ORIGINAL ARTICLE

UTILITY OF INFLAMMATORY MARKERS FOR TOCILIZUMAB IN COVID-19 PATIENTS: A SINGLE-SITE RETROSPECTIVE STUDY

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Background: Studies are available on the use of inflammatory biomarkers for profiling and patient prognosis, but literature for tocilizumab monitoring parameters is scarce. Thus, we aim to evaluate different inflammatory markers that can relate to the effectiveness of tocilizumab in hospitalized patients suffering from severe covid. **Method:** We analyzed a retrospective cohort of 227 patients who were admitted due to SAR-Co2 in one of the largest hospitals in Pakistan, Lady Reading Hospital. Using in-hospital mortality as the primary parameter for the effectiveness of tocilizumab. Wilcoxon rank-sum test, chi-square test and ROC curve analysis were performed to evaluate inflammatory biomarkers. The p- values less than 0.05 were considered statistically significant. **Result:** A total of 1639 tests were identified from 227 patients admitted to the hospital. CRP (28%), LDH (27.3%) were the most commonly prescribed and 40% of the total test were prescribed pre-dose. D-dimer, ferritin and CRP were found to have a clinically significant impact of the dose. **Conclusion:** D-dimer, ferritin and LDH do not seem to relate proportionally with tocilizumab effectiveness. CRP can be utilized for monitoring tocilizumab effectiveness.

Keywords: Tocilizumab; COVID-19; D-Dimer; CRP, Ferritin; Inflammatory markers; Pakistan; dexamethasone

Citation: Amir M, Ali U, Javed A, Ahmad J. Utility of inflammatory markers for tocilizumab in COVID-19 patients: A single-site retrospective study. J Ayub Med Coll Abbottabad 2022;34(4):777–81.

DOI: 10.55519/JAMC-04-10565

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-C0V-2), the causative agent of Coronavirus Disease 2019 (COVID-19), had been a major reason for global concern since December 2019.[1] Mortality rate among mild to moderate remained low, however, a significant number of patients will progress to a severe stage requiring hospitalization. These patients are at high risk of mortality.[2] It was later identified that the severity of the disease was linked with cytokine release syndrome (CRS) due to elevated interleukin-6 (IL-6).[3] Thus, it was strongly proposed that halting the progression of CRS or its treatment may contribute to reducing the mortality rate in such patients.[4] Inflammatory markers such as D-dimer, ferritin, C-reactive protein (CRP) have been found to be elevated in non-survival patients of COVID-19.[5] The levels of these serum biomarkers are highly correlated with the production of IL-6, thus causing failure of different organs in particular to lungs resulting in acute respiratory distress syndrome (ARDS). Thus, respiratory failure remains the major reason for mortality in COVID patients.[6] Now many hospitals utilize these inflammatory markers to observe the progression of CRS in COVID-19 patients.

The realization of the role of IL-6 in COVID-19 played a vital role in the introduction of anti-cytokines or immunomodulatory agents as its treatment. Different IL-6 inhibitors were trialed to observe their effectiveness. These included sarilumab, bevacizumab, fingolimod, eculizumab, and tocilizumab.[7] Tocilizumab, a recombinant humanized monoclonal antibody, inhibits IL-6 to bind with its receptors. The initial successful studies resulted in its approval as compassionate use for COVID-19 in many countries. Initially, it was not known which patients would benefits thus many small trials were conducted. Few of these trials suggested its use while few had reservations. Regardless, limited data were available to develop a clear guideline for its usage.[8] Our pilot study, TOCIPAK Trial, allowed optimized use of Tocilizumab in the hospitalized patients. The result of the study helped to develop our new drug use protocol, i.e., patients with oxygen requirements but not on invasive respiratory therapy and high inflammatory markers were eligible for Tocilizumab.[9] Later, the RECOVERY trial cemented the evidence of the beneficial impact of tocilizumab in the early stage of disease in hospitalized severe to critical patients.[10] However, we still faced the difficulty when to initiating tocilizumab and how to monitor tocilizumab effectiveness.

During the second phase and third wave, the established protocol of the institution allowed tocilizumab for patients not requiring invasive respiratory therapy and who have high inflammatory markers such as D-Dimer, ferritin, IL-6, CRP and

procalcitonin. However, the utility of inflammatory markers was not definite for the initiation of tocilizumab or monitoring of the drug's effectiveness. Studies are available on the use of inflammatory biomarkers for profiling and patient prognosis, but literature for tocilizumab monitoring parameters is scarce.[11] Limited resources and low availability of the drug highlight the significance of the prognostic tool. A tool that could assist in evaluating patients' eligibility for tocilizumab and assist in monitoring its effects. Thus, we aim to evaluate different inflammatory markers that can relate to the effectiveness of tocilizumab in hospitalized patients.

MATERIAL AND METHODS

We analyzed a retrospective cohort of 227 patients admitted due to SAR-Co2 in Lady Reading Hospital-MTI. This 1770 bed health facility is a public hospital serving a population of 2,273,000 inhabitants. Around 250 beds in high-dependency units which provided care to moderate to severe patients requiring high-intensity oxygen therapy and 25 beds in the intensive critical unit were assigned for critically ill patients requiring invasive respiratory support or cardiac support.

Clinical, demographic, laboratory, treatment, and outcome data were extracted manually from electronic medical records using a standardized data collection form. The form was developed to ensure maximum data retrieval. To increase the accuracy of the data collection, two researchers were involved in data extraction simultaneously. One researcher was responsible for data extraction from the electronic health record and the second researcher was responsible for the entry of the data in the form. Read back technique was used to validate the data. Most authors contributed to data retrieval, data entry, and data verification.

The study included patients admitted as positive COVID-19 if the virus SARS-CoV-2 by PCR (polymerase chain reaction) of nasopharyngeal swab specimens or RAT Test resulted positive. Patients with highly suggestive of COVID infection based on clinical and radiological evaluation were also included in the study. Other inclusion criteria included age greater than 18 years, at least a single dose of tocilizumab and at least one inflammatory marker. Patients with a terminal illness, ventilated patients were excluded from the study. Patients were classified according to the severity based on Pakistan National Guideline.[12]

The study was approved by the Institutional Review Committee of Lady Reading Hospital. All patients provided verbal, not written, informed consent because of isolation precautions.

A standard treatment guideline was developed by the institutional committee which comprises physicians relating to critical care, pulmonology, internal medicine; nurse, and pharmacist. The standard treatment included anticoagulants, gastric protecting agents, steroid (dexamethasone) and an antibiotic if required. Medication relating to co-morbid were also prescribed. To standardize the treatment, a treatment set was established in the electronic record. Interventional therapies such as hydroxychloroquine, azithromycin, oseltamivir, and montelukast were not part of institution guideline. A multi-disciplinary team of physicians and pharmacist was responsible for recommending tocilizumab based on the levels of inflammatory markers, and the patient's condition.

The Kolmogorov-Smirnov and Shapiro-Wilk test was used to assess the normality of the distribution of investigated parameters. The data demonstrated non-significant skewness and kurtosis thus non-parametric tests were employed to analyze the data. Wilcoxon rank-sum test was done to compare the impact of tocilizumab on pre and post results of biomarkers. A Chi-square test was carried out to compare categorical data. Receiver Operator Characteristics (ROC) curve analysis was performed to identify the optimal level of inflammatory biomarkers. Using in-hospital mortality as the primary parameter for the effectiveness of tocilizumab, the acceptable cut-off point for ROC curve analysis is 0.7 whereas cut-off point below 0.7 will be considered unacceptable. Logistic regression was performed to identify different levels of inflammatory markers with mortality. The p- values less than 0.05 were considered statistically significant. Statistical analysis was completed using software (SPSS 23.0; SPSS; Chicago, IL)

RESULTS

A total of 1639 tests were identified from 227 patients admitted from 27 November 2020 to 15 June 2021. The demographic information of the patients is available in Table-1. Sample selected has equivalent gender representations. 72 % of the sample lies in the age bracket of 36–65, whereas only 5% of sample were below 36 and 14 % above 65 years. 21.9% of the patients received a second dose of the drug.

Table-2 provide details regarding the test evaluated. CRP (28%), LDH (27.3%) were the most commonly prescribed tests followed by D-dimer (14.3%). IL-6 was the least prescribed. With respect to pre-dose and follow-up tests, CRP was the most common followed by LDH and ferritin.

Table-3 depicts the Wilcoxon Sign Rank Test analysis of all the inflammatory markers. The result shows that tocilizumab has a c significant impact on D-dimer, ferritin, and CRP. However, reducing trends of levels in the follow-up tests were found in ferritin and

CRP but were not found in the post-administration test of D-dimers. Table 4 provide information ROC curve analysis was carried out for all biomarkers. Most of test result showed AUC around 0.5 with insignificant *p*-value. Table-5 provides information relating the logistic regression analysis. The result shows that levels of different inflammatory markers at different timing had no statistically significant impact on mortality.

Table-1: Demographic information of patients who received tocilizumab

Demographic Information	*F*	%
Gender		
Male	136	59.9
Female	91	40.1
Age		
18-25	3	1.4
26-35	9	3.9
36-45	32	14
46-55	59	25.9
56-65	76	33.3
> 65	49	21.5
COVID Status		
Positive	98	43
Suspected	89	39
Co-Morbid		
Single Co-morbid	4	1.8
2-3 Comorbid	130	57.8
> 3 Comorbid	91	40.4
Total Doses of Tocilizumab		
Single Dose	227	100
Two Doses	50	21.9
Survival		
Discharged	90	40%
In-hospital Mortality	137	60%

Table-2: Detail of Tests and its characteristics

Total Test	*F*	%
Types of Tests Evaluated		
D-dimer	234	14.3
Ferritin	393	24.0
LDH	447	27.3
CRP	462	28.2
IL-6	9	0.5
Procalcitonin	94	5.7
Total Test	1639	100.0
Timing of Tests		
Pre-Dose Test	623	40.3
1st Post-Dose Test	568	36.7
2nd Post Dose Test	356	23.0
Details of Test and its timing		
Pre-D-dimer Test	86	37.7
1st Post D-dimer Test	90	39.5
2nd Post D-dimer Test	55	24.1
Pre-Ferritin Test	160	70.2
1st Post Ferritin Test	143	62.7
2nd Post Ferritin Test	88	38.6
Pre-LDH Test	179	78.5
1st Post LDH Test	159	69.7
2nd Post LDH Test	106	46.5
Pre-CRP Test	188	82.5
1st Post CRP Test	170	74.6
2nd Post CRP Test	101	44.3
Pre-IL-6 Test	7	3.1
Post-IL-6 Test	2	0.9

Table-3: Pre and Post Evaluation of Inflammatory markers

	Positive Rank	Negative Rank	*Z*	*p*-value
Pre-D-dimer Test-1st Post D-dimer Test	80	60	-2.885	0.004
1st Post D-dimer Test-2nd Post D-dimer Test	55	41	-1.55	0.121
Pre-Ferritin Test-1st Post Ferritin Test	34	61	-2.262	0.024
1st Post Ferritin Test -2nd Post Ferritin Test	36	42	-0.112	0.911
Pre-LDH Test-1st Post LDH Test	67	66	-1.105	0.269
1st Post LDH Test-2nd Post LDH Test	55	46	0.562	0.574
Pre-CRP Test-1st Post CRP Test	60	80	-2.855	0.004
1st Post CRP Test-2nd Post CRP Test	41	55	-1.55	0.121

Table-4: ROC Curve Analysis of Inflammatory Biomarkers

Test	AUC	*p*-value
Pre-D-dimer Test	0.576	0.45
1st Post D-dimer Test	0.577	0.45
Pre-Ferritin Test	0.496	0.38
1st Post Ferritin Test	0.58	0.48
Pre-LDH Test	0.463	0.36
1st Post LDH Test	0.583	0.49
Pre-CRP Test	0.545	0.45
1st Post CRP Test	0.5449	0.45

Table-5 Logistic regression analysis of inflammatory marker

		Mortality		*P* value
	Time of level	Yes	No	
D-Dimer	Pre-tocilizumab	34	52	0.1
	Post-tocilizumab	34	56	0.2
Ferritin	Pre-tocilizumab	63	97	0.6
	Post-tocilizumab	54	85	0.1
LDH	Pre-tocilizumab	75	104	0.4
	Post-tocilizumab	56	92	0.1
CRP	Pre-tocilizumab	109	79	0.6

DISCUSSION

Numerous studies are present evaluating inflammatory markers' relationship with the severity of COVID-19 and its prognosis. However, literature relating utility of inflammatory markers to monitor tocilizumab effectiveness is scarce. The study described providing the analysis of different inflammatory markers with the effectiveness of tocilizumab in the hospitalized patient due to severe to critical patients of COVID-19. The aim was to identify a parameter that can be used for monitoring drug effectiveness. To our knowledge, this is the first focused study evaluating different parameters with drug effectiveness. Moreover, the study have also provided the insight regarding the effectiveness of combination of tocilizumab with dexamethasone, first in the context of Pakistan.

The elevated level of inflammatory markers d-dimer, IL-6, ferritin, LDH and CRP have been directly identified a high risk of mortality in patients of COVID-19, but many studies have shown that no or low impact of tocilizumab with d-dimer, IL-6, and LDH. The relationship between ferritin and CRP has been found in these studies.[13] Our study showed the relationship of D-dimer, CRP, and ferritin with tocilizumab dose but no relationship was found for LDH. The follow-up tests of d-dimer, ferritin, and CRP after tocilizumab were found to be lowered by the dose. We evaluated the inflammatory markers for the optimal cutoff for survival and non-survival in COVID patients. Another study tried to identify the cut-off values of inflammatory biomarkers but was not conclusive.[14] Likewise, our study was not able to identify any inflammatory markers as a diagnostic tool for tocilizumab survival.

Various health agencies have now recommended tocilizumab. IDSA recommends it for severe to critical COVID-19 patients.[15] However, NICE and NIH guidelines have provided more clear criteria for its use. These guidelines included a marker, i.e., CRP of 75 mg/L and time, i.e., 48 hours of starting high-flow nasal oxygen, continuous positive airway pressure, non-invasive ventilation, or invasive mechanical ventilation.[17,18] The recommendation of CRP is indistinct as beneficial effect below the level of 75 mg/L is still unknown.[19]

With respect to effectiveness of the combination of tocilizumab and dexamethasone in COVID-19 patients, different researches have shown no additional benefit in term of mortality. Our study has also shown similar result when the survival rate of current study is compared to the non-critical patients in TOCIPAK Trial in which only tocilizumab was used alone (40% vs 50%).

The study has few strengths and limitations. In the first wave of the pandemic, the investigational use of registered medications was commonly observed throughout the world. Drugs such as azithromycin, ivermectin, montelukast, oseltamivir along with few antivirals were trialed for the treatment of SAR Co-V Infection.[15] Like other institutions, such medications were also prescribed in our institution. However, their use was limited due to the constant updating of institutional guidelines. Thus, our study remained free from confounding bias that may have been created due to the administration of these drugs. Secondly, all patients received dexamethasone as recommended by the RECOVERY Trial.

The limitations of this study are those inherent to an observational study due to the absence of randomization and a control group. Secondly, the effectiveness of tocilizumab was determined by in-hospital mortality rather than long-term. The rationale behind the selection of in-house mortality is that most of the patients enrolled in the study belong to low economic status and resides in far distant places. Inaccessibility to patients remained a major hindrance for follow-up.

CONCLUSION

This study showed that the dose of tocilizumab has impact on the level of D-dimer, ferritin and CRP, however, the association between the effectiveness of the drug and their level was not identified. Thus, have no value as clinical tool. Early administrating tocilizumab found to have greater impact on survival rate of these patients.

Recommendations:

Inflammatory markers pose an additional burden on the treatment of COVID patients financially. Since the study does not show high relevancy of these markers with tocilizumab use and survival rate, it is recommended that the use of tocilizumab should be based on days of admissions. For monitoring its impact CRP can be utilized because its level is proportional to IL-6 and low-cost value.

AUTHORS' CONTRIBUTION

MA developed the idea, conducted the analysis and wrote the manuscript., AK and JA collected the data. UA collected the data and supervised the data collection.

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Submitted: January 9, 2022	*Revised: May 7, 2022*	*Accepted: July 12, 2022*

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