https://doi.org/10.33472/AFJBS.6.2.2024.200-212

African Journal of Biological Sciences

Research Paper Open Access

Tocilizumab, as a promising therapeutic tool for cytokine release syndrome in COVID-19 patients, mediated by interleukin-6, and its effect on hemodynamics, particularly temperature, for the diagnosis of sepsis

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Article History

Volume 6, Issue 2, March 2024

Received:10 March 2024

Accepted: 24 March 2024

Published: 30 March 2024

doi: 10.33472/AFJBS.6.2.2024.200-212

Abstract: Tocilizumab is an interleukin-6 inhibitor approved for use in patients with moderately to severely active rheumatoid arthritis, adults with giant cell arteritis, children ages two and above with Polyarticular Juvenile Idiopathic Arthritis or Systemic Juvenile Idiopathic Arthritis and recently for patients severely affected by COVID-19. It has reduced mortality and duration of mechanical ventilation but the effects of Tocilizumab on routinely monitored hemodynamic parameters in the critically ill COVID-19 population are unclear. Tocilizumab started being administered to acutely deteriorating COVID-19 patients, presenting with cytokine release syndrome, evident by clinical deterioration and increased IL6 level, in June 2020. This study aims to assess the effect of Tocilizumab as a therapeutic tool on cytokine release syndrome, on the level of Interleukin 6 post Tocilizumab administration as well as its effects on hemodynamics, particularly temperature, especially after the development of secondary bacterial infection as evident by clinical deterioration, increase in oxygen requirements, worsening of chest imaging and positive cultures/biofires. Methods:50 patients who received Tocilizumab and developed secondary bacterial infection were recruited in this study. Routine laboratory workup as well as routine monitoring of vital signs was recorded every 2 hours during the whole ICU stay. Interleukin 6 (IL6) level was measured before TCZ therapy, after increase in oxygen requirements (cytokine storm) and after TCZ therapy. ELISA kits for the measurement of human Interleukin 6 were used. The normal level of Interleukin 6 in the blood varies between 0 and 43.5 pg/ml. Cultures were withdrawn once secondary bacterial infection was suspected (around Day 10 from Tocilizumab administration). Non-parametric univariate analysis was used for statistical analysis. Results: IL6 levels were significantly lower after Tocilizumab therapy. Temperature is significantly affected by administration of Tocilizumab, remaining within normal values even after the development of secondary bacterial infection. Other vital signs such as blood pressure, heart rate and respiratory rate were not affected. Conclusion: Tocilizumab may be used as a therapeutic tool in Cytokine Release Syndrome mediated by Interleukin 6 in COVID-19 patients. Fever may not be a reliable indicator of bacterial superinfection in severe COVID-19 pneumonia patients who have been given Tocilizumab.

Keywords: COVID-19 patients, Tocilizumab, interleukin-6, sepsis

Introduction: The corona virus disease 2019(COVID-19) pandemic has swept across the world since 2019 **[1,2].**

Risk factors for developing severe infection include advanced age, male gender, diabetes, obesity, a history of heart disease, immunosuppression, smoking, and substance abuse– these risk factors are very common in many populations **[3].**

One of the most common complications of COVID-19 is adult respiratory distress syndrome (ARDS) **[4],** which involves diffuse alveolar inflammation and viral particles within type II pneumocytes **[5,6]**. A cytokine storm, in which interleukin-6 (IL-6) is believed to play an important role, is believed to underly the pathogenesis of ARDS in COVID-19 **[7,8].**

Tocilizumab (TCZ), a well-established treatment in rheumatoid arthritis, has emerged as a potential treatment for severe COVID-19, being a monoclonal antibody against the IL-6 receptor with the potential to modulate the cytokine storm **[9,10].**

Early in 2020, small studies demonstrated the survival advantages of TCZ in severe COVID-19 **[9,11]**. The largest trial was the REMAP-CAP trial published in late February 2021, which aimed at assessing the efficacy of TCZ when compared to sarilumab (another IL-6 receptor inhibitor) or to no anti-interleukin treatment **[12,13]**. Patients given IL-6 receptor inhibitors fared better than the control group, with 10 fewer median organ support-free days in the TCZ group and 11 fewer days in the sarilumab group. An analysis at three months also demonstrated improved survival in treated patients compared with controls **[14]**.

As studies continue to be published confirming the positive effect of TCZ on survival, clinicians turned their attention of potential harm from TCZ.

Known side effects include neutropenia, thrombocytopenia, immunosuppression, and liver damage **[15]**.

However, we suggest another indirect effect on clinical management might be more significant and should be studied in more detail. Bacterial coinfection on admission was detected in early samples taken from approximately 20% of COVID admissions; during the weeks on intensive care up to 50% of COVID admissions could also suffer from hospital-acquired sepsis **[16,17].**

There was some data before the pandemic that TCZ, as an approved therapeutic modality in rheumatological diseases, can independently lower all markers of infection used by clinicians to monitor bacterial sepsis onset and prognosis and decide on escalation or de-escalation of antimicrobial therapy **[18-20].**

An interesting question regarding the use of TCZ in the management of severe COVID-19 infection and other rheumatological diseases is related to its effects on biochemical and clinical markers of infection.

Common infection markers used include CRP, procalcitonin (PCT) and white cell count (WCC) **[21-23].**

There is even less data available on how TCZ affects these infection markers. This only became standard therapy for COVID disease after June2020 **[24].**

Jain and Sharma **[25]** published a study in which they considered the effect of TCZ on fever. This review did suggest that the suppression of fever by tocilizumab render this parameter unusable for diagnosing an underlying bacterial infection **[25]**.

Following UK guidance on TCZ in COVID disease issued in January 2021, the Egyptian Ministry of Health and Population recommended to start TCZ to patients who exhibit a cytokine storm diagnosed by sudden deterioration in condition after being diagnosed with COVID-19 pneumonia **[26,27].** Sudden deterioration was determined by an acute increase in oxygen requirements and intensive care admission as well as an elevated levels of IL6.

Patients in whom the deterioration was more gradual were not considered for TCZ therapy. In suitable candidates, TCZ is given as a single dose of 8mg/kg intravenously up to a maximum dose of 800mg **[27,28].**

Methods

This study was a prospective cohort study that was carried out from March 2021 to June 2022 at the Internal Medicine intensive care unit- Kasr El Aini hospital and Kasr El Ainy New Teaching Hospital.

The protocol was approved by the critical care medicine department ethical committee, the research ethics committee of Faculty of Medicine (Protocol #: MD-291-2021), Cairo university and informed consent was obtained from the patients or their next of kin.

50 patients were recruited in our study. Patients with COVID 19 infection who received Tocilizumab were followed closely for any clinical or laboratory evidence of secondary bacterial infection & sepsis, patients who developed sepsis were recruited in the study.

Blood panel results for PCT, CRP, WCC, neutrophils and lymphocytes at 6 am each day were recorded at day 0, day 3 and day 6 of admission and day after day from the day on which TCZ was given until discharge from the ICU or death if this occurred earlier. The first set of parameters recorded in this group were generally taken on admission and just before the dose of TCZ is administered.

Interlukin 6 (IL6) level was measured before TCZ therapy, after increase in oxygen requirements (cytokine storm) and after TCZ therapy.

Pan cultures and sensitivity and/or biofires (Blood, urine, sputum, wound, CVC, ect…) were obtained when secondary bacterial infection was suspected.

Statistical analysis

Data were coded and entered using the statistical package for the Social Sciences (SPSS) version 28 (IBM Corp., Armonk, NY, USA). Data was summarized using mean, standard deviation, median, minimum and maximum in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. For comparison of serial measurements within each patient the non-parametric Friedman test and Wilcoxon signed rank test were used [345]. For comparing categorical data, Chi square ($\chi 2$) test was performed. Exact test was used instead when the expected frequency is less than 5. For comparison of serial measurements within each patient the non-parametric Cochran's Q test and Marginal Homogeneity Test were used [346]. P-values less than 0.05 were considered statistically significant.

The main outcome of the study was to was to investigate whether the administration of TCZ in critically ill patients with COVID-19 pneumonia had a significant effect on PCT levels as well as on other commonly recorded clinical and biochemical markers in subsequent days, especially after the development of secondary bacterial infection.

Results

The demographic data of the studied population is shown in Table 1.

Table 1: Baseline demographic characteristics of the studied group :

Age (years)	Mean±SD Median (min-max)	58.76 ±14 59.5 (25-85)
Gender	Type Male Female	Number (%) 34 (68%) 16 (32%)

Distribution of the studied cases regarding the course during hospitalization in relation to the onset of symptoms is shown in Table 2.

Table 2 : Distribution of the studied cases regarding the course during hospitalization:

	Mean	SD
Day of first positive swab	4.74	2.19
Day of starting Tocilizumab	9.98	4.48
Day of hospitalization	7.54	4.00
Day of increased O2 requirements	9.54	4.51
1st dose of Tocilizumab (day)	9.98	4.48
2nd dose Tocilizumab (day)	10.42	3.97
Duration between doses (h)	23.04	3.29
Clinical worsening (day)	19.00	5.99
	126.31	160.56
	26.32	6.67
	27.82	8.70
	19.42	8.10

Distribution of the studied cases regarding the timing of administration of Tocilizumab in relation to increased oxygen requirements is shown in Table 3

Table 3 : Distribution of the studied cases regarding the timing of Tocilizumab.

Timing	Number	Percentage
Same day	39	78%
1 day	5	10%
2 days	3	6%
3 days	2	4%
4 days	1	2%

Distribution of the studied cases regarding outcome is shown in Table 4.

Table 4 : Distribution of the studied cases regarding outcome:

Outcome	Number	Percentage
Died	28	56.0%
Discharged	22	44.0%

Comparing the level of Interleukin 6 before and after Tocilizumab therapy is shown in Table 5

Table 5 : Comparing IL6 level before and after TCZ therapy

	Mean	SD	Median	Minimum	Maximum
IL6 before TCZ	126.31	160.56	90.50	10.00	1043.00
IL6 after TCZ	11.92	4.094	11.00	6.00	20.00

Comparing before Tocilizumab, 24 and 48 hours after Tocilizumab administration and then after the development of secondary bacterial infection regarding hemodynamics is shown in Table 6-9.

1. Blood pressure (BP):

Table 6 : Comparing before TCZ, 24h and 48h after administration and after the development of secondary bacterial infection regarding BP:

	Shocked		stable		P value compared to before
	Count	%	Count	%	
BP before Tocilizumab	0	0.0%	50	100.0%	----
BP 24 h after Tocilizumab	0	0.0%	50	100.0%	1
BP 48 h after Tocilizumab	1	2.0%	49	98.0%	1
BP after clinical deterioration	18	36.0%	32	64.0%	< 0.001

The change in BP after Tocilizumab was statistically insignificant with a P value of >0.005, while there was a statistically significant drop in the BP after the development of 2ry bacterial infection, with a P value of <0.001.

2. Heart rate (HR):

Table 7 : Comparing before TCZ, 24h and 48h after administration and after the development of secondary bacterial infection regarding HR:

	Brady		Tachy		Normal		P value compared to before
	Count	%	Count	%	Count	%	
HR before TCZ	1	2.0%	11	22.0%	38	76.0%	----
HR 24 h after TCZ	1	2.0%	6	12.0%	43	86.0%	0.059
HR 48 h after TCZ	1	2.0%	5	10.0%	44	88.0%	0.034
HR after clinical deterioration	0	0.0%	32	64.0%	18	36.0%	0.008

The change in HR after Tocilizumab was statistically insignificant with a P value of >0.005, while there was a statistically significant increase in the HR after the development of 2ry bacterial infection, with a P value of 0.008.

3. Respiratory rate (RR):

Table 8 : Comparing before TCZ, 24h and 48h after administration and after the development of secondary bacterial infection regarding RR:

	Tachy		Normal		P value
	Count	%	Count	%	
RR before Tocilizumab	45	90.0%	5	10.0%	----
RR 24 h after Tocilizumab	43	86.0%	7	14.0%	0.537
RR 48 h after Tocilizumab	36	72.0%	14	28.0%	0.005
RR after clinical deterioration	41	82.0%	9	18.0%	0.217

There was a statistically significant decrease in the RR after 48 hours of Tocilizumab administration, with a P value of 0.005, , while the change in RR after 24 hours of Tocilizumab administration and after the development of 2ry bacterial infection was statistically insignificant with a P value of >0.005.

4. Temperature:

Table 9 : Comparing before TCZ, 24h and 48h after administration and after the development of secondary bacterial infection regarding temperature:

	Fever		Normal		P value compared to before
	Count	%	Count	%	
Temp before Tocilizumab	35	70.0%	15	30.0%	----
Temp 24 h after Tocilizumab	10	20.0%	40	80.0%	<0.001
Temp 48 h after Tocilizumab	6	12.0%	44	88.0%	<0.001
Temp after clinical deterioration	14	28.0%	36	72.0%	<0.001

There was a statistically significant decrease in the temperature after 24 and 48 hours of Tocilizumab administration, as well as after the development of secondary bacterial infection with a P value of <0.001.

Comparing before Tocilizumab, 24 and 48 hours after Tocilizumab administration and then after the development of secondary bacterial infection regarding oxygen requirements is shown in Table 10.

Table 10: Comparing before TCZ, 24h and 48h after administration and after the development of secondary bacterial infection regarding oxygen requirements:

	Room air		**nasal canula**		**Oxygen face mask**		**High Flow Non-rebreather mask (HFNR)**		**High Flow Nasal Cannula (HFNC)**		**Non-Invasive Ventilation (NIV)**		**Invasive mechanical ventilation**		**P value compared to before**
	n	**%**	**n**	**%**	**n**	**%**	**N**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	
O2 before TCZ	0	0.0 %	4	8.0 %	7	14.0 %	27	54.0 %	1	2.0 %	10	20.0 %	1	2.0 %	----
O2 24 h after TCZ	0	0.0 %	3	6.0 %	7	14.0 %	24	48.0 %	1	2.0 %	14	28.0 %	1	2.0 %	0.105

O2 48 h after TCZ	0	0.0 %	6	12.0 %	3	6.0 %	27	54.0 %	1	2.0 %	11	22.0 %	2	4.0 %	0.558
O2 after clinical deterioration	1	2.0 %	2	4.0 %	1	2.0 %	6	12.0 %	3	6.0 %	19	38.0 %	18	36.0 %	<0.001

Although there was an improvement in the oxygen requirements after Tocilizumab administration, this change was statistically insignificant with a P value of >0.005.

There was a statistically significant increase in oxygen requirements after the development of 2ry bacterial infection, with a P value of <0.001.

Discussion

CRS is an acute systemic inflammatory syndrome characterized by fever and multiple organ dysfunction that is associated with increased levels of inflammatory mediators, such as IL6, in blood. We investigated the effect of TCZ as a therapeutic modality in the treatment of CRS monitored by the improvement in clinical condition and the level of IL6. Also, the suspicion of infection in a critically ill patient is triggered by fever. However, since the introduction of immunomodulatory therapy, the value of these clinical data is unclear.

We investigated the hemodynamic parameters, particularly temperature, in critically ill COVID-19 patients treated with Tocilizumab and assessed the value of these clinical data to detect secondary bacterial infections.

The aim of this study was to investigate whether the administration of TCZ in critically ill patients with COVID-19 pneumonia had a significant effect on cytokine storm as well as on the commonly recorded clinical data, particularly temperature, in subsequent days, especially after the development of secondary bacterial infection.

Our results show that IL6 levels were significantly decreased following TCZ therapy and that fever subsided after 48 hours from TCZ therapy and temperature did not rise after the development of secondary bacterial infection, as most of the cases showed normal value after TCZ therapy and throughout all the hospital stay, even after developing secondary bacterial infection as evident by clinical deterioration, increase in oxygen requirements and positive cultures/biofires.

Similar to our study, Robert and colleagues studied the effectivess of TCZ therapy in Treatment of Chimeric Antigen Receptor (CAR) T Cell-Induced Severe or Life-Threatening

Cytokine Release Syndrome and concluded that, in agreement with our study, the risks of using tocilizumab appear to be outweighed by the potential benefit for patients with severe or life-threatening CAR T cell-induced CRS.

Similar to our study, Xu and colleagues studied the effective treatment of severe COVID-19 patients with tocilizumab on a total of 19 COVID-19 patients who received TCZ and reported that, fever returned to normal on the first day, and other symptoms improved remarkably within a few days (RR) **[30].**

Jain & Sharma studied the rational use of Tocilizumab in COVID-19 and reported that, in agreement with our study, the suppression of fever by tocilizumab render this parameter unusable for diagnosing an underlying bacterial infection **[31].**

Hirao and colleagues studied laboratory and febrile features after joint surgery in patients with rheumatoid arthritis treated with tocilizumab and reported that, in agreement with our study, Tocilizumab partially, but significantly, suppressed the increase in body temperature on postoperative days 1 and 2 **[32]**.

Ma Chaoyi and colleagues studied Tocilizumab therapy for persistent high-grade fever in systemic lupus erythematosus in two female Chinese patients who presented with Systemic Lupus Erythematosuss and high-grade fever, with raised inflammatory markers including C-reactive protein, erythrocyte sedimentation rate, and IL-6, but no signs of opportunistic infections.and whom their fever and other symptoms responded poorly to broad-spectrum antibiotics, antifungals, antivirals, and glucocorticoids. And reported that, in agreement with our study, body temperatures returned to normal after treatment with TCZ, and other symptoms, including arthralgia, gradually improved **[33].**

Luo and colleagues studied Tocilizumab treatment in COVID-19 and reported that, in agreement with our study, the fever subsided after the first dose of TCZ **[34].**

Cáceres and colleagues studied the effect of tocilizumab on cytokine release syndrome in COVID-19 patients in a retrospective, descriptive and observational study of 75 COVID-19 patients who received TCZ and reported that, in agreement with our study, treatment with TCZ was associated with a statistically significant reduction in fever ($p < 0.01$) on day 5 after its administration **[35].**

Kuwahara and colleagues studied the effect of Tocilizumab treatment on patients with Coronavirus disease 2019 and bacteremia and reported that, unlike our study, Tocilizumab does not suppress fever **[36].** However, the limitations of this study were, the small sample size (bacteremia was observed in only 13 patients of the studied population who received Tocilizumab) and that the data were limited to a single center and may not generally reflect the same observation across all patients of COVID-19 who developed bacteremia.

Limitations of this study include the relatively small sample size which may affect the generalizability of our findings. In addition, the fact that only one center was included.
However, further studies are required into the consequences of TCZ administration and the best way to prevent complications of bacterial superinfection in these patients. Larger trials and more prolonged studies are needed to better elucidate the exact effects and mechanisms of TCZ function in severe COVID-19, prior to officially encouraging a change in clinical practice.

Summary

Tocilizumab, an interleukin-6 inhibitor often prescribed for rheumatoid arthritis, has shown survival benefit and a reduction in ventilation time in patients with severe COVID-19 pneumonia.
This treatment started being used in June 2020 for patients with COVID-19 pneumonia that exhibited a cytokine storm evident by sudden deterioration in condition, as indicated by rapid increase in oxygen requirements, need for admission to intensive care and increased level of IL6.
Little data is available in the literature about the effect Tocilizumab has on common clinical markers of infection in this population of patients, and how the interpretation of these values may need to be altered in patients who have received the drug.
50 patients were recruited in this prospective, cohort study, which was conducted in a tertiary intensive care unit treating adult COVID-19 patients using Tocilizumab as well as the conventional COVID-19 therapy at that time.
We recorded the routine full laboratory profile, IL6, as well as hemodynamics. Cultures were withdrawn once secondary bacterial infection was suspected (around D10 from Tocilizumab administration).
We reported that Tocilizumab significantly lowers the levels of IL6 and temperature in severely ill COVID-19 patients in the days following its administration and throughout the whole hospital stay even after developing secondary bacterial infection as evident by clinical deterioration, increased oxygen requirements and positive cultures/biofires.
This demonstrates that caution is required when relying on this clinical marker to diagnose a secondary or worsening infection, since it may be falsely low.

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