCZ: tocilizumab.

[a]Bold characters indicate variables included in the multivariate model.

[b]Hosmer-Lemeshow test $P = .990$.

[c]To the first tocilizumab dose (day 0).

[d]CRP levels available for 86 patients at day 0.

[e]IL-6 levels available for 55 patients at day 0.

[f]Ferritin levels available for 43 patients at day 0.

12.8% (5/39) of patients with baseline category 4 were dead (Figure S3).

4 | DISCUSSION

As highlighted by the Infectious Disease Society of America,[15] the efficacy and safety of therapies for COVID-19 should be evaluated in the setting of a randomized clinical trial (RCT). Unfortunately, the labor- and time-consuming nature of such type of study poses an inherent difficulty due to the urgency of the current pandemic. Until results from RCTs become available, well-designed observational studies may be valuable for guiding therapeutic decisions. In the present single-center experience with 88 COVID-19 patients consecutively treated with TCZ, the rates of clinical improvement by days 7 and 14 were 44.3% and 73.9%, and as much as 81.8% scored 3 or lower on the ordinal scale at day 14. No safety signals were observed. These results may be regarded as encouraging considering that all patients had clinical, radiological, or laboratory features at baseline known to be associated with poor outcomes in COVID-19. In comparison, only 24.5% of patients recruited in the compassionate use study of remdesivir were on room air or required low-flow oxygen by day 7.[16]

Similarly to SARS, COVID-19 may lead to a hyperinflammatory state characterized by the rise of IL-2, IL-6, IL-7, and other cytokines and chemokines that contribute to lung damage, ARDS, and death.[2,5,17] IL-6 is a pleiotropic cytokine involved in inflammation and immune regulation. Among other functions, IL-6 mediates systemic effects such as fever and acute phase protein response, contributes to local inflammation, and participates in immunoregulation by promoting effector/regulatory T-cell balance and B-cell functions.[18] This theoretical rationale underlies the proposal for blocking IL-6 signaling as a therapeutic strategy in COVID-19.[7] In their seminal study Xu et al[12] reported outcomes in 21 severe or critically ill patients treated with TCZ, with body temperature, SpO_2, serum CPR, and lymphocyte count returning to normal ranges within the first 5 days. Most patients (19 out of 21) were discharged from the hospital, with rates of clinical and radiological improvement above 90.0%.[12] Sciascia et al[19] conducted an open-label, noncomparative multicentre study involving 63 adult patients, which experienced significant improvements in ferritin, CRP and D-dimer levels, as well as PaO_2/FiO_2 ratio.[19] A small retrospective case-control study reported that the use of TCZ was associated with lower mortality and/or admission rate to the intensive care unit (ICU), despite the higher comorbidity burden and more severe COVID-19 forms exhibited by these patients as compared to the control group.[20] No attributable adverse events were reported in none of these studies,[10,12,19,20] although the development of acute hypertriglyceridemia has been observed in two COVID-19 patients treated with TCZ (with laboratory findings consistent with acute pancreatitis in one of them).[21]

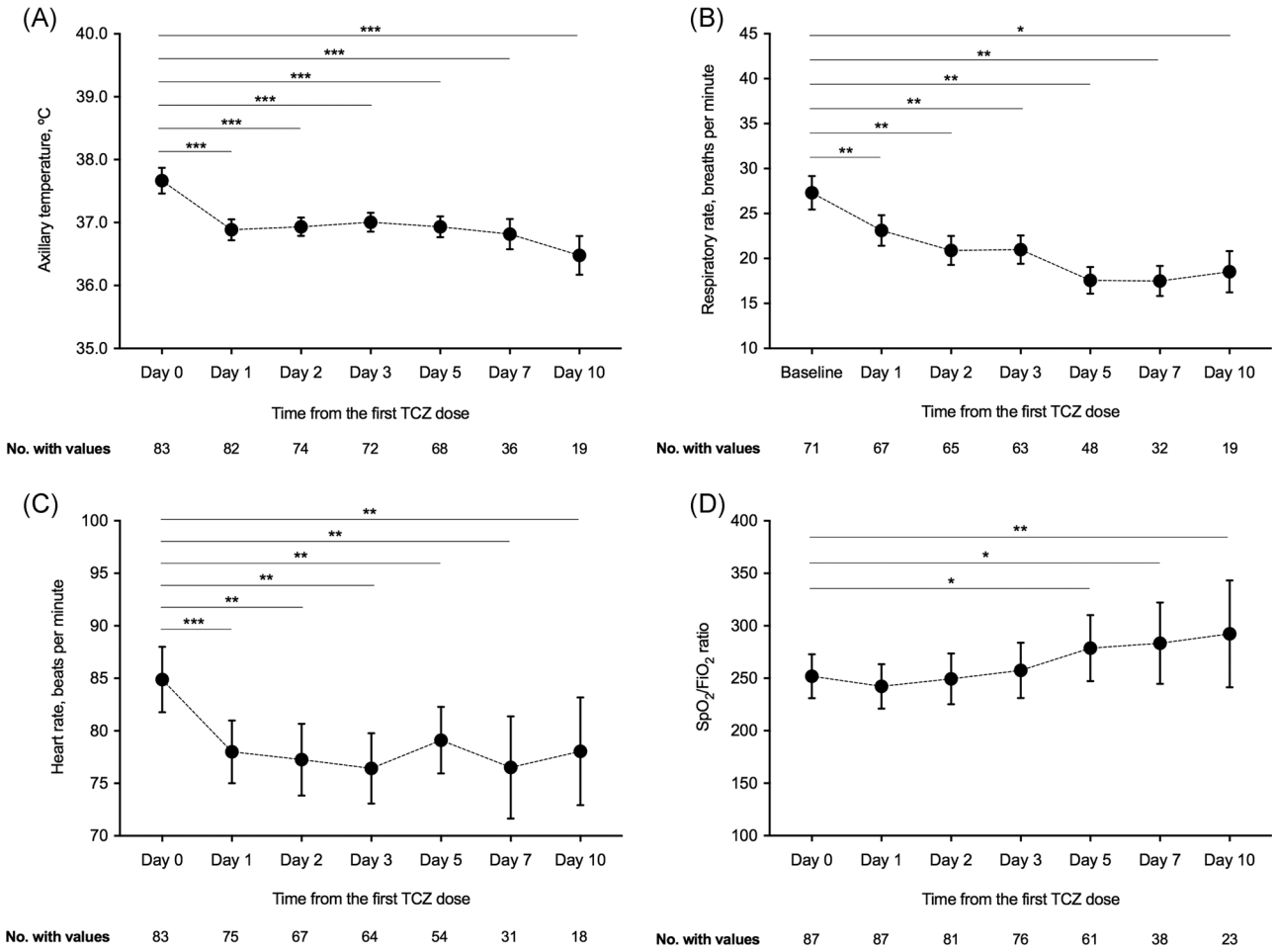

FIGURE 2 Evolution of vital signs following the administration of the first TCZ dose: (A) axillary temperature; (B) respiratory rate; (C) heart rate; and (D) SpO_2/FiO_2 ratio. $^*P < .05$; $^{**}P < .01$; $^{***}P < .0001$ (statistical test for repeated measures was used). SpO_2/FiO_2, pulse oximetry oxygen saturation/fraction of inspired oxygen; TCZ, tocilizumab

Preliminary results have been also reported for the anti-IL-6 monoclonal antibody siltuximab, although sample size was again small (n = 21). Serum CRP levels returned to the normal range by day 5, and one-third of patients experienced a decrease in their requirements for respiratory support. Nevertheless, 24.0% of them eventually underwent IMV.[22]

The dosing regimen of TCZ used in the present study was modeled on that approved for CAR T-cell therapy-induced CRS.[6] Only a minority of patients (8.0%) required a third infusion after 24 hours, suggesting that response may be achieved with only one or two doses. This observation is valuable to plan drug supply in the face of potential shortages. Nevertheless, Luo et al[23] observed in a small case series that, although CPR levels usually decreased rapidly following TCZ therapy, three out of four critically ill patients that received a single dose dead, whereas the CRP level in the remaining patient (that experienced disease aggravation) failed to return to the normal range.[23] Thus, the administration of repeated TCZ doses should be still considered for patients with advanced stages of COVID-19 and persistent elevation of acute-phase reactants.

The presence of serum LDH levels more than 450 U/L at day 0 was identified as an independent factor negatively associated with clinical improvement by day 7. In addition, LDH levels decreased (negative Δ) during the first 72 hours among patients with a response to TCZ in contrast to those not experiencing improvement. It is well known that high serum LDH may reflect cell damage and inflammation in lung tissues, as observed in patients with *Pneumocystis jirovecii* pneumonia.[24,25] Serial assessment of serum LDH may offer a readily available approach to guide the selection of candidates for TCZ therapy and dosing regimens. On the other hand, the use of IFN-β exerted a negative impact, although this finding must be taken with caution due to potential confounding by indication. The role of type I IFN responses in the natural history of SARS is unclear, and it has been proposed that hyperinnate immune responses at early stages of SARS-CoV infection—hallmarked by the robust expression of

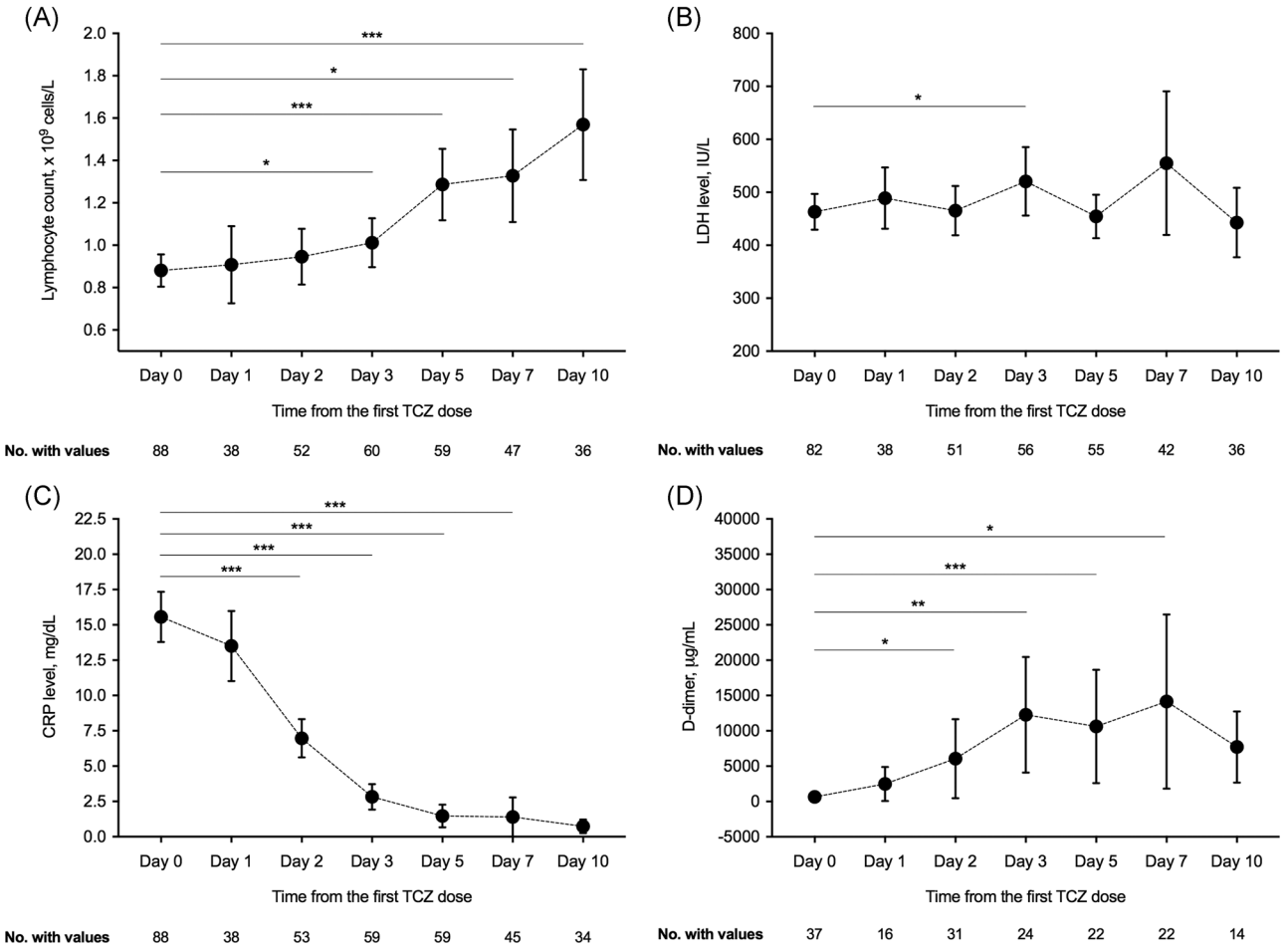

FIGURE 3 Evolution of laboratory values following the administration of the first TCZ dose: (A) lymphocyte count; (B) LDH level; (C) CRP level; and (D) D-dimer level. *$P < .05$; **$P < .01$; ***$P < .0001$ (statistical test for repeated measures was used). CRP, C-reactive protein; LDH, lactate dehydrogenase; TCZ, tocilizumab

IFN-α, IFN-γ, and IFN-stimulated genes—may preclude the development of effective adaptive immunity.[26] In addition to its proinflammatory activity, IL-6 plays a role in mounting adaptive T-cell and humoral responses.[27] It could be hypothesized that the sequential blockade of IL-6 signaling in the setting of ongoing IFN-β therapy and absence of effective antiviral treatment would abrogate immune control of SARS-CoV-2 replication and contribute to poorer outcomes.

Although previous studies have analyzed all-cause mortality and/or ICU admission as primary outcome,[12,19,20,28,29] none of them assessed dynamic changes in clinical status or baseline factors predictive of therapeutic response. The present experience would suggest that the early initiation of TCZ, when patients still do not require supplemental oxygen or receive low-flow oxygen therapy, might be associated with better outcomes as compared to those at more advanced phases. Our attempt of initiating IL-6 blockade in patients with bilateral lung infiltrates and increased inflammatory parameters but not yet requiring invasive respiratory support is in contrast with other experiences, since 19.0% and 10.0% of patients in the study by Xu et al[12] were critically ill or required mechanical ventilation at the time of therapy.[12] Alattar et al[28] reported outcomes in 25 patients admitted to the ICU at the time of receipt of the first TCZ dose. Only 36% of participants achieved the primary endpoint of being discharged alive from ICU by day 14, whereas half of them were still in the ICU and 12% had dead.[28] On the contrary, the administration of TCZ within the first 6 days from hospital admission was associated with an increased likelihood of survival in a further study.[19] In a recent retrospective study performed in three Italian centers, Guaraldi et al[29] reported that the use of TCZ (either IV or SC) reduced the risk of IMV or death as compared to standard care alone.[29] It should be noted that the patients included in that study were substantially older than ours (median age of 61 vs 45.5 years, respectively) and had higher

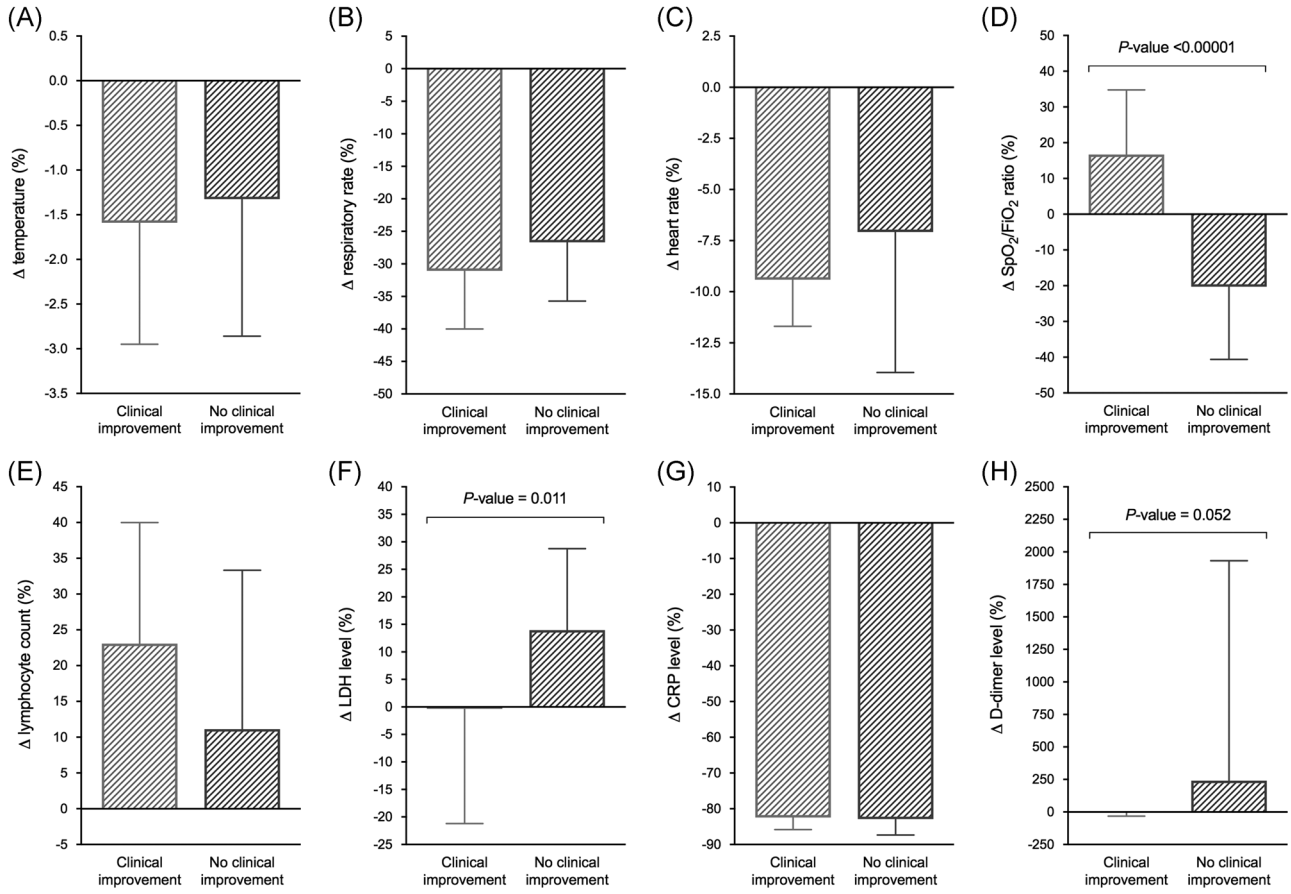

FIGURE 4 Median variations (Δ) with 95% confidence intervals for vital signs and laboratory values between days 0 and 3 after the administration of the first TCZ dose in patients with and without clinical improvement at day 7: (A) axillary temperature; (B) respiratory rate; (C) heart rate; (D) SpO_2/FiO_2 ratio; (E) lymphocyte count; (F) LDH level; (G) CRP level; and (H) D-dimer level. CRP, C-reactive protein; LDH, lactate dehydrogenase; SpO_2/FiO_2, pulse oximetry oxygen saturation/fraction of inspired oxygen; TCZ, tocilizumab

disease severity at the time of therapy (as suggested by differences in baseline PaO_2/FiO_2 ratios). Optimal timing for immunomodulation in COVID-19 remains to be assessed, and we cannot rule out that the potential benefit derived from TCZ may be more evident among patients at later stages of the disease.

Quite unexpectedly, the interval from symptom onset to the initiation of therapy was higher in patients with clinical improvement as compared to those without, with no differences in the time elapsed from the appearance of dyspnoea. Again, this observation may be subject to confounding by indication, since those with a more severe course would have been prompted to seek care earlier. On the other hand, it has been suggested that patients with COVID-19 would not feel dyspnoea even at advanced stages of the disease as a distinct neurological manifestation of SARS-CoV-2 infection.[30]

The present study has a number of limitations, the most relevant of which is the lack of a control group. Indeed, we cannot exclude that the overall favorable results observed for TCZ-treated patients would have been explained by the natural course of the disease. Nevertheless, it should be noted that all patients had baseline factors predictive of poor outcome and progression to ARDS. In addition, the rapid improvement in vital signs and laboratory values would substantiate the biological effect of therapy. Due to drug restrictions, strict criteria had to be applied to select candidates to TCZ. Therefore, the present cohort may not be representative of the entire COVID-19 patient population. Finally, no multivariate adjustment could be carried out for factors predicting clinical improvement by day 14 due to insufficient sample size.

In conclusion, early initiation of TCZ appears to be useful and safe for patients with COVID-19 pneumonia and poor prognosis factors (such as bilateral involvement, respiratory failure, or increased inflammatory parameters). High baseline or increasing LDH levels and previous or concurrent use of SC IFN-β identified patients less likely to benefit from this therapy. Although these results must be confirmed by RCTs, this experience suggests that immunomodulatory therapy with TCZ may have a role during the hyperinflammatory state of SARS-CoV-2 infection.

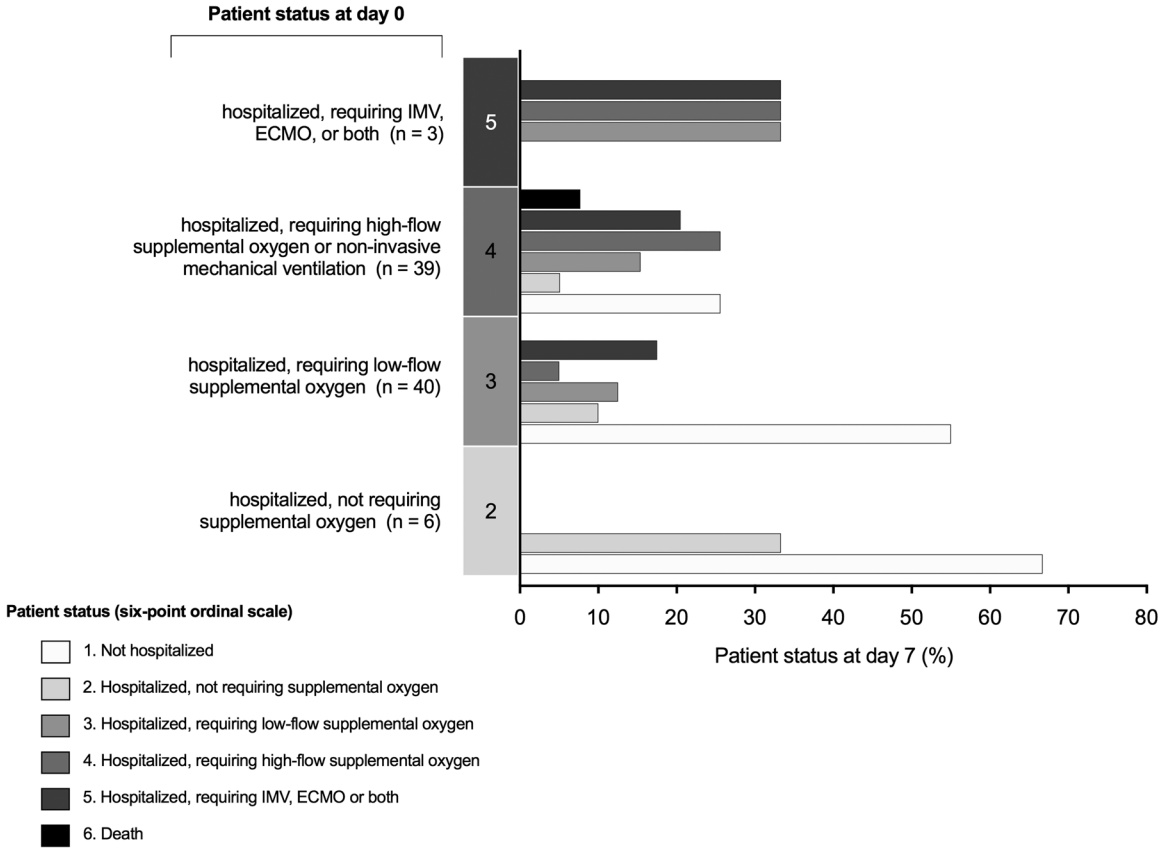

FIGURE 5 Impact of patient status according to the 6-point ordinal scale at day 0 on the clinical outcome by day 7 after the administration of the first tocilizumab dose. ECMO, extracorporeal membrane oxygenation; IMV, invasive mechanical ventilation

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CONFLICT OF INTERESTS

MFR holds a research contract "Miguel Servet" (CP 18/00073) from the Instituto de Salud Carlos III, Spanish Ministry of Science and Innovation. The other authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

MFR, FLM, and JMA designed the study; MFR and FLM analyzed the data; MFR and FLM wrote the manuscript; RLG performed immunological assays (ie, IL-6 measurement); CL and JMA were in charge of overall direction and planning; MAPJA, GMC, HB, JMCT, MC, CC, RGG, CG, RLG, ML, JML, JO, JLP, MR, RSJ, HT, and CL substantially contributed to the implementation of research, data analysis and interpretation, and critical review of the manuscript.

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REFERENCES

1. Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med*. 2020;382:727-733.
2. Wu C, Chen X, Cai Y, et al. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. *JAMA Intern Med*. 2020;180:1-11.
3. Arentz M, Yim E, Klaff L, et al. Characteristics and outcomes of 21 critically ill patients with COVID-19 in Washington State. *JAMA*. 2020;323:1612-1614.
4. Yang X, Yu Y, Xu J, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med*. 2020;8:475-481.
5. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020;395:497-506.
6. Kotch C, Barrett D, Teachey DT. Tocilizumab for the treatment of chimeric antigen receptor T cell-induced cytokine release syndrome. *Expert Rev Clin Immunol*. 2019;15:813-822.
7. Liu B, Li M, Zhou Z, Guan X, Xiang Y. Can we use interleukin-6 (IL-6) blockade for coronavirus disease 2019 (COVID-19)-induced cytokine release syndrome (CRS)? *J Autoimmun*. 2020;111:102452.
8. Zhang C, Wu Z, Li JW, Zhao H, Wang GQ. The cytokine release syndrome (CRS) of severe COVID-19 and interleukin-6 receptor (IL-6R) antagonist Tocilizumab may be the key to reduce the mortality. *Int J Antimicrob Agents*. 2020;55:105954.
9. Fontana F, Alfano G, Mori G, et al. Covid-19 pneumonia in a kidney transplant recipient successfully treated with tocilizumab and hydroxychloroquine. *Am J Transplant*. 2020;20:1902-1906.
10. Di Giambenedetto S, Ciccullo A, Borghetti A, et al. Off-label use of tocilizumab in patients with SARS-CoV-2 infection. *J Med Virol*. 2020. https://doi.org/10.1002/jmv.25897
11. Cellina M, Orsi M, Bombaci F, Sala M, Marino P, Oliva G. Favorable changes of CT findings in a patient with COVID-19 pneumonia after treatment with tocilizumab. *Diagn Interv Imaging*. 2020;101:323-324.
12. Xu X, Han M, Li T, et al. Effective treatment of severe COVID-19 patients with tocilizumab. *Proc Natl Acad Sci USA*. 2020;117:10970-10975.
13. Rice TW, Wheeler AP, Bernard GR, et al. Comparison of the SpO_2/FIO_2 ratio and the PaO_2/FIO_2 ratio in patients with acute lung injury or ARDS. *Chest*. 2007;132:410-417.
14. Ministerio de Sanidad, Centro de Coordinación de Alertas y Emergencias Sanitarias. Documento técnico de manejo clínico de pacientes con enfermedad por el nuevo coronavirus (COVID-19) (versión 3 de Marzo de 2020) [in Spanish]. Available at: https://www.mscbs.gob.es/profesionales/saludPublica/ccayes/alertasActual/nCov-China/documentos/Protocolo_manejo_clinico_COVID-19.pdf. Accessed April 1, 2020.
15. Bhimraj A, Morgan RL, Hirsch Shumaker A, et al. Infectious Diseases Society of America Guidelines on the treatment and management of patients with COVID-19 (Version 1.0.4). Available at: https://www.idsociety.org/globalassets/idsa/practice-guidelines/covid-19/treatment/idsa-covid-19-gl-tx-and-mgmt-v1.0.4.pdf. Accessed April 26, 2020.
16. Grein J, Ohmagari N, Shin D, et al. Compassionate use of remdesivir for patients with severe Covid-19. *N Engl J Med*. 2020;382:2327-2336.
17. Lagunas-Rangel FA, Chavez-Valencia V. High IL-6/IFN-gamma ratio could be associated with severe disease in COVID-19 patients. *J Med Virol*. 2020. https://doi.org/10.1002/jmv.25900
18. Kimura A, Kishimoto T. IL-6: regulator of Treg/Th17 balance. *Eur J Immunol*. 2010;40:1830-1835.
19. Sciascia S, Aprà F, Baffa A, et al. Pilot prospective open, single-arm multicentre study on off-label use of tocilizumab in patients with severe COVID-19. *Clin Exp Rheumatol*. 2020;38:529-532.
20. Klopfenstein T, Zayet S, Lohse A, et al. Tocilizumab therapy reduced intensive care unit admissions and/or mortality in COVID-19 patients. *Med Mal Infect*. 2020. https://doi.org/10.1016/j.medmal.2020.05.001
21. Morrison AR, Johnson JM, Ramesh M, Bradley P, Jennings J, Smith ZR. Acute hypertriglyceridemia in patients with COVID-19 receiving tocilizumab. *J Med Virol*. 2020. https://doi.org/10.1002/jmv.25907
22. Gritti G, Raimondi F, Ripamonti D, et al. Use of siltuximab in patients with COVID-19 pneumonia requiring ventilatory support. *medRxiv*. 2020. https://doi.org/10.1101/2020.04.01.20048561
23. Luo P, Liu Y, Qiu L, Liu X, Liu D, Li J. Tocilizumab treatment in COVID-19: a single center experience. *J Med Virol*. 2020;92:814-818.
24. Drent M, Cobben NA, Henderson RF, Wouters EF, van Dieijen-Visser M. Usefulness of lactate dehydrogenase and its isoenzymes as indicators of lung damage or inflammation. *Eur Respir J*. 1996;9:1736-1742.
25. Smith RL, Ripps CS, Lewis ML. Elevated lactate dehydrogenase values in patients with *Pneumocystis carinii* pneumonia. *Chest*. 1988;93:987-992.
26. Cameron MJ, Ran L, Xu L, et al. Interferon-mediated immunopathological events are associated with atypical innate and adaptive immune responses in patients with severe acute respiratory syndrome. *J Virol*. 2007;81:8692-8706.
27. Kopf M, Baumann H, Freer G, et al. Impaired immune and acute-phase responses in interleukin-6-deficient mice. *Nature*. 1994;368:339-342.
28. Alattar R, Ibrahim TBH, Shaar SH, et al. Tocilizumab for the treatment of severe coronavirus disease 2019. *J Med Virol*. 2020. https://doi.org/10.1002/jmv.25964
29. Guaraldi G, Meschiari M, Cozzi-Lepri A, et al. Tocilizumab in patients with severe COVID-19: a retrospective cohort study. *Lancet Rheumatol*. 2020. https://doi.org/10.1016/S2665-9913(20)30173-9
30. Bertran Recasens B, Martinez-Llorens JM, Rodriguez-Sevilla JJ, Rubio MA. Lack of dyspnea in Covid-19 patients; another neurological conundrum? *Eur J Neurol*. 2020. https://doi.org/10.1111/ene.14265

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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