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Research article

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Efficacy of tocilizumab treatment in COVID-19 patients with cytokine release syndrome

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Ethics Committee Approval
The study was conducted with the permission of The Medical Research Ethics Committee of the Bakirkoy Dr. Sadi Konuk Training and Research Hospital, 30/04/2020, 2020/162.
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

Conflict of Interest
No conflict of interest was declared by the authors.

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Abstract

Background/Aim: Cytokine release syndrome, a potentially life-threatening condition in SARS-CoV-2 (COVID-19) patients, plays a critical role that may lead to the prioritization of tocilizumab (TCZ) in the treatment of this disease. We aimed to present our TCZ-treated SARS-CoV-2 patients' data that might help and guide clinicians in dealing with this infectious disease in their daily practice and research.
Methods: This is a retrospective multicenter cohort study from two Turkish pandemic centers. A total of 5165 patients' data who were hospitalized due to SARS-CoV-2 pneumonia from March 16 to May 20, 2020 were screened and 72 patients treated with TCZ were included in the study. We evaluated patients' demographic data, laboratory and imaging studies, and clinical outcomes and the effect of TCZ treatment on patients' laboratory and clinical results.
Results: O_2 saturation levels significantly increased, and fever significantly decreased on the 5th day after TCZ therapy compared to before its initiation (P=0.001, P=0.010, respectively). The decrease in troponin-I, creatinine, LDH, fibrinogen, CRP, procalcitonin, CK, and ferritin levels after TCZ therapy were significant (P<0.05 for all). There was no significant difference in mortality rates with regards to CT results, duration of hospitalization, and the location of initiation of TCZ therapy (clinic vs. ICU) (P>0.05 for all). Importantly, we found a significant increase in mortality rates in patients who received azithromycin, oseltamivir, and ascorbic acid treatments compared to those who did not receive those treatments (P<0.05 for all).
Conclusion: Our results showed that TCZ treatment may improve the SpO_2 levels, fever and laboratory findings and repress further deterioration of severe SARS-CoV-2 patients. TCZ treatment can be given to the patients in non-ICU clinical beds. It is obvious that randomized controlled studies are needed to observe the efficacy of tocilizumab treatment in COVID-19 patients more clearly.

Keywords: COVID-19, tocilizumab, SARS-CoV-2, cytokine release syndrome

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Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) outbreak was announced by the World Health Organization (WHO) on 30 January 2020 as a public health emergency of international concern (PHEIC) and a pandemic on 11 March 2020. There have been 37,109,851 laboratory-confirmed cases and 1,070,355 deaths globally as of October 11, 2020 [1].

In its most severe form, SARS-CoV-2 (COVID-19) causes life-threatening pneumonia and acute respiratory distress syndrome, which has a mortality rate of up to 40-50 percent [2,3]. Although the mechanisms of SARS-CoV-2 induced lung injury are still being elucidated, studies showed that the cytokine release syndrome (CRS) -a.k.a. cytokine storm- contributes to the mortality of SARS-CoV-2. CRS, a potentially life-threatening condition mediated by overproduction of proinflammatory cytokines is observed in most critical patients infected with SARS-CoV-2 [4,5]. The pathophysiology of the hyperinflammation caused by SARS-CoV-2 has not been well studied, but first studies regarding the pathological alterations in the peripheral blood of these patients, which showed increased differentiation of CD4 T cells to proinflammatory CCR6 + Th17 cells, excessive activation of T cells, and high cytotoxicity of CD8 T cells, partly explain the severe immune injury in these patients [6]. This excessive and dysregulated host immune response may contribute to the development of ARDS in patients infected with SARS-CoV-2 [7, 8].

The cytokine profile in SARS-CoV-2 patients resembles Macrophage Activation Syndrome / secondary hemophagocytic lymphohistiocytosis (MAS/sHLH) and is characterized by increased serum levels of interleukin (IL)-1β, IL-2, IL-6, IL-7, IL-10, IL-18, granulocyte-colony stimulating factor, interferon-γ inducible protein 10, monocyte chemoattractant protein 1, macrophage inflammatory protein 1-α, and tumor necrosis factor-α [5, 9, 10].

A recent retrospective, multicenter study of 150 confirmed SARS-CoV-2 cases showed an association between mortality and elevated serum IL-6 levels of the patients. All these findings suggest that mortality might be due to SARS-CoV-2 driven severe immune injury [11]. The increased levels of proinflammatory cytokines in patients infected with SARS-CoV-2 and the relationship between mortality and hyperinflammation in these patients brought up immunosuppressive therapy. The similarity of cytokine profile of SARS-CoV-2 patients and MAS/sHLH patients and the opinion that IL-6 levels may play a critical role in these patients led to the prioritization of tocilizumab (TCZ) in the treatment of immunosuppression [12-14].

TCZ, a recombinant monoclonal antibody against the human IL-6 receptor (IL-6R), specifically binds both soluble IL-6R and membrane IL-6R, and blocks signaling pathways involving IL-6 [15]. Currently, TCZ is not approved for use by the Food and Drug Association (FDA) in patients with COVID-19-induced CRS but it is already approved for adult and pediatric patients for the treatment of CRS caused by chimeric antigen receptor (CAR) T -cells [16, 17]. It is used for rheumatoid arthritis and the studies conducted on animals regarding long- term toxicity showed it was well tolerated. Also, in other clinicopathological studies of this drug, no substantial abnormalities were detected [18, 19].

Early identification and management of the CRS are of crucial importance for patients infected with SARS-CoV-2. However, there are limited real-life data about the effect of TCZ on the inflammatory activity in newly observed SARS-CoV-2 patients [20]. In this retrospective observational study, we aimed to provide treatment-related outcomes associated with TCZ use in COVID-19 patients as well as guidance to clinicians.

Materials and methods

Study design and patients

This is a retrospective, multicenter cohort study from two pandemic state hospitals of Turkey. The study data were gathered from the records of those patients who were treated with at least a single dose of 400 mg TCZ infusion. The only available TCZ drug and form in our country is Actemra® (400 mg/20 cc flacon) (Roche Pharma [Schweiz] Ltd, B2084B21). The study flow chart was shown in Figure 1.

The Turkish Ministry of Health's Coronavirus Scientific Advisory Board recommendations were followed by the clinicians dealing with the management of SARS-CoV-2 infection at Turkish hospitals. The advised contraindications to this drug in SARS-CoV-2 infected patients were as follows: Presence of pregnancy, other active other viral infections (such as viral hepatitis, HIV), active or suspicious bacterial infection(s), having an absolute neutrophil count <500 /mm^3, platelet count <50000 /mm^3, and a history of diverticulitis. Before treatment, patients were screened and questioned for contraindications. Patients whose outcome, laboratory and/or clinical finding data could not be obtained and those younger than 18 years of age were excluded from the study. Receiving TCZ treatment between March 16 to May 20, 2020 for CRS was considered the inclusion criteria. To prevent selection bias, different researcher groups gathered and evaluated the patient data.

The Medical Research Ethics Committee of the University of Health Sciences Bakirkoy Dr. Sadi Konuk Training and Research Hospital approved the study (Approval number: 2020/162–30/04/2020). We are committed to protecting patient privacy and comply with the Helsinki Declaration.

This cross-sectional study is reported according to the STROBE statement (http://www.strobe-statement.org).

Diagnosis of CRS

CRS diagnosis was made by a consensus between the pulmonologists, rheumatologists, internal medicine, and infectious disease physicians in relevant hospitals according to The Turkish Ministry of Health's Coronavirus Scientific Advisory Board recommendations and the guideline prepared by scientific committee of both hospitals.

Treatment

All patients participating in our study received standard care according to the National Guideline for the Diagnosis and Treatment Protocol for SARS-CoV-2 Infection [21], including hydroxychloroquine (HCQ), favipiravir, azithromycin (AZT), low-molecular-weight heparin (LMWH), methylprednisolone, other symptom relievers, and oxygen therapy. Patients who were

diagnosed with CRS received 400 mg TCZ intravenously once a day for a single or two consecutive days.

Laboratory studies

Complete blood count (CBC), procalcitonin, ferritin, C reactive protein (CRP), D-dimer, activated partial thromboplastin time (APTT), prothrombin time (PT), international normalized ratio (INR) and routine biochemical tests were obtained before the initiation of and 5 days after TCZ treatment. Patients whose laboratory data were not available before or after TCZ administration were considered study dropouts.

All laboratory tests were performed at the central laboratories of the hospitals. Both are accredited laboratories with standardized internal quality control and external quality assurance measures to monitor the accuracy and precision of the performed tests. All biochemical tests including alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH), gamma-glutamyl transferase (GGT), lipase, total bilirubin, direct bilirubin, ferritin, triglycerides (TG), D-dimer, Troponin-I, procalcitonin, CRP, creatine kinase (CK), magnesium (Mg), phosphorus (P), sodium (Na), and potassium (K) levels were determined using Beckman Coulter AU5800 clinical chemistry analyzer (Beckman Coulter, Brea, CA, USA). CBC was analyzed with ADVIA 2120i hematology autoanalyzer (Siemens Healthcare Diagnostics, Erlangen, Germany). For coagulation assay (Fibrinogen), blood samples were collected into (0.105 mol/L) trisodium citrate- containing test tubes. The samples were centrifuged at 2000g for 15 minutes. All analytical procedures were performed on a random- access coagulation analyzer (Beijing Succeeder Technology Inc. China) and the reagents were used according to manufacturer's protocol.

Radiological evaluation

Patients were scanned with spiral computerized tomography (CT) on admission using a low-dosage, 64-slice, helical CT scanner (Somatom Somatom 64, Siemens Healthcare, Forchheim, Germany). Whole-lung CT images were evaluated and reported by at least one experienced radiologist. Radiological findings of SARS-CoV-2 pneumonia were classified into four types (mild involvement, moderate involvement, severe involvement, and no radiological finding) according to chest computed tomography severity score (CT-SS) published by Yang et al. [22].

Patients' demographic data and clinical outcome

The demographic, comorbidity, and clinical outcome data of the patients were gathered from the medical records of hospitals. Clinical outcomes were evaluated by overall survival. The lowest value of O_2 saturation and the highest value of fever before TCZ administration were selected as baseline values before TCZ therapy and those obtained 5 days after TCZ administration were considered after TCZ therapy values.

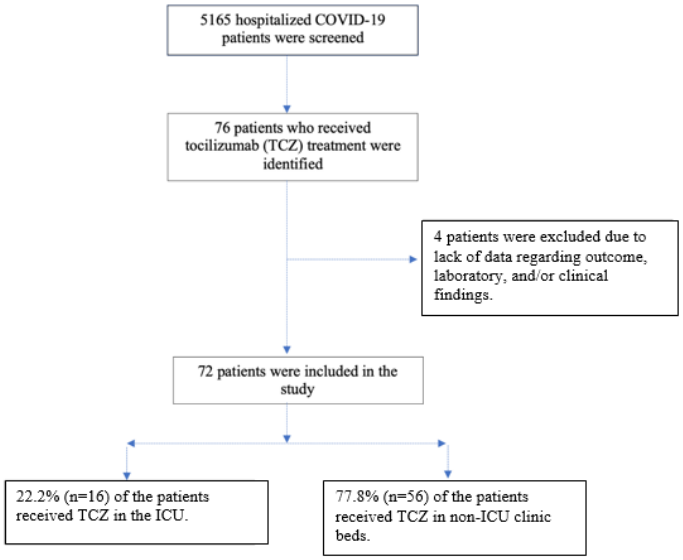
Statistical analysis

To determine the number of patients that should be included in the study, the data of the study conducted by Aomar-Millan et al. [23] were used. It was concluded that forty-five patients should be included in the sample for maximum power. However, considering that patient data may be missing, all seventy-two patients who could be reached were included in the study. NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA) program was used for statistical analysis. Descriptive statistics were used when evaluating the study data. The suitability of quantitative data to normal distribution was assessed by Shapiro-Wilk test and graphical evaluations. Student's t-test or Mann Whitney U was used for comparing two groups. In the comparison of qualitative data, Pearson Chi-Square test, Fisher-Freeman-Halton Exact test and Fisher's Exact test were used. Paired Sample t-test was used for pre- and post-drug comparisons of normally distributed parameters, and Wilcoxon Signed Ranks test was utilized for non-normally distributed parameters. No imputation was made for missing data. Significance was evaluated at the level of $P<0.05$.

Results

A total of 5165 patients were retrospectively screened, out of which seventy-six patients had received TCZ. Four patients were excluded based on the exclusion criteria and seventy-two patients' data were included in this study. The study flow chart is presented in Figure 1.

Figure 1: Study Flow Chart



Demographics and clinical characteristics of the patients

Among 72 patients who received TCZ, 22.2% (n=16) were females and 77.8% (n=56) were males. The mean age of the study population was 54.58 (11.45) (min-max: 22-73) years. While 41.7% (n=30) of the patients did not have any comorbidities, 58.3% (n=42) of them had at least one comorbid disease (CD). The fever on the admission of the patients ranged between 36 to 40 °C, with an average of 37.11 (0.86) °C, and the SpO_2 measurements on admission were between 67% and 99% with an average of 90.27% (6.66). Regarding the CT results of the patients, 11.1% (n=8) of the cases had mild involvement, 33.3% (n=24) had moderate involvement and 55.6% (n=40) had severe involvement. While 22.2% (n=16) of all cases received TCZ therapy in ICU, 77.8% (n=56) received TCZ therapy in non-ICU clinic beds. Demographics and clinical characteristics of the patients are presented in Table 1.

Follow-up and clinical outcomes of the patients

The duration of hospitalization ranged from 4 to 50 days with an average of 18.82 (9.36) days (median: 17 days). While 44.4% (n=32) died, 55.6% (n=40) were discharged from the hospital. Intensive care unit (ICU) admission was observed in

66.7% (n=48) of the cases before, after, or at the time of TCZ treatment (Table 1).

Table 1: Demographics, baseline characteristics, and clinical outcomes of the patients

		n	%
Age (year)	Min – Max (Median)	22 - 73 (56)	
	Mean (SD)	54.58 (11.45)	
Sex	Female	16	22.2
	Male	56	77.8
Participating hospitals	Bakirkoy Dr. Sadi Konuk Training and Research Hospital	43	59.7
	Bagcilar Training and Research Hospital	29	40.3
Comorbidity	No	30	41.7
	Yes	42	58.3
# of comorbid diseases	No	30	41.7
	1	20	27.8
	2	10	13.9
	3	10	13.9
	4	2	2.8
Fever on admission (°C)	Min – Max (Median)	36 - 40 (37)	
	Mean (SD)	37.11 (0.86)	
SpO2 on admission (%)	Min – Max (Median)	67 - 99 (91)	
	Mean (SD)	90.27 (6.66)	
CT results	Mild involvement	8	11.1
	Moderate involvement	24	33.3
	Severe involvement	40	55.6
ICU admission	No	24	33.3
	Yes	48	66.7
The location of initiation of TCZ therapy	ICU	16	22.2
	Clinic	56	77.8
Outcome	Dead	32	44.4
	Discharged	40	55.6
Duration of hospitalization (day)	Min – Max (Median)	4 - 50 (17)	
	Mean (SD)	18.82 (9.36)	

CT: Computerized Tomography, ICU: Intensive Care Unit, TCZ: Tocilizumab

Treatments other than TCZ of the patients

While all patients (n=72) received HCQ, 97.2% (n=70) received LMWH, 97.2% (n=70) received favipiravir, 87.5% (n=63) received AZT, 75.0% (n=54) received acetylsalicylic acid (ASA), 68.1% (n=49) received oseltamivir, and 51.4% (n=37) received ascorbic acid. The treatments of the patients are shown in Table 2.

Comparison of demographic and clinical findings of the patients according to clinical outcome

There was no statistically significant difference in mortality rates by age and gender. While there was a significant correlation between the presence of CD and mortality (P=0.037), no significant correlation was found between the number of CDs and mortality (see Table 3 for p values). There was no significant difference in mortality rates according to the location of the initiation of TCZ therapy (clinic vs. ICU), CT results, and the duration of hospitalization (P>0.05 for all). The fever on admission was significantly higher in patients who died than those discharged (P=0.032), however, there was no such significance with regards to SpO_2 levels on admission (P=0.331). The mortality rate was significantly higher in patients admitted to the ICU than those who were not (P=0.001). Comparison of demographic and clinical findings of the patients according to clinical outcomes are shown in Table 3.

Evaluation of the effect of the treatments other than TCZ on clinical outcome

Since all our cases received HCQ, LMWH, Favipiravir, and TCZ treatments, there was no significant difference between mortality rates and these drugs, as expected. No significant difference was found in mortality rates between patients who did and did not receive ASA (P=0.584). Importantly, we found a significant increase in mortality rates in patients who received AZT, oseltamivir, or ascorbic acid treatments compared to those who did not (P<0.05 for all) (Table 4).

Table 2: Treatments those patients received other than TCZ

			n	%
HCQ	Receiving Status	Not received	0	0
		Received	72	100
	Days taken	Min – Max (Median)	4 - 14 (9)	
		Mean (SD)	8.65 (2.71)	
	Initiation day of the therapy after admission	Min – Max (Median)	1 - 14 (1)	
		Mean (SD)	1.33 (1.70)	
AZT	Receiving Status	Not received	9	12.5
		Received	63	87.5
	Days taken	Min – Max (Median)	2 - 10 (5)	
		Mean (SD)	5.30 (1.23)	
	Initiation day of the therapy after admission	Min – Max (Median)	1 - 8 (1)	
		Mean (SD)	1.29 (1.20)	
Oseltamivir	Receiving Status	Not received	23	31.9
		Received	49	68.1
	Days taken	Min – Max (Median)	0 - 10 (5)	
		Mean (SD)	4.67 (1.92)	
	Initiation day of the therapy after admission	Min – Max (Median)	0 - 6 (1)	
		Mean (SD)	1.08 (0.73)	
LMWH	Receiving Status	Not received	2	2.8
		Received	70	97.2
	Days taken	Min – Max (Median)	2 - 40 (15)	
		Mean (SD)	16.33 (7.77)	
	Initiation day of the therapy after admission	Min – Max (Median)	1 - 10 (1)	
		Mean (SD)	2.14 (2.05)	
Favipiravir	Receiving Status	Not received	2	2.8
		Received	70	97.2
	Days taken	Min – Max (Median)	4 - 9 (5)	
		Mean (SD)	5.31 (0.93)	
	Initiation day of the therapy after admission	Min – Max (Median)	1 - 15 (3.5)	
		Mean (SD)	3.74 (2.72)	
TCZ	Receiving Status	Not received	0	0
		Received	72	100
	Days taken	Min – Max (Median)	1 - 3 (2)	
		Mean (SD)	1.92 (0.44)	
	Initiation day of the therapy after admission	Min – Max (Median)	1 - 25 (7)	
		Mean (SD)	8.43 (4.91)	
ASA	Receiving Status	Not received	18	25.0
		Received	54	75.0
	Days taken	Min – Max (Median)	1 - 32 (10)	
		Mean (SD)	11.11 (6.23)	
	Initiation day of the therapy after admission	Min – Max (Median)	1 - 35 (6)	
		Mean (SD)	6.07 (5.77)	
Ascorbic acid	Receiving Status	Not received	35	48.6
		Received	37	51.4
	Days taken	Min – Max (Median)	1 - 25 (6)	
		Mean (SD)	7.95 (5.91)	
	Initiation day of the therapy after admission	Min – Max (Median)	1 - 16 (5)	
		Mean (SD)	5.41 (3.22)	

HCQ: Hydroxychloroquine, AZT: Azithromycin, LMWH: Low-Molecular-Weight-Heparin, TCZ: Tocilizumab, ASA: Acetylsalicylic acid

Comparison of clinical and laboratory findings before and 5 days after the tocilizumab therapy

The O_2 saturation levels significantly increased, and fever significantly decreased on the 5th day after TCZ treatment (mean estimate of difference: 4.97 [95% CI 2.49 to 7.46, P=0.001]) and 0.34 [95% CI 0.08 to 0.61, P=0.010], respectively). While the change in AST, GGT, urea, D-dimer, WBC, NEU, HB, and APTT levels after TCZ treatment was not significant, ALT, lipase, lymphocyte, and PLT levels significantly increased after TCZ administration (see Table 5 for p values). The decreases in troponin-I, creatinine, LDH, CRP, procalcitonin, CK, ferritin, PTZ, and INR levels after TCZ therapy were significant (see Table 5 for P-values and the comparison results of rest of the laboratory findings).

Table 3: Evaluation of the relationship between mortality and demographic and clinical features

		Outcome		Test value	*P*-value
		Discharged (n=40) n (%)	Death (n=32) n (%)		
Age (year)	Min – Max (Median)	22 - 73 (54.5)	24 - 72 (57.5)	t: 0.254	[c]0.800
	Mean (SD)	54.28 (10.93)	54.97 (12.24)		
Sex	Female	7 (17.5)	9 (28.1)	χ^2: 1.161	[d]0.281
	Male	33 (82.5)	23 (71.9)		
Comorbidity	No	21 (52.5)	9 (28.1)	χ^2: 4.346	[d]0.037*
	Yes	19 (47.5)	23 (71.9)		
# of comorbid diseases	No	21 (52.5)	9 (28.1)	χ^2: 7.200	[d]0.066
	1	11 (27.5)	9 (28.1)		
	2	5 (12.5)	5 (15.6)		
	≥3	3 (7.5)	9 (28.2)		
Fever on admission (°C)	Min – Max (Median)	36 – 38.5 (36.8)	36 - 40 (37.2)	t: 2.182	[c]0.032*
	Mean (SD)	36.91 (0.79)	37.35 (0.90)		
SpO2 on admission (%)	Min – Max (Median)	71 - 99 (91.5)	67 - 98 (91)	t: -0.978	[c]0.331
	Mean (SD)	90.95 (6.39)	89.39 (7.01)		
CT results	Mild involvement	3 (7.5)	5 (15.6)	χ^2: 5.780	[e]0.054
	Moderate involvement	18 (45.0)	6 (18.8)		
	Severe involvement	19 (47.5)	21 (65.6)		
ICU admission	No	24 (60.0)	0 (0)	χ^2: 28.800	[d]0.001**
	Yes	16 (40.0)	32 (100)		
The location of initiation of TCZ therapy	ICU	7 (17.5)	9 (28.1)	χ^2: 1.161	[d]0.281
	Clinic	33 (82.5)	23 (71.9)		
Duration of hospitalization (day)	Min – Max (Median)	5 - 50 (17)	4 - 37 (16)	Z: -1.056	[f]0.291
	Mean (SD)	20.20 (10.39)	17.09 (7.71)		

[c] Student t Test, [d] Pearson's chi-squared test, [e] Fisher Freeman Halton Exact Test, [f] Mann Whitney U Test, * *P*<0.05, ** *P*<0.01, CT: Computerized Tomography, ICU: Intensive Care Unit, TCZ: Tocilizumab

Table 4: Evaluation of the relationship between mortality and treatments that patients received other than TCZ

		Outcome		Test value	*P*-value
		Discharged (n=40) n (%)	Death (n=32) n (%)		
HCQ	Not received	0	0	-	-
	Received	40 (100)	32 (100)		
AZT	Not received	8 (20.0)	1 (3.1)	χ^2: 4.629	[g]0.037*
	Received	32 (80.0)	31 (96.9)		
Oseltamivir	Not received	17 (42.5)	6 (18.8)	χ^2: 4.613	[d]0.032*
	Received	23 (57.5)	26 (81.3)		
LMWH	Not received	0	2 (6.3)	χ^2: 2.571	[g]0.194
	Received	40 (100)	30 (93.8)		
Favipiravir	Not received	0	2 (6.3)	χ^2: 2.571	[g]0.194
	Received	40 (100)	30 (93.8)		
TCZ	Not received	0	0	-	-
	Received	40 (100)	32 (100)		
ASA	Not received	11 (27.5)	7 (21.9)	χ^2: 0.300	[d]0.584
	Received	29 (72.5)	25 (78.1)		
Ascorbic acid	Not received	26 (65.0)	9 (28.1)	χ^2: 9.677	[d]0.002**
	Received	14 (35.0)	23 (71.9)		

[d] Pearson's chi-squared test, [g] Fisher's Exact Test, * *P*<0.05, ** *P*<0.01, HCQ: Hydroxychloroquine, AZT: Azithromycin, LMWH: Low-Molecular-Weight-Heparin, TCZ: Tocilizumab, ASA: Acetylsalicylic acid

Table 5: Comparison of laboratory findings and clinical characteristics before and 5 days after the TCZ therapy

		n	Min – Max (Median)	Mean (SD)	Test value	*P*-value
SpO2	Before	54	64 - 99 (89)	88.07 (7.88)	t:-4.022	[a]0.001**
	After	54	65 - 100 (94.5)	93.06 (6.19)		
Fever (°C)	Before	60	34 - 39 (36.5)	36.65 (0.97)	t:-2.656	[a]0.010*
	After	60	36 – 37.3 (36.1)	36.30 (0.36)		
AST (U/L) (range: 0-40)	Before	60	22 - 296 (58.5)	77.05 (52.73)	Z:-0.286	[b]0.775
	After	60	16 - 561 (55.5)	88.83 (101.93)		
ALT (IU/L) (range: 0-41)	Before	60	13 - 341 (46.5)	67.32 (67.25)	Z:-2.970	[b]0.003**
	After	60	12 - 736 (72.5)	105.73 (117.93)		
GGT (U/L) (range: <55)	Before	58	14 - 478 (47)	83.16 (92.49)	Z:-1·878	[b]0.060
	After	58	19 - 609 (61)	118.34 (133.78)		
Direct Bilirubin (mg/dL) (range: 0-0.2)	Before	60	0 – 1.7 (0.2)	0.25 (0.24)	Z:-2.664	[b]0.008**
	After	60	0 – 1.5 (0.1)	0.19 (0.21)		
Indirect Bilirubin (mg/dL) (range: 0-1.2)	Before	61	0.1 – 1.3 (0.5)	0.48 (0.23)	Z:-1.288	[b]0.198
	After	61	0 – 1.4 (0.4)	0.44 (0.28)		
Lipase (U/L) (range: 0-67)	Before	45	1.8 - 186 (32.5)	50.21 (42.40)	Z:-2.799	[b]0.005**
	After	45	12.5 - 493 (60)	92.85 (91.41)		
Troponin-I (pg/mL) (range: 0-17.5)	Before	58	0 – 2984.9 (12)	129.97 (433.07)	Z:-3.350	[b]0.001**
	After	58	0 - 1268 (6.7)	66.55 (192.61)		
Creatinine (mg/dL) (range: 0.7-1.2)	Before	60	0.3 – 7.4 (0.8)	1.08 (1.17)	Z:-3.353	[b]0.001**
	After	60	0.2 – 6.3 (0.7)	0.90 (0.85)		
Urea (mg/dL) (range: 17-43)	Before	60	9 – 295.6 (38.4)	49.27 (40.54)	Z:-1.823	[b]0.068
	After	60	18 – 225.2 (39.2)	55.62 (42.60)		
LDH (U/L) (range: 135-248)	Before	60	225 - 1512 (544.5)	582.42 (251.85)	Z:-2.102	[b]0.036*
	After	60	6.1 - 1717 (495)	519.37 (261.46)		
D-dimer (µg FEU/mL) (range: 0-0.5)	Before	60	0 – 8.6 (0.8)	2.05 (2.64)	Z:-1.351	[b]0.177
	After	60	0 – 7.8 (1.8)	2.24 (1.84)		
Fibrinogen (mg/dL) (range: 200-400)	Before	43	108 - 905 (607)	588.70 (189.40)	Z:-5.020	[b]0.001**
	After	43	150 - 905 (340)	345.70 (137.03)		
Triglyceride (mg/dL) (range: 0-200)	Before	35	52 - 355 (113)	147.47 (83.72)	Z:-3.292	[b]0.001**
	After	35	82 - 614 (184)	222.31 (127.6)		
# WBC (10e3/uL) (range: 3.7-10.1)	Before	60	1.9 – 31.9 (8.1)	9.81 (5.60)	Z:-0.015	[b]0.988
	After	60	1.6 – 39.8 (7.9)	9.73 (6.42)		
# LYM (10e3/uL) (range: 1.09-2.99)	Before	60	0 – 3.6 (0.8)	0.89 (0.56)	Z:-3.087	[b]0.002**
	After	60	0.1 – 4.9 (1)	1.28 (0.95)		
# NEU (10e3/uL) (range: 1.63-6.96)	Before	60	0.5 – 28.8 (6.9)	8.16 (5.38)	Z:-0.226	[b]0.821
	After	60	1 – 34.7 (6.5)	7.78 (6.07)		
# PLT (10e3/uL) (range: 155-366)	Before	60	24 - 657 (227)	254.67 (131.19)	Z:-3.386	[b]0.001**
	After	60	27 - 634 (323)	314.40 (139.78)		
Hb (g/dL) (range: 12.9-15.9)	Before	60	6.3 – 15.4 (12.3)	11.89 (2.00)	t:1.144	[b]0.257
	After	60	6.9 – 16.6 (11.6)	11.68 (2.26)		
CRP (mg/L) (range: <5)	Before	60	1.3 – 422.6 (183.6)	185.24 (109.54)	Z:-6.670	[b]0.001**
	After	60	0.7 – 250.6 (11)	27.26 (47.37)		
Procalcitonin (ng/mL) (range: <0.5)	Before	60	0 - 66 (0.3)	2.50 (8.94)	Z:-5.416	[b]0.001**
	After	60	0 – 15.9 (0.1)	0.63 (2.31)		
Ferritin (µg/L) (range: 23.9-336.2)	Before	60	101.1 - 6903(1025.5)	1165.71 (966.93)	Z:-4.627	[b]0.001**
	After	60	54.6 - 3554 (581.6)	744.62 (638.76)		
Magnesium (mg/dL) (range: 1.8-2.6)	Before	54	1.6 – 4.5 (2.2)	2.27 (0.44)	t:0.444	[a]0.659
	After	54	1.6 – 4.2 (2.2)	2.23 (0.38)		
Sodium (mmol/L) (range: 136-145)	Before	60	123 - 156 (136)	137.03 (6.47)	t:-2.842	[a]0.006**
	After	60	126 - 157 (139)	139.30 (6.56)		
Potassium (mmol/L) (range: 3.5-5.1)	Before	60	0.5 – 5.7 (4.1)	4.08 (0.76)	t:-1.763	[a]0.083
	After	60	3 – 5.7 (4.3)	4.30 (0.62)		
Phosphorus (mg/dL) (range: 2·5-4·5)	Before	60	0.5 – 9.6 (3)	3.11 (1.32)	t:-1.118	[a]0.268
	After	60	1.5 – 7.3 (3.1)	3.38 (1.16)		
APTT (sec.) (range: 27-45)	Before	60	24.1 – 60.8 (38)	38.78 (6.99)	t:0.915	[a]0.364
	After	60	19.6 – 74.8 (36.4)	37.85 (9.13)		
PT (sec.) (range: 11-15)	Before	41	12.3 - 22 (15.7)	16.12 (2.33)	t:3.680	[a]0.001**
	After	41	11.4 – 21.8 (14.6)	14.73 (1.90)		
INR (range: 0.8-1.2)	Before	42	1 – 1.8 (1.2)	1.24 (0.18)	Z:-3.978	[b]0.001**
	After	42	0.9 – 1.7 (1.1)	1.13 (0.13)		
CK (U/L) (range: 0-171)	Before	59	16 - 5316 (134)	409.03 (847.23)	Z:-3.569	[b]0.001**
	After	59	0.2 - 2406 (69)	199.02 (402.59)		

[a] Paired Samples, t Test, [b] Wilcoxon Signed Ranks Test,* *P*<0.05, ** *P*<0.01, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, GGT: Gamma Glutamyl Transferase, LDH: Lactate dehydrogenase, WBC: White Blood Cells Count, LYM: Lymphocyte count, NEU: Neutrophil count, PLT: platelet count, Hb: Hemoglobin, CRP: C-reactive protein, APTT: Activated Partial Thromboplastin Time, PT: Prothrombin Time, INR: International Normalized Ratio, CK: Creatine Kinase

Discussion

In this study, we retrospectively evaluated the efficacy of TCZ treatment in critically ill SARS-CoV-2 patients. The overall mortality rate of SARS-CoV-2 pneumonia was 4.9% in the hospitals who participated in the study (unpublished data) whereas the overall mortality rate of our study population who received TCZ therapy was 55% over a median follow-up of 17 days. The initiation rate of TCZ was 1.3%. While the true mortality rate of SARS-CoV-2 infection is not known, studies published so far indicate that the crude death rate (the number of reported deaths divided by the reported cases) of SARS-CoV-2 infection is around 4% [13]. Although the definition of 'critically-ill patient' has not been clearly determined in SARS-CoV-2 patients, mortality rates among patients with worsened clinical and laboratory findings, despite the recommended standard treatments, can reach up to around 66% [2,24]. Because effective treatment is yet to be determined in critically ill patients, high mortality rates among these patients indicate that we need treatments that can prevent the development of critical illness and findings that can predict this course. In previous studies, mortality rates have been reported up to 78% in severe cases who need invasive ventilation, admission to ICU, or both [25-31]. The wide variation in these mortality rates may be due to lack of standardized national and/or international criteria guidelines for intensive care admission of SARS-CoV-2 infected patients. These criteria vary from country to country, even from hospital to hospital. For example, early invasive ventilation is recommended in some places, while in others, delaying invasive ventilation and early intensive care follow-up are recommended. Therefore, although the patients in these studies appear in a similar category, indeed they are not so. They include patients at different critical levels.

In our study, we observed a significant decrease in troponin-I, LDH, CRP, and ferritin levels along with an increase in O_2 saturation percent and a decrease in fever values on the 5^{th} day after TCZ treatment. These findings show that TCZ treatment provides clinical and laboratory improvement in these patients. Since we did not have a control group in our study, we could not determine whether TCZ treatment has a definite effect on mortality and median follow-up time. Among all patients, 77.8% received TCZ treatment in non-ICU clinical beds. The discharge rates among patients who received TCZ in non-ICU and ICU beds were 59% and 43%, respectively. It should be taken into consideration that one of the reasons for the higher mortality rate in ICU may be due to the delayed initiation of TCZ treatment to those patients. These findings suggest that TCZ treatment can be given safely to the patients in non-ICU clinical beds. If these findings could be supported with new randomized controlled studies, it might be of paramount importance to clinicians dealing with such patients (especially in centers lacking sufficient ICU beds).

In our study, we did not find a relationship between mortality, age and gender in patients receiving TCZ treatment. While there was a correlation between the presence of CD and mortality, there was no such correlation between the number of CDs and mortality. Although we observed that the fever level on admission was higher in patients who died (in comparison to patients who survived), the same relationship was not detected with SpO_2. Similarly, we did not find a relationship between mortality and CT results on admission.

Since all our patients received HCQ, LMWH, Favipiravir, and TCZ treatments, their relationship with mortality was out of evaluation, but ASA treatment did not affect mortality. Interestingly, mortality was significantly higher in patients receiving AZT, oseltamivir, and ascorbic acid therapies. In our study, it would not be rational to decide the effects of these treatments on mortality alone due to the insufficient number of patients receiving these treatments alone. Therefore, studies involving more patients are needed at this point.

Among the important limitations of our study are the limited number of patients and the absence of comparison between our study population and a control group. Our study is an observational study, and a significance bias might exist. Also, not including the safety outcomes, such as adverse events or infections constitutes another limitation. Still, the findings of this study might guide the more detailed and randomized studies in this field.

Future directions

There are currently ongoing global phase III trials including the COVACTA, EMPACTA, and REMDACTA trials that aim to show the efficacy of TCZ in COVID-19 patients.

COVACTA trial, which aimed to improve the clinical situation in COVID-19 patients with pneumonia and reduce patient mortality, could not reach its neither primary nor secondary endpoints. However, the study is ongoing, and researchers are determined to further investigate TCZ in other treatment settings, including in combination with an antiviral agent [32].

Phase III EMPACTA study showed 44% less mechanical ventilation or death in COVID-19-associated pneumonia patients receiving TCZ treatment in addition to standard care when compared to patients receiving placebo plus standard care [33]. EMPACTA also showed that non-ICU patients can receive TCZ during COVID-19.

Another ongoing, phase III, randomized, double-blind, multicenter study, REMDACTA, aims to investigate the efficacy and safety of the combination of remdesivir plus tocilizumab in hospitalized patients with severe COVID-19 pneumonia [34].

Conclusion

Our study results show that TCZ treatment may improve SpO_2, fever levels and laboratory findings, and may repress the deterioration of severe SARS-CoV-2 patients. Since there was no control group in our study, we could not determine whether TCZ treatment had a definite effect on mortality and median follow-up time. If these findings could be supported with new randomized controlled studies, it might be of paramount importance to clinicians dealing with such patients (especially in centers lacking sufficient ICU beds). It is obvious that to see the efficacy of tocilizumab treatment in COVID-19 patients with cytokine release syndrome more clearly, the results of the ongoing studies and further randomized large studies are needed.

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