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Treatment with Tocilizumab in Adult Patients with Moderate to Critical COVID-19 Pneumonia: A Single-Center Retrospective Study

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ABSTRACT

Objectives: This study aimed to assess if tocilizumab (TCZ) timing is associated with improved survival.
Material and methods: Data obtained from adult patients with moderate/severe/critical COVID-19 and treated with TCZ, who were admitted to the Teaching Hospital of Infectious Diseases, Cluj-Napoca, Romania (April 2020-April 2021), were retrospectively analyzed. The database included demographics, clinical data, computed tomography scan results, the kinetics of IL-6, laboratory variables, and the outcome until discharge.
Results: A total of 221 patients received dexamethasone, antivirals, anticoagulants, and 1-2 doses of TCZ, 8 mg/kg. In 2021, more patients received high-flow oxigen/non-invasive ventilation compared to those hospitalized in 2020, but demographics, in-hospital mortality, and laboratory data did not differ significantly. In-hospital mortality was associated with age, disease severity, lung damage, intensive care unit (ICU) admission, cardiovascular comorbidities, and IL-6>100 pg/mL at TCZ administration. In multivariate analysis the risk of death was significantly higher in patients with a persistent inflammatory state, adjusted odds ratio (aOR) 16.6 (95% CI 3.07-108.96); lung damage>40%, aOR 11.68 (95% CI 2.05-224.98); and cardiovascular comorbidities>2, aOR 3.65 (95% CI 1.06-12.53). TCZ initiation at ≤3 days after admission showed improved survival, odds ratio (OR)=0.39 (95% CI 0.16-0.9). Severe infections were found in 11 (4.9%) patients.
Conclusion: Early initiation of TCZ seems beneficial and safe in patients with moderate to critical COVID-19 pneumonia.

1. Introduction

The ongoing pandemic of coronavirus disease 2019 (COVID-19) caused a substantial death toll despite an incredible search for effective treatment regimens. Cytokine release syndrome (CRS), especially that involving the release of interleukin-6 (IL-6), plays a major role in mediating acute lung injury, leading to poor clinical outcomes (Xu et al., 2020; Mehta et al., 2020). The administration of corticosteroids has been proven to reduce mortality among patients requiring respiratory support (Fernandez-Ruiz et al., 2021, WHO, 2021 a).

In an attempt to enhance the intervention toward cytokines storm, the use of IL-6 inhibitors has been shown to be a potential option in moderate or severe COVID-19 pneumonia. The humanized anti–IL-6 receptor monoclonal antibody tocilizumab (TCZ) is being used in patients with moderate or severe COVID-19 (including Romania) on the basis of the previous experience with immune-mediated conditions and known safety profile (Ministry of Health, 2020, 2021; WHO, 2020; WHO, 2021a, b). The National COVID-19 Protocol included intravenous administration of TCZ 8 mg/kg in 1-2 doses (total dose – maximum 800 mg) at the physician's discretion (Ministry of Health, 2020; Price et al., 2020; Somers et al., 2021; WHO, 2021b).

Like other biological agents, TCZ can be associated with an increased risk of serious infections and other significant adverse events (neutropenia, thrombocytopenia, liver impairment, and hypersensitivity reactions) (WHO, 2021b; Campbell et al., 2011; Salama et al., 2021).

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As of July 2021, the World Health Organization (WHO) recommended TCZ in severe patients with COVID-19, being the second drug recommended by the WHO for COVID-19 treatment after dexamethasone (WHO, 2021a, WHO, 2021b).

This study aimed to evaluate if early treatment with TCZ is associated with a lower risk of in-hospital death and the interaction with personal characteristics, severity of disease, and systemic inflammation together with safety issues.

2. Materials and Methods

This study was performed in the Teaching Hospital of Infectious Diseases Cluj-Napoca, Romania, from 23 April 2020 (when TCZ was available) to 30 April 2021. We analyzed data from all adult patients with moderate to critical SARS-CoV-2 infection, who received TCZ and concomitant medication against SARS-CoV-2 (dexamethasone, low molecular weight heparin, remdesivir/favipiravir/hydroxychloroquine) (Ministry of Health, 2020). Inclusion criteria included the following: all patients with signs of respiratory worsening consisting of tachypnea, dyspnea, or increasing oxygen requirements over 24 hours, classified according to the WHO Clinical Progression Scale (CPS) for SARS-CoV-2 infection with scores between 5 and 8, plus 2 or more of the following predictors for severe disease: IL-6>10 pg/mL (upper normal value [UNV] 6.4 pg/mL), C-reactive protein (CRP)>75 mg/L (UNV 10 mg/L), ferritin>500 ng/mL (UNV 306.8 ng/mL), D-dimer>0.5 mg/L (UNV 0.55 mg/L), and lactate dehydrogenase (LDH)>200 U/L (UNV 250 U/L) (WHO, 2021a; 2021b; Ministry of Health, 2020). SARS-CoV-2 infection was confirmed by reverse transcriptase–polymerase chain reaction (RT-PCR) tests performed on nasopharyngeal swabs and other respiratory specimens.

Data retrieved from our hospital's electronic database included demographics, clinical data, comorbidities, chest computerized tomography (CT) images, intensive care unit (ICU) admission, IL-6, and other laboratory variables in dynamics (at admission and on days 0, 1, 3, and 7 after TCZ administration) according to the hospital protocol, and the outcomes were in-hospital mortality or discharged clinically improved.

In our clinical routine, the French Society of Thoracic Imaging Recommendations for grading lung involvement was implemented on the basis of visual assessment: minimal (<10%), moderate (10-25%), extensive (25-50%), severe (50-75%), and critical (>75%), combined with Örebro COVID-19 Scale (ÖCoS) for the parenchymal pattern (A. Only ground-glass opacities [GGO]; B. Predominantly GGO with some consolidations; C. Approximately equal amounts of GGO and consolidations; D. Predominantly or exclusively consolidations) (Ahlstrand et al., 2021). Of 12 321 patients with COVID-19 admitted during the study period, 221 cases fulfilled the inclusion criteria.

We evaluated the patients' characteristics in the second and third waves of the pandemic (2020 vs 2021) and the risk factors for in-hospital mortality, not always directly attributable to COVID-19. Data were collected on complications possibly related to TCZ treatment, including complex respiratory disease (bacterial, fungal pneumonia), bloodstream infections, and liver toxicity. The occurrence of bacterial complications was confirmed by the physicians on the basis of clinical, imaging, and microbiological tests (blood cultures and deep respiratory samples). Acute liver injury was defined as alanine aminotransferase (ALT)>150 U/L (UNV 50 U/L).

Access to patient data was authorized by our Teaching Hospital Ethical Committee. In the view of the retrospective design of this study, the requirement for patient consent was disregarded.

Statistical analysis

Apart from the descriptive analyses of clinical and laboratory tests, log2-transformed data were used for CRP and IL-6 kinetics. Hypothesis testing was performed using odds ratio (OR) with 95% confidence interval (CI) and p-value from Fisher or Mann-Whitney tests, as appropriate, followed by logistic regression for the outcome by certain variables. A cumulative distribution plot and a log-rank test were used for the outcome by TCZ administration timing. Statistical analysis was performed with R version 4.0 (R Core Team, 2020). p-Values of <.05 were considered to be statistically significant.

3. Results

3.1. The dataset included 221 adult patients, mean age (SD) 63.96±13.1 years, with moderate to critical COVID-19; 108 were admitted in 2020 and 113 in 2021, in which almost two-thirds were classified with CPS 6-7. All patients received dexamethasone, antibiotics, prophylactic or therapeutic low molecular heparin, and 1 dose of TCZ 8 mg/kg (201/221, 91%) or 2 administrations (12 hours apart) with a median (interquartile range [IQR]) total dose of 560 (400-800) mg. The total or the first dose was administered at a median (IQR) of 3 (1-6) days after admission. Regarding antiviral treatments, remdesivir was considered in 52.5% of patients (116/221, mostly in 2021), favipiravir in 20.3% (45/221), and hydroxychloroquine in 27% (60/221); only 4% (9/221) patients underwent invasive mechanical ventilation (IMV). Patient characteristics, relevant laboratory data in dynamics, and treatment options are presented in Table 1 (2021 vs 2020).

Compared with patients admitted in 2020, more patients received high-flow oxygen/non-IMV, most of them, 64% (142/221) in the medical wards, remdesivir as antiviral treatment, and TCZ was administered earlier in 2021. Age, sex, in-hospital mortality, length of stay, comorbidities (except obesity), and laboratory data (white blood cell count, absolute lymphocyte count, CRP, ferritin, LDH, ALT) did not differ significantly in 2021 compared with 2020. In all patients, a sharp decrease in CRP and almost normal ALT values were observed on days 3 and 7 after TCZ administration (Table 1, Figure 1).

Among this study's patients, the in-hospital mortality was 11.3% (25/221). In patients younger than 60 years (37.1%, 82/221), the in-hospital mortality was 9.7% (8/82). By age decades, in the 50-59 years patients the rate was higher 13.5% (7/52) compared with the overall rate while in patients aged 60-79 years, the mortality rate was 8.9% (10/112). The highest rate, 26% (7/27) was observed in patients > 80 years, although without statistical significance. In univariate analysis, the risk of in-hospital mortality was associated with age, CPS≥6, >40% lung damage, ICU admission, >2 cardiovascular, and other comorbidities, IL-6>100 pg/mL. TCZ administration at fewer than 3 days after admission was associated with decreased in-hospital mortality, OR=0.39 (95% CI, 0.16-0.9) (p = .034) (Table 2, Figure 2).

A cumulative distribution plot and a log-rank test were used for the outcome depending on TCZ timing and IL-6 dynamics on days 0, 1, 3, and 7. At TCZ initiation, the IL-6 levels were significantly higher in non-survivors with similar kinetics in all patients (Table 2, Figure 3).

Laboratory data in survivors and deceased are presented in dynamics at days 0, 3, and 7 after TCZ administration in Table 3. A sharp decrease in CRP values was found in all patients, but in non-survivors, LDH and D-dimers were 2-3–fold and 6-fold higher, respectively (Table 3, Figure 1). Remdesivir showed some benefit albeit without statistical significance (Table 2).

In multivariate analysis, the risk of in-hospital mortality was associated with a persistent inflammatory state (CRP>75 mg/L in day 3), aOR 16.6 (95% CI 3.07-108.96, p=.002); lung damage >40%, aOR 11.68 (95% CI 2.05-224.98, p=.024); cardiovascular comorbidities>2, aOR 3.65 (95% CI 1.06-12.53, p=.037); and TCZ administration at more than 3 days after admission, aOR 3.76 (95% CI 1.06-15.14, p=.047).

Table 1
Univariate analysis of selected variables by year in patients with COVID-19 (2021 vs 2020).

Variable **Year**	**Details**	**2021** **113 (51.1%)**	**2020** **108 (48.9%)**	**Total** **221**	**Statistics** p-value
Age	**N (%)**	**113 (100%)**	**108 (100%)**	**221 (100%)**	
(years)	μ±SD	62.75±12.2	65.22±13.9	63.96±13.1	p=.162
Gender Male		60 (53.1%)	66 (61.1%)	126 (57.0%)	p=.23
Deceased		11 (9.7%)	14 (13.0%)	25 (11.3%)	p=.43
Length of stay (days) M (IQR)		12 (9-17)	12 (8-18)	12 (8-17)	MW: p=.647
WHO CPS	≥6	87 (77.0%)	64 (59.3%)	151 (68.3%)	**p=.0048**
ICU admission		25 (22.12%)	40 (37.3%)	65 (29.4%)	**p=**0.0136
Lung damage (%)	M (IQR)	60 (40-70)	50 (30-70)	50 (40-70)	MW: p=.211
Lung damage >40%		74 (65.5%)	68 (63.0%)	142 (64.3%)	p=.69
Diabetes mellitus II		37 (32.7%)	39 (36.1%)	76 (34.4%)	p=.59
Cardiovascular comorbidities (>2)		21 (18.6%)	27 (25.0%)	48 (21.7%)	p=.24
Hypertension		74 (65.5%)	77 (71.3%)	151 (68.3%)	p=.35
Obesity		60 (53.1%)	43 (39.8%)	103 (46.6%)	**p=.048**
Admission to TCZ administration (days)	M (IQR)	3 (1-4)	4 (1-7)	3 (1-6)	**MW: p=.011**
TCZ>3 days after admission		42 (37.2%)	55 (50.9%)	97 (43.9%)	**p=.041**
Sepsis/pneumonia		3 (2.7%)	8 (7.4%)	11 (5.0%)	p=.10
Remdesivir use		76 (67.3%)	40 (37.0%)	116 (52.5%)	**p<.0001**
Day 0-TCZ administration: CRP (mg/L)	M (IQR)	90.9 (53.3-151)	92.2 (47.7-133.6)	91.2 (48.5-145.7)	MW: p=.558
Day 3-TCZ administration: CRP (mg/L)	M (IQR)	24.8 (10.8-41.9)	19.6 (11-34.6)	21 (10.9-39.7)	MW: p=.355
Day 7-TCZ administration: CRP (mg/L)	M (IQR)	3.9 (2-5.4)	4.2 (2.2-7.3)	3.9 (2-6.3)	MW: p=.250
Day 0-TCZ administration: ALT (U/L)	M (IQR)	42 (26.75-70.75)	41 (22.75-64)	42 (25-66.25)	MW: p=.228
Day 3-TCZ administration: ALT (U/L)	M (IQR)	60 (38-96)	54 (27-106)	59 (36-101.75)	MW: p=.610
Day 7-TCZ administration: ALT (U/L)	M (IQR)	68 (44-99)	62.5 (44.75-90.5)	65 (44-94)	MW: p=.520

μ, mean; ALT, alanine aminotransferase; CI, confidence interval; CPS, Clinical Progression Scale (WHO); CRP, C-reactive protein; IQR, interquartile range; M (min:max), median (minimum:maximum); MW, Mann-Whitney test; OR, odds ratio; TCZ, tocilizumab; WHO, World Health Organization.
OR (95% CI) and p-value from Fisher test.

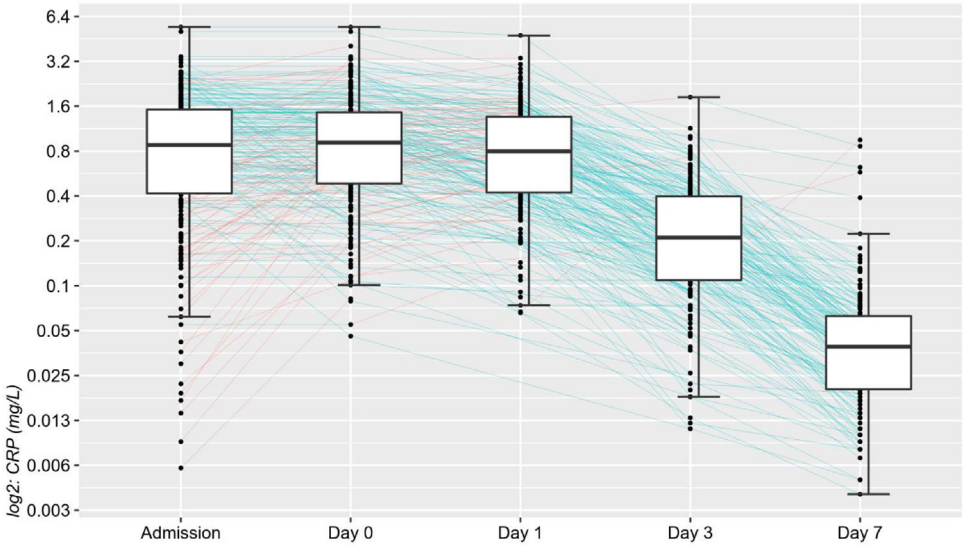

Figure 1. CRP kinetics: log2-transformed data in all patients, at admission and on days 1, 3, and 7 after TCZ administration (in green, decreasing trend). CRP, C-reactive patient; TCZ, tocilizumab.

Globally, we did not find a significant increase in ALT levels after TCZ administration (Table 3).

Sepsis and bacterial or fungal pneumonia were present in 11 (4.9%) patients (aged 64.5±13.1 years with >70% lung damage); all were admitted in ICU, but only 2 patients were mechanically ventilated. Eleven patients (4.9%) with severe hypoxemic respiratory failure (>70% lung damage) were admitted in the ICU and later developed sepsis and bacterial or fungal pneumonia. Three survived sepsis with *Enterococcus faecium* and *Methicillin-resistant Staphylococcus epidermidis* (MRSE), respectively, and the third one presented sepsis with *Enterocccus faecalis* and MRSE. Among the eight nonsurvivors (two were mechanically ventilated), six patients developed sepsis with Acinetobacter baumanii, in one case mixed etiology was found (*Enterococcus faecium* and *Klebsiella pneumoniae* carbapenemase (KPC)-producing bacteria) and the latter developed *Aspergillus fumigatus* pneumonia. All strains identified in sepsis and pneumonia had Extensively Drug-Resistant profiles: *Klebsiella pneumoniae* (meropenem 59%, amikacin 27%, colistin 75.9%), *Acinetobacter baumanii* (meropenem/imipenem-relebactam 100%, amikacin 76.4%, colistin 0%), *Pseudomonas aeruginosa* (piperacillin/tazobactam 75%, meropenem 77%, amikacin 72%, colistin 8.6%), *Enterococcus faecium* (vancomycin 37%, linezolid 3.8%, gentamicin-high 85%) and coagulase-negative staphylococci (two strains vancomycin susceptible). Four patients were aged less than 60 years, of whom 2 were profoundly immunosuppressed (1 was a recent kidney transplant recipient and 1 had advanced pancreatic cancer).

Table 2
Univariate analysis by COVID-19 death.

Variable Deceased	Details	Yes 25 (11.3%)	No 196 (88.7%)	Total 221	Statistics
Age	μ±SD	68.88±13.5	63.33±13.0	63.96±13.1	**T test: p=.046**
Age 50-59 years	Reference	7 (28%)	45 (23%)	52 (23.5%)	
Age 60-69 years		3 (12%)	58 (29.6%)	61 (27.6%)	OR=0.33 (0.09, 1.32) p=.18
Age 70-79 years		7 (28%)	44 (22.4%)	51 (23.07%)	OR=1.02 (0.35, 2.92) p=.99
Age ≥80 years		7 (28%)	20 (10.2%)	27 (12.21%)	OR= 2.25 (0.72, 6.96) p=.21
Gender	M	16 (64.0%)	110 (56.1%)	126 (57.0%)	**P=.45**
WHO CPS	≥6	**25 (100%)**	**126 (64.3%)**	**151 (68.3%)**	**OR=28.42 (1.70, 473.98) (p<.001)**
Length of stay (days)	M (IQR)	17 (11-23)	12 (8-17)	12 (8-18)	MW: p=.067
Lung damage CT scan (%)	M (IQR)	**80 (70-90)**	**50 (30-60)**	**50 (40-70)**	**MW: p<.001**
Lung damage >40%		**24 (96.0%)**	**118 (60.2%)**	**142 (64.3%)**	**OR=15.86 (2.10, 119.68) (p<.001)**
ICU admission		**17 (68.0%)**	**48 (24.5%)**	**65 (29.4%)**	**OR=6.55 (2.66, 16.13) (p<.001)**
Diabetes mellitus II		9 (36.0%)	67 (34.2%)	76 (34.4%)	OR=1.08 (0.45, 2.58) (p=.827)
Obesity		8 (32.0%)	95 (48.5%)	103 (46.6%)	OR=0.50 (0.21, 1.21) (p=.139)
Cardiovascular comorbidities (>2)		**12 (48.0%)**	**36 (18.4%)**	**48 (21.7%)**	**OR=4.10 (1.73, 9.73) (p=.002)**
Hypertension		20 (80.0%)	131 (66.8%)	151 (68.3%)	OR=1.98 (0.71, 5.53) (p=.254)
Atrial fibrillation		**5 (20.0%)**	**14 (7.1%)**	**19 (8.6%)**	**OR=3.25 (1.06, 9.97) (p=.047)**
Coronary heart disease		10 (40.0%)	48 (24.5%)	58 (26.2%)	OR=2.06 (0.87, 4.88) (p=.145)
Valvulopathies		5 (20.0%)	17 (8.7%)	22 (10.0%)	OR=2.62 (0.87, 7.86) (p=.085)
Congestive heart failure		**7 (28.0%)**	**17 (8.7%)**	**24 (10.9%)**	**OR=4.07 (1.49, 11.12) (p=.010)**
Stroke history		3 (12.0%)	11 (5.6%)	14 (6.4%)	OR=2.28 (0.59, 8.81) (p=.203)
Chronic kidney disease		**5 (20.0%)**	**8 (4.1%)**	**13 (5.9%)**	**OR=5.88 (1.75, 19.68) (p=.009)**
Chronic obstructive pulmonary disease		3 (12.0%)	13 (6.6%)	16 (7.2%)	OR=1.92 (0.51, 7.27) (p=.401)
Asthma		1 (4.0%)	7 (3.6%)	8 (3.6%)	OR=1.12 (0.13, 9.54) (p > .999)
Cancer		4 (16.0%)	25 (12.8%)	29 (13.1%)	OR=1.30 (0.41, 4.11) (p=.752)
Onset of symptoms to admission (days)	M (IQR)	**7 (4-10)**	**7 (5-10)**	**7 (5-10)**	**MW: p=.053**
Onset of symptoms to administration of TCZ (days)	M (IQR)	**12 (8.5-16)**	**10 (8-14)**	**11 (8-14)**	**MW: p=.0132**
Admission to administration of TCZ (days)	M (IQR)	**5 (2-7)**	**3 (1-5)**	**3 (1-6)**	**MW: p=.023**
IL-6 at TCZ administration	M (IQR)	**146 (101.4-332)**	**88 (53-153.3)**	**94 (58-160)**	**P=.0001**
IL-6>100 pg/mL at administration of TCZ		**19 (76%)**	**87 (44.4%)**	**47 (48%)**	**OR= 3.96 (1.57, 9.97) (p=.0049)**
Administration of TCZ ≤3 days after admission		**9 (36.0%)**	**115 (58.7%)**	**124 (56.1%)**	**OR=0.39 (0.16, 0.9) (p=.034)** **Reciprocal OR=2.54 (1.1-6.14)**
Remdesivir use		17 (68.0%)	99 (50.5%)	116 (52.5%)	OR=0.48 (0.20, 1.11) (p=.136)
Sepsis/pneumonia		**8 (32.0%)**	**3 (1.5%)**	**11 (5.0%)**	**OR=30.27 (7.34, 124.81) (p<0.001)**

μ, mean; CI, confidence interval; CPS, Clinical Progression Scale (WHO); CT, computerized tomography; IQR, interquartile range; M (min:max), median (minimum:maximum); MW, Mann-Whitney test; OR, odds ratio; TCZ, tocilizumab; WHO, World Health Organization.
OR (95% CI) and p-value from Fisher test.

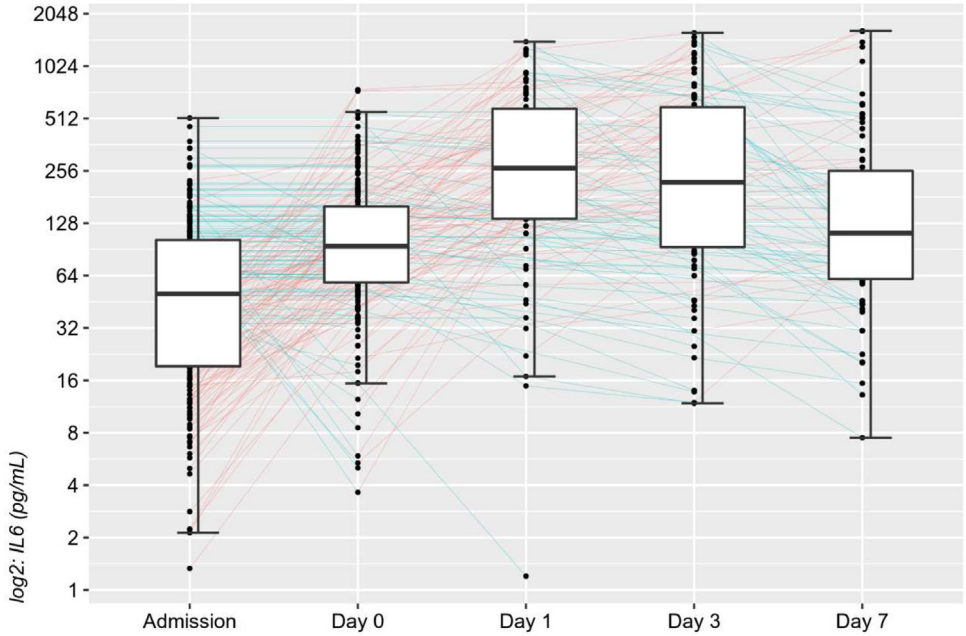

Figure 2. IL-6 kinetics: log2-transformed data in all patients, at admission and on days 1, 3, and 7 after TCZ administration (in green, decreasing trend). TCZ, tocilizumab.

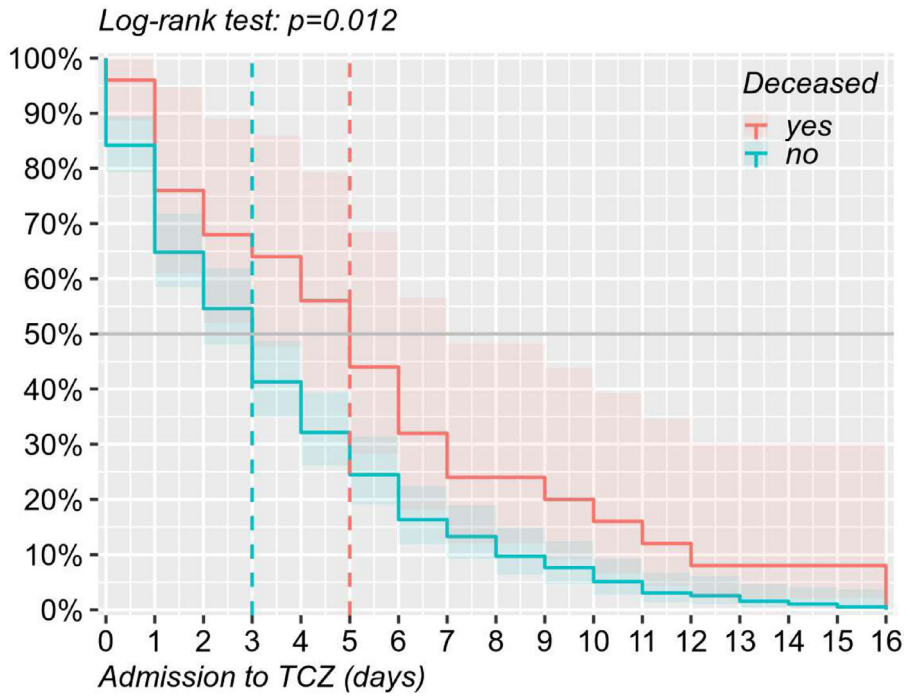

Figure 3. Cumulative distribution plot, TCZ administration by time and outcome, and log-rank test. TCZ, tocilizumab.

Table 3
Univariate analysis of some laboratory variables by COVID-19 death on days 0, 3, and 7 after TCZ administration.

Variable Deceased	Details	Yes 25 (11.3%)	No 196 (88.7%)	Total 221	Statistics
Day 0: D-dimer (mg/L)	M (IQR)	**1.22 (0.86-3.73)**	**0.72 (0.43-1.51)**	**0.82 (0.46-1.68)**	**MW: p=.002**
D- dimer>1 mg/L		**16 (64%)**	**70 (35.7%)**	**86 (38.9%)**	**OR=3.2 (1.38-7.69)** (p=.0085)
CRP (mg/L)	M (IQR)	111.1 (57.8-145.7)	89 (48.2-145.4)	91.2 (48.5-145.7)	MW: p=.258
CRP>75 mg/L		16 (64.0%)	112 (57.1%)	128 (57.9%)	OR=1.33 (0.56, 3.16) (p=.668)
Ferritin (ng/mL)	M (IQR)	761 (444.1-1448)	575.65 (281.88-894.72)	584 (303.7-938.5)	MW: p=.075
ALT (U/L)	M (IQR)	39 (28-88.5)	42 (25-65.5)	42 (25-66.25)	MW: p=.489
LDH (U/L)	M (IQR)	**488 (386.75-588.75)**	**362 (277.5-479.5)**	**379 (289.5-490)**	**MW: p<.001**
LDH>400 U/L		**17 (68%)**	**61 (36%)**	**78 (35.3%)**	**OR=4.7 (1.79-8.47) (p=.0006)**
Day 3: D-dimer (mg/L)	M (IQR)	**3.79 (1.63-11.68)**	**0.94 (0.57-2.13)**	**1.03 (0.58-2.57)**	**MW: p=.001**
D- dimer>1 mg/L		**14 (56%)**	**65 (33.16%)**	**79 (35.74%)**	**OR=2.56 (1.11-5.67)** (p=.044)
CRP (mg/L)	M (IQR)	30.9 (14.9-73.5)	20.7 (10.8-36.8)	21 (10.9-39.7)	MW: p=.057
CRP>75 mg/L		**5 (25.0%)**	**5 (3.2%)**	**10 (5.6%)**	**OR=10.13 (2.63, 39.02) (p=.002)**
Ferritin (ng/mL)	M (IQR)	716.45 (629.68-935.02)	676 (352.6-1021.7)	682.9 (355.7-1021.7)	MW: p=.372
ALT (U/L)	M (IQR)	38.5 (24.75-80)	61 (38.25-105)	59 (36-101.75)	MW: p=.108
LDH (U/L)	M (IQR)	**684.5 (497.25-862.25)**	**377 (292.75-538)**	**417 (313-583)**	**MW: p<.001**
LDH>400 U/L		**14 (56%)**	**48 (24.5%)**	**62 (28%)**	**OR=3.92 (1.66-8.83) (p=.0018)**
Day 7: D-dimer (mg/L)	M (IQR)	**3.27 (2.03-5.67)**	**1.02 (0.51-2.16)**	**1.15 (0.53-2.96)**	**MW: p<.001**
D- dimer>1 mg/L		**14 (56%)**	**64 (32.6%)**	**78 (39.7%)**	**OR=2.62 (1.13-5.8)** (p=.026)
CRP (mg/L)	M (IQR)	**7.4 (3.2-15.9)**	**3.8 (2-5.7)**	**3.9 (2-6.3)**	**MW: p=.037**
CRP>75 mg/L		**2 (11.8%)**	0	**2 (1.4%)**	OR=41.77 (1.92-910.5) (p =.013)
Ferritin (ng/mL)	M (IQR)	719.9 (376.97-976.45)	536.8 (275.4-795.95)	547.5 (291.45-804.8)	MW: p=.281
ALT (U/L)	M (IQR)	**54 (29-70)**	**67.5 (45-99.25)**	**65 (44-94)**	**MW: p=.020**
LDH (U/L)	M (IQR)	**780 (498-823.5)**	**346 (268-421.25)**	**358 (274-503)**	**MW: p<.001**
LDH>400 U/L		**15 (60%)**	**25 (12.75%)**	**62 (18%)**	**OR=10.2 (4.04-26.24) (p<.0001)**

μ, mean; ALT, alanine aminotransferase; IQR, interquartile range; LDH, lactate dehydrogenase; M (min:max), median (minimum:maximum); MW, Mann-Whitney test; OR, odds ratio; CRP, C-reactive protein
OR (95% CI) and p-value from Fisher test.

4. Discussion

During the study period, Romania reported 1 055 265 COVID-19 cases and Cluj county registered 56 054 patients, ranked the second after the capital Bucharest (and surroundings). In the second COVID-19 pandemic wave, there were up to 10 000 cases/day (November 2020), thereafter decreasing until the end of February 2021 to less than 3 000 cases/day. The third wave reached a peak in the mid-March with 4 500 cases/day, later decreasing to 1 800 cases until the end of April (INSP, 2020; INSP, 2021). Whole-genome sequencing was accomplished in Romania from February 2021 and in our hospital from the end of March 2021, with almost all selected samples being positive for the Alpha/B.1.1.7 variant by the end of April 2021. Therefore, we appreciate that in the second wave the wild variant was predominant while in the third one, the Alpha/ B.1.1.7 variant was prevailing (INSP, 2021; ECDC, 2021).

Despite the different SARS-CoV-2 variants, the patients treated with TCZ were astonishingly similar by age, gender, comorbidities, lung damage, and even laboratory data. Non-invasive ventilation with high-flow oxygen equipment/non-invasive ventilation (CPS 6)

was more commonly considered in 2021 reflecting treatment escalation and maximum degree of organ support toward severe cases rather than increased severity along with earlier TCZ treatment and more common use of remdesivir. In the United Kingdom, Frampton et al. showed that at the end of 2020, patients infected with the variant B.1.1.7, representing ~60% (198/341) of severe hospitalized COVID-19 cases, had the same mortality rate as those infected with the non-B.1.1.7. variant (Frampton et al., 2021). We also suggest that COVID-19 severity was similar regardless of the SARS-Co-V2 variant.

Individual characteristics and comorbidities were extensively studied in COVID-19, showing that advanced age, male gender, and certain comorbidities are associated with increased mortality compared with non–COVID-19 deaths. In a large cohort study within the OpenSAFELY platform from England (February 2020 to November 2020), older age, male gender, deprivation, obesity, and some comorbidities (diabetes, recently diagnosed cancer, hematological malignancy, reduced kidney function) were more strongly associated with COVID-19 death than non–COVID-19 death (for those aged 80 years vs those aged 50-59 years). Instead, smoking, history of cancer, and chronic liver disease were more likely associated with non–COVID-19 death (Williamson et al., 2020; Bhaskaran et al., 2021). In another large study performed in 302 United Kingdom healthcare facilities in 80 388 patients, in-hospital complications were evaluated, particularly in survivors.

Higher rates of complications were found in younger patients with no comorbidities and in those aged >60 years. Regarding in-hospital mortality, increasing age and male gender were the most relevant risk factors, but the complications were more deleterious in younger patients, in the presence of comorbidities, and a poor outcome was more frequently observed than in older patients (Drake et al., 2021). In this study, we also found the highest in-hospital mortality in patients aged >80 years, and yet, in patients aged 50-59 years, the rate was higher than in the group aged 60-79 years (although not statistically significant), suggesting that severe disease, comorbidities, and complications might be more important in younger patients than in older patients.

Early reports suggested that IL-6 blockade with TCZ is beneficial in the treatment of severe COVID-19 (Rosas et al., 2021; RECOVERY Collaborative Group 2021; Martínez-Sanz et al., 2021). TCZ is a humanized antibody that blocks both soluble and membrane-bound forms of the IL-6 receptor (IL-6R), preventing ligand binding. In fact, IL-6 is mainly eliminated via IL-6R mediated clearance. TCZ binding to IL-6R inhibits receptor-mediated clearance of IL-6, which led to its accumulation in serum (Mathew et al., 2020). This is the plausible explanation for the paradoxical increase in IL-6 serum levels after TCZ treatment. Thus, TCZ-mediated blockade of IL-6R signaling leads to a gradual decrease in circulating inflammatory mediators, resulting in stabilization or improvement of the clinical outcome. Using the baseline IL-6 and CRP values are helpful to better discriminate the patients particularly benefiting from TCZ treatment, but repeated IL-6 measurements were not found relevant for outcome prediction because we observed a significant increase by day 3 and 7 in most patients (Galván-Román et al., 2021). Therefore, CRP is a more reliable marker in dynamics than IL-6; still, if available, IL-6 measurement should complete the baseline evaluation in severe cases. Galvan-Ramon et al. (Galván-Román et al., 2021) considered the IL-6 value of 30 pg/mL as a good prognostic marker for IMV and the need for TCZ blockade. In all patients, IL-6 values (Median [IQR]=94 [58-160]) were markedly increased demonstrating the overt CRS.

We also found a significant difference in IL-6 value (at TCZ initiation) in non-survivors versus survivors, and for the threshold of 100 pg/mL (the median value in all patients), a significantly higher risk of death was observed.

Several authors evaluated the proinflammatory and prothrombotic state owing to severe COVID-19 showing that treatment with TCZ was associated with a decrease in CRP, D-dimer, and ferritin levels (Martínez-Sanz et al., 2021; Moreno Diaz et al., 2021). Likewise, we found that TCZ reduced the proinflammatory state with a sharp decrease in CRP from the baseline by days 3 and 7.

Many studies reported that LDH increase during the disease is associated with a poor prognosis indicating severe lung and tissue injury (Nuñez-Ramos et al., 2021). In our study, we found a 3-fold increase of the upper normal value (UNV) by day 7 in non-survivors, in survivors, and in all assessments.

Regarding the ferritin kinetics, it has been shown that its level could be correlated with IL-6 serum level (Conrozier et al., 2020). However, ferritin also depends on IL-18 levels, explaining why ferritin and CRP did not decrease at the same rate after IL-6 blockade. This observation is consistent with our results as we did not observe a significant decrease in the ferritin level within 7 days, neither in survivors nor in non-survivors.

Comparable with Toniatti et al. (Toniati et al., 2020), we found that the hypercoagulation state was not positively influenced in non-survivors, showing a 6-fold increase of D-dimer values by day 7 after TCZ administration, and even in survivors, no decrease was observed, suggesting that TCZ was able to act only on the inflammatory syndrome with no effect on down-regulating hypercoagulation.

Moreno Diaz et al., (Moreno Diaz et al., 2021) raised a question about optimal timing of TCZ administration, suggesting that early treatment is associated with decreased mortality and the decreased need for mechanical ventilation. A meta-analysis on 27 eligible trials evaluating the association between administration of IL-6 antagonists showed a lower 28-day all-cause mortality (OR 0.83 [95% CI 0.74-0.92; p<.001]) in patients receiving TCZ. In addition, the best results were obtained in patients receiving corticosteroids and not requiring IMV at randomization (WHO, 2021a). Our results are concordant; early initiation of TCZ in patients treated with dexamethasone and without IMV had a better outcome with a survival rate of 88.7% (196/ 221).

Early studies and the previously mentioned meta-analysis showed a high rate of superinfections (~22%) in the TCZ-treated patients but no difference in the case fatality rate among TCZ-treated patients with or without superinfection. As opposed, we found a low rate of serious superinfections (4.9%) with multidrug-resistant bacteria being responsible for sepsis and pneumonia in patients admitted in ICU (Somers et al., 2021; WHO, 2021).

Similar to other authors (Hermine et al., 2021; Tleyjeh et al., 2021), we did not observe severe liver injury except a mild increase of ALT values by day 7.

4.1. Strengths and limitations

The strength of our study was a comprehensive assessment at admission and repeatedly after TCZ administration. Although we lacked a control group, all patients had baseline factors predictive of poor outcome, and because concomitant treatments were similar (all receiving dexamethasone, low molecular heparin, and antivirals), it is highly probable that TCZ made the difference. We were able to assess disease severity related to presumably different SARS-CoV-2 variants by comparing patients admitted in the second and third waves of the pandemic.

This study has a number of limitations, inherent for an observational compared with a randomized controlled trial, and thus, we cannot preclude that the overall favorable results observed for TCZ-treated patients may be explained by the natural course of the disease and other factors. We were not able to continue the follow-up in survivors till 28 days, but the discharge was considered only in those with a predictable good outcome.

5. Conclusions

Our study suggests that early immunomodulatory therapy with TCZ is beneficial and safe during the COVID-19 cytokine storm in patients with moderate to critical COVID-19 pneumonia. Poor prognosis was associated with cardiovascular comorbidities, extended lung damage, respiratory failure, persistent hyperinflammatory, and prothrombotic profiles.

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Author Contributions

Conceptualization, A.R and M.M; methodology A.R and M.M; software, A.R and A.I; validation, A.R and M.M; formal analysis, A.R and A.I; investigation, A.R. and M.M; data curation, A.R, M.M, and A.I; writing, original draft preparation, A.R and M.M; writing, review and editing, A.R and M.M; supervision, A.R and M.M. All authors have read and agreed to the published version of the manuscript.

Ethical approval statement

This study was approved by the Teaching Hospital Ethical Committee, and all patients signed an informed consent form.

Conflict of Interest

The authors declare no conflict of interest.

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