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ORIGINAL ARTICLE

The effect of tocilizumab on procalcitonin and other biochemical and clinical markers in severe COVID-19 infection: Time to rethink our interpretation of results?

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Background

Tocilizumab (TCZ) is an interleukin-6 (IL-6) inhibitor approved for use in patients severely affected by COVID-19, which has been shown to reduce mortality but has as yet undetermined effects on procalcitonin (PCT) and C-reactive protein (CRP).

In Malta, TCZ started being administered to COVID-19 patients who experience worsening symptoms or increased oxygen requirements over a period of hours in January 2021. This study aimed to assess the effect of TCZ on PCT primarily, and white cell count (WCC), lymphocyte and neutrophil counts, neutrophil to lymphocyte ratio (NLR), CRP and PaO2/FiO2 (P/F) ratio as secondary measures.

Methods

Fifty patients who received tocilizumab were recruited to the treatment group along with a matched control group of 50 patients who did not receive the drug. Serum PCT and other biochemical markers were recorded daily for both groups and differences in the values for the two groups extracted. Outcome measures included differences between the biomarkers at 5, 10 and 15 days.

Results

PCT and CRP were significantly lowered by administration of TCZ on Day 5. WCC, lymphocyte and neutrophil counts and P/F ratios were not affected. There was no difference in positive blood culture results between the two groups.

Conclusion

PCT and CRP may not be reliable indicators of bacterial superinfection in severe COVID-19 pneumonia patients who have been given TCZ.

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Introduction

The coronavirus disease 2019 (COVID-19) pandemic has swept across the world since 2019, with thirty thousand cases and 462 recorded deaths in Malta at the time of writing.[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 commonest 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 to date 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]

The TOCIVID-19 trial is another large phase 2 single arm trial involving multiple centres, dedicated to assessing the effects of tocilizumab in critically ill COVID-19 patients.[13] This trial is still underway and expected to be completed in December 2022.[15] Primary outcomes will be mortality at fourteen and at thirty days post-administration. Secondary outcomes will include time to death, time to initiation of mechanical ventilation, time to extubation, time until invasive ventilation was no longer necessary, duration of hospital stay, and trends in clinical and biochemical markers such as IL-6, C-reactive protein (CRP), SOFA score, lymphocyte counts, radiological evidence of response to treatment and PaO2/FiO2 (P/F) ratio.[13]

As studies continue to be published confirming the positive effect of TCZ on survival, clinicians turned their attention to potential harm from TCZ. Known side effects include neutropenia, thrombocytopenia, immunosuppression and liver damage.[16] 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.[17-18] There was some data before the pandemic that TCZ 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.[19-21] An interesting question regarding the use of TCZ in the management of severe COVID-19 infection 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).[22-24] There is even less data available on how TCZ affects infection markers in a population on regular corticosteroid therapy given this only became standard therapy for COVID disease after June 2020.[25]

Vu et al[26] reported an increase in P/F ratio after administration of single dose TCZ in a group of sixty patients. A reduction in CRP was observed for 10 days following drug administration, subsequently rising again. The team postulated that this may indicate the need for further doses of TCZ. It is worth noting that in this study 32 out of 60 patients were treated concomitantly with glucocorticoids.[26]

Neutrophil to lymphocyte ratio (NLR) has been discovered to be a predictor of COVID-19 severity. Elevated NLR has been associated with poorer outcomes in terms of disease progression and mortality.[27] In a study by Hartog et al[28] patients who were administered TCZ in the setting of severe COVID-19 and whose NLR did not immediately show a downward trend were found to have poorer outcomes compared to those who responded swiftly to anti-IL6 treatment. Elevated NLR may therefore also indicate a likelihood for resistance to TCZ therapy.[28] This study did not compare the effect of TCZ on WCC or NLR to a control group.

Finally, Hariyanto and Kurniawan[29] published a short review including 9 studies and 577 patients in which they considered the effect of TCZ on multiple biochemical infective markers. CRP, ferritin and D-dimer were reduced following the administration of TCZ and lymphocytes were increased. This review did suggest that PCT was also found to decrease after TCZ was given.[29]

Following UK guidance on TCZ in COVID disease issued in January 2021, local infectious disease physicians in Malta started prescribing TCZ to patients who exhibit a sudden deterioration in condition after being diagnosed with COVID-19 pneumonia.[30-31] Sudden deterioration was determined by an acute increase in oxygen requirements and intensive care admission. Patients in whom the deterioration was more gradual or patients who have a serum alanine aminotransferase level 5 times above the upper limit of normal, 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.[31-32]

The main objective of our study is to monitor effects of TCZ on PCT levels, CRP, WCC, neutrophil and lymphocyte specific counts, NLR and P/F ratio from day of admission until discharge from intensive care in a population of patients admitted for COVID pneumonia receiving regular dexamethasone.

Methods

This was a retrospective single centre study, in a general intensive therapy unit (ITU) in a tertiary University-affiliated hospital, with over 1,000 admissions per year. Data protection and ethical approval were obtained from the relevant authorities. This trial was registered on the clinicaltrials.gov (NCT05035589).

The first 50 consecutive COVID-19 patients admitted to ITU given TCZ were recruited to the treatment group. The control group for this study consisted of 50 patients, all admitted to ITU due to COVID-19, who were not given TCZ. They were matched with the treatment group for gender, age, length of stay in ITU and type of respiratory support (high flow nasal oxygen (HFNO) or mechanical ventilation) required.

Blood panel results for PCT, CRP, WCC, neutrophils, lymphocytes and the P/F ratio at 6am each day were recorded from the day on which TCZ was given until twenty days post-administration, or until discharge from the ITU 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.

Statistical Analysis

Results were analysed statistically using R Studio (version 1.4.1106) with R statistical package (version 4.0.4). The data was checked for normality and skewness using visual methods and using Shapiro-Wilk normality tests. When appropriate, t-tests, Mann-Whitney U tests, Kruskal-Wallis test and chi-squared tests were used for univariate analysis. With a p-value of 0.05 or less, the result was taken to be significant.

The main outcome of the study was difference of PCT on Day 5, 10 and 15 between the two groups, using univariate tests. A linear mixed model was also used to compare the trajectories of individual patients in the two groups. The same methodology was then repeated for CRP, WCC, Neutrophil and Lymphocyte counts and the P/F ratio.

Furthermore, the incidence of positive blood cultures, and the date of first positive culture was compared between the two groups.

Results

The demographic data of the two groups are shown in Table 1. Given the small sample size, and that there were high levels of skewness in the data, median values with interquartile ranges are shown. There were no statistical differences between the two groups at baseline.

Table 1: Baseline demographic characteristics of the two groups of patients. Results reported as median values [IQR].

	Control Group	Treatment Group	p-value
n	50	50	
Age (years)	68.5 [63.0 - 74.0]	66.0 [60.0 - 72.0]	0.22
Male (n)	40 (80%)	41 (82%)	1.00
Respiratory support			
- HFNO	18 (36%)	17 (34%)	1.00
- Intubation	32 (64%)	32 (64%)	
- Non-rebreather mask	0 (0%)	1 (2%)	
WCC	10.2 [7.9 - 12.5]	9.6 [7.3 - 12.6]	0.46
Neutrophils	9.1 [6.9 – 10.9]	8.5 [5.4 – 10.9]	0.36
Lymphocytes	0.6 [0.4 – 0.7]	0.6 [0.5 – 0.8]	0.55
N/L Ratio	15.7 [9.3 – 21.1]	13.0 [8.7 – 18.5]	0.26
PCT	0.4 [0.1 - 0.9]	0.4 [0.2 - 1.6]	0.48
CRP on Day 0	127 [77.9 - 227.3]	140 [80.1 – 224]	0.99
P/F Ratio	144 [112 - 192]	137 [104 - 195]	0.91
Survived (n)	30 (60%)	38 (76%)	0.13

Table 2 summarises the differences observed in the values of all biochemical and clinical markers studied at day 5, day 10 and day 15, along with the p-values obtained in order to determine statistical significance. Below is a breakdown of the findings from data analysis for each biochemical and clinical marker.

Procalcitonin

On univariate analysis, the PCT level was influenced by TCZ administration, on Day 5 with PCT values for the treatment group being 0.18 in the treatment group and 0.37 in the control group. The p-value obtained for this data was 0.19. On day 10 and day 15, there still was a marked difference, but this was not statistically significant since the p-values were 0.06 and 0.08 respectively. The values obtained for the two groups on these days were 0.31 for the treatment group on day 5 compared to 0.76 in the control group, and 0.37 in the treatment group at day 15 versus 0.77 in the control group,

Table 2: Difference in biochemical and clinical markers observed between the two groups at Day 5, 10 and 15, with corresponding p-values

Parameter	Day:	Treatment Group	Control Group	p-Value
PCT	5	0.18 [0.10 - 0.41]	0.37 [0.15 - 1.22]	**0.019**
	10	0.31 [0.11 - 0.72]	0.76 [0.23 - 1.12]	0.08
	15	0.37 [0.26 - 0.47]	0.77 [0.31 – 1.7]	0.06
CRP	5	15.4 [9.0 - 22.9]	107.0 [51.3 - 181.0]	**<0.001**
	10	8.9 [3 - 33.8]	109.2 [65.1 - 178.7]	**<0.001**
	15	31.4 [11.9 - 65.1]	215.6 [84.8- 260.3]	**0.0011**
WCC	5	9.6 [7.4 - 13.1]	11.3 [9.1 - 14.3]	0.20
	10	11.7 [9.5 - 18.7]	12.0 [9.9 - 14.5]	0.92
	15	8.4 [7.8 – 17.0]	10.4 [8.6 - 12.92]	0.49
Neutrophils	5	7.7 [6.2 - 10.8]	9.7 [7.3 - 11.3]	0.16
	10	9.9 [7.4 - 15.2]	9.9 [8.7 - 13.0]	0.96
	15	6.8 [6.3 - 13.4]	8.4 [6.9 – 10.9]	0.34
Lymphocytes	5	0.70 [0.40 - 0.93]	0.53 [0.40 - 0.79]	0.22
	10	0.80 [0.46 - 1.37]	0.74 [0.41 - 0.95]	0.32
	15	1.09 [0.54 - 1.39]	0.68 [0.55 - 1.14]	0.30
NLR	5	11.0 [5.4 - 26.2]	17.3 11.5 – 26.9]	0.11
	10	11.1 [5.9 - 21.52]	14.7 [9.7 - 27.8]	0.21
	15	6.3 [5.2 - 14.3]	10.5 [7.7 - 18.4]	0.11
P/F ratio	5	189 [131- 219]	158 [138 - 193]	0.20
	10	187 [158- 240]	169 [136- 219]	0.071
	15	195 [174- 251]	186 [135- 232]	0.34

Following TCZ administration, the median levels of PCT decreased until day 6, and started to increase again by day 7.

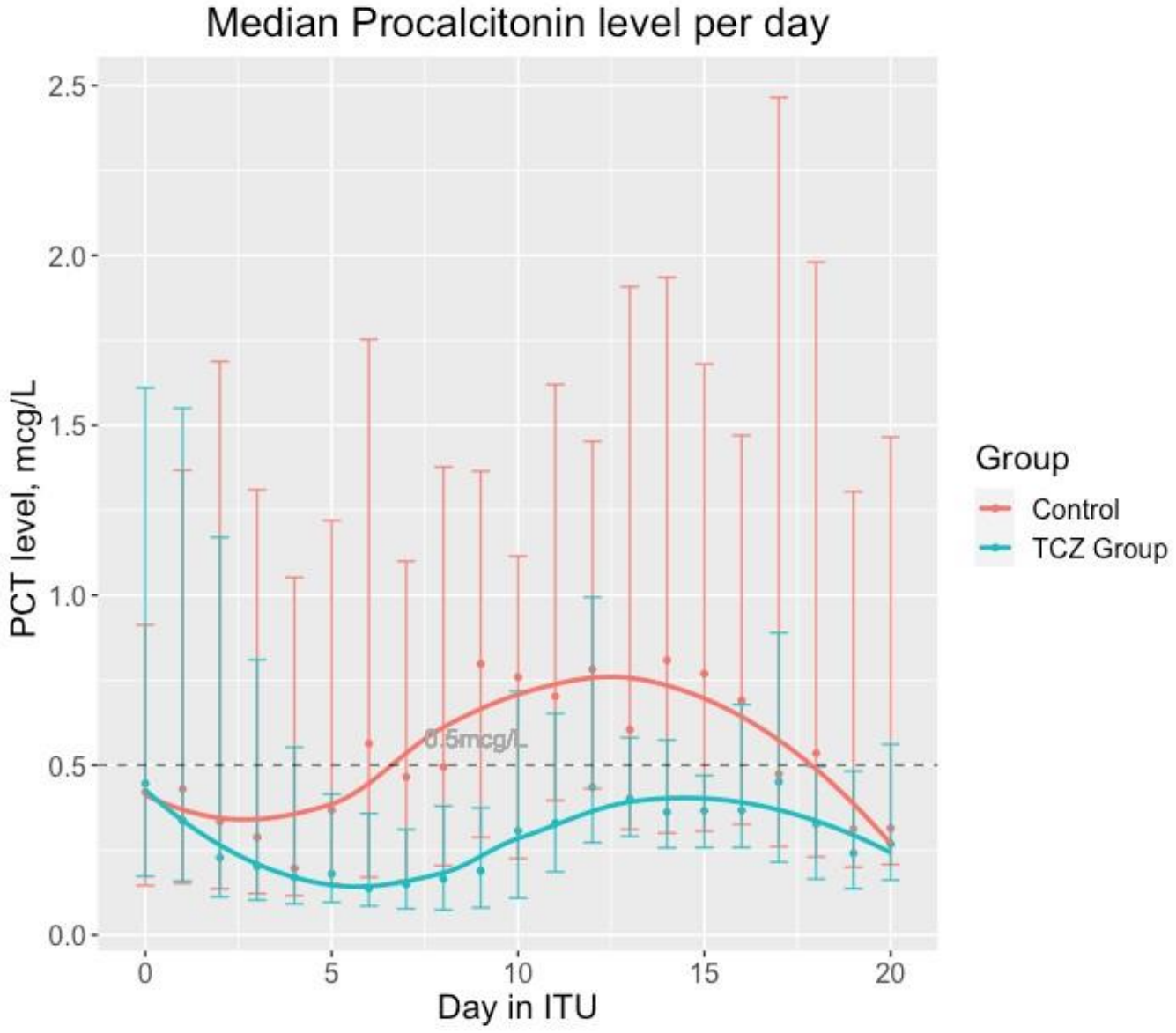
A linear mixed effects analysis, to account for the repeated measures, showed that the TCZ administration affected the trajectory of PCT levels (Estimate -1.93, p-value 0.03).

In fact, throughout the observed period, the median levels of PCT in the treatment group never exceeded 0.5mcg/L, which is the cut-off level suggestive of infection.[33]

Five patients (10%) who received TCZ had an increase in their PCT after the initial decrease. This started after an average of 5.2 days (range: 3 – 10 days).

Figure 1 depicts the described variations in PCT levels following administration of TCZ when compared to the control group.

Figure 1: PCT variation over twenty days in ICU admission, difference between tocilizumab test group and control group. Data presented as median values, with error-bars indicating IQR.



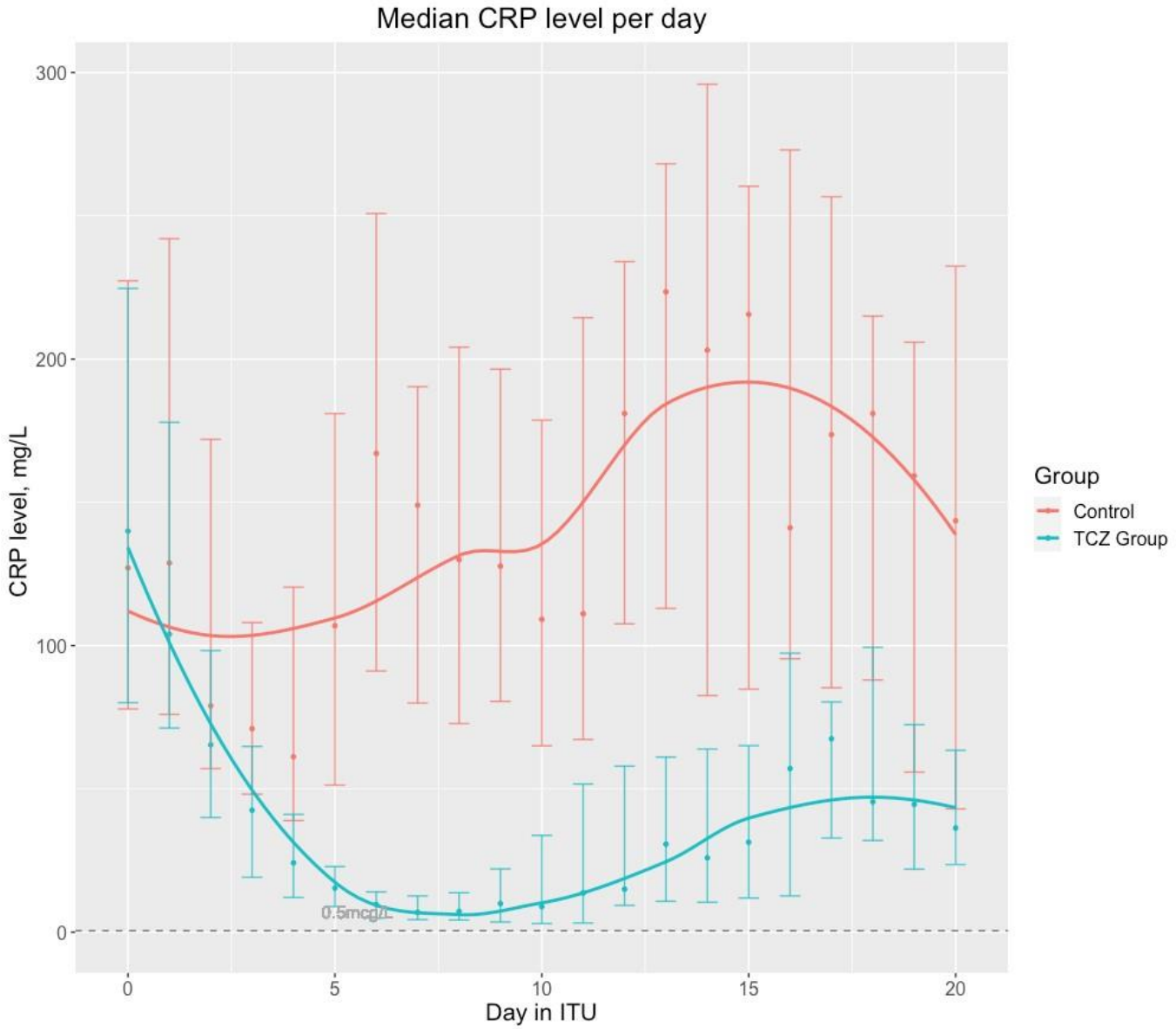
C-Reactive Protein

As seen in Table 1, CRP was significantly higher in patients who had not received TCZ at all points investigated. On day 5, the CRP in the treatment group was 15.4 compared to 107 in the control group. On day 10, the CRP in the treatment group dropped to 8.9 but that in the control group increased marginally to 109, and on day 15, the CRP value for the treatment group was 31.4, and that for the control group was 215.6. The p-values for all this data were below the 0.05 cut-off.

The median levels of CRP decreased significantly over 8 days in the treatment group, then started to rise slowly again, but remained lower than 75mg/L. This is depicted in figure 2.

Analysis using a linear mixed effect model confirms the above, with a marked effect of TCZ on CRP levels (Estimate: -63.8, p<0.0001).

Figure 2: CRP variation over twenty days in ICU admission, difference between tocilizumab test group and control group.



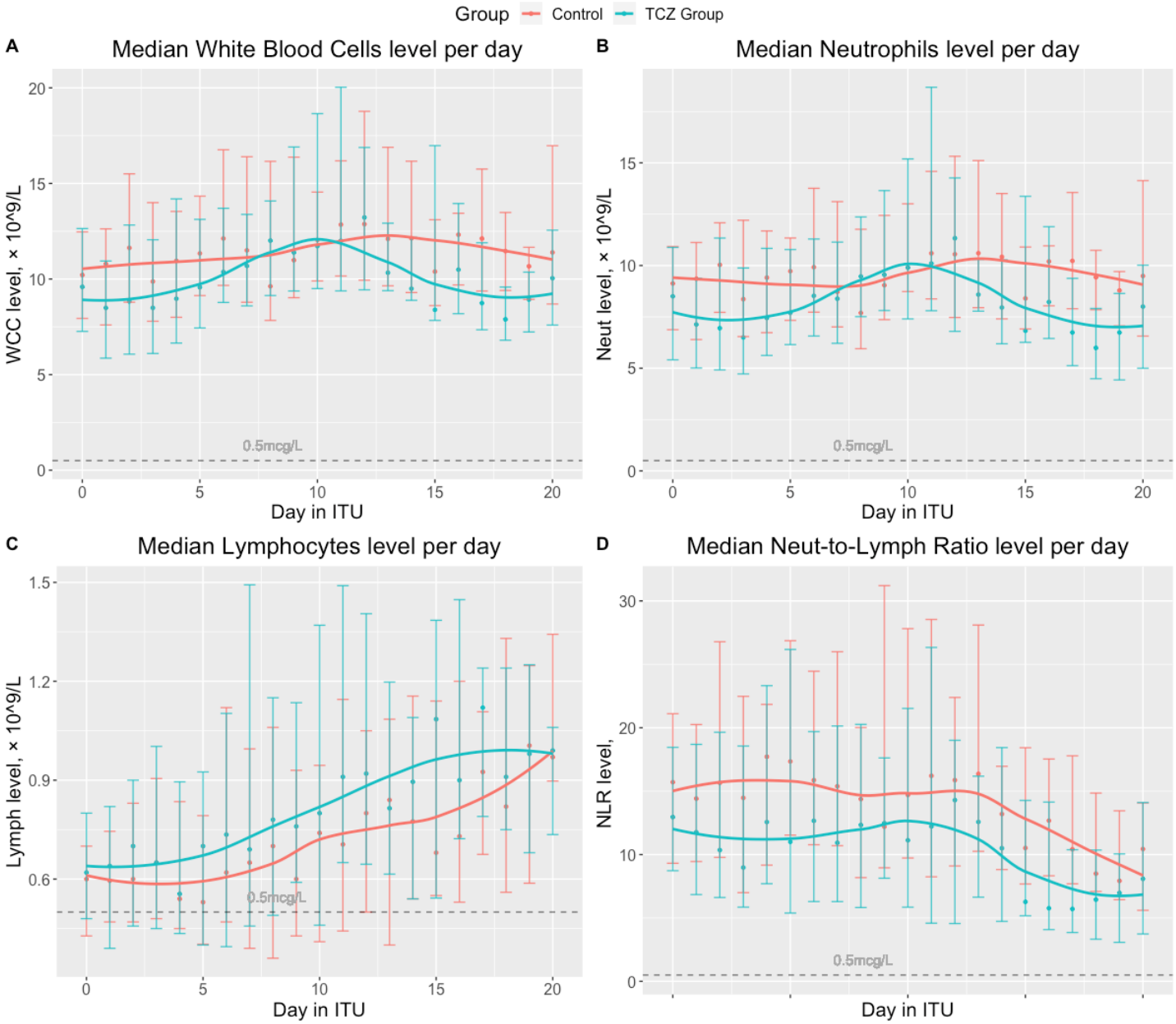
White Cell Count, Neutrophil Count, Lymphocytic Count

Figure 3 shows the median counts for White Cells, neutrophils, lymphocytes and the NLR.

Although on Day 5 and 15, the total WCC and the neutrophil counts were higher in patients in the control group, this was not statistically significant. This was confirmed with analysis by a linear mixed effects model.

Lymphocyte counts and the NLR were not different between the two groups.

Figure 3: WCC variation over twenty days in ICU admission, difference between tocilizumab test group and control group.



PaO2 / FiO2 ratio

As shown in Figure 4, there was no difference between the median P/F ratio in the first 20 days between the two groups. This was confirmed on a linear mixed model analysis.

Microbiology

Table 3 provides details of blood culture results for both bacterial and fungal cultures in the TCZ group as well as the control group, with the corresponding p-values.

There was little difference in the number of patients who had a positive blood culture (excluding contaminants) between the two groups (tocilizumab group: 48% vs Controls: 40%, p-value= 0.55). Positive cultures tended to occur earlier during the ITU stay in patients treated with TCZ, but this was not statistically significant (TCZ group: 6.5 days vs Controls: 9 days, p-value=0.16). This is shown in Table 2.

There was no difference in the incidence of fungal cultures in either group.

Figure 4: P/F ratio variation over twenty days in ICU admission, difference between tocilizumab test group and control group.

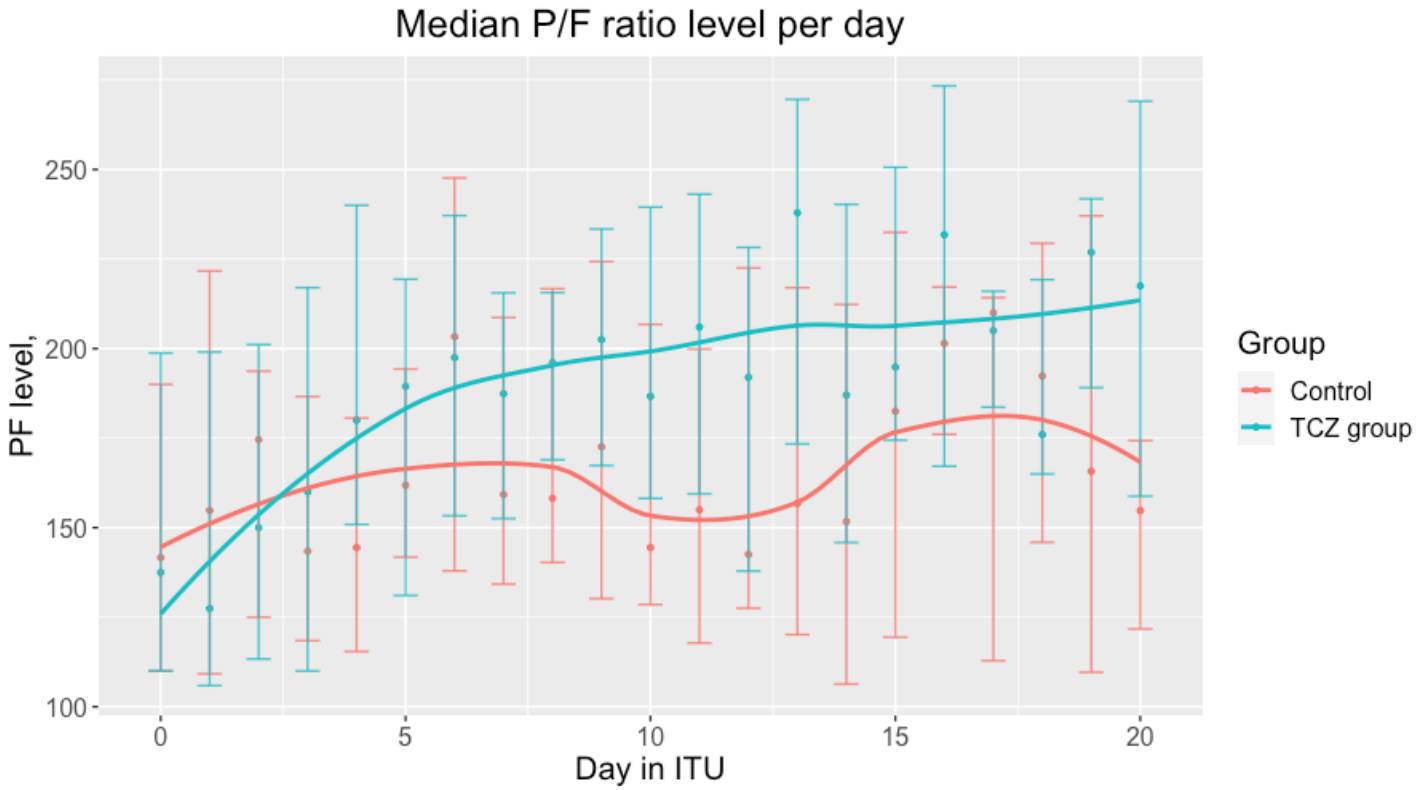


Table 3: Number of positive Blood culture results in both groups, median day of occurrence, and number of positive Mycology results. These values are excluding cultures thought to be due to contamination.

	Control Group	Treatment Group	p-value
n	50	50	
Any positive blood culture	20 (40%)	24 (48%)	0.55
Day of occurence	9 [6 - 11.3]	6.5 [4 - 9]	0.16
Fungal cultures	14 (28%)	10 (20%)	0.48

Discussion

The primary 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 PCT levels as well as on other commonly recorded clinical and biochemical markers in subsequent days.

Our results show that PCT is significantly lowered following TCZ administration until the sixth day following dosing. After this point, the median PCT tended to be lower, but the difference was not statistically significant.

Secondary aims of this study were the assessment of TCZ effects on other biochemical markers, namely CRP, WCC, lymphocytes and neutrophils, NLR and finally P/F ratio and positive blood cultures as clinical markers.

CRP is lowered to a significant extent following TCZ administration. This decrease is sustained throughout all twenty days included in this study. Other parameters, mainly WCC, neutrophil and lymphocyte count, NLR were not significantly affected by the use of TCZ therapy, although they tended to be lower at all times during the study. P/F ratios and the rate of positivity of blood cultures (excluding contaminants) were also found to demonstrate no statistically significant difference between the treatment group and the control group.

Some international studies have already hinted at results similar to those obtained from our cohort. PCT and CRP have been significantly lowered with TCZ administration, including in. patients who did not require mechanical ventilation, as was reported in the COVIDOSE trial.[26,34-35]

With regards to other parameters, these are less extensively studied and conflicting reports seem to exist. An Indonesian study showed that NLR was improved in patients treated with TCZ.[36] Rana et al[37] reported improvement in the P/F ratio of patients treated with TCZ.[37] Salvarani et al[38] found no benefit of TCZ treatment in patients with mild ARDS with regards to P/F ratio and CRP.[38] Other studies described in the introductory section of this paper demonstrated various effects on inflammatory markers such as a lowering of PCT and D-dimer,[29] as well as an overall positive effect on survival outcomes and weaning off mechanical ventilation.[14]

Studies that focus on PCT response in TCZ treatment are few and far between.

The significance of this study is that it demonstrates that PCT and CRP may no longer be reliable markers of superinfection in the setting of severe COVID-19 pneumonia in patients treated with TCZ.

TCZ itself is still associated with a risk of bacterial infection, although in COVID-related ARDS the benefits of its use seem to outweigh the risk in most cases.[39] However, when monitoring for the development of infection, other markers such as WCC and bacterial cultures may have a more important role to play than the commonly used PCT and CRP values. Whether prophylactic antibiotics should be administered with TCZ in order to reduce the possibility of undetected superinfection is still a topic for ongoing discussion, since microbial resistance must be considered.[40]

Limitations of this study include the relatively small sample size and the fact that only one centre was included. All patients received dexamethasone, so this could be a confounding factor that might have minimised bigger differences between the two groups.

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 practise.

SUMMARY

What is Currently Known about the Subject:

- 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.
- In Malta, this treatment started being used in January 2021 for patients with COVID-19 pneumonia that exhibited a sudden deteriorated in condition, as indicated by rapid increase in oxygen requirements and need for admission to intensive care.
- Little data is available in the literature about the effect tocilizumab has on common biochemical and 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.

The New Findings in this Study:

- Tocilizumab significantly lowers the levels of procalcitonin and C-reactive protein in severely ill COVID-19 patients in the days following its administration.
- This demonstrates that caution is required when relying on these inflammatory markers to diagnose a secondary or worsening infection, since they may be falsely low.
- Other markers such as white cell count, neutrophil and lymphocyte count and PaO2/FiO2 ratio, as well as results of blood cultures are unaffected by this drug, and can therefore still be used as accurate markers for infective conditions.

References

1. [1] "COVID Live Update: 177,419,908 Cases and 3,838,675 Deaths from the Coronavirus - Worldometer." https://www.worldometers.info/coronavirus/ (accessed Jun. 16, 2021).
2. [2] "Malta COVID: 30,582 Cases and 419 Deaths - Worldometer." https://www.worldometers.info/coronavirus/country/malta/ (accessed Jun. 16, 2021).
3. [3] "Certain Medical Conditions and Risk for Severe COVID-19 Illness | CDC." https://www.cdc.gov/coronavirus/2019-ncov/need-extra-precautions/people-with-medical-conditions.html (accessed Jun. 16, 2021).
4. [4] S. J. Tzotzos, B. Fischer, H. Fischer, and M. Zeitlinger, "Incidence of ARDS and outcomes in hospitalized patients with COVID-19: A global literature survey," *Critical Care*, vol. 24, no. 1. BioMed Central, Aug. 21, 2020, doi: 10.1186/s13054-020-03240-7.
5. [5] Z. Xu *et al.*, "Pathological findings of COVID-19 associated with acute respiratory distress syndrome," *Lancet Respir. Med.*, vol. 8, no. 4, pp. 420–422, Apr. 2020, doi: 10.1016/S2213-2600(20)30076-X.
6. [6] H. Li *et al.*, "SARS-CoV-2 and viral sepsis: observations and hypotheses," *The Lancet*, vol. 395, no. 10235. Lancet Publishing Group, pp. 1517–1520, May 09, 2020, doi: 10.1016/S0140-6736(20)30920-X.
7. [7] J. P. Hussman, "Cellular and Molecular Pathways of COVID-19 and Potential Points of Therapeutic Intervention," *Front. Pharmacol.*, vol. 11, Jul. 2020, doi: 10.3389/fphar.2020.01169.
8. [8] M. Hoffmann *et al.*, "SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor," *Cell*, vol. 181, no. 2, pp. 271-280.e8, Apr. 2020, doi: 10.1016/j.cell.2020.02.052.
9. [9] C. Zhang, Z. Wu, J. W. Li, H. Zhao, and G. Q. Wang, "Cytokine release syndrome in severe COVID-19: interleukin-6 receptor antagonist tocilizumab may be the key to reduce mortality," *Int. J. Antimicrob. Agents*, vol. 55, no. 5, May 2020, doi: 10.1016/j.ijantimicag.2020.105954.
10. [10] L. J. Scott, "Correction to: Tocilizumab: A Review in Rheumatoid Arthritis (Drugs, (2017), 77, 17, (1865-1879), 10.1007/s40265-017-0829-7)," *Drugs*, vol. 78, no. 2. Springer International Publishing, p. 285, Feb. 01, 2018, doi: 10.1007/s40265-017-0856-4.
11. [11] X. Xu *et al.*, "Effective treatment of severe COVID-19 patients with tocilizumab," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 117, no. 20, pp. 10970–10975, May 2020, doi: 10.1073/pnas.2005615117.
12. [12] "Sarilumab | Side-effects, uses, time to work." https://www.versusarthritis.org/about-arthritis/treatments/drugs/sarilumab/ (accessed Jun. 18, 2021).
13. [13] P. Chiodini, L. Arenare, M. C. Piccirillo, F. Perrone, and C. Gallo, "A phase 2, open label, multicenter, single arm study of tocilizumab on the efficacy and tolerability of tocilizumab in the treatment of patients with COVID-19 pneumonia (TOCIVID-19 trial): Statistical analysis plan," *Contemp. Clin. Trials Commun.*, vol. 20, Dec. 2020, doi: 10.1016/j.conctc.2020.100665.
14. [14] "Interleukin-6 Receptor Antagonists in Critically Ill Patients with Covid-19," *N. Engl. J. Med.*, vol. 384, no. 16, pp. 1491–1502, Apr. 2021, doi: 10.1056/nejmoa2100433.
15. [15] F. Perrone *et al.*, "Tocilizumab for patients with COVID-19 pneumonia. The single-arm TOCIVID-19 prospective trial," *J. Transl. Med.*, vol. 18, no. 1, Dec. 2020, doi: 10.1186/s12967-020-02573-9.
16. [16] N. Wadud *et al.*, "Improved survival outcome in SARs-CoV-2 (COVID-19) Acute Respiratory Distress Syndrome patients with Tocilizumab administration," *medRxiv*, p. 2020.05.13.20100081, May 2020, doi: 10.1101/2020.05.13.20100081.
17. [17] G. G *et al.*, "Hospital-Acquired Infections in Critically Ill Patients With COVID-19," *Chest*, vol. 160, no. 2, pp. 454–465, Aug. 2021, doi: 10.1016/J.CHEST.2021.04.002.
18. [18] A. Elabbadi, M. Turpin, G. T. Gerotziafas, M. Teulier, G. Voiriot, and M. Fartoukh, "Bacterial coinfection in critically ill COVID-19 patients with severe pneumonia," *Infection*, vol. 49, no. 3, p. 1, Jun. 2021, doi: 10.1007/S15010-020-01553-X.

19. [19] M. Berman *et al.*, "The effect of tocilizumab on inflammatory markers in patients hospitalized with serious infections. Case series and review of literature," *Life*, vol. 11, no. 3, 2021, doi: 10.3390/life11030258.

20. [20] F. H *et al.*, "Masked early symptoms of pneumonia in patients with rheumatoid arthritis during tocilizumab treatment: a report of two cases," *Mod. Rheumatol.*, vol. 19, no. 1, pp. 64–68, 2009, doi: 10.1007/S10165-008-0111-7.

21. [21] Y. Yanagawa, Y. Hirano, H. Kato, and T. Iba, "Unusual presentation of more common disease/injury: The absence of typical pneumonia symptoms in a patient with rheumatoid arthritis during tocilizumab and steroid treatment," *BMJ Case Rep.*, vol. 2012, 2012, doi: 10.1136/BCR.02.2012.5835.

22. [22] G. C and K. I, "Acute-phase proteins and other systemic responses to inflammation," *N. Engl. J. Med.*, vol. 340, no. 6, pp. 448–454, Feb. 1999, doi: 10.1056/NEJM199902113400607.

23. [23] M. M, "Update on procalcitonin measurements," *Ann. Lab. Med.*, vol. 34, no. 4, pp. 263–273, 2014, doi: 10.3343/ALM.2014.34.4.263.

24. [24] M. L *et al.*, "Comparison between white blood cell count, procalcitonin and C reactive protein as diagnostic and prognostic biomarkers of infection or sepsis in patients presenting to emergency department," *Clin. Chem. Lab. Med.*, vol. 52, no. 10, pp. 1465–1472, Oct. 2014, doi: 10.1515/CCLM-2014-0210.

25. [25] "Corticosteroids | COVID-19 Treatment Guidelines." https://www.covid19treatmentguidelines.nih.gov/therapies/immunomodulators/corticosteroids/ (accessed Jul. 19, 2021).

26. [26] C. A. Vu *et al.*, "Effects of Tocilizumab in COVID-19 patients: a cohort study," *BMC Infect. Dis.*, vol. 20, no. 1, pp. 1–10, Dec. 2020, doi: 10.1186/s12879-020-05701-4.

27. [27] A. P. Yang, J. ping Liu, W. qiang Tao, and H. ming Li, "The diagnostic and predictive role of NLR, d-NLR and PLR in COVID-19 patients," *Int. Immunopharmacol.*, vol. 84, p. 106504, Jul. 2020, doi: 10.1016/j.intimp.2020.106504.

28. [28] N. L. Hartog, A. T. Davis, J. W. Prokop, A. Walls, and S. Rajasekaran, "Monitoring neutrophil-to-lymphocyte ratio in patients with coronavirus disease 2019 receiving tocilizumab," *Ann. Allergy, Asthma Immunol.*, vol. 126, no. 3, pp. 306–308, Mar. 2021, doi: 10.1016/j.anai.2020.12.014.

29. [29] T. Ivan Hariyanto and A. Kurniawan, "Tocilizumab administration is associated with the reduction in biomarkers of coronavirus disease 2019 infection," *J. Med. Virol.*, vol. 93, no. 3, pp. 1832–1836, Mar. 2021, doi: 10.1002/jmv.26698.

30. [30] N. I. F. H. and C. E. (NICE), "COVID-19 rapid Guideline: Managing COVID-19," 2021. https://app.magicapp.org/#/guideline/L4Qb5n/section/ERY1zn (accessed Mar. 25, 2021).

31. [31] "Interim Clinical Commissioning Policy: Tocilizumab for hospitalised patients with COVID-19 pneumonia (adults) Commissioning position," 2021, Accessed: Jul. 19, 2021. [Online]. Available: https://coronavirus-yellowcard.mhra.gov.uk/.

32. [32] "Your responsibility," 2021, Accessed: Jul. 19, 2021. [Online]. Available: https://www.nice.org.uk/terms-and-.

33. [33] C. Rhee, "Using Procalcitonin to Guide Antibiotic Therapy," *Open Forum Infect. Dis.*, vol. 4, no. 1, Jan. 2017, doi: 10.1093/OFID/OFW249.

34. [34] D. A. Hofmaenner, P. D. Wendel Garcia, C. C. Ganter, S. D. Brugger, P. K. Buehler, and S. David, "What every intensivist should know about Tocilizumab," *Crit. Care 2021 251*, vol. 25, no. 1, pp. 1–3, Jul. 2021, doi: 10.1186/S13054-021-03696-1.

35. [35] G. W. Strohbehn *et al.*, "COVIDOSE: Low-dose tocilizumab in the treatment of Covid-19," *medRxiv*, p. 2020.07.20.20157503, Jul. 2020, doi: 10.1101/2020.07.20.20157503.

36. [36] A. Widysanto *et al.*, "Experience of using tocilizumab for treatment in Indonesian patients with severe COVID-19," *Cytokine*, vol. 138, p. 155393, Feb. 2021, doi: 10.1016/j.cyto.2020.155393.

37. [37] M. A. Rana *et al.*, "Tocilizumab is associated with reduction in inflammation and improvement in P/F ratio in critically sick COVID19 patients," *medRxiv*. Cold Spring Harbor Laboratory Press, p. 2020.10.20.20210195, Oct. 21, 2020, doi: 10.1101/2020.10.20.20210195.

38. [38] C. Salvarani *et al.*, "Effect of Tocilizumab vs Standard Care on Clinical Worsening in Patients Hospitalized with COVID-19 Pneumonia: A Randomized Clinical Trial," *JAMA Intern. Med.*, vol. 181, no. 1, pp. 24–31, Jan. 2021, doi: 10.1001/jamainternmed.2020.6615.
39. [39] W. Alam and A. R. Bizri, "Efficacy of tocilizumab in COVID-19: A review of the current evidence:," *https://doi.org/10.1177/00368504211030372*, vol. 104, no. 3, Jul. 2021, doi: 10.1177/00368504211030372.
40. [40] "COVID-19 & Antibiotic Resistance | CDC." https://www.cdc.gov/drugresistance/covid19.html (accessed Oct. 24, 2021).