TOCILIZUMAB IN THE TREATMENT OF PATIENTS WITH MODERATE TO SEVERE COVID-19 PNEUMONIA: A SINGLE-CENTRE EXPERIENCE

Dr. Chintal Vyas[1*], Dr. Nachiketa Parmar[2], Dr. Amee Pandya[3], Dr. Nilay Suthar[4], Dr. Sapan Pandya[5], Dr. Atul Patel[6]

[1]Associate Professor, [2]Resident doctor, Department of Medicine, SVP Hospital SMT NHL Municipal Medical College, Ahmedabad. [3]Resident doctor, Department of Pharmacology, SMT NHL Municipal Medical College, Ahmedabad. Department of Medicine, [4]Professor, LG Hospital, AMCMET Medical College, Ahmedabad. [5]Assistant Professor, Department of Rheumatology, SVP Hospital SMT NHL Municipal Medical College, Ahmedabad.[6]Chief consultant and director, Infectious Disease Clinic, Vedanta Institute of Medical Sciences, Ahmedabad

Abstract

Background and objectives: Patients with COVID-19 infection may develop respiratory failure following cytokine release storm associated hyper-inflammatory state. Tocilizumab (TCZ) can reduce this response by blocking IL-6 receptors and may improve survival. Here we aimed to study the efficacy of TCZ in patients with moderate to severe COVID-19 pneumonia. **Methods:** In this retrospective observational study, all COVID-19 RT PCR confirmed patients who received TCZ from May 1st through May 31st, 2020 at a tertiary care teaching hospital in Gujarat, were analysed. The primary outcome was mortality on day 7 and day 28 after TCZ. Secondary outcomes were changes in laboratory and respiratory parameters after TCZ. **Results:** Out of sixty-one treated patients (M: 45, F: 16, Median age 60 years), 75.4% had associated co-morbidity. The overall mortality was 31.05%(n=18) while 68.96% (n=40) patients were discharged by day 28. Old age, high ferritin, high baseline SOFA score, and low P/F ratio were important risk factors for mortality. Polymorphonuclear leucocytosis and persistent higher CRP level after TCZ were associated with reduced survival and may probe towards secondary bacterial infection. We observed significant improvement in lymphocyte count, ferritin and CRP (<0.05) along with rising WBC count after TCZ. **Conclusion:** In conclusion, TCZ can be used in patients with moderate to severe COVID 19 pneumonia with cytokine storm as an anti-inflammatory agent. We saw improvement in laboratory markers of inflammation, lymphocyte count, and P/F ratio. As this is a single-arm retrospective observational study, future prospective randomised controlled trials are needed to confirm the efficacy of this drug.

Keywords: COVID-19, Mortality, P/F ratio, Tocilizumab

*Corresponding author:
Dr Chintal Vyas
E-mail:chintalvyas@gmail.com

Introduction

A novel coronavirus, referred to as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), was first reported in China at the end of December 2019 as the cause of an acute viral illness that led to respiratory distress and mortality in a few [1,2]. The WHO declared a pandemic of the same on 11th March 2020 [3,4].

Some patients with COVID-19 pneumonia develop severe respiratory failure, the reason for which remains unknown. These patients have laboratory evidence of a hyper-inflammatory state which is similar to cytokine release syndrome (CRS)[5,6]. Early

studies of highly pathogenic human coronaviruses (hCoVs) infection like SARS-CoV and MERS-CoV had shown that compared to individuals with mild disease, patients with severe disease had high serum levels of pro-inflammatory cytokines (IFN-γ, IL-1, IL-6, IL-12) and chemokines (CCL2, CXCL10, CXCL9, and IL-8) [7-10]. Similar hyper-inflammatory states are also reported in patients with severe COVID-19 pneumonia[11].

Tocilizumab (TCZ), a humanized monoclonal antibody- targeting the IL-6 receptor, is already used to treat rheumatological conditions such as rheumatoid arthritis[12] and other auto-inflammatory conditions[13], and in patients with severe cytokine release syndrome (CRS) induced by chimeric antigen receptor T-cell (CAR-T) therapy [14]. IL-6 is one of the key cytokines involved in infection-induced cytokine storms. So timely inhibition of the IL-6 pathway by TCZ may reduce hyper-inflammatory response and can potentially be clinically effective in selected patients[5,6,15]. It has been used to treat complicated SARS-CoV2 infection with variable results[16-24].

Here, we aimed to study the outcome of patients with moderate to severe COVID-19 pneumonia receiving TCZ and to study changes in respiratory and laboratory parameters in these patients.

Materials and Methods

Study population and study design

In this retrospective observational study, all the SARS CoV-2 RT-PCR confirmed patients with age > 18 years treated with TCZ from May 1st through May 31st, 2020 at a tertiary care hospital in Ahmedabad, Gujarat, were included. Institutional criteria for TCZ in COVID-19 treatment are $SpO_2 \leq 93\%$ or PaO_2/FiO_2(P/F ratio) <300 mmHg, with indication of cytokines storm (alone or any combination of): CRP >15 xUpper Limit of Normal (ULN), D-dimer >2.5 mcg/ml or ferritin >500ng/ml. Patients with known allergy to TCZ or active tuberculosis (TB) infection, suspected active bacterial, fungal, viral, or other infection (besides COVID-19), pregnant and breastfeeding patients, patients with transaminitis with SGPT or SGOT > 5×ULN, Absolute neutrophil count (ANC) < 1000/ml, Platelet count < 50,000/ml (except where these were thought to be likely due to macrophage activation syndrome) were excluded. A single dose TCZ was administered at a dosage of 8 mg/kg (max 800 mg) by intravenous infusion to all patients satisfying inclusion criteria.

All the patients received a standard institute protocol of treatment including antibiotic (azithromycin 500 mg 1 PO OD for 5 days or ceftriaxone 1gm IV BD), hydroxychloroquine (400 mg PO BD on Day 1 followed by 400 mg PO OD for 4 days), low molecular weight heparin (1 mg/kg SC BD), methylprednisolone (80mg IV OD for day 1 followed by 40mg IV BD for 2-5 days) and oxygen therapy according to patients' clinical and laboratory parameters. The study was approved by the Institutional ethics committee.

Data Retrieval

Data in the form of demographics, co-morbidities, serial laboratory parameters, respiratory parameters, treatments, and clinical outcomes, were obtained from medical records. The patient's daily clinical status was recorded from the hospital progress notes using the 8 categories WHO ordinal scale [25]. Improvement/Worsening in clinical status was defined as a one-point change in the WHO ordinal scale. All laboratory parameters were recorded on the day of admission (baseline), on the day of TCZ administration (day 0), and on day 3 and day 7 after TCZ administration.

Baseline Sequential Organ Failure Assessment Score (SOFA Score) and day 7 SOFA Score were calculated to identify patients with multi-organ involvement. Absolute neutrophil count and need for higher antibiotics after TCZ were recorded in all patients for suspected and confirmed secondary bacterial infection.

Outcome

The primary outcome was mortality on day 7 and day 28 after TCZ. Secondary outcomes included changes in laboratory parameters [CRP, D-dimer, NLR (neutrophil-lymphocyte ratio), absolute lymphocyte and neutrophil counts, serum ferritin, ALT, AST], and changes in respiratory parameters [PaO2/FiO2 (P/F Ratio) and WHO ordinal scale] after TCZ.

Statistical analysis

Data were analysed using SPSS version 23.0. Continuous variables were reported as mean and standard deviations. Categorical variables were reported as numbers and percentages. Shapiro Wilk test was used to categorize the normality of data. Data were summarised using number (percentage) or median (range), as appropriate. Clinical improvement rates on subsequent days were expressed as a percentage. Repeated measure ANOVA was used to compare changes in laboratory parameters on subsequent days. Chi-square test and Unpaired t-test were used to compare the characteristics and outcomes before and after TCZ administration in discharged and deceased patients. All p-values with a value of <0.05 were considered statistically significant.

Results

Characteristics of COVID-19 Patients Treated with TCZ

Sixty-one COVID-19 patients treated with TCZ were included in this study. The characteristics of these patients are summarised in Table 1. The median age was 60 years. The most common chest X-ray finding on admission was bilateral pulmonary infiltrates seen in 47 (77.04%) patients.

Mortality and Outcome

Table 2 shows the change in the Modified Ordinal Scale of patients after TCZ administration.At the time of TCZ administration, 32 (52.45%) patients were on NRBM/oxygen mask/ nasal prongs and 20 (32.7%) patients were on non-invasive ventilation/ high flow nasal cannula. Initially, on the day of TCZ administration, 55 (90.16%) patients did not require invasive ventilator support while 6(9.84%) patients were on invasive ventilation. Eventually, 16(29.8%) out of 55 patients required invasive ventilation during their period of hospitalisation.

By day 3, 15(25.8%) patients improved according to the modified ordinal scale whereas 13(22.4%) patients worsened and the status of 33(56.9%) patients remained the same. By day 7, 30(51.7%) patients had improved and 19(32.7%) patients' respiratory status deteriorated out of which 9 (15.5%) patients died. Onday 28, the overall mortality was 31.03%(n=18), while 68.96% (n=40) patients were discharged.(Table 2).

Changes in Laboratory Parameters after TCZ

The laboratory findings of the patients are summarised in Table 3.At the time of TCZ administration, most patients (77.04%) presented with lymphopenia (<1000cells/mm^3) and high levels of inflammatory markers, including CRP, ferritin, and D-dimer (Table 3). Severe lymphopenia was corrected with TCZ therapy in 53.4% of patients on the 7th day in the present

study. After the TCZ administration, a rise in WBC count was also seen in these patients (p=0.014) while no significant changes were seen in ALT/SGPT levels.

Table 1: Characteristics of COVID-19 patients treated with TCZ

Characteristics	**Patients (n=61)**
Mean Age (range), years	58.11±14.07 years (25-88)
Gender Ratio [M: F] Male Female	2.81:1 45 (73.8%) 16 (26.2%)
The duration between onset of symptoms and hospitalisation	5.04± 2.6 days
Patients with Co-morbidities • Diabetes Mellitus Type II • Hypertension • Ischaemic Heart Disease Patients with >1 co-morbidities	46/61 (75.4%) 30/46 (65.2%) 33/46 (71.7%) 7/46 (15.2%) 20/61 (32.7%)
Symptoms: 1. Fever 2. Shortness of breath 3. Cough 4. Others: o Sore throat o Headache o Diarrhoea o Giddiness o Body ache o Chest pain o Myalgia o Vomiting	 49 (80.3%) 34 (55.7%) 29 (47.5%) 13 (21.3%) 6 (9.8%) 4 (6.5%) 1 (1.63%) 1 (1.63%) 1 (1.63%) 1 (1.63%) 1 (1.63%)
Baseline Laboratory Parameters (On Admission) 1. Neutrophil: Lymphocyte ratio (1-3) 2. Lymphocyte count (1000-4000 cells/mm^3) 3. S. Ferritin (10-282 ng/ml) 4. CRP(<0.6mg/l) 5. D-Dimer (<0.5 mcg/ml)	 6.69±5.11 1038.97±427.28 cells/µl 561.57±461.19 ng/ml 11.43±11.73 mg/l 1.05±0.92 mcg/ml
Baseline P/F Ratio (On Admission)	302.94±108.63
Baseline SOFA score (On Admission)	1.52±1.68
TCZ started on the day (Mean) From symptom onset (days) After admission to hospital (days)	 8.81±3.78 3.77±2.87
Steroid use: No. of patients receiving Methylprednisolone Mean Duration of Steroids, days	 46 (75.4%) 4.29±2.46
Mean Duration of ICU Stay, days (range)	10.6±5.32 (0-27)
Mean Duration of Hospitalization, days (range)	15.1±6.11 (2-32)

Table 2: Modified Ordinal Scale of patients after TCZ therapy

Scoring Points	Scoring Variables	day 0 (n =61)	day 3 (n=58)	day 7 (n=58)	day 10 (n=58)	day 14 (n=58)	day 28* (n=58)
Ambulatory							
1	No Limitation of activities	0	0	1(1.7%)	9(15.5%)	19(32.7%)	38 (65.5%)
2	Ambulatory with Limitation of activities	0	0	1(1.7%)	1(1.7%)	0	0
Hospitalised and Mild disease							
3	No O_2 therapy	3(4.9%)	13(22.4%)	24(41.4%)	20(34.4%)	16(27.6%)	0
4	O_2 by Nasal prong/mask	32(52.4%)	19(32.7%)	10(17.2%)	7(12.1%)	4(6.9%)	0
Hospitalised and Severe disease							
5	NIV/HFNC	20(32.7%)	17(29.3%)	9(15.5%)	5(8.6%)	1(1.7%)	0
6	Intubated	3(4.9%)	4(6.9%)	1(1.7%)	2(3.4%)	2(3.4%)	0
7	Intubated plus additional supports	3(4.9%)	3(5.2%)	3(5.2%)	2(3.4%)	1(1.7%)	2 (3.4%)
Death							
8	Deceased	0	2(3.4%)	9(15.5%)	12(20.7%)	15(25.8%)	18(31.03%)

*Three patients were transferred to other hospitals by day 28.NIV - Non-invasive ventilation, HFNC – High flow nasal cannula

Changes in Respiratory Parameters

Respiratory parameters were assessed by P/F ratio on day 0, day 3, and day 7(Table 4).

Comparison between Survivors and Non-survivors:

Table 5 shows the comparison of characteristics between patients who improved and were discharged with those who died.

Out of 18 patients who died, 5 patients had bacterial bloodstream infection. Among them, two had *Acinetobacterbaumanii complex* infection while one each had *Enterococcus faecium*, *Klebsiella pneumoniae* and *Escherichia coli* infection.

Table 3: Change in laboratory parameters of patients after TCZ therapy

Parameter	day 0 n=61	day 3 n=58	day 7 n=58	p-value
Neutrophil: Lymphocyte ratio(1-3)	12.55±8.62	11.69±9.80	8.83±9.96	0.112
Total WBC count(5200-12400 cells/mm^3)	9166.77±4021.49	10707.19±5392.82	12424.28±7066.73	0.01*
Neutrophil count (2500-8000 cells/mm^3)	7840.64±3777.47	9018.82±5127.63	10288.35±7112.92	0.064
Lymphocyte count (1000-4000 cells/mm^3)	793.98±419.48	1037.60±548.82	1332.14±824.70	0.0001*
S. Ferritin (10-282 ng/ml)	845.17±519.37	671.57±425.51	589.44±375.37	0.014*
CRP (<0.6mg/l)	11.80±9.65	2.63±2.70	1.37±3.10	0.0001*
SGPT (10-49 U/L)	56.73±69.82	67.62±48.12	67.85±48.27	0.532
SGOT (0-34 U/L)	50.09±40.08	50.29±23.86	46.09±29.51	0.793
D-Dimer (<0.5 mcg/ml)	2.23±1.73	2.75±1.92	2.78±1.79	0.485

*values <0.05 are considered statistically significant (Repeated measure ANOVA)

Table 4: Change in P/F Ratio of patients after TCZ therapy

P/F Ratio (mean± SD)	day 0	day 3	day 7	p-value
All patients (n=58)	275.35±113.67	282.70±142.21	332.59±146.42	0.065
InSurvivors (n=40)	295.93±117.92	327.24±133.99	388.25±108.47	0.005†
In Non-Survivors (n=18)	224.13±92.60	182.49±106.79	160.80±106.59	0.276

† values <0.05 are considered statistically significant (Repeated measure ANOVA)

Table 5: Characteristics of Survivors and Non-survivors

Characteristics	Improved/ discharged (n=40)	Deceased (n=18)	p-value
Mean age of patients (Years)	54.5±14.18 (25-88)	65.83±14.13 (36-84)	0.0067*
Male Female	28 (70%) 12 (30%)	15 (83.3%) 3 (16.6%)	0.273
Requirement of ICU (n)	40 (100%)	18 (100%)	-
Duration of ICU Stay (days)	11.05±5.93	13.6±5.91	0.125
Duration of hospitalisation (days)	17.37±6.48	13.7±6.45	0.050
The duration between onset of symptoms and hospitalisation (days)	5.17±2.60	4.38±2.03	0.257
Co-morbidities: • Diabetes Mellitus Type II • Hypertension • Ischaemic Heart Disease	28 (70%) 19(47.5%) 21(52.5%) 2(5%)	15 (83.3%) 10(55.5%) 10(55.5%) 4(22.2%)	0.283 0.570 0.826 0.046
Patients with 2 co-morbidities	14(35%)	3 (16.6%)	0.14
Patients with all 3 co-morbidities	0	3 (16.6%)	-
Symptoms: • Fever • Shortness of breath • Cough	 33(82.5%) 22(55%) 21(52.5%)	 14(77.7%) 11(61.1%) 7(38.8%)	 0.671 0.654 0.316
TCZ started on the day : From onset of illness (days) After admission (days)	 9.27±3.04 3.57±2.59	 8.61±3.97 4.38±3.62	 0.490 0.336
SOFA Score	0.83±1.24	2.83±2.03	0.0001*
Need for Higher Antibiotics	9 (22.5%)	10 (55.5%)	0.013*
No. of patients receiving Methylprednisolone	29 (72.5%)	15 (83.3%)	0.378

*values <0.05 are considered statistically significant (Chi-square test and Unpaired t-test)

Table 5: Characteristics of Survivors and Non Survivors (continued)

Characteristics	Improved/ discharged (n=40)	Deceased (n=18)	p-value
On the day of TCZ Administration			
Neutrophil: Lymphocyte ratio	11.70±9.07	15.15±10.87	0.212
Total WBC count	8534.7±3281	10265.29±3471.26	0.073
Neutrophil count	7069.12±3293.75	8909.1±3224.04	0.052
Lymphocyte count	991.73±563.39	745.37±350	0.089
S. Ferritin	518.69±238.56	857.71±571.27	0.002*
CRP	11.56±11.70	10.74±6.37	0.761
SGPT	48.00±40.43	49.71±38.01	0.880
SGOT	42.77±11.21	60.77±39.41	0.009*
D-Dimer	2.42±1.89	2.01±1.64	0.412
P/F Ratio	295.93±114.68	224.13±92.60	0.023*
After TCZ (day 7)			
Neutrophil: Lymphocyte ratio	4.9±4.13	16.14±10.71	0.0001*
WBC Total count	8432.66±2751.56	19647.3±7218.77	0.0001*
Neutrophil count (2500-8000 cells/mm^3)	5862.25±2720.28	17859.7±6984.23	0.0001*
Lymphocyte count (1000-4000 cells/mm^3)	1875.11±1018.17	994.72±421.17	0.0009*
S. Ferritin (10-282 ng/ml)	552.5±351.87	626.2±490.97	0.518
CRP	0.63±0.27	4.25±4.92	0.0001*
SGPT	62.12±36.96	41.37±23.39	0.029*
SGOT	41.66±13.21	44.66±43.57	0.690
D-Dimer	1.87±1.53	3.65±2.61	0.0019*
P/F Ratio	388.25±145.03	182.49±106.79	0.0001*
SOFA Score	0.37±0.72	4.70±3.12	0.0001*

*values <0.05 are considered statistically significant (Chi-square test and Unpaired t-test)

According to ROC curve analysis, Day 3 CRP [(AUC= 0.81 (95% CI: 0.66 to 0.96)] and Day 3 NLR [AUC= 0.72 (95% CI: 0.55 to 0.89)] were able to identify patients with higher risk of death among COVID-19 pneumonia patients treated with TCZ with CRP ≥3.5mg/L (Sensitivity 71.4%, Specificity 80.6%) and NLR ≥11.5 (Sensitivity 78.6%, Specificity 71%) as the optimal cut-off (Figure 1). Similarly, ROC curve analysis for Day 7 CRP [(AUC= 0.66 (95% CI: 0.41 to 0.91)] and Day 7 NLR [AUC= 0.87 (95% CI: 0.74 to 0.99)] were able to identify patients with higher risk of death among COVID-19 pneumonia patients treated with TCZ with CRP ≥2.5mg/L (Sensitivity 44.4%, Specificity 100%) and NLR ≥9.5 (Sensitivity 88.9%, Specificity 72.7%) as the optimal cut-off (Figure 2).

Figure 1: ROC Curve of Association between CRP and NLR of Day-3 with Adverse Outcome

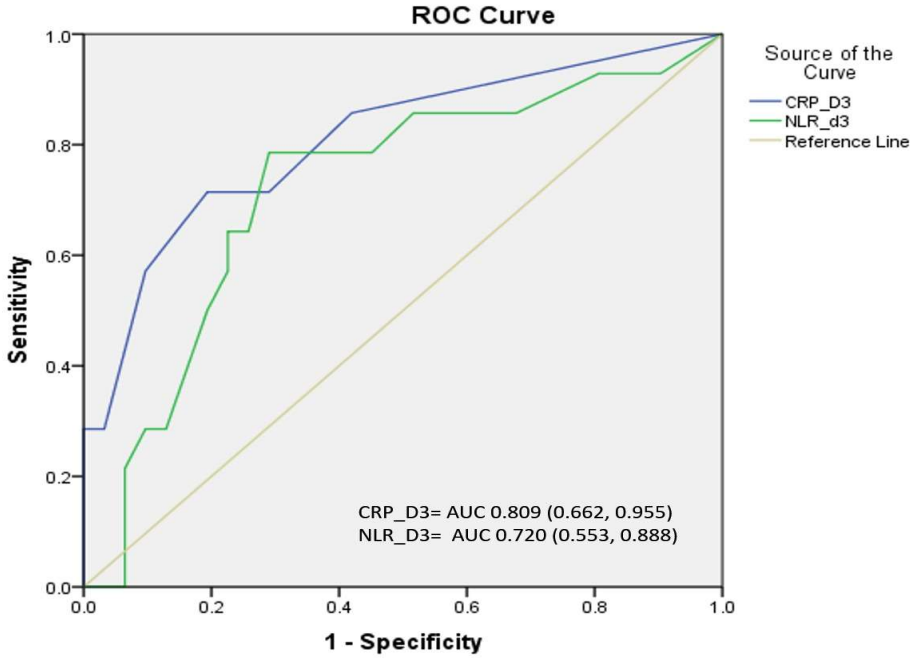
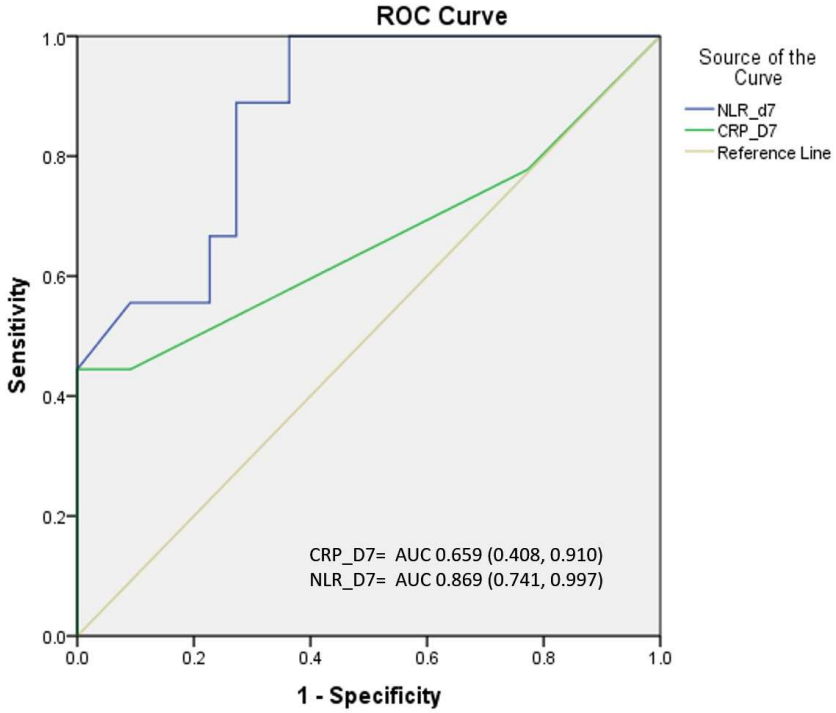


Figure 2: ROC Curve of Association between CRP and NLR of Day-7 with Adverse Outcome



We created several contingency tables to analyse the Odds ratio among groups of patients treated with TCZ to check the Odds ratio of adverse outcomes (Death) in this group. We found

a 9.105 of Odds ratio of developing adverse outcomes in patients aged>49 years of age. Other parameters have shown the Odds ratio of Adverse Outcomes (Death) 1.0 to 3.8 (table 6).

Table 6: Association between various parameters and Death in patients treated with TCZ

	Death (numbers)	Odds Ratio	Confidence interval		P value
Age>49yrs	17	9.105	1.683	119.216	0.024
Male	15	1.935	0.474	7.907	0.518
Ischemic Heart Disease	4	3.810	0.757	19.173	0.105
Hypertension	10	1.087	0.360	3.285	0.883
DM	10	1.438	0.476	3.345	0.520
NLR>6.9	13	1.815	0.502	6.561	0.363

Discussion

There is a dearth of published data on the clinical benefit of TCZ for the treatment of COVID-19 associated CRS from our country.

In our study, 65.57% of patients improved and could be discharged over the next 28 days. We noted a mortality of 31.03%. Looking at the Hospital database, we found overall mortality of 33% in April when TCZ was not used at our centre. The data on TCZ in COVID 19 pneumonia has been contradictory. The study from Rossotti R et al reported better overall survival with TCZ therapy (HR 0.499 [95% CI 0.262–0.952], p=0.035) compared to controls. They also described patients requiring a longer hospital stay (HR 1.658 [95% CI 1.088–2.524], p=0.019) in the TCZ arm mainly due to biochemical, respiratory, and infectious adverse events [23]. In the study by Biran et al, patients with COVID-19 requiring ICU support who received TCZ had reduced mortality (HR 0.64, 95% CI 0.47–0.87; p=0.0040)[26]. Similar associations with TCZ were noted among subgroups requiring mechanical ventilatory support and with baseline C-reactive protein of 15 mg/dL or higher. In the study by Guaraldi et al,TCZ treatment was associated with a reduced risk of invasive mechanical ventilation or death (adjusted hazard ratio 0.61, 95% CI 0.40–0.92; p=0.020)[27]. On the other hand, the randomised control trial COVACTA showed no statistically significant difference in clinical status between TCZ and placebo group patients assessed using a 7-category ordinal scale at week four (p=0.36; odds ratio [95% CI] = 1.19 [0.81, 1.76]) while in EMPACTA trial, patients with COVID-19 associated pneumonia who received TCZ plus standard of care were 44% less likely to progress to mechanical ventilation or death compared to patients who received placebo plus standard of care (log-rank p-value = 0.0348; HR [95% CI] = 0.56 [0.32, 0.97])[28].

Amongst factors associated with reduced survival in our study were older age and higher baseline SOFA score ($p<0.05$). Hence it can be inferred that a higher baseline SOFA Score reduced the success of treatment with TCZ. The reason behind the poor outcome with TCZ therapy in such cases with higher baseline SOFA score might be the multi-organ involvement or advanced disease spectrum.High ferritin levels, high AST, and low P/F ratio on the day of TCZ administration were identified as markers of poor prognosis in our study ($p<0.05$). Among survivors and non-survivors, no significant difference was found in duration from symptom onset to admission and from symptom onset to TCZ administration. Surprisingly at the time of administration of TCZ (day 0), CRP was higher although not statistically significant in the survivor group. Similar observation was also reported by Chen Jet al[29]. In the study by Biran

et al, reduced mortality was found in a subgroup of patients with baseline C-reactive protein of 15 mg/dL or higher.

After TCZ, patients who died had increased total white cell counts with predominant polymorphonuclear leucocytosis and persistently high CRP which could be due to secondary bacterial infection. Among non-survivors, five patients had bloodstream infections. The need for higher antibiotics was statistically significantly higher in patients who died (p=0.042). The study from Somers EC et al reported a survival benefit with the use of TCZ in mechanically ventilated COVID-19 patients but with higher superinfection[24].In the study by Guaraldi et al, 24 (13%) of 179 patients treated with tocilizumab were diagnosed with new infections, versus 14 (4%) of 365 patients treated with standard of care alone (p<0.0001).Clinicians should be vigilant about the risk of secondary bacterial infection especially in those who have polymorphonuclear leucocytosis and persistently high CRP following TCZ.

Severe lymphopenia was corrected with TCZ therapy in 53.4% of patients on the 7th day in the present study. Similar findings were also reported in a study done by Xu X et al [16]. In our study, after TCZ therapy, CRP and ferritin levels normalised and lymphocyte counts increased (p<0.05). Similar results were observed in other studies[11,18,20]. Although D-Dimer levels increased after TCZ therapy in our patients, statistically it did not come out to be significant (p=0.581). Similar findings were reportedby Toniati P et al[18]. This suggests that TCZ can curtail hyper-inflammatory response but may not have an effect on the intravascular coagulopathy[30].

In our study, we observed that the P/F ratio improved from day 0 to day 7, though statistically not significant. Rossotti R et al observed rapid deterioration in P/F ratio and increased requirement of non-invasive/invasive ventilation within 24 hrs after TCZ administration followed by significant improvement in P/F ratio by day 5 to day 7[23]. This might be due to increased IL-6 levels after TCZ administration which is inversely related to P/F ratio [2,6-10]. So, clinicians should be watchful for rapid worsening of respiratory status soon after TCZ administration. A rapid decrease in oxygen requirement and invasive ventilation requirement was also noted in our study which is similar to other studies[16,18].

In our study, according to ROC curve analysis, high CRP and high NLR on day 3 and on day 7 were significantly associated with risk of death among COVID-19 pneumonia patients treated with TCZ. Among various risk factors, age>49 years was significantly associated with adverse outcome (odds ratio = 9.105) in these patients.

Although we found clinical and laboratory improvement in 2/3rd of our patients given TCZ, our results have to be interpreted with caution. This was a single-arm retrospective observational study. Therefore, in the absence of a control group a robust inference cannot be established. Forty-six (75.4%) of these patients also received concomitant corticosteroid therapy, which is also a potent anti-inflammatory agent. Also, unlike other studies, we did not use the 2nd bolus of TCZ in any of our patients.

Conclusion

TCZ can be used in patients with moderate to severe COVID-19 pneumonia with cytokine storm as an anti-inflammatory agent. We saw improvement in laboratory markers of inflammation, lymphocyte count, and P/F ratio.As this is a single-arm retrospective observational study, future randomised controlled trials are needed to confirm the efficacy of this drug.

References

1. Guo YR, Cao QD, Hong ZS, *et al.* The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak—An update on the status. *Mil. Med. Res.* 2020,7:1–10.
2. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult in patients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020;395(10229): 1054-1062.
3. World Health Organization. Coronavirus (COVID-19) Events as They Happen. 2020. Available online: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/events-as-they-happen (Accessed on 20 April 2020).
4. Ghebreyesus TA. WHO Director-General's Opening REMARKS at the media Briefing on COVID-19–11 March 2020. Available online: https://www.whoint/dg/speeches/detail/who-director-general-s-opening- remarks-atthe-media-briefing-on-covid-19–11-March-2020 (Accessed on 20 April 2020).
5. Zhang C, Wu Z, Li JW, Zhao H, Wang GQ. Cytokine release syndrome in severe COVID-19: interleukin-6 receptor antagonist tocilizumab may be the key to reduce mortality. *Int J Antimicrob Agents*. 2020;55(5):105954. doi:10.1016/j.ijantimicag.2020.105954
6. Park WY, Goodman RB, Steinberg KP, et al. Cytokine balance in the lungs of patients with acute respiratory distress syndrome. *Am J Respir Crit Care Med* 2001; 164:1896-1903
7. Chien JY, Hsueh PR, Cheng WC, Yu CJ, Yang PC. Temporal changes in cytokine/chemokine profiles and pulmonary involvement in severe acute respiratory syndrome. Respirology. 2006;11:715–22.
8. Wang CH, Liu CY, Wan YL, Chou CL, Huang KH, Lin HC, Lin SM, Lin TY, Chung KF, Kuo HP. Persistence of lung inflammation and lung cytokines with high-resolution CT abnormalities during recovery from SARS. Respiratory research. 2005 Dec;6(1):1-2.
9. Wong CK, Lam CW, Wu AK, et al. Plasma inflammatory cytokines and chemokines in severe acute respiratory syndrome. *Clin Exp Immunol* 2004;136(1):95–103.
10. Zhang Y, Li J, Zhan Y, Wu L, Yu X, Zhang W et al. Analysis of serum cytokines in patients with severe acute respiratory syndrome. *Infect Immun* 2004; 72(8):4410–4415.
11.Conrozier T, Lohse A, Balblanc JC, et al. Biomarker variation in patients successfully treated with tocilizumab for severe coronavirus disease 2019 (COVID-19): results of a multidisciplinary collaboration. *Clin Exp Rheumatol*. 2020; 38(4): 742- 747.
12. Scott LJ. Tocilizumab: A Review in Rheumatoid Arthritis. Drugs 2017, 77:1865–1879.
13. Schoels MM, Van Der Heijde D, Breedveld FC, et al. Blocking the effects of interleukin-6 in rheumatoid arthritis and other inflammatory rheumatic diseases: systematic literature review and meta-analysis informing a consensus statement. *Ann. Rheum. Dis.* 72, 583–589 (2013).
14. Kotch C, Barrett D, Teachey DT. Tocilizumab for the Treatment of Chimeric Antigen Receptor T Cell-Induced Cytokine Release Syndrome. *Expert Rev. Clin. Immunol*. 2019, 15:813–822.
15. Wang Z, Han W. Biomarkers of cytokine release syndrome and neurotoxicity related to CAR-T cell therapy. *Biomark Res*. 2018;6:4.
16. Xu X, Han M, Li T, et al. Effective treatment of severe COVID-19 patients with tocilizumab. *Proc Natl AcadSci USA*. 2020;117(20):10970-10975.

17. Campochiaro C, Della-Torre E, Cavalli G, et al. Efficacy and safety of tocilizumab in severe COVID-19 patients: a single-centre retrospective cohort study. *Eur J Intern Med*. 2020;76:43-49.
18. Toniati P, Piva S, Cattalini M, et al. Tocilizumab for the treatment of severe COVID-19 pneumonia with hyperinflammatory syndrome and acute respiratory failure: A single-centre study of 100 patients in Brescia, Italy. *Autoimmun Rev*. 2020;19(7):102568.
19. Colaneri M, Bogliolo L, Valsecchi P, et al. Tocilizumab for Treatment of Severe COVID-19 Patients: Preliminary Results from SMAtteo COvid19 Registry (SMACORE).*Microorganisms*. 2020;8(5):695.
20. Lohse A, Klopfenstein T, Balblanc JC, et al. Predictive factors of mortality in patients treated with tocilizumab for acute respiratory distress syndrome related to coronavirus disease 2019 (COVID-19). *Microbes Infect*. 2020 Oct; 22(9): 500–503.
21. Sciascia S, Aprà F, Baffa A, et al. Pilot prospective open, single-arm multicentre study on off-label use of tocilizumab in patients with severe COVID-19. *ClinExpRheumatol*. 2020;38(3):529-532.
22. Luo P, Liu Y, Qiu L, Liu X, Liu D, Li J. Tocilizumab treatment in COVID-19: A single centre experience. *J Med Virol*. 2020;92(7):814-818.
23. Rossotti R, Travi G, Ughi N, et al. Safety and efficacy of anti-il6-receptor tocilizumab use in severe and critical patients affected by coronavirus disease 2019: a comparative analysis. *J Infect*. 2020 Oct; 81(4): e11–e17.
24.Somers EC, Eschenauer GA, Troost JP, et al. Tocilizumab for treatment of mechanically ventilated patients with COVID-19. *Clin Infect Dis*. 2020 Jul 11:ciaa954.
25. World Health Organization. WHO R&D blueprint: novel coronavirus: COVID-19 therapeutic trial synopsis. Accessed September 15, 2020. https:// www. who.int/ blueprintzpriority-diseases/key-action/COVID19_Treatment_Trial Design_Master_ Protocol_ synopsis_Final_18022020.pdf
26. Biran N, Ip A, Ahn J, et al. Tocilizumab among patients with COVID-19 in the intensive care unit: a multicentre observational study. Lancet Rheumatol. 2020 Oct;2(10):e603-e612.
27. Guaraldi G, Meschiari M, Cozzi-Lepri A, et al. Tocilizumab in patients with severe COVID-19: a retrospective cohort study. *Lancet Rheumatol*. 2020;2(8):e474-e484.
28. Roche. Roche provides an update on the phase III COVACTA trial of Actemra/RoActemra in hospitalised patients with severe COVID-19 associated pneumonia. [Date assessed 15 September 2020]Available from: https://www.roche.com/investors/updates/inv-update-2020-07-29.htm
29. Chen J, Wang X, Zhang S, Liu B, Wu X, Wang Y, et al. Characteristics of Acute Pulmonary Embolism in Patients With COVID-19 Associated Pneumonia From the City of Wuhan. *Clin Appl Thromb Hemost.* 2020 Jan-Dec; 26: 1076029620936772.
30. González-Gay Ma, Mayo J, Castañeda S, Cifrián Jm, Hernández-Rodríguez J: Tocilizumab: from the rheumatology practice to the fight against COVID-19, a virus infection with multiple faces. *Expert Opin Biol Ther*. 2020 Jul;20(7):717-723