Received: 28 May 2020 | Accepted: 5 June 2020

DOI: 10.1002/jmv.26149

JOURNAL OF MEDICAL VIROLOGY WILEY

SHORT COMMUNICATION

Higher levels of IL-6 early after tocilizumab distinguish survivors from nonsurvivors in COVID-19 pneumonia: A possible indication for deeper targeting of IL-6

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Abstract

Introduction: The most serious COVID-19 deriving from severe acute respiratory syndrome coronavirus 2 causes a cytokine release storm and it is associated with worse outcomes. In COVID-19 patients, interleukin-6 (IL-6) levels are significantly elevated. Blocking IL-6 preliminarily resulted in the improvement of this hyperinflammatory state. It is unknown which patients could require higher doses of tocilizumab to get out of the cytokine storm.

Materials and Methods: Twenty-four patients affected by COVID-19 pneumonia were included. All the patients underwent tocilizumab 8 mg/kg intravenously and were tested for serum IL-6 24 to 48 hours before and 12 to 48 hours after tocilizumab infusion. Comparisons between survivors and nonsurvivors were performed.

Results: Eighteen patients were discharged, while six patients died, with no clinical or laboratory differences between the two groups at baseline. IL-6 was not different at baseline ($P = .41$), while 24 to 48 hours post-tocilizumab IL-6 serum levels were significantly higher in nonsurvivors than in survivors (2398.5 [430.5-9372] vs 290.5 [58.5-1305.5] pg/mL, $P = .022$). Serum IL-6 post-tocilizumab showed a good predictive ability to discriminate survivors from nonsurvivors (area under the curve, 0.815; 95% confidence interval, 0.63-0.99, $P = .02$).

Conclusion: Repeated measurement of the serum level of IL-6 early after tocilizumab may distinguish nonsurvivors from survivors and support the choice of deeper targeting IL-6 in COVID-19 pneumonia.

KEYWORDS

coronavirus, COVID-19, cytokine, interleukin-6, tocilizumab

1 | INTRODUCTION

At the end of 2019, a novel coronavirus was identified as the cause of a cluster of pneumonia cases in Wuhan, China. It rapidly spread, resulting in an epidemic throughout China, followed by an increasing number of cases in other countries throughout the world. In February 2020, the World Health Organization designated the disease as COVID-19, which stands for coronavirus disease 2019.[1] The virus that causes COVID-19 is designated severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Some patients with severe COVID-19 have laboratory evidence of an exuberant inflammatory response, similar to cytokine release syndrome, with persistent fever, elevated inflammatory markers (eg, D-dimer, ferritin), and elevated pro-inflammatory cytokines, such as interleukin-6 (IL-6) and IL-1; these laboratory abnormalities have been associated with critical and fatal illnesses.[2-5]

Treatments selectively targeting the downstream inflammation have recently shown some effectiveness in the most critically ill patients.[6-8] Serum IL-6 levels are significantly higher in patients severely ill and needing tocilizumab than in patients recovering with the standard of care for COVID-19 pneumonia,[9] and serum IL-6 levels could effectively help the therapeutic choice of the clinicians.[7-9] Protocols on the efficacy and safety of intravenous tocilizumab for COVID-19 include the possibility of a second infusion at 12 to 24 hours from the first one in patients with no adequate response.[6] However, clinicians often find this choice difficult in the absence of objective laboratory parameters, which could early support the clinical choice for a second infusion of tocilizumab. To answer this unmet need, a cohort of patients undergoing tocilizumab for COVID-19 pneumonia was studied by looking at the serum IL-6 levels before and soon after tocilizumab (ie, within 48 hours from the first infusion), in survivors and nonsurvivors. The serum levels of IL-6 were significantly higher in patients dying than in survivors, thus supporting the early dosage (ie, by 24-48 hours from tocilizumab infusion) of IL-6 as a useful biomarker for subsequent therapeutic choice.

2 | MATERIALS AND METHODS

Twenty-four patients affected by COVID-19 pneumonia were included in this study (Table 1). All these patients underwent tocilizumab 8 mg/kg intravenously for COVID-19 pneumonia and all of them were tested for serum IL-6 24 to 48 hours before and within 48 hours after tocilizumab infusion. Only one of them received two tocilizumab infusions 2 days apart.

Serum IL-6 was measured by CE_IVD electrochemiluminescence immunoassay (Elecsys IL-6, Cobas, physiological range <7 pg/mL).[7]

Variables were reported as mean and standard deviation or median and interquartile range (IQR), as appropriate, or frequency rates and percentages if categorical; consequently, comparisons between survivors and nonsurvivors were made by parametric tests (*t* test for two independent samples) or no parametric tests (Mann-Whitney test) for continuous variables. Proportions were compared by χ^2 test or Fisher's exact test. For unadjusted comparisons, a two-sided α of less than .05 was considered statistically significant. A receiver operating characteristic curve (ROC curve) was made to

TABLE 1 Main comparisons between groups at hospital admission

Feature	Survivors (N = 18)	Nonsurvivors (N = 6)	*P* value
Age, y	65.8 ± 8.2	68.8 ± 9.4	.45
Gender, male, %	15 (83.3)	4 (66.7)	.1
Days from onset to tocilizumab	8.5 ± 3.6	7.7 ± 4.1	.65
Weight, kg	84.1 ± 11.7	88.7 ± 15.1	.47
Hypertension, %	10 (55.6)	4 (66.7)	1.0
Charlson's index ≥ 2, %	3 (16.7)	0	.55
Antivirals[a], %	18 (100)	6 (100)	-
Antimalarials[b], %	17 (94.4)	6 (100)	1.0
Glucocorticoids[c], %	5 (27.8)	3 (50)	.36
LMWH (%)	13 (72.2)	4 (66.7)	1.0
Antibiotics[d], %	14 (77.8)	5 (83.3)	1.0
WBC count, cells/μL	6221.2 ± 2435.1	6761.7 ± 4395.9	.71
Neutrophil count, cells/μL	5332.3 ± 2524.3	5623.3 ± 4190.8	.85
Lymphocytes, cells/μL	782.3 ± 355.9	711.7 ± 300.2	.68
Neutrophil/lymphocyte ratio	8.6 ± 5.4	10.2 ± 9.8	.72
Platelet count, cells/μL	174 187.5 ± 56 520.5	179 500 ± 51 960.6	.84
CRP, mg/L	145.3 ± 93.5	176 ± 95.6	.49
Procalcitonin, ng/mL	0.14 (0.07-0.28)	0.28 (0.07-0.75)	.45
LDH, IU/L	726.6 ± 361.2	707 ± 299.5	.91
IL-6, pg/mL	63.5 (50.7-140)	171 (30.5-626.5)	.41

Abbreviations: CRP, C-reactive protein; IL-6, interleukin-6; LDH, lactate dehydrogenase; LMWH, low molecular weight heparin; WBC, white blood cells.

[a]Lopinavir/ritonavir (L/R) or darunavir/cobicistat (D/C); remdesivir as second- or third-line treatment.

[b]Hydroxychloroquine or chloroquine.

[c]Glucocorticoids were always administered intravenously at the dose of 1 mg/kg of methylprednisolone in the first 2 days, then steroids were tapered and finally suspended in 7 days.

[d]As prophylactic treatment, before tocilizumab therapy.

identify the optimal cut-off value for serum IL-6 post-tocilizumab that was able to distinguish survivors and nonsurvivors. Ethical approval for this study was given by "Comitato Etico Unico Regionale (CEUR)," with the following registration number: CEUR-2020-Os-102. The study was conducted in accordance with the ethical principles of the Helsinki Declaration. Patients' consent for using data for research purpose was obtained at the time of hospital admission.

3 | RESULTS

Eighteen patients recovered and were discharged, while six patients died. There were no differences between the two groups regarding sex, age, weight, time to tocilizumab infusion from onset, Charlson's index, and other clinical and laboratory baseline features as reported in Table 1.

Notably, there was no difference between the two groups as regards baseline IL-6 levels ($P = .41$) (Table 1), while 24 to 48 hours post-tocilizumab, IL-6 serum levels were significantly higher in nonsurvivors than in survivors (2398.5 [430.5-9372] vs 290.5 [58.5-1305.5] pg/mL, $P = .022$). In addition, the C-reactive protein (CRP) level was significantly higher in nonsurvivors than in survivors (114.5 ± 83.6 vs 54.5 ± 34.9 pg/mL, $P = .04$), while procalcitonin did not differ (0.69 [0.36-1.13] vs 0.07 [0.04-0.92] ng/mL, $P = .21$). Lactate dehydrogenase, total white blood cell count, lymphocyte count, and platelet count did not differ between survivors and nonsurvivors early after tocilizumab (data not shown). The course of serum IL-6, CRP, and procalcitonin before and after tocilizumab in survivors and nonsurvivors is depicted in Figure 1.

Serum IL-6 post-tocilizumab showed a good predictive ability to discriminate survivors from nonsurvivors (area under the curve, 0.815; 95% confidence interval, 0.63-0.99, $P = .02$), the value of 442.5 pg/mL being the discriminant between the two groups, with a sensitivity of 0.83 and a specificity of 0.67. The cut-off value of 336.5 pg/mL yielded a sensitivity of 1.0 and a specificity of 0.56. The only patient who received two subsequent tocilizumab infusions recovered from COVID-19 and showed the following values of serum IL-6: 130 pg/mL before tocilizumab, 433 pg/mL 24 hours after, and 51 pg/mL after 1 week.

4 | DISCUSSION

Recent data suggest that severe COVID-19 causes cytokine release storm and it is associated with worse clinical outcomes.[10] IL-6 plays a pivotal role in this clinical scenario. In fact, in COVID-19 patients treated with tocilizumab, IL-6 levels are significantly elevated, which are supportive of the cytokine storm.[11] It is plausible that blocking IL-6 resulted in the improvement of this hyperinflammatory state, especially in patients with baseline higher levels of IL-6.[7,9,12] Ongoing randomized control trials will allow for further evaluation of this promising therapy. It is known that following initiation of tocilizumab, there is an elevation in the IL-6 levels due to saturation of the IL-6 receptors by the drug.[13] The results herein reported are in line with a

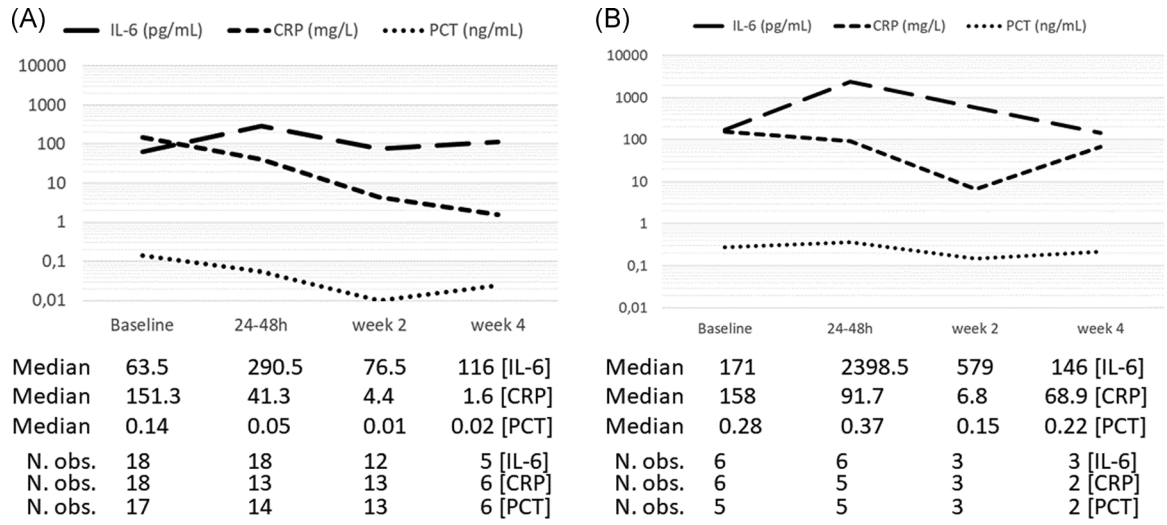

FIGURE 1 On a logarithmic scale, this figure reports the value of interleukin-6 (IL-6), C-reactive protein (CRP), and procalcitonin (PCT) over time in survivors (A) and nonsurvivors (B) as median. Below the graphs, the median and the number of available observations at each time are indicated. For completeness, the interquartile ranges [25%-75% IQR], which have been omitted in the figure, are as follows: for survivors (A), baseline IL-6 (pg/mL): 63.5 [52.2-136], IL-6 24 to 48 hours: 290.5 [76.7-1119.7], IL-6 week 2: 76.5 [47-198.75], IL-6 week 4: 116 [66-124]; baseline CRP (mg/L): 150.6 [66.5-210], CRP 24 to 48 hours: 41.3 [26.9-63.8], CRP week 2: 4.4 [1.5-6.4], CRP week 4: 1.6 [0.2-5.1]; baseline PCT (ng/mL): 0.14 [0.08-0.28], PCT 24 to 48 hours: 0.06 [0.04-0.09], PCT week 2: 0 [0-0.04], PCT week 4: 0.03 [0-0.04]. For nonsurvivors (B), baseline IL-6 (pg/mL): 171 [51-523.5], IL-6 24 to 48 hours: 2398.5 [594-6819.7], IL-6 week 2: 579 [338-820], IL-6 week 4: 146 [80-212]; baseline CRP (mg/L): 158 [116-255.5], CRP 24 to 48 hours: 91.7 [54.5-116.5], CRP week 2: 6.8 [3.9-160.6], CRP week 4: 68.9 [60.9-77]; baseline PCT (ng/mL): 0.28 [0.09-0.46], PCT 24 to 48 hours: 0.37 [0.36-1.01], PCT week 2: 0.15 [0.09-0.63], PCT week 4: 0.22 [0.14-0.29]

recent published individual patient data systematic review that summarized the baseline characteristics and clinical outcomes of COVID-19 patients who received tocilizumab.[11] The increase of IL-6, as well as the dramatic changes of other laboratory features, seem to characterize the subset of patients carrying the highest risk of death.[9,12]

In addition, the present study preliminarily demonstrated, with an appropriate follow-up and methodology,[7] that this early elevation is significantly higher in patients showing the worst outcome than in patients who recovered. Luo et al[14] previously reported a similar clinical observation in a smaller cohort of patients, in which all the three patients who died showed increasing levels of serum IL-6 after tocilizumab until death. It may be argued that in older people there is a dysregulated innate immunity and damage from too much IL-6,[15] even if the virus is killed. However, nonsurvivors were not older than survivors in this study, as well as in the study by Luo et al.[15] CRP values showed similar differences between survivors and nonsurvivors as seen for IL-6, even if less significant. Nonsurvivors showed a less clear reduction in CRP levels, and interestingly, procalcitonin appeared not to decrease after tocilizumab. Tocilizumab increases the risk of secondary infections, as other biological agents do,[16] and it could mask infections by inhibiting CRP production.[17] Importantly, procalcitonin, as well as white blood cell count, did not differ between the two groups, thus the higher levels of IL-6 or CRP in nonsurvivors were unlikely secondary to a superimposed sepsis, while they probably mirror a higher hyperinflammatory state, and may support the hypothesis of an intrinsically more resistant disease in a subset of patients.[7] In fact, in an autoinflammatory disease such as Adult Onset Still's Disease, that similarly shows high levels of IL-6 and IL-1, procalcitonin can increase in the absence of any infection.[18] The absence of differences in the baseline clinical features and in the therapeutic approach between the two groups further support this observation. Furthermore, patients at a higher risk of death may produce the highest levels of IL-6, and tocilizumab, by rapidly saturating IL-6 receptors, may reveal the greatest amount of circulating IL-6 in those patients. Thus, monitoring IL-6 serum levels soon after tocilizumab may be of major value in COVID-19 pneumonia evolving into cytokine storm syndrome, as this feature could support clinicians in the difficult choice of continuing to target IL-6 soon after the first tocilizumab administration. Notably, transient elevated IL-6 levels have been implicated in Chimeric Antigen Receptor (CAR) neurotoxicity, which can occur independently from Cytokine Release Syndrome (CRS).[19] Thus, a second infusion of tocilizumab might be hazardous, while other strategies targeting IL-6, which are currently under evaluation in clinical trials,[20] might be more suitable. Siltuximab is a monoclonal antibody directly targeting IL-6 and the siltuximab-IL-6 complex is unlikely to cross the blood brain barrier. Thus, it may be a safer choice as second-line treatment targeting IL-6 after tocilizumab in COVID-19.[19]

The serum IL-6 cut-off value of 442.5 pg/mL appeared to best differentiate survivors and nonsurvivors. The open question whether a second infusion of tocilizumab, as in the case herein reported, following the protocol for CAR T Cell-induced CRS, is better than using other drugs directly targeting IL-6, such as siltuximab, needs to be addressed.

This study has some limitations: first, the retrospective nature, secondly, the absence of corrections for confounding factors and the small number of patients included; therefore, it needs confirmation by larger studies, and a more precise definition of the cut-off value of serum IL-6.

To conclude, though preliminary, this study may provide a useful indication for clinicians who are facing the most serious complication of COVID-19. Repeated measurement of serum level of IL-6 early after tocilizumab may identify patients carrying the highest risk of death and possibly support the choice of further indirectly or directly targeting IL-6 to deeper reduce the hyperinflammatory state. Randomized studies by stratifying patients according to different levels of inflammatory state would be more insightful regarding the therapeutic effect and the best schedule of tocilizumab in this setting.

CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest.

AUTHOR CONTRIBUTIONS

L.Q., C.T., A.S. conceived the study. A.S., D.P., M.P., M.F. collected the data. All the authors analyzed the data. L.Q. wrote the manuscript. All the authors revised the manuscript and approved the final version.

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How to cite this article: Quartuccio L, Sonaglia A, Pecori D, et al. Higher levels of IL-6 early after tocilizumab distinguish survivors from nonsurvivors in COVID-19 pneumonia: A possible indication for deeper targeting of IL-6. *J Med Virol*. 2020;1–5. https://doi.org/10.1002/jmv.26149