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UTICAJ TOCILIZUMABA NA TOK I ISHOD LEČENJA PACIJENATA SA COVID-19 PNEUMONIJOM – ISKUSTVO BOLNICE SEKUNDARNOG NIVOA

UVOD

Teški akutni respiratorni sindrom koronavirus 2 (SARS-CoV-2) identifikovan je kao uzrok pandemije COVID-19 2019. godine (1).

Bolest ima širok klinički spektar, od asimptomatske infekcije do teških oblika sa smrtnim ishodom. Oko jedne četvrtine obolelih razvija tešku kliničku sliku sa komplikacijama i multiorganskim oštećenjem i zahteva lečenje u jedinicama intenzivne nege (2).

Progresija bolesti uslovljena je inflamatornim i imunološkim procesima posredovanim faktorom nekroze tumora (TNF), Janus kinazom (JAK) i interleukinima, pri čemu interleukin-6 (IL-6) ima najznačajniju ulogu (3).

Studije su pokazale da se dobar terapijski efekat može postići primenom blokatora proinflamatornih medijatora. Takav blokator je tocilizumab (TCZ) (4). Bezbednost primene i dobar terapijski odgovor na TCZ pokazani su i kod lečenja blizanačke trudnoće (5).

Tocilizumab je monoklonsko antitelo koje se vezuje za IL-6 receptore i na taj način sprečava prenos signala koji aktivira Janus kinazu, što je preduslov za nastanak citokinske oluje (6).

Cilj ovog istraživanja bio je da se analiziraju efekti primene tocilizumaba na klinički tok bolesti, laboratorijske parametre inflamacije i radiografske promene kod bolesnika sa COVID-19 pneumonijom, kao i da se utvrde prediktori nepovoljnog ishoda bolesti.

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BOLESNICI I METODI

U istraživanje je uključeno 340 bolesnika sa COVID-19 pneumonijom starijih od 18 godina, lečenih na odeljenju ili u jedinici intenzivne nege (JIN) Opšte bolnice u Užicu, tokom 2021. godine.

Kriterijum za prijem u JIN bilo je pogoršanje respiratorne funkcije koje je zahtevalo neinvazivnu (NIV), ili mehaničku ventilaciju (MV).

Dijagnoza COVID-19 postavljena je na osnovu detekcije virusa SARS-CoV-2 u nazofaringealnom brisu.

Bolesnici su lečeni prema Nacionalnom protokolu za lečenje COVID-19. Kod svih bolesnika primenjena je terapija kiseonikom, antikoagulantna, kortikosteroidna (metilprednizolon), gastroprotektivna, antivirusna (favipiravir) i antibiotska terapija. Svi ispitani bolesnici su primili TCZ.

Indikacije za primenu TCZ bile su vrednosti IL-6 > 40 pg/mL, CRP > 50 mg/L, ili njegovo trostruko povećanje u toku 48 časova (ukoliko IL-6 nije mogao biti određen), uz znake ekstenzivne COVID-19 pneumonije praćene respiratornom frekvencijom > 25/min, kiseoničnom saturacijom (SpO2) < 93% na sobnom vazduhu i parcijalnim pritiskom kiseonika u arterijskoj krvi (PaO_2) < 8,66 kPa.

Tocilizumab je primenjivan intravenski, 8 mg/kg, podeljeno u dve doze u razmaku od 12h (maksimalno do 800 mg po dozi).

Iz istraživanja su isključeni bolesnici sa stanjima kontraindikovanim za primenu TCZ: tuberkulozom, HIV infekcijom, autoimunskim bolestima, virusnim hepatitisima, malignim bolestima, rizikom od gastrointestinalnog krvarenja, trombocitopenijom (< 50×10^9/L) i neutropenijom (< $0{,}5 \times 10^9$/L).

Kiseonik je primenjivan putem nazalne kanile, Venturi maske, NIV, ili MV.

Rendgenski snimci pluća rađeni su digitalnom anteroposteriornom radiografijom pri punoj inspiraciji, mobilnim aparatom(Vision M, Visaris). Radiografske promene opisivane su terminima Glosar Fleischnerovog društva (7).

Težina promena na rendgenskom snimku grudnog koša (CXR-SS) određivana je na osnovu RALE skora (Radiographic Assessment of Lung Edema). Svako plućno krilo bodovano je od 0 do 4 prema procentu zahvaćenosti promenama (0 = bez promena; 1 = <25%; 2 = 25–50%; 3 = 50–75%; 4 = >75%), a ukupni skor dobijen je sabiranjem skorova oba pluća (8).

Prikupljeni su podaci o polu i starosti, kliničkom toku bolesti i ishodu, prisustvu i vrsti komorbiditeta, razvoju bolničkih infekcija (BI), proceni respiratorne funkcije, laboratorijskim parametrima i radiografskim promenama. Podaci o respiratornoj funkciji, laboratorijski i radiografski podaci prikupljani su pre primene i petog dana nakon primene TCZ.

Dobijeni podaci statistički su obrađeni pomoću SPSS softvera verzije 25.0. Za statističku obradu korišćene su metode deskriptivne statistike, Hi-kvadrat (χ^2) test, kao i neparni t-test i Mann–Whitney test za kvantitativne varijable. Statistički značajne

varijable uključene su u univarijantni i multivarijantni logistički regresioni model. Vrednost $p < 0,05$ smatrrana je statistički značajnom.

REZULTATI

Od ukupno 340 ispitanika, 68,5% je bilo starije od 65 godina. Prosečna starost iznosila je 66,22 ± 11,37 godina (raspon 24–95 godina). Značajno veći broj ispitanika činili su muškarci (66,4%) (Tabela 1).

Većina bolesnika imala je komorbiditete (65,4%). Bolničke infekcije registrovane su kod 29 bolesnika (8,5%), uključujući 10 bakterijskih pneumonija, 10 urinarnih infekcija, 5 slučajeva pseudomembranoznog kolitisa, 7 plućnih embolija i 5 cerebrovaskularnih inzulta. *Acinetobacter baumannii* izolovan je iz bronhijalnog aspirata kod pet bolesnika, *Klebsiella* spp. kod četiri, a *Streptococcus pneumoniae* kod jednog bolesnika.

Najčešći način oksigenacije bio je NIV (64,1%), dok je MV bila potrebna kod gotovo četvrtine bolesnika (24%). Ukupno 78,2% bolesnika otpušteno je iz bolnice, dok je kod 21,8% bolest završila smrtnim ishodom tokom hospitalizacije.

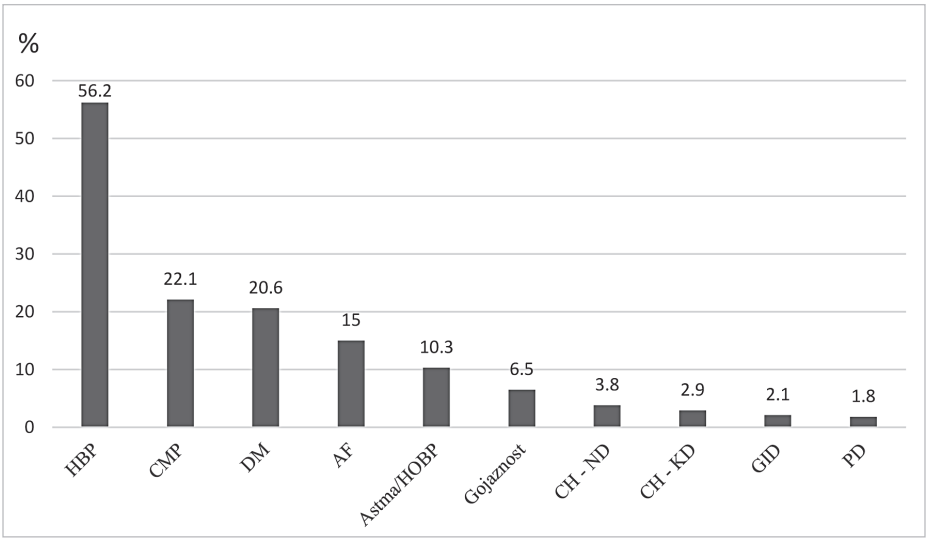
Starost, pol, prisustvo komorbiditeta, bolničke infekcije, način oksigenacije i ishod bolesti prikazani su u Tabeli 1.

Tabela 1. Karakteristike bolesnika sa COVID-19 lečenih tocilizumabom

Parametri		N % 340 100	P
Uzrast	>65 ≤ 65	199 (58.5) 141 (41.5)	0.089
Pol	Muški Ženski	226 (66.4) 114 (33.5)	<0.001
Komorbiditeti	Da Ne	222 (65.3) 118 (34.7)	0.002
Bolničke infekcije	Da Ne	29 (8.5) 311 (91.5)	0.00
Način oksigenacije	Nazalna kanila/maska Neinvazivna ventilacija Invazivna mehanička ventilacija	41 (12.1) 218 (64.1) 81 (23.8)	0.00
Ishod lečenja	Otpušteni Preminuli	266 (78.2) 74 (21.8)	0.00

Grafik 1 prikazuje vrste komorbiditeta i njihovu učestalost.

Grafik 1. Komorbiditeti kod bolesnika sa COVID-19 lečenih tocilizumabom



Najčešći komorbiditet bila je hipertenzija (56,2%), a slede kardiomiopatija (22,1%), dijabetes melitus (20,6%), atrijalna fibrilacija (15%), astma/hronična opstruktivna bolest pluća (HOBP) (10,3%), gojaznost (indeks telesne mase > 30) (6,5%), kao i hronične neurološke, bubrežne, psihijatrijske i gastrointestinalne bolesti sa učestalošću od 3,8%, 2,9%, 2,1% i 1,8%, respektivno.

Broj leukocita, broj trombocita, biohemijski inflamatorni parametri (laktat-dehidrogenaza (LDH), CRP i IL-6), parametar oksigenacije PaO_2/FiO_2 (P/F) i skor rendgenskih promena pluća (CXR) upoređivani su pre i petog dana nakon primene TCZ, kao što je prikazano u Tabeli 2.

Tabela 2. Karakteristike bolesnika sa COVID-19 pre i posle primene tocilizumaba

Parametri		Na dan primene TCZ N % 340 100	Petog dana nakon TCZ N % 340 100	P
WBC	<4 x 10^9/L >10 x 10^9/L	98 (28.8) 205 (60.3)	50 (14.7) 183 (53.8)	0.130
PLT	<100 x 10^9/L	42 (12.4)	21 (6.2)	0.156
LDH	>245 U/L	304 (89.4)	187 (55.0)	0.004
CRP	>50 mg/L	309 (90.9)	191(56.2)	0.004
IL-6	>40 pg/mL	340 (100)	149 (43.8)	<0.001

Parametri		Na dan primene TCZ N % 340 100	Petog dana nakon TCZ N % 340 100	P
HAIs	da	29 (8.5)	31 (9.1)	0.888
P/F odnos	250-300 200-250 <200	39 (11.5) 220 (64.7) 81 (23.8)	87 (25.6) 111 (32.6) 54 (15.9)	<0.001
CXR-SS	1 2 3 4 5 6 7 8	0 0 0 5 (1.5) 28 (8.2) 215 (63.2) 42 (12.4) 50 (14.7)	0 0 10 (2.9) 69 (20.3) 73 (21.5) 128 (37.6) 41 (12.1) 19 (5.6)	<0.001
CXR-SS (ukupno)	Umeren (3, 4) Težak (5, 6) Vrlo težak (7, 8)	5 (1.6) 243 (71.5) 92 (27.1)	79 (23.2) 201 (59.1) 60 (17.6)	<0.001

Nije uočena statistički značajna razlika u broju leukocita i trombocita u odnosu na primenu TCZ. Zabeleženo je značajno smanjenje vrednosti LDH, CRP i IL-6 nakon primene TCZ.

Svi bolesnici su pre primene TCZ imali $SpO_2 < 90\%$ i $PaO_2 < 60$ mmHg. Kontrolne vrednosti petog dana pokazale su značajno poboljšanje P/F odnosa.

Ukupni CXR-SS bio je težak kod 71,5% i vrlo težak kod 27,1% bolesnika pre primene TCZ. Nakon terapije TCZ, značajno veći procenat bolesnika imao je umeren CXR-SS. Nije utvrđena statistički značajna razlika u učestalosti vrlo teškog CXR-SS nakon primene TCZ.

Dalja poređenja podataka izvršena su prema ishodu bolesti. Rezultati su prikazani u Tabeli 3.

Tabela 3. Karakteristike bolesnika sa COVID-19 lečenih tocilizumabom u odnosu na ishod bolesti

Parametri		Otpušteni N (%) 266 100	Preminuli N (%) 74 100	P vrednost
Uzrast	>65	141 (53.0)	58 (78.3)	0.027
Pol	Muški Ženski	178 (66.9) 88 (33.1)	48 (64.9) 26 (35.2)	0.862 0.799
Komorbiditeti	Da	167 (62.8)	73 (98.6)	0.005
Bolničke infekcije	Da	15 (5.6)	14 (18.9)	0.007
Način oksigenacije	Invazivna mehanička ventilacija	20 (7.5)	61 (82.4)	0.00
WBC	<4 x 10^9/L >10 x 10^9/L	76 (28.6) 158 (59.4)	22 (29.7) 47 (63.5)	0.928
PLT	<100 x 10^9/L	32 (12.0)	10 (13.5)	0.767
LDH	>245 U/L	198 (74.4)	62 (83.8)	0.455
CRP	>50 mg/L	243 (91.4)	66 (89.2)	0.869
IL-6	>80 pg/mL	217 (81.6)	72 (97.3)	0.240
CXR-SS (ukupno)	Umeren (4) Težak (5, 6) Vrlo težak (7, 8)	5 (1.9) 225 (84.6) 36 (13.5)	0 18 (24.3) 56 (75.7)	 0.00 0.00

U odnosu na ishod bolesti, nije uočena statistički značajna razlika u raspodeli po polu. Smrtnost je bila veća kod bolesnika starijih od 65 godina (78,3%), uz veću učestalost komorbiditeta (98,6%). Jedanaest bolesnika (14,9%) je imalo tri ili više komorbiditeta. Preminuli bolesnici su češće imali bolničke infekcije (18,9%) (9 pneumonija, 4 plućne embolije i 2 cerebrovaskularna inzulta). Značajno veći procenat preminulih bolesnika (82,4%) zahtevao je mehaničku ventilaciju. Uporedne vrednosti CRP, LDH i IL-6 nisu pokazale statistički značajne razlike. U pogledu CXR-SS, težak skor bio je najčešći kod preživelih bolesnika (84,6%), dok je vrlo težak skor bio značajno češći kod preminulih bolesnika (75,7%).

Tabela 4 prikazuje rezultate univarijantne linearne regresije za starost, prisustvo komorbiditeta, pridružene infekcije, mehaničku ventilaciju i stepen radiografskih promena kao nezavisne faktore povezane sa smrtnim ishodom COVID-19 pneumonije lečene TCZ.

Tabela 4. Prediktivni faktori smrtnog ishoda kod bolesnika sa COVID-19 pneumonijom lečenih tocilizumabom

Parametri	Smrtni ishod				
	B	S.E.	Exp(B)	95% CI	P
Starost > 65	1.167	0.308	3.214	1.757-5.877	0.000
Komorbiditeti	3.768	1.015	43.275	5.922-316.248	0.000
Pridružene infekcije	1.362	0.398	3.904	1.788-8.525	0.001
Mehanička ventilacija	3.743	0.372	42.234	20.372-87.555	0.000
CXS-SS	2.971	0.324	19.510	10.331-36.844	0.000

Multivarijantna analiza pokazala je da su najznačajniji prediktori smrtnog ishoda bili potreba za mehaničkom ventilacijom (B = 3,195, SE = 0,454, P = 0,000) i veoma izražene radiografske promene (B = 2,150, SE = 0,450, P = 0,000).

DISKUSIJA

Klinički tok i ishod COVID-19 pneumonije zavise od karakteristika samog virusa i imunološkog odgovora domaćina. Spektar različitih proteina SARS-CoV-2 doprinosi njegovoj visokoj patogenosti i virulenciji, kao i sposobnosti da izbegne imunološku odbranu domaćina. Nove varijante virusa, nastale stalnim mutacijama, povećavaju njegovu otpornost na humoralni imunski odgovor. Funkcionalan imunski odgovor obezbeđuje blaži klinički tok i povoljan ishod bolesti. Stoga je od ključnog značaja identifikovati neadekvatan imunski odgovor i intervenisati efikasnom terapijom (9).

U ovom istraživanju analizirali smo efekte primene TCZ kod bolesnika sa COVID-19 pneumonijom, kao i različite faktore koji mogu uticati na tok i ishod lečenja.

Naši bolesnici imali su prosečnu starost od 66 godina. U skladu sa drugim studijama, starost preko 65 godina bila je značajan prediktor smrtnosti (10, 11).

Većina ispitanika bili su muškarci, iako muški pol nije bio prediktivni faktor smrtnog ishoda. Neki autori navode slične rezultate (11, 12). U drugim studijama muški pol je bio češće zastupljen među obolelima od COVID-19 i predstavljao je prediktor teškog oblika bolesti i smrtnog ishoda, što se objašnjava socioekonomskim faktorima, navikama i prirodom radnog mesta (13, 14). Nismo analizirali ove faktore, ali su žene u našem istraživanju imale značajno više udruženih bolesti. Komorbiditeti su, kao i u drugim studijama, bili prisutni kod većine bolesnika sa teškim oblikom COVID-19 infekcije (15, 16). S obzirom na to da njihovo prisustvo doprinosi razvoju teškog kliničkog oblika bolesti, ovaj nalaz je očekivan. Prisustvo komorbiditeta, posebno dva ili više, bilo je značajan prediktor smrtnosti u našem istraživanju, kao i kod drugih autora. I naši ispitanici su najčešće imali hipertenziju i srčana oboljenja (10, 13, 17).

Smrtni ishod bio je povezan sa prisustvom bolničkih infekcija. Schmidt i saradnici takođe su identifikovali nozokomijalne infekcije kao značajan prediktor smrtnosti, pri čemu je najčešća bila pneumonija izazvana bakterijom *Acinetobacter baumannii* (10). Kod naših bolesnika su iz sputuma izolovani i *Acinetobacter baumannii* i *Klebsiella pneumoniae.* Ovaj nalaz je očekivan, imajući u vidu visoku učestalost multirezistentnih sojeva ovih bakterija u jedinicama intenzivne nege u našem regionu (18).

Poremećena produkcija IL-6 i drugih proinflamatornih citokina dovodi do citokinske oluje, koja ima ključnu ulogu u patogenezi teške pneumonije. Razaranje alveolarnih ćelija povećava propustljivost i dovodi do plućnog edema, čime se narušava razmena gasova i nastaje hipoksemija (19). TCZ poboljšava respiratornu funkciju, naročito kod teških oblika COVID-19 pneumonije (20).

Naše iskustvo potvrđuje poboljšanje respiratorne funkcije, procenjivano pomoću P/F odnosa i načina oksigenacije. Nismo uočili povećanu učestalost pridruženih bakterijskih i gljivičnih infekcija, kako su navodili drugi autori (21). Skoro četvrtina naših bolesnika bila je na mehaničkoj ventilaciji pre primene TCZ, što je, kao i u drugim studijama, predstavljalo značajan prediktor smrtnosti (22).

Efekat TCZ praćen je i analizom radiografskih nalaza. Uočili smo značajnu regresiju plućnih promena, u skladu sa regresijom inflamatornih parametara i poboljšanjem respiratorne funkcije. Naši rezultati podržavaju nalaze Lacedonije i saradnika (23). Najveća regresija primećena je kod bolesnika sa nižim vrednostima CXR-SS, što ukazuje na bolji efekat TCZ kada se primeni u ranijim fazama bolesti. CXR-SS kategorije „vrlo težak" bio je značajan prediktor smrtnosti naših ispitanika. I drugi autori su ukazali na značaj radiografije u predviđanju smrtnosti. Preživljavanje je bilo povezano sa zahvatanjem pluća manjim od 70% ili 50%, a analiza dominantne konsolidacije i prisustva pleuralnog izliva imala je još veću prediktivnu vrednost (10, 17, 24).

Zabeležili smo smanjenje vrednosti IL-6, CRP i LDH, ali bez značajnih promena u broju leukocita i trombocita. Na osnovu ovih hematoloških i biohemijskih parametara nismo mogli predvideti smrtni ishod, što je u skladu sa nalazima drugih autora (12, 17, 25).

Važno je uzeti u obzir uticaj drugih lekova na ove parametre i tok bolesti. Kortikosteroidi i antivirusni lekovi imaju antiinflamatorno dejstvo, pa u sinergiji sa TCZ smanjuju hiperinflamatorni odgovor i time doprinose smanjenju smrtnosti (26).

Često se razmatra blagovremena primena TCZ, posebno u vezi sa povoljnim efektom inhibicije IL-6 u aktivnoj fazi inflamacije. Neki autori smatraju da TCZ ima najbolji efekat ako se primeni pre razvoja teške respiratorne insuficijencije, između 9. i 12. dana od pojave simptoma, ili u prvih pet dana od pojave dispneje (10, 27). Kaya H. i saradnici navode da je smrtnost manja ukoliko se TCZ primeni pre šestog dana bolesti (11).

Svi naši bolesnici lečeni su kortikosteroidima, antivirusnim lekovima i TCZ. Stopa smrtnosti iznosila je 22%, u poređenju sa 18% u jednoj drugoj srpskoj studiji (13). Za razliku od pomenute studije, naši bolesnici su bili stariji, većina je imala

tešku kliničku sliku, a 24% je zahtevalo mehaničku ventilaciju. Radimo u sekundarnoj zdravstvenoj ustanovi koja prima kritično obolele pacijente iz manjih bolnica u regionu. Zbog toga za mnoge bolesnike nismo imali precizne podatke o prethodnoj kortikosteroidnoj terapiji. Takođe, nismo imali pouzdane podatke o trajanju simptoma pre prijema, što je važan prediktivni faktor ishoda bolesti (26). Za razliku od nekih studija, u našem istraživanju uočeni su povoljni efekti TCZ (21, 28). TCZ poboljšava klinički ishod i smanjuje inflamatorne markere. Saglasni smo da odluka o terapiji treba da bude individualizovana, u skladu sa kliničkim i biohemijskim parametrima svakog bolesnika (29, 30).

ZAKLJUČAK

Teška COVID-19 pneumonija bila je češća kod muškaraca sa kardiovaskularnim komorbiditetima. Primena tocilizumaba imala je povoljan efekat na smanjenje inflamatornih biohemijskih parametara, poboljšanje respiratorne funkcije i regresiju radiografskih promena na plućima. Nepovoljan ishod bolesti bio je povezan sa starošću preko 65 godina, prisustvom komorbiditeta i razvojem bolničkih infekcija. Izražene radiografske promene i potreba za mehaničkom ventilacijom predstavljali su najznačajnije prediktivne faktore smrtnosti.

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THE EFFECT OF TOCILIZUMAB ON THE COURSE AND OUTCOME OF THE DISEASE IN PATIENTS WITH COVID-19 PNEUMONIA – EXPERIENCE OF A SECONDARY-LEVEL HOSPITAL

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was identified as the cause of the COVID-19 pandemic that occurred in 2019 (1).

The disease had a broad clinical spectrum, ranging from asymptomatic to severe with fatal outcomes. A quarter of the affected individuals presented with a severe clinical picture, including complications and multiorgan damage, and were treated in intensive care units (2).

Disease progression is conditioned by inflammatory and immune processes mediated by Tumor Necrosis Factor, Janus Kinase (JAK), and interleukins, with interleukin 6 (IL-6) playing the most significant role (3).

Studies have shown that a good therapeutic effect can be achieved with the use of pro-inflammatory mediator blockers, such as tocilizumab (TCZ) (4). Safety in application and good therapeutic response to TCZ was also demonstrated in the example of a treated twin pregnancy (5).

Tocilizumab is a monoclonal antibody that binds to IL-6 receptors. In this way, the transmission of the signal that activates Janus kinase is prevented. Janus kinase activation is a condition for the onset of cytokine storm (6).

The aim of this research was to analyze the effects of tocilizumab administration on the clinical course, laboratory parameters of inflammation and radiographic chan-

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ges of patients with COVID-19 pneumonia, as well as to determine the predictors of unfavorable disease outcome.

PATIENTS AND METHODS

The study included 340 patients over the age of 18, with COVID-19 pneumonia, treated in the ward or in the intensive care unit (ICU) of the General Hospital in Užice, during 2021.

ICU admission criteria were worsening respiratory function requiring non-invasive ventilation (NIV) or mechanical ventilation (MV).

The diagnosis of COVID-19 was made based on the detection of SARS-CoV-2 virus in the nasopharyngeal swab.

Patients were treated according to the National COVID-19 treatment protocol. Oxygen therapy, anticoagulant therapy, corticosteroid therapy (methylprednisolone), gastro-protective, antiviral (favipiravir) and antibiotic therapy were administered to all examined patients. All patients received TZC, too. The indication for the use of TCZ was an IL-6 value over 40 pg/mL and/or an increase in C-reactive protein (CRP) over 50 mg/L, or a three-fold increase in co-concentration within 48 hours (if IL-6 cannot be determined) with signs of extensive COVID-19 pneumonia followed by more than 25 respirations/minute, saturation (pulse oximetry) less than 93% without oxygen replacement, pO2 less than 8.66 kPa. It was administered in a dose of 8 mg/kg intravenously, divided into two doses with an interval of 12 hours (maximum up to 800 mg per dose).

The study excluded patients with tuberculosis, human immunodeficiency virus infection, autoimmune diseases, viral hepatitis, malignancies, risk of gastrointestinal bleeding, thrombocytopenia (<50x109/L), and neutropenia (<0.5x109/L), as these were contraindications for the use of TCZ.

Oxygen was administered via nasal cannula, Venturi oxygen masks, NIV or MV.

Lung radiographs were obtained by digital anteroposterior bedside chest radiography at full inspiration, using a portable radiography unit (Vision M, Visaris). Pulmonary radiographic changes were described following the Fleischner Society glossary (7).

Chest X-ray severity score (CXR-SS) is determined based on the Radiographic Assessment of Lung Edema (RALE) score. The maximum score was obtained by adding the scores of both lungs.Changes in each lung were scored from 0 to 4 (0 = no involvement; 1 = <25%; 2 = 25–50%; 3 = 50–75%; 4 = >75%) (8).

Data on gender and age, clinical course and disease outcome, presence and type of comorbidities, development of Healthcare associated infections (HAIs), respiratory function assessment, laboratory parameters, and radiographic changes were collected. Respiratory function data, laboratory and radiographic data were collected before and 5 days after TCZ administration.

The obtained data were statistically analyzed using SPSS software v. 25.0. The methods of descriptive statistics, Chi-square ($\chi 2$) test, and for quantitative variables unpaired t test and Mann-Whitney test were used for statistical processing of the obtained values. Significant variables were entered into a univariate and multivariate logistic regression model. A probability value of $P < 0.05$ was considered significant.

RESULTS

Out of a total of 340 participants, 68.5% were over the age of 65 years. The mean age was 66.22 ± 11.37 years (range 24–95 years). The examined patients were significantly more often male (66.4%) (Table 1).

A significant majority had comorbidities (65.4%). Healthcare associated infections (HAIs) occurred in 29 (8.5%) (10 bacterial pneumonias, 10 urinary infections, 5 pseudomembranous colitis, 7 pulmonary embolisms, 5 cerebrovascular insults). *Acinetobacter baumannii* was isolated in bronchial aspirate cultures in 5 patients, *Klebsiella spp*. in 4, and *Streptococcus pneumoniae* in one patient. The most common way of oxygenation was NIV (64.1%). MV was necessary for almost a quarter of patients (24%). A significant majority (78.2%) were discharged from the hospital. In 21.8% of patients, the disease ended in death before discharge.

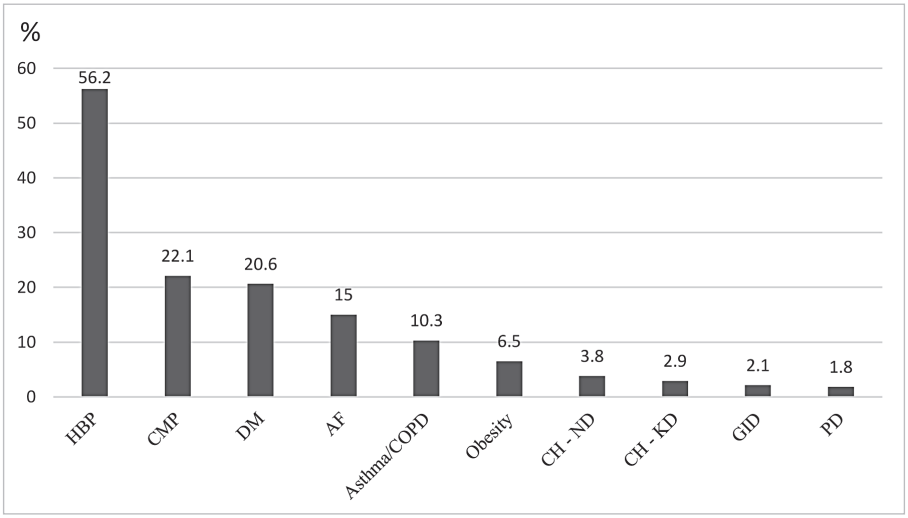
Age, sex, presence of comorbidities, HAIs, method of oxygenation, and disease outcomes are presented in Table 1.

Table 1. Characteristics of patients with COVID-19 treated with tocilizumab

Parameters		N % 340 100	P value
Age group	>65 ≤ 65	199 (58.5) 141 (41.5)	0.089
Gender	Men Women	226 (66.4) 114 (33.5)	<0.001
Comorbidity	Yes No	222 (65.3) 118 (34.7)	0.002
HAIs	Yes No	29 (8.5) 311 (91.5)	0.00
Method of oxygenation	Nasal prongs/face mask Noninvasive ventilation Invasive mechanical ventilation	41 (12.1) 218 (64.1) 81 (23.8)	0.00
Treatment outcome	Dismissed In-hospital mortality	266 (78.2) 74 (21.8)	0.00

Figure 1 shows the types of comorbidities and their frequencies.

Figure 1. Comorbidities in patients with COVID-19 treated with tocilizumab



The most common comorbidity was hypertension (56.2%), followed by cardiomyopathy (22.1%), diabetes mellitus (20.6%), atrial fibrillation (15%), asthma/chronic obstructive pulmonary diseases (COPD) (10.3%), obesity (body mass index > 30) (6.5%), and chronic neurological, renal, psychiatric, gastrointestinal/liver, and psychiatric disorders in 3.8%, 2.9%, 2.1%, and 1.8%, respectively.

Leukocyte count, platelet count, biochemical inflammatory parameters (lactate dehydrogenase (LDH), CRP, IL-6), oxygenation parameter PaO2/FiO2 (P/F) ratio, and chest X-ray (CXR) score were compared before and 5 days after TCZ administration, as shown in Table 2.

Table 2. Characteristics of patients with COVID-19 before and after tocilizumab

Parameters		At the day of TCZ treatment N % 340 100	The fifth day of TCZ treatment N % 340 100	P value
WBC	<4 x 10^9/L >10 x 10^9/L	98 (28.8) 205 (60.3)	50 (14.7) 183 (53.8)	0.130
PLT	<100 x 10^9/L	42 (12.4)	21 (6.2)	0.156
LDH	>245 U/L	304 (89.4)	187 (55.0)	0.004
CRP	>50 mg/L	309 (90.9)	191(56.2)	0.004
IL-6	>40 pg/mL	340 (100)	149 (43.8)	<0.001

HAIs	yes	29 (8.5)	31 (9.1)	0.888
P/F ratio	250-300 200-250 <200	39 (11.5) 220 (64.7) 81 (23.8)	87 (25.6) 111 (32.6) 54 (15.9)	<0.001
CXR-SS	1 2 3 4 5 6 7 8	0 0 0 5 (1.5) 28 (8.2) 215 (63.2) 42 (12.4) 50 (14.7)	0 0 10 (2.9) 69 (20.3) 73 (21.5) 128 (37.6) 41 (12.1) 19 (5.6)	<0.001
CXR-SS (total)	Moderate (3, 4) Severe (5, 6) Very severe (7, 8)	5 (1.6) 243 (71.5) 92 (27.1)	79 (23.2) 201 (59.1) 60 (17.6)	<0.001

There was no significant difference in leukocyte and platelet counts relative to TCZ therapy. A significant regression of LDH, CRP, and IL-6 levels was observed following TCZ administration.

All patients had peripheral oxygen saturation (SpO2) < 90% and a partial pressure of oxygen in arterial blood (PaO2) < 60 mmHg prior to TCZ treatment. The control values on day 5 showed a significant improvement in the P/F ratio.

The total CXR-SS was severe in 71.5% and very severe in 27.1% of patients prior to TCZ therapy. A significantly higher proportion of patients had a moderate CXR-SS after TCZ treatment. There was no statistically significant difference in the frequency of very severe CXR-SS following TCZ therapy.

Further comparisons were made between the data prior to TCZ administration and the disease outcome. The results are presented in Table 3.

Table 3. Characteristics of patients with COVID-19 treated with tocilizumab in relation to the outcome of the disease

Parameters		Dismissed N (%) 266 100	Deceased N (%) 74 100	P value
Age group	>65	141 (53.0)	58 (78.3)	0.027
Gender	Men Women	178 (66.9) 88 (33.1)	48 (64.9) 26 (35.2)	0.862 0.799

Parameters		Dismissed N (%) 266 100	Deceased N (%) 74 100	P value
Comorbidity	Yes	167 (62.8)	73 (98.6)	0.005
HAIs	Yes	15 (5.6)	14 (18.9)	0.007
Method of oxygenation	Invasive mechanical ventilation	20 (7.5)	61 (82.4)	0.00
WBC	<4 x 10^9/L >10 x 10^9/L	76 (28.6) 158 (59.4)	22 (29.7) 47 (63.5)	0.928
PLT	<100 x 10^9/L	32 (12.0)	10 (13.5)	0.767
LDH	>245 U/L	198 (74.4)	62 (83.8)	0.455
CRP	>50 mg/L	243 (91.4)	66 (89.2)	0.869
IL-6	>80 pg/mL	217 (81.6)	72 (97.3)	0.240
CXR-SS (total)	Moderate (4) Severe (5, 6) Very severe (7, 8)	5 (1.9) 225 (84.6) 36 (13.5)	0 18 (24.3) 56 (75.7)	 0.00 0.00

Regarding disease outcome, there was no significant difference in sex distribution. Mortality was higher in those over 65 years old (78.3%), with a higher frequency of comorbidities (98.6%). Eleven (14.9%) patients had three or more comorbidities. HAIs were also more common in the deceased group (18.9%) (9 pneumonias, 4 pulmonary embolisms, and 2 cerebrovascular insults). A significantly higher proportion of deceased patients (82.4%) required mechanical ventilation. Comparative values of CRP, LDH, and IL-6 did not show significant differences. Regarding the CXR-SS, the severe score was most common in the surviving group (84.6%), while the very severe score was significantly more frequent in the deceased group (75.7%).

Table 4 presents the results of univariate linear regression for age, presence of comorbidities, associated infections, mechanical ventilation, and radiographic severity score as independent factors related to the fatal outcome of COVID-19 pneumonia treated with TCZ.

Table 4. Predictive factors of fatal outcome in patients with COVID-19 pneumonia treated with tocilizumab

Parameters	Fatal outcome				
	B	S.E.	Exp(B)	95% CI	P
Age > 65	1.167	0.308	3.214	1.757-5.877	0.000
Comorbidities	3.768	1.015	43.275	5.922-316.248	0.000
Associated infection	1.362	0.398	3.904	1.788-8.525	0.001
Mechanical ventilation	3.743	0.372	42.234	20.372-87.555	0.000
CXS-SS	2.971	0.324	19.510	10.331-36.844	0.000

Multivariate analysis showed that the most significant predictors of a fatal outcome were the need for mechanical ventilation (B = 3.195, SE = 0.454, P = 0.000) and very severe radiographic changes (B = 2.150, SE = 0.450, P = 0.000).

DISCUSSION

The clinical course and outcome of COVID-19 pneumonia are influenced by the characteristics of the virus itself and the host's immune responses. The spectrum of different proteins of SARS-CoV-2 contributes to its high pathogenicity and virulence, as well as its ability to evade the host's immune defense. New variants of the virus, arising from continuous mutations, increase its resistance to the humoral immune response. A functional immune response ensures a milder clinical course and a favorable disease outcome. Therefore, it is crucial to identify inadequate immune responses and intervene with effective therapy (9).

In this study, we analyzed the effects of tocilizumab (TCZ) administration in patients with COVID-19 pneumonia and various factors that may affect the course and outcome of such treatment.

Our patients had an average age of 66 years. Consistent with other studies, we also found that age over 65 years was a significant predictor of mortality (10, 11).

The majority of the subjects were male, although male sex was not a predictive factor for fatal outcomes. Some authors report similar findings (11, 12). In other studies, male sex was more frequently observed in COVID-19 infections and was also a predictor of severe disease and fatal outcomes, explained by socio-economic factors, habits, and nature of the workplace (13, 14). We did not analyze these factors, but women in our cohort had significantly more comorbidities compared to men, particularly three or more. Comorbidities were, as in other studies, present in most patients with severe COVID-19 infection (15, 16). Since comorbidities contribute to the severe clinical form of the disease, this finding was expected. The presence of

comorbidities, especially two or more, was a significant predictor of mortality in our patients, as reported in other studies. Accordingly, hypertension and heart disease were the most common comorbidities in our cohort (10, 13, 17).

A fatal outcome was associated with the presence of hospital-acquired infections (HAIs). Schmidt and colleagues also identified nosocomial infections as a significant predictor of mortality, with patients most commonly having pneumonia caused by *Acinetobacter baumannii* (10). Both *Acinetobacter baumannii* and *Klebsiella pneumoniae* were isolated from the sputum of our patients. This finding was expected, given the proven high prevalence of multidrug-resistant strains of these bacteria in intensive care units in our region (18).

Abnormal IL-6 production and other pro-inflammatory cytokines lead to a cytokine storm, which plays a key role in the pathogenesis of severe pneumonia. Destruction of alveolar cells increases permeability and results in pulmonary edema. This impairs gas exchange and leads to hypoxemia (19). TCZ improves respiratory function, particularly in severe cases of COVID-19 pneumonia (20).

Our experience confirms the improvement of respiratory function, assessed based on the P/F ratio and methods of oxygenation. We did not observe the increased incidence of concomitant bacterial and fungal infections, as reported by others (21). Nearly a quarter of our patients were on mechanical ventilation prior to receiving TCZ, which, as in other studies, was a significant predictor of mortality (22).

The effect of TCZ was also monitored by analyzing radiographic findings. We observed a significant regression of pulmonary changes, consistent with the regression of inflammatory parameters and improvement in respiratory function. Our findings support those of Lacedonia and colleagues (23). The greatest regression was noted in patients with lower CXR-SS values, suggesting a good effect of TCZ when administered in the early stages of the disease. A CXR-SS of "very severe" was a significant predictor of mortality in our cohort. Other authors also observed the utility of radiography in predicting mortality. Survival was associated with less than 70% or 50% lung involvement, and the analysis of consolidation dominance and pleural effusion presence had even greater predictive value (10, 17, 24).

We observed a regression of IL-6, CRP, and LDH values, but no significant changes in leukocyte and platelet counts. Based on these hematological and biochemical parameters, we could not predict the fatal outcome. This is in agreement with other authors (12, 17, 25).

It is important to consider the impact of other medications used on these parameters and the disease course. Corticosteroids and antiviral drugs have anti-inflammatory effects and, in synergy with TCZ, reduce the hyperinflammatory response, leading to a reduction in mortality (26).

The timely administration of TCZ has been discussed, particularly regarding the good effect of IL-6 inhibition in the active phase of inflammation. Some authors

suggest that TCZ has the best effects when given before the onset of severe respiratory failure, estimating this to be between days 9 and 12 from the onset of symptoms, or within the first five days of dyspnea onset (10, 27). Kaya H. and colleagues believe that mortality is lower if TCZ is administered before day 6 of the disease (11).

All of our patients were treated with corticosteroids, antiviral drugs, and TCZ. The mortality rate was 22%, compared to 18% in another Serbian study (13). Unlike those studies, our patients were older, the majority had severe disease, and 24% required MV for oxygenation. We work in a secondary hospital that receives patients in critical condition from smaller hospitals in the region. Therefore, for many patients, we did not have precise information about previous therapy, such as corticosteroid doses and duration. Additionally, we lacked reliable data on symptom duration before admission to our facility, which is an important predictive factor for disease outcome (26). In contrast to some studies, we observed the benefits of TCZ (21, 28). It improves clinical outcomes and reduces inflammatory markers. We agree that the decision on therapy should be individualized, based on each patient's clinical and biochemical parameters (29, 30).

CONCLUSION

Severe COVID-19 pneumonia was more common in men with cardiovascular comorbidities. The administration of tocilizumab had a beneficial effect on reducing inflammatory biochemical parameters, improving respiratory function, and leading to regression of radiographic pulmonary changes. Unfavorable disease outcomes were associated with age over 65 years, the presence of comorbidities, and the development of hospital-acquired infections. Pronounced radiographic changes and the need for mechanical ventilation were the most important predictive factors for mortality.

The authors declare no relevant conflicts of interest.

The study was approved by the Ethics Committee of the Užice Health Center, decision No. 14560, adopted on December 9, 2024.

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