TOCILIZUMAB IN SEVERE TO CRITICAL CONFIRMED COVID-19: A CASE SERIES AT ULIN REFERRAL HOSPITAL OF SOUTH KALIMANTAN

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ABSTRACT

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a new type of coronavirus that causes pneumonia. The clinical severity of COVID-19 is related to the presence of a cytokine storm that causes overproduction of inflammatory mediators such as interleukin (IL)-6. Tocilizumab (TCZ), as an IL-6 inhibitor, is the subject of major studies as a potential therapeutic agent. This study reported 20 cases of COVID-19 patients being treated with the IL-6 inhibitor TCZ besides standard therapy. Patients were followed up on clinical, laboratory, and chest x-rays before and after the therapy administration, which was report descriptively. The oxygen saturation of patients who survived shows rapid improvements. The laboratory results showed that CRP decreased after administration TCZ immediately. Meanwhile, other markers improve slowly, such as leucocytes, Neutrophil Lymphocyte Ratio (NLR), Absolute Lymphocyte Count (ALC), and Lactate Dehydrogenase (LDH), but ferritin was tended to fluctuate. In chest X-rays, infiltrate bilateral on admission began to diminish until almost disappeared on the 14th day after TCZ. From 20 patients, 80% of patients survived with improvement in clinical, laboratory, and chest X-rays. At the same time, the rest death with a good response on the first therapy but fluctuated and worsened before death. Tocilizumab can be considered to provide clinical improvements in severe and critical COVID-19 patients.

Keywords: COVID-19; CRP; Interleukin-6; Tocilizumab

INTRODUCTION

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a new type of coronavirus that causes pneumonia, first reported in Wuhan, China, on December 31, 2019.[1] Analysis from the isolation of the lower respiratory tract shows a new type of coronavirus, which is named Coronavirus disease 2019 (COVID-19) by the World Health Organization (WHO). The coronavirus is the etiology of COVID-19, belongs to the genus betacoronavirus, round with some pleomorphic shape, and 60-140 nm in diameter. The results of phylogenetic analysis show that this virus in the same subgenus as the coronavirus caused the SARS outbreak in 2002-2004, namely Sarbecovirus. The International Committee on Taxonomy of Viruses (ICTV) named the cause of COVID-19 as SARS-CoV-2.[2]

On May 03, 2021, WHO recorded 152 million confirmed cases of COVID-19, with 3,1 thousand confirmed deaths people worldwide. In Indonesia, statistical data as of May 2021 1,691,658 cases with 46,349 deaths. As one of the provinces in Indonesia, South Kalimantan reports the number of confirmed positive cases of 33,148 and 995 cases of death. [1,3]

The clinical severity of COVID-19 is related to the presence of a "cytokine storm" that causes the overproduction of inflammatory mediators. The great majority of the inflammatory cells infiltrating the lungs are monocytes and macrophages. When COVID-19 infection, monocytes, and macrophages are increased, which may explain elevated levels of pro-inflammatory cytokines such as interleukin (IL)-6, IL-1, tumor necrosis factor (TNF)α, and IL-8, which in some patients turn out to be a

cytokine storm. [4] In particular, IL-6 emerged as a treatment target because of its strong association with disease severity. IL-6 more than 55 pg/mL resulted in a high risk of severe COVID-19. Meanwhile, the cut-off value of more than 80 pg/ml is related to patient mortality. [5]

The current treatment of COVID-19 is supportive therapy, and there is no definite clinical trial data that support preventive or therapeutic drugs. Current management guidelines in various countries rely to a large extent on some evidence from small studies or an interim analysis. Tocilizumab (TCZ) is an example of an IL-6 inhibitor, which is currently the subject of major studies besides sarilumab, siltuximab, and clazakizumab. The drug is currently considered one of the additional immunomodulatory therapies that can benefit in severe or critical COVID-19. [6]

The use of tocilizumab has been studied in several other places: China, Italy, France, Qatar, and the USA. Most of them provided a fairly good clinical improvement for people with COVID-19. Some showed less significant changes.[7] However, considering the variants of COVID-19 that vary in several places and there are many differences such as race, ethnicity, and geography in various research subjects may give different results.[8,9] Meanwhile, research in Indonesia on the use of this drug is still limited.

The inflammatory response that occurs due to COVID-19 infection can be observed indirectly with inflammatory markers such as C-Reactive Protein (CRP), Lactate Dehydrogenase (LDH), and Ferritin reported to increase in cases of COVID-19. In addition, it can also be observed from the body's immune response, such as the number of leucocytes, Neutrophil Lymphocyte Ratio (NLR), and Absolute Lymphocyte Count (ALC). [10]

Case reports in two moderate to severe COVID-19 patients showed satisfactory results with the administration of TCZ. Studies of COVID-19 patients in China also show that TCZ is an effective therapy in severe and critical COVID-19 COVID-19 patients. In a retrospective study in 51 COVID-19 patients, TCZ was associated with a significantly shorter duration of vasopressor administration than in patients not given TCZ. [11,12] This study will report the clinical features of 20 severe and critical patients with confirmed COVID-19 who were given TCZ as an additional therapy.

MATERIAL AND METHODS

This case study is from the medical records of 20 COVID-19 confirmed patients treated in the Ulin General Hospital Banjarmasin isolation room with a severe-critical case and received TCZ besides standard therapy from Mei to September 2020. A confirmed case of COVID-19 was defined by a positive result on a reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assay of a specimen collected on a nasopharyngeal swab. Severe cases are defined as dyspnea, respiratory frequency $\geq$ 30 breaths/minute, oxygen saturation [SpO2] $\leq$ 93%, and critical case are defined as acute respiratory distress syndrome, septic shock, and multiorgan dysfunction or failure.

Descriptive statistics were used in this study to summarize data and results reported in median and interquartile ranges where appropriate. Categorical variables were summarized as counts and percentages. No imputations are made for the lost data. Data were processed and illustrated using Graphs with GraphPad Prism 8.0.1.

RESULTS
Demographic and Clinical Characteristic

In this case, we provide TCZ adjunct therapy to patients with severe and critical conditions. The median age of this report was 50 (26-70) years. Patients were male 70%, with 55% obese and 30% overweight. A history of smoking was found in 3 of 20 cases (15%). The patient's previous medical history was hypertension (25%) and diabetes mellitus (25%). The initial complaints experienced by most patients were shortness of breath (95%), cough (90%), and fever (85%). The length of admission days from a patient hospitalized until reverse transcriptase-polymerase chain reaction (RT-PCR) result negative or death

was between 20-57 days with a median of 28.5 days (Table 1).

Table 1. Baseline Demographic and Clinical Manifestation of COVID-19 Patients

Variable	N	%
Age in years, median (range)	50 (26-70)	
Gender		
Male	14	70.0
Female	6	30.0
Body Mass Index		
Normal (18,5-25)	3	15.0
Overweight (25,1-27)	6	30.0
Obesity (>27)	11	55.0
History of Smoking	3	15.0
Comorbidities		
Diabetes Mellitus	5	25.0
Hypertension	5	25.0
Clinical Manifestation		
Fever/ History of Fever	16	85.0
Shortness of Breath	19	95.0
Cough	18	90.0
Sore Throat	1	5.0
Cold	2	10
Anosmia	2	10
Nausea/ Vomiting	6	30
Diarrhea	4	20
Fatigue	6	30
Admission days, median (range)	28,5 (20-57)	

The development of oxygen saturation (SpO_2) of 20 patients in this report is shown in Figure 1. In the survivor group (figure 1A), the SpO_2 of 16 patients varied from 70% before TCZ. It can be seen that the improvement of oxygen saturation and more homogeneous in all patients until day 14 after administration of TCZ, which is all the oxygen saturation is 95% and above. Oxygen saturation increased in the first days after the treatment with tocilizumab and returned to normal in most of the patients who survived on the 11th day.

Meanwhile, in the four patients who died (Figure 1B), all the oxygen saturation before therapy was below 90%, then increased on the first days but fluctuated after that. On the first to the third day, there was a good response which the increase of saturation then fluctuated and experienced a gradual decrease until death.
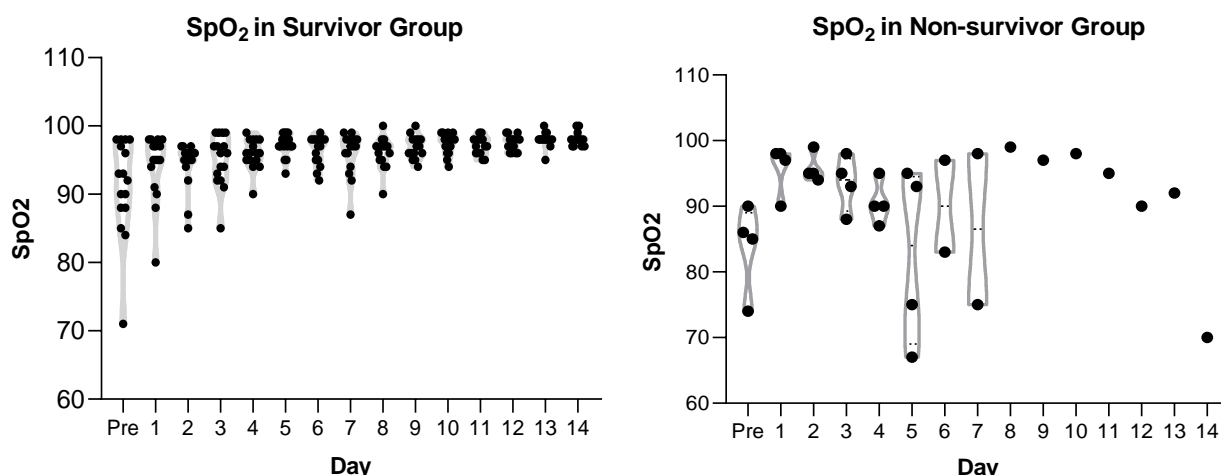
Figure 1. Oxygen saturation in survivor (A) and non-survivor (B) patients before and after TCZ administration. (A) Oxygen saturation increased in the first days after the treatment with tocilizumab and returned to normal in most patients. (B) Oxygen saturation increased in the first days after TCZ but fluctuated on third days until it decreased near death.

Laboratory Findings

The laboratory results were presented in median and interquartile (Table 2) to assess their overall progress in all patients. From routine blood tests on leucocytes and different counts of leucocytes, we found that leucocytosis appears before TCZ administration (Figure 2A). The incidence of leucocytosis was common and generally stable until the 8th day. On the 12th and 14th days, the median leucocytes values tended to fall to the normal range. The NLR value (Figure 2B) gradually decreased on the fifth day of TCZ administration.

Lymphocytopenia (Figure 2C), in which ALC obtained less than 1500/ µL, is common in patients in this study. Since the fifth day of TCZ administration, the ALC value showed fluctuation tended to increase until the 14th day. LDH (Figure 2D) fluctuated until five days of therapy, then gradually decreased from 8th to 14th after TCZ administration. CRP (Figure 2E) values were decreased since the third day of TCZ administration and continue to improve. Ferritin (Figure 2F) shows a change in the median ferritin value after three days of therapy, then fluctuated.

Table 2. **Laboratory Findings of COVID-19 Patients Before and After TCZ**

| Variable	Before TCZ	Day-3	Day-5	Day-8	Day-12	Day-14
	Median (IQR)					
Leucocytes, $\times 10^9$/L	10.65 (8.45)	10.4 (8.93)	10.35 (8.975)	9.9 (12.28)	8.7 (18.6)	7.2 (14)
NLR	6.97 (6.507)	7 (9.4)	5.63 (11.5)	5.9 (10.867)	2.92 (6.16)	2.5 (8.04)
ALC	1027 (923)	1003 (1011.7)	1473 (1126.8)	1570 (834.6)	1459 (1060)	1539 (1003)
LDH, U/L	641.5 (326)	569.5 (835.5)	692 (584)	515 (662)	256.5 (140.2)	247 (300)
CRP, mg/dl	92.4 (131.05)	9.2 (50.1)	3.95 (11.45)	2.8 (7.225)	1.2 (8)	3.95 (7.725)
Ferritin, ng/ml	2000 (1370.3)	1009 (1497.4)	1065 (1253.1)	623.5 (892.9)	815.7 (1175.4)	822.3 (1132.6)
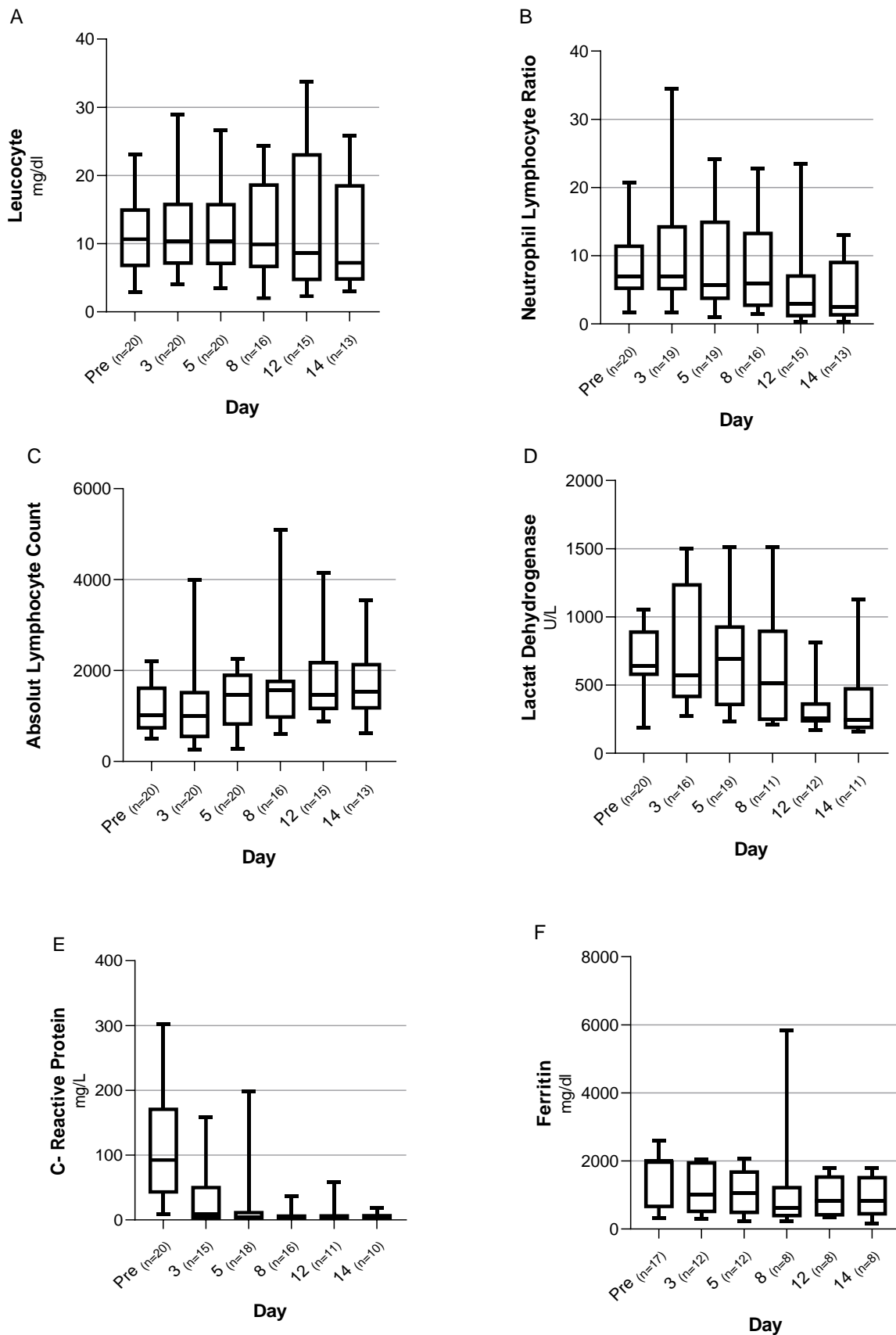
Figure 2. The values of leucocytes(A), NLR (B),
ALC(C), LDH (D), CRP (E), and Ferritin (F) before and after the treatment with tocilizumab in
severe – critical confirmed COVID-19.

Chest X-rays

All cases in this study had abnormal chest X-rays (CXR), ranging from minimal consolidation to the typical ground-glass-opacity, common in pneumonia COVID-19. Below is attached a case with good progress before and after IL-6 inhibitor therapy (Figure 3). Before administering therapy, a chest X-ray of the patient with infiltrating bilateral began to diminish on the 7th day after therapy and almost disappeared on the 14th day after therapy.

| A. CXR before TCZ | B. CXR 7th days after TCZ | C. CXR 14th days after TCZ |
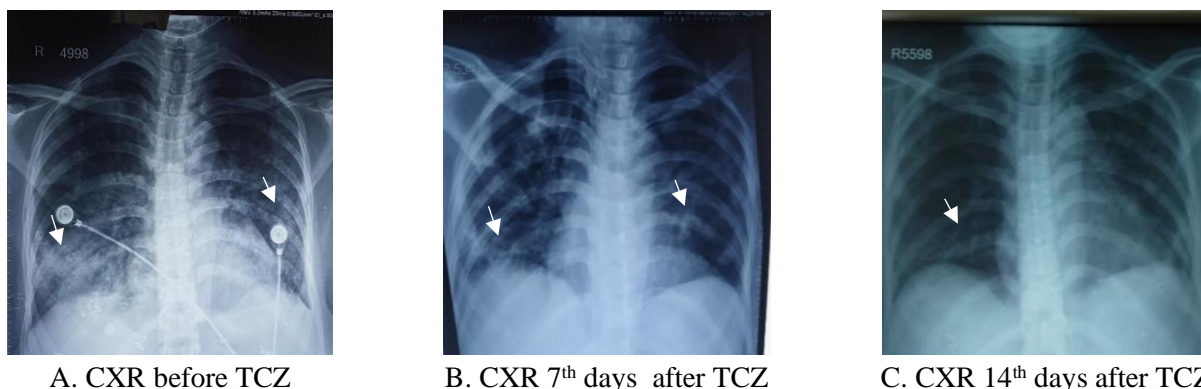
Figure 3. Chest X Rays (A) infiltrate (arrows) bilateral before TCZ, (B) infiltrate (arrows) began to diminish on 7th days after TCZ, and (C) infiltrate (arrow) almost disappeared on 14th days after TCZ.

DISCUSSION

We studied 20 patients administered TCZ according to the standard of therapy. Currently, criteria for ideal candidates for IL-6 therapy have not been agreed upon. In general, in the case series by Xu et al., the criteria for patients who can be candidates for IL-6 inhibitor therapy as follows:[11]

- Severe pneumonia, with hypoxemia, SpO2 <94% in room air, tachypnea (RR> 30 x/m) or PaO2 / FiO2 ratio <300.
- Critical pneumonia, i.e., patients require assisted mechanical ventilation or in shock and multiorgan failure requiring ICU care.

Based on the above, all of the patients in this study were included in the criteria for a candidate. All of the patients in this study could be given IL-6 inhibitor therapy. In this study, we conducted observations on clinical symptoms such as peripheral oxygen saturation and laboratory markers of inflammation which are based on the literature associated with cytokine storms, namely leucocytes, NLR, ALC, LDH, CRP, and ferritin. The outcome of this study was the patient's status at the end of treatment died or experienced clinical improvement with reverse transcriptase-polymerase chain reaction (RT-PCR) result negative.

Oxygen saturation is an observable feature of clinical improvement. In this study, there was an increase in SpO_2 since the start of therapy. The SpO2 increase continued to improve, especially in survivor patients. Otherwise, in the dead patient, fluctuating and worsening happen since the third day. This assessment of SpO_2 is important to see and assess the response after TCZ administration. When there is a worsening condition, the doctor must understand the direction of the disease progression and explore other possible causes of worsening in the patient's condition.

The results of this study are in line with Xiaoling Xu et al. at Anhui Provincial Hospital, China, about the effective treatment of COVID-19 with TCZ. They also reported 15 patients (75%) have significant improvement in patients' peripheral oxygen saturation after TCZ administration within the

fifth day.[13] Similar results were found in a randomized, double-blind, placebo-controlled trial conducted by J.H Stone et al. at Boston Hospital. The median time of discontinuation of supplemental oxygen in patients with TCZ was five days (Interquartile range 3.8-7.6). Although it was mentioned not significantly different from the placebo group. [14]

From routine blood tests on leucocytes and different counts of leucocytes, we found a slow improvement. Leucocyte decreased on 8th days while NLR and ALC improve on 5th days. COVID-19 infection activates both the immediate and adaptive immune systems. In conditions of increased inflammation, there will be an increase in the number of leukocytes and neutrophils and a decrease in the number of lymphocytes. Lymphocytopenia is currently a predictor that can be used to assess patient outcome. [15–17]. The mechanisms underlying lymphocytopenia are unclear. Previous reports explained that several other viruses, such as dengue and SARS-CoV, infect cells through antibody-dependent enhancement (ADE), where the virus infects cells via IgG Fc or complement receptors. However, whether lymphocytopenia is associated with this severity in relation to ADE still needs further research. [18]

Lactate dehydrogenase (LDH) is one of the inflammation markers, and its values fluctuated until five days of therapy, then gradually decreased on the 8th to 14th after TCZ administration. Another study described that LDH values decreased in the first 24 hours after TCZ then fluctuated until the seventh day. [19] LDH is one of the predictors of inflammation, where in previous studies, it was shown to be one of the predictors of outcomes with Area Under the Curve (AUC) of 0.878, and the cut-off value was 344.5. [20] This LDH indicates tissue damage in patients, and it becomes a marker of inflammation. LDH was said to increase during acute and severe lung damage, and this increase in LDH has also been associated with other interstitial pulmonary infections. [21,22]

CRP values were conclusively significantly decreased since the third day of TCZ administration from a fairly extreme CRP rate in this study. In accordance with the target of action of TCZ as an IL-6 inhibitor which is directly related to CRP synthesis. IL-6 is reported to be the main inducer of CRP gene expression, with IL-1 enhancing the effect. However, although IL-6 is necessary for CRP gene induction, it is insufficient to achieve this alone. Many factors can change the baseline CRP level, including age, gender, smoking status, body weight, lipid levels, and blood pressure. [23]

Therefore, the effect of TCZ administration on CRP can be seen more directly than other markers of inflammation. This is comparable to several studies in other countries. Research by Xioling Xu et al. also assessed the markers of inflammation after TCZ administration, showed a trend of marker improvement, and occurred rapidly on days 2-3.[13] The research results by Keske et al. also showed an improvement in inflammatory markers after TCZ administration, which occurred more quickly two days post-therapy. [24] The results, which are not much different from those in China, may be due to research by Ansori et al. showing that there is no significant difference between the SARS-CoV-2 spike glycoprotein gene sequences found in Indonesia and the Wuhan-Hu-1 isolate from China. However, this is a preliminary study that may differ as the pandemic progresses.[25] In previous studies, it was mentioned that CRP was one of the markers related to outcomes. [26], interestingly, TCZ decreases CRP and CRP related to prognosis, which can have an impact on outcomes.

But, these results and virulence of COVID-19 are influenced by several factors such as host, agent, and environmental factors. The number of different variants of COVID-19 in various regions has recently been one of the influencing factors. [8] Several studies have also shown differences in host factors, such as finding differences in race, ethnicity, and geography. [9] Some have linked this with the differences in the SARS-CoV-2 receptor mentioned. Even though Angiotensin-converting enzyme (ACE)2 is

expressed, it turns out that this virus cannot always use it as a receptor. The ability of SARS-CoV-2 to utilize ACE2 was predicted by observing a few essential single amino acid (A.A.) variant sites.[27]

In ferritin, we found a change in the median ferritin value that has fluctuated. However, the distribution was tended not to have a difference. It should be noted that the result at our center of ferritin value is presented with a relatively small number of samples. Comparable to other studies that showed fluctuating changes in ferritin until the seventh day after TCZ administration. [19] Ferritin and IL-6 also showed higher values in the group with death. [28] During inflammation, ferritin is actively produced. Macrophages are thought to be responsible for ferritin secretion, which produces cytokines and immune cells in the lung parenchyma. Ferritin synthesis can also be induced by cytokines such as interleukin-6. Tocilizumab is an IL-6 inhibitor agent that may affect ferritin changes as well. [29]

From 20 patients, 20% of patients in this study died, and 80% experienced improvement. An oxygen saturation improvement, an increase in ALC, and a decrease in CRP are markers that appear to have changed after the administration of TCZ therapy. Other laboratories had changed, but during the 14-day observation, they were fluctuated until showing improvement.

This post-therapy improvement was related to the mechanism of infection SARS-CoV-2 causing COVID-19 triggers a hyperinflammatory state driven by multiple cells and mediators such as interleukin (IL)-1, IL-6, IL-12, and IL-18, tumor-necrosis-factor-alpha (TNFα), etc. which cause a state called a cytokine storm. Cytokine storms cause ARDS and multiple organ failure, resulting from macrophage activating syndrome (MAS) in both sepsis and COVID-19. This condition can be observed directly by examining the plasma IL-6 levels as one of the potent cytokines that cause cytokine storms. [30–32]

In addition, the work of TCZ as an IL-6 inhibitor is considered to be one of the therapeutic targets of the cytokine storm mechanism that occurs in severe and critical COVID-19 patients. Elevated IL-6 levels characterize the cytokine storm that occurs. TCZ is an anti-human monoclonal antibody directed against soluble and membrane-bound IL-6 receptors. TCZ works by binding to IL-6 receptors (IL-6R) and preventing interactions with IL-6. This IL-6 elevation interrupts the downstream signal transduction cascade, which would release additional cytokines and chemokines. Therefore, it can reduce cytokine storms.[33,34]

However, the IL-6 assessment was not performed in this study because it was limited to our center. Other markers described the evaluation of the TCZ effect. The marker that appears to be the most rapid improvement is CRP, whose expression of this gene is also mainly induced by IL-6. [23] Meanwhile, other markers tended to improve slowly, such as leucocytes on 12th days, NLR, ALC on the fifth day, LDH on 8th days, and ferritin fluctuated. Clinically, the patient's oxygen saturation has improved gradually, especially in 80% of surviving cases.

CONCLUSION

This study shows the response to tocilizumab in patients with severe to critical COVID-19. From 20 patients, 80% of patients showed improvement in clinical such as oxygen saturation, laboratory and chest X-rays. CRP was a rapidly changing marker that can be checked immediately after administration to assess response to treatment. TCZ is an IL-6 inhibitor that can be considered a potential therapeutic agent in the management of COVID-19. However, this research was a descriptive and single-center study. We would suggest further research with more prominent and multicenter data.

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