Original Article

A serum interleukin-6-based analysis of patients with nasopharyngeal swab reverse-transcriptase polymerase chain reaction negative for SARS-COV-2 infection with CO-RADS 4 and 5 on computed tomography of the chest

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Abstract

Background: Serum interleukin 6 (IL-6) levels have been studied in the diagnostic evaluation of patients with severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) disease (COVID-19).

Methods: We studied the utility of treatment with tocilizumab in COVID-19 patients ($n=19$) with a negative nasopharyngeal swab real time reverse transcriptase polymerase chain reaction (RT-PCR) test for SARS-CoV-2 who had suggestive computed tomography (CT) findings, namely, COVID-19 Reporting and Data System (CO-RADS) 4,5.

Results: Receiver operator characteristic (ROC) curve analysis showed that serum IL 6 at a cut-off of >56.9 pg/L was a predictor of mortality in nasopharyngeal swab RT-PCR negative patients with suggestive CT findings. Tocilizumab had no significant effect on the mortality.

Conclusions: In nasopharyngeal swab RT-PCR negative patients with suggestive chest CT findings, elevated serum IL-6 levels > 56.9 pg/L predicted mortality. However, treatment with tocilizumab had no effect on mortality.

Keywords: Computed tomography, CO RADS, negative, reverse transcriptase polymerase chain reaction, SARS COV 2 infection, serum interleukin 6, tocilizumab

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Submitted: 16-Jan-2021 **Accepted:** 26-May-2021 **Published:** 12-Dec-2022

INTRODUCTION

Early in the pandemic of severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) disease (COVID-19), the concept of COVID-19-related cytokine storm syndrome (COVID-CSS) emerged. This concept helped explain why some patients exposed to this virus become critically ill with acute respiratory distress syndrome,

Access this article online	
Quick Response Code:	Website: www.jcsr.co.in
	DOI: 10.4103/jcsr.jcsr_10_21

How to cite this article: Reddy KVK, Kumar P, Raju SS, Gaddam S, Sailaja SL, Priyanka A, *et al.* A serum interleukin-6-based analysis of patients with nasopharyngeal swab reverse-transcriptase polymerase chain reaction negative for SARS-COV-2 infection with CO-RADS 4 and 5 on computed tomography of the chest. J Clin Sci Res 2023;12:45-50.

multi-organ failure and death.[1] The term CSS was first coined to describe the hypercytokinaemia in graft versus host disease after allogeneic stem cell transplant.[2] Experts[3] define CSS as a clinical phenotype consisting of (i) immune dysregulation characterised by perpetuated activation of lymphocytes and macrophages; (ii) resulting in secretion of large quantities of cytokines; and (iii) leading to overwhelming systemic inflammation and multi-organ failure with high mortality.[1]

The patients with COVID-CSS are a subset of patients who demonstrate excessive immune activation characterised by markedly elevated inflammatory markers such as C-reactive protein (CRP), interleukin-6 (IL-6) and ferritin, as well as lymphopenia and a spectrum of end-organ damage from isolated respiratory dysfunction to multi-organ failure.

Two studies[4,5] have shown that serum IL-6 is superior to CRP, serum ferritin and liver enzymes, for predicting clinical outcomes such as respiratory failure and death.

Tocilizumab is a monoclonal antibody to IL-6 receptor and is in widespread use for several auto-immune diseases particularly for rheumatoid arthritis. As tocilizumab is widely used in the treatment of IL-6-mediated cytokine release syndrome, it is being tried as an off-label treatment for moderate-to-severe COVID-19 disease.[6]

The real-time polymerase chain reaction assay (RT-PCR) test is considered the standard for the diagnosis of COVID-19, but in several patients, it can have false-negative results.[7] False-negative results may depend on several pre-analytical and analytical issues.[8]

In a study[9] of 213 patients from whom 205 throat swabs, 490 nasal swabs, 142 sputum and 29 bronchoalveolar lavage fluid (BALF), the BALF showed 100% positive rate followed by the sputum (88.9%), followed by 119 nasal swabs (73.3%) and then the throat swabs (60.0%).All specimens were derived from severely ill patients.[9]

In a study[10] of 1014 patients, 601 (59%) had positive realtime reverse transcriptase polymerase chain reaction (RT-PCR) results and 888 of 1014 (88%) had positive chest CT scans. The sensitivity of chest CT in suggested COVID-19 was 97% (95% confidence interval: 95%–98%; 580 of 601 patients) based on positive RT-PCR results. In the 413 patients with negative RT-PCR results, 308 (75%) had positive chest computed tomography (CT) findings. Of those 308 patients, 48% (103 of 308) were considered as highly likely and 33% (103 of 308) as probable.[10]

For symptomatic patients with suspected COVID-19, chest imaging has sometimes been used for the diagnostic workup of COVID-19 when RT-PCR test is not available, RT-PCR test is available, but results are delayed and initial RT-PCR testing is negative, but with high clinical of suspicion of COVID-19.[11]

The coronavirus disease 2019 (COVID-19) Reporting and Data System (CO-RADS) of reporting chest computed tomography (CT) findings is a standardised reporting system for patients with suspected COVID-19 infection developed for a moderate-to-high prevalence setting. CO-RADS 4 suggests abnormalities suspicious for COVID-19 and CO-RADS 5 suggests typical of COVID-19.

In this background, we studied the following outcomes of interest: (i) serum IL-6 as a prognostic marker for mortality in patients with nasopharyngeal swab RT-PCR negative and CO-RADS 4 and 5 on CT scan chest; (ii) to identify the discriminatory cut-off value of serum IL-6 as a determinant of mortality; and (iii) to study the effect of injection tocilizumab in CSS in patients with nasopharyngeal swab RTPCR negative and CO-RADS 4 and 5 on CT of the chest.

MATERIAL AND METHODS

Sri Venkateswara Institute of Medical Sciences-Sri Padmavathi Medical College for Women hospital had been ordained as the State COVID Hospital in March 2020. Separate wards in this hospital were earmarked for the patients who are nasopharyngeal swab RTPCR assay negative for SARS-COV-2 infection, and CT of the chest revealed CO-RADS 4 and 5.

All patients had serum creatinine, blood urea, serum sodium and serum potassium, complete haemogram, liver function tests, prothrombin time and partial thromboplastin time, serum procalcitonin, serum ferritin, CRP and serum D-dimer sent on the first day of admission.

Serum IL-6 estimation was done and tocilizumab was prescribed when the patients had following features: need for non-invasive ventilation from the day of admission, failure to wean from non-invasive ventilation, deterioration of hypoxia and need for non-invasive ventilation, clinical features such as persistent fever, splenomegaly, hepatomegaly, lymphadenopathy, neurological symptoms, progression to acute lung injury and acute respiratory distress syndrome with radiological abnormalities, acute kidney injurym congestive cardiac failure and laboratory

abnormalities such as pancytopenia, anaemia, leukopenia, thrombocytopenia, deranged renal function and liver function tests, raised CRP, serum ferritin and procalcitonin and coagulation abnormalities.

The serum IL-6 levels were estimated by chemiluminescence assay using reagents from Beckman Coulter, USA on ccess 2 Immunoassay System, Beckman Coulter, USA immunoassay. The laboratory reference value of serum IL-6 was <7.5 pg/mL. An article published early in the pandemic had suggested that the single dose of injection tocilizumab might be expected to benefit these seriously ill patients with 10 times or more elevated IL-6. Moreover, the moderately ill patients with an extremely higher level of IL-6, almost 90 times of normal, could also benefit from repetitive tocilizumab therapy.[12] Tocilizumab was used accordingly.

Contraindications for use of tocilizumab included platelet count <100,000/mm3, neutrophil count <2000/mm3 and alanine aminotransferase or aspartate aminotransferase >5 times the upper limit of normal range.

Injection tocilizumab was administered intravenously at 8 mg/kg per dose, up to a maximum dose of 400 mg which was repeated one more time.

In all patients receiving tocilizumab, a thorough history and examination was done, chest radiograph was obtain to exclude the current tuberculosis and any other bacterial or fungal infection.

All patients were treated from admission on injection remdesivir 200 mg on the day 1, followed by 100 mg from day 2 to day 10, injection dexamethasone 8 mg/day intravenous 10 days for and low-molecular-weight heparin and vitamins.

Written informed consent was obtained from the study participants as tocilizumab is still an investigational drug. Intravenous paracetamol and injection pheniramine premedication to before administering tocilizumab. All patients were observed for new onset of fever, raised total leucocyte count or new onset of consolidation on chest radiograph.

For comparing continuous variables and categorical variables, Student's t-test and Chi-square tests were used, respectively. For survival analysis, Kaplan–Meier analysis was done. Serum IL-6 cut-off to predict cytokine storm was studied using the receiver-operator characteristic (ROC) curve. Univariate analysis was used to predict the risk factors for mortality. Statistical analysis was done using an online statistical tool MedCalc (available at https://www.medcalc.org/).

We calculated the sample size at the required absolute precision level for sensitivity and specificity by Buderer's formula.[13] The required sample size was 86 for the sensitivity of 90%.

RESULTS

From March 18, 2020, to December 10, 2020, we managed 5486 patients with the nasopharyngeal swab RT-PCR assay positive. We also managed 1470 nasopharyngeal swab RT-PCR negative for SARS-COV-2 infection, in whom CT of the chest revealed CO-RADS 4 and 5. Of these patients, the serum IL-6 was sent for 58 patients. Injection tocilizumab was prescribed for 27 patients [Figure 1].

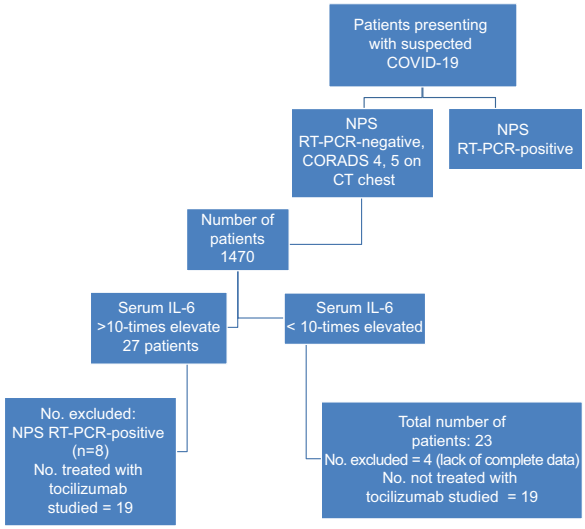

Figure 1: Study design NPS RT-PCR = Nasopharyngeal swab real time reverse transcriptase polymerase chain reaction; CORADS = coronavirus disease 2019 (COVID-19) Reporting and Data System (CO-RADS); IL=Interleukin

Table 1 shows the comparative data of patients at presentation. There was no significant difference between the two groups in age, prevalence of diabetes mellitus, hypertension, chronic or acute renal failure, obesity and CT chest severity. Furthermore, there were no significant differences in the laboratory parameters.

Table 2 depicts comparison of serum IL-6 in survivors and non-survivors.

Table 1: Data of patients at presentation

Parameter	Serum IL-6 >10x elevated, tocilizumab treated (*n*=19) No. (%)	Serum IL-6 <10-times elevated; tocilizumab not treated (*n*=19) No. (%)	*P* - value
Age (years)*	49.15*	53.16	0.4372
Diabetes mellitus†	12 (63.15)	15 (78.94)	0.2832
Hypertension†	17 (89.47)	18 (94.74)	0.5475
CKD/AKI†	1 (5.26)	-	
Obesity†	13 (68.42)	14 (73.68)	0.7324
CT severity score*	14.99±5.4	15.05±5.65	0.9538
Haemoglobin (g/dL)*	13.1±1.7	12.1±2.3	0.086
Total leucocyte count (/mm^3)*	11347.3±5185.4	10227.7±3902.8	0.473
Platelet count (/mm^3)*	2.1±0.94	2.3±1.04	0.702
Serum urea (mg/dL)*	42.7±22.1	52.5±44.5	0.437
Serum creatinine (mg/dL)*	1.0±0.5	1.41±1.8	0.444
Total serum protein (g/dL)*	6.3±0.7	6.5±0.8	0.365
Serum albumin (g/dL)*	2.9±0.4	3.3±0.4	0.056
Serum bilirubin (mg/dL)*	0.95±0.84	0.79±0.4	0.492
SGOT (IU/L)*	48.4±31.18	120.6±192.02	0.143
SGPT (IU/L)*	35.2±27.19	91.2±164.20	0.182
Serum ALP (IU/L)*	105.1±51.9	106.7±51.93	0.932
CRP (g/L)*	112.14±106.05	95.61±79.02	0.603
Serum ferritin (ng/L)*	555.6±287.2	453.9±288.3	0.310

*Data are presented as mean ± standard deviation
†Data are presented as No. (%)
IL-6=Interleukin-6; CKD=Chronic kidney disease; AKI=Acute kidney injury; CT=Computed tomography; SGOT=Serum glutamic oxaloacetic transaminase; SGPT=Serum glutamic pyruvic transaminase; ALP=Alkaline phosphatase; CRP=C-reactive protein

Table 2: Comparison of serum IL-6 in survivors and non-survivors

Serum IL-6 (pg/mL)	Survivors (*n*=25)	Non-survivors (*n*=13)	*P*-value
>75	9	10	19
<75	16	3	0.040205

The laboratory reference value of serum IL-6 = <7.5 pg/mL
IL-6=Interleukin

Kaplan–Meier survival analysis (Figure 2) (Table 3) revealed that in the group which received injection tocilizumab, the last death after injection tocilizumab happened on 27th day and the patient survived after injection tocilizumab till 90th day.

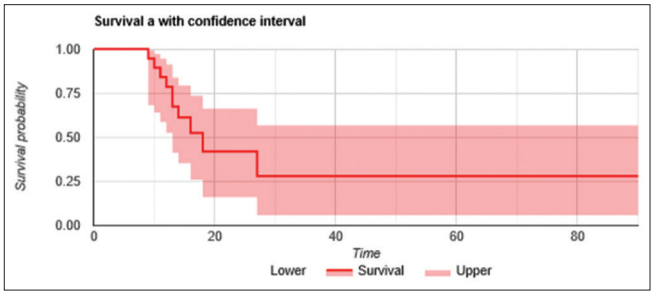

Figure 2: Kaplan-Meier survival analysis

On univariate linear regression analysis to identify the predictors of mortality Table 3 none of the variables had significant effect on the mortality.

Table 3: Univariate linear regression analysis

Model	Unstandardised coefficients *B*	Standardised coefficients β	SE	*t*	*P*-value
Constant	−1.008		0.819	−1.231	0.306
Diabetes mellitus	0.465	0.449	0.548	0.849	0.458
Hypertension	−0.349	−0.214	0.672	−0.519	0.639
CKD/AKI	1.721	0.77	1.597	1.077	0.36
Haemoglobin	−0.5	−0.224	0.498	−1.004	0.389
Total leucocyte count	0.767	0.759	0.461	1.664	0.195
N:L ratio=4.5	−0.605	-0.372	0.778	−0.777	0.494
Platelet count	0.093	0.068	0.509	0.183	0.867
Serum creatinine	1.767	1.086	1.098	1.61	0.206
Total serum protein	−0.5	−0.224	0.498	−1.004	0.389
Serum albumin	0.698	0.51	0.902	0.774	0.495
Serum bilirubin	−0.419	−0.306	0.729	−0.575	0.606
Serum CRP	2.93	1.31	1.481	1.979	0.142
Serum ferritin	0.279	0.172	0.588	0.474	0.668
Serum IL-6	−1.008	−0	0.819	−1.231	0.306
Use of tocilizumab	−1.008	−0	0.819	−1.231	0.306

IL-6=Interleukin; SE=Standard error; CKD=Chronic kidney disease; AKI=Acute kidney injury; N:L ratio = Neutrophil lymphocyte ratio; CRP=C-reactive protein

On ROC analysis (Figure 3) serum IL-6 at a cut-off >56.9 pg/mL predicted mortality with a sensitivity of 90% and specificity of 65.4% (Figure 3).

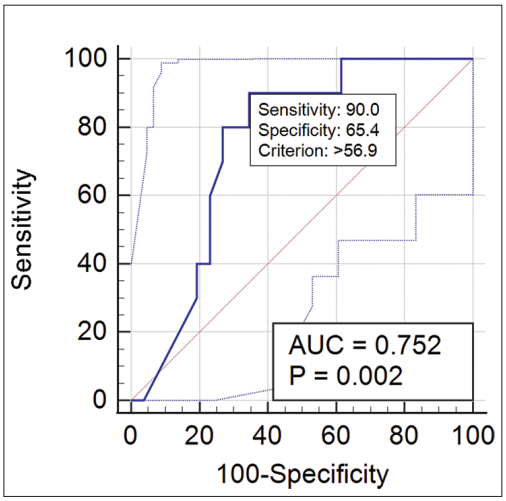

Figure 3: Receiver operator characteristic curve to identify serum IL-6 cut-off for predicting mortality. IL-6=Interleukin-6; AUC=Area under the curve

DISCUSSION

Sparse published data are available on serum IL-6 and use of tocilizumab in patients with nasopharyngeal swab RT-PCR negative for SARS-CoV-2 infection, and CT of the chest revealed CO-RADS 4 and 5. The published studies on the use of injection tocilizumab are on patients with nasopharyngeal swab RTPCR assay positive for SARS-CoV-2 infection.[14] These studies had limitations such as most had small sample sizes, there was high or moderate risk of bias, mostly due to confounding, varied in dosing (single or double) and had drug availability issues emerged in some centres, which may have influenced both sample sizes and study designs.[14]

In the present study tocilizumab was administered when serum IL-6 levels were elevated more than 10-fold the reference range. Our study revealed serum IL-6 >56.9 pg/mL as a discriminatory cut-off value. Several studies have given different cut-off values. The cut-off values ranged from 35 pg/mL to 86 pg/mL.[4,5,15-17]

Our study has a few limitations. The study is not a randomised comparison, and therefore unmeasured confounding cannot be ruled out. The sample size is small. This is a short follow-up and single-centre study. We lacked on the data of other inflammatory markers than serum IL-6.

Our findings show that in nasopharyngeal swab RT-PCR negative patients with suggestive chest CT findings, elevated serum IL-6 levels > 56.9 pg/L predicted mortality. However, treatment with tocilizumab had no effect on mortality.

Acknowledgements

The authors wish to thank Department of Biochemistry, Sri Venkateswara Institute of Medical Sciences, Tirupati, for their timely help in carrying out the serum interleukin-6 assay.

Financial support and sponsorship

Nil.

Conflicts of interest

The authors are faculty members/residents of Sri Venkateswara Institute of Medical sciences, Tirupati, of which Journal of Clinical and Scientific Research is the official Publication. The article was subject to the journal's standard procedures, with peer review handled independently of these faculty and their research groups.

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