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IL-6 serum levels predict severity and response to Tocilizumab in COVID-19: an observational study

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Corresponding Author:	Jose Maria Galvan-Roman, MD Hospital Universitario de la Princesa Madrid, Madrid SPAIN
First Author:	Jose Maria Galvan-Roman, MD
Order of Authors:	Jose Maria Galvan-Roman, MD
	Sebastián de la Cruz Rodríguez-García
	Emilia Roy-Vallejo
	Ana Marcos-Jiménez
	Santiago Sánchez-Alonso
	Carlos Fernández-Díaz
	Ana Alcaraz-Serna
	Tamara Mateu-Albero
	Pablo Rodríguez-Cortes
	Ildefonso Sánchez-Cerrillo
	Laura Esparcia
	Pedro Martínez-Fleta
	Celia López-Sanz
	Ligia Gabrie
	Luciana del Campo Guerola
	Carmen Suarez
	Julio Ancochea
	Alfonso Canabal
	Patricia Albert
	Diego Anibal Rodríguez-Serrano
	Juan Mariano Aguilar
	Carmen del Arco
	Ignacio de los Santos
	Lucio García-Fraile
	Rafael de la Camara
	José María Serra
	Esther Ramírez
	Tamara Alonso

	Pedro Landete
	Joan B Soriano
	Enrique Martín-Gayo
	Arturo Fraile Torres
	Nelly Daniela Zurita Cruz
	Rosario García-Vicuña
	Laura Cardeñoso
	Francisco Sánchez-Madrid
	Arantzazu Alfranca
	Cecilia Muñoz-Calleja
	Isidoro González-Álvaro
Manuscript Region of Origin:	SPAIN
Abstract:	Background COVID-19 patients can develop a cytokine release syndrome that eventually leads to acute respiratory distress syndrome (ARDS) requiring invasive mechanical ventilation (IMV). Since interleukin-6 (IL-6) is a relevant cytokine in ARDS, the blockade of its receptor with Tocilizumab (TCZ) could reduce mortality and/or morbidity in severe COVID-19. Objective To determine whether baseline IL-6 serum levels can predict the need for IMV and the response to TCZ. Methods Retrospective observational study performed in hospitalized patients diagnosed of COVID-19. Clinical information and laboratory findings, including IL-6 levels, were collected approximately 3 and 9 days after admission to be matched with pre- and post-administration of TCZ. Multivariable logistic and linear regressions, and survival analysis were performed depending on outcomes: need for IMV, evolution of arterial oxygen tension/fraction of inspired oxygen ratio (PaO 2 /FiO 2) or mortality. Results One hundred and forty-six patients were studied, predominantly male (66%); median age was 63 years. Forty-four patients (30%) required IMV, and 58 patients (40%) received treatment with TCZ. IL-6 levels>30 pg/ml was the best predictor for IMV (OR:7.1; p<0.001). Early administration of TCZ was associated with improvement of oxygenation (PaO 2 /FiO 2) in patients with high IL-6 (p=0.048). Patients with high IL-6 not treated with TCZ showed high mortality (HR: 4.6; p=0.003), as well as those with low IL-6 treated with TCZ (HR: 3.6; p=0.016). No relevant serious adverse events were observed in TCZ-treated patients. Conclusion Baseline IL-6>30 pg/ml predicts IMV requirement in patients with COVID-19 and contributes to establish an adequate indication for TCZ administration.

Dr. Cezmi A. Akdis, MD
Dr. Zuhair K. Ballas
Co-Editors-in-Chief
Journal of Allergy & Clinical Immunology

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Madrid, September 7th, 2020

Dear Dr Akdis and Dr Ballas,

We completely agree with your comment regarding research on COVID-19 being a continuously evolving scenario. We consider that unpublished data provided in press releases from Roche (COVACTA study, NCT04320615) and Sanofi (ex-US study, NCT04327388) are relevant enough to be discussed within the manuscript. In light of these results, we understand that this is the right time to highlight the possible role that measuring IL-6 serum levels can play in selecting patients that can benefit from IL-6 blockade as derived from our results.

We believe our results are of importance for guiding clinicians and scientists to better select patients that can benefit from IL-6 blockade. Therefore, we resubmit our final revised manuscript entitled “IL-6 serum levels predict severity and response to Tocilizumab in COVID-19: an observational study” (#JACI-D-20-01110R3) by Dr Galvan-Roman et al., according to the editors' recommendations.

Editor's comments:

#1 "The revision addressed the previous concerns adequately."

Response: Thank you for recognizing our efforts fulfilling all of the reviewers' demands.

#2 "However, since then there have been new developments in the COVID area that need to be addressed. In particular, the COVACTA Phase III trial, a controlled trial, showed that anti-IL-6 failed to show statistically significant difference. The authors will need to address this issue. The editors believe that this manuscript offers additional data not in COVACTA; that is the correlation of the level of serum IL-6 with potential response. The editors that suggest that this finding be expanded further."

Response: We fully agree with this statement. The fact that the phase III clinical trials with tocilizumab and sarilumab for patients with severe COVID-19 did not reach statistical significance does not invalidate our results at all. In fact, we think that this lack of significance could be due to a non-optimal selection of patients, just as our work indicates. As we have commented in the discussion of our work, and highlighted in this new version, only patients with high levels of interleukin 6 respond to treatment with tocilizumab, not being effective for those with low baseline levels, despite presenting data on systemic inflammation. In accordance we have modified the following sections of the manuscript:

a) Abstract Conclusion in line 143: "… to establish an adequate indication for TCZ administration"
b) Capsule summary in line 177: "… and should be used to guide…"
c) Discussion in lines 450-61: "…mortality. These observations pose the question whether it is possible that severe patients with low IL-6 included in phase 3 trials with Tocilizumab and Sarilumab account for the failure to meet their primary endpoints (29, 30). Therefore, our results support the measurement of baseline IL-6 levels in hospitalized COVID-19 patients, since in those severe or critical patients with low IL-6 levels, other cytokines such as IL-1 or tumor necrosis factor (TNF) could be driving the exacerbated inflammatory response in lungs (25). Probably, this specific group could benefit from receiving other anti-inflammatory agents such as IL-1 or TNF blockers. In this regard, the need for biomarkers of response to TCZ has been recently highlighted (31) and

neither COVACTA study (Tocilizumab trial; NCT04320615) nor ex-US Sarilumab clinical trial (NCT04327388) listed increased baseline IL-6 serum levels within the inclusion criteria. Nevertheless, both clinical trials reported a decrease in duration of hospital stay in the active arm, that was statistically significant in COVACTA (29, 30).”

d) Discussion in lines 470-1: “use of TCZ was safe and it did not increase the number of serious bacterial infections. These findings are consistent with the results of both the COVACTA and Sarilumab trials (29, 30).”
e) Discussion in line 491: can instead of may
f) References in lines 639-51: As a consequence of the inclusion of the press releases of Roche and Sanofi as references 29 and 30, the previous references 29 and 30 are now 31 and 32 respectively.

#3 In particular, supplementary figure 4 should probably be in the body of the manuscript rather than in an online repository.

Response: We think that the editors have probably misinterpreted figure numbering, since there is no supplementary figure 4. In fact, we think that the Figure 4 displaying survival depending on IL-6 levels and need for TCZ is the most important figure. On the initial submission, it was panel E in figure 3. However, following the indications of reviewer 1 in the first review of the work, we gave more importance to this figure being the unique panel in figure 4. Since version R1, the importance of measuring IL-6 in order to prescribe TCZ is clearly shown in this one panel figure.

Other possibility is that editors refer to Supplementary Table 4. However, the most interesting results of this table, the effect of TCZ on PaFiO2 depending on IL-6 levels, are displayed in a more visual way at panel C of Figure 3.

All in all, we believe that the manuscript has been significantly improved as a result of the changes we have incorporated, and hope it is now suitable for publication in the Journal of Allergy and Clinical Immunology. We are convinced that our article provides very important information for physicians to guide an early and proper prescription of IL-6R antibodies in COVID-19 patients.

Thank you for your kind consideration.

Sincerely,

Jose María Galvan-Roman — Department of Internal Medicine

Isidoro González-Alvaro — Department of Rheumatology

Hospital Universitario La Princesa (ISS/IP) – Madrid (Spain)

TITLE

IL-6 serum levels predict severity and response to Tocilizumab in COVID-19: an observational study

AUTHORS

José María Galván-Román[1*] MD, Sebastián C. Rodríguez-García[2*] MD, Emilia Roy-Vallejo[1] MD, Ana Marcos-Jiménez[3] MSc, Santiago Sánchez-Alonso[3] MSc, Carlos Fernández-Díaz[2]MD, Ana Alcaraz-Serna[3] MD, Tamara Mateu-Albero[3] MSc, Pablo Rodríguez-Cortes[1] MD, Ildefonso Sánchez-Cerrillo[3] MD, Laura Esparcia[3] BSc, Pedro Martínez-Fleta[3] MSc, Celia López-Sanz[3] BSc, Ligia Gabrie[3] MD, Luciana del Campo Guerola[3] MD, Carmen Suarez[1] MD Prof, Julio Ancochea[4] MD Prof, Alfonso Canabal[5] MD/PhD, Patricia Albert[5] MD/PhD, Diego A. Rodríguez-Serrano[5] MD/PhD, Juan Mariano Aguilar[6] MD/PhD, Carmen del Arco[6] MD/PhD, Ignacio de los Santos[1] MD/PhD, Lucio García-Fraile[1] MD, Rafael de la Camara[7] MD/PhD, José María Serra[8] Pharm, Esther Ramírez[8] PharmD, Tamara Alonso[4] MD, Pedro Landete[4] MD/PhD, Joan B Soriano[4] MD/PhD, Enrique Martín-Gayo[3] PhD, Arturo Fraile Torres[9] Pharm, Nelly Daniela Zurita Cruz[9] Pharm, Rosario García-Vicuña[2] MD/PhD, Laura Cardeñoso[9]MD/PhD, Francisco Sánchez-Madrid[3] Prof, Arantzazu Alfranca[3$] MD/PhD, Cecilia Muñoz-Calleja[3$] Prof, Isidoro González-Álvaro[2$] MD/PhD on behalf of REINMUN-COVID group (see Acknowledments).

[1]Internal Medicine Service; [2]Rheumatology Service; [3]Immunology Service; [4]Pneumology Service; [5]Intensive Care Unit; [6]Emergency Service; [7]Hematology Service, [8]Hospital Pharmacy Service, [9]Microbiology Service. Hospital Universitario de la Princesa. Universidad Autónoma Madrid. Instituto de Investigación Sanitaria Princesa (IIS-IP). [10]CIBER CV.

* These authors have contributed equally

$ These authors share senior authorship

AUTHOR FOR CORRESPONDENCE

Isidoro González-Alvaro.

Rheumatology Service. Hospital Universitario de la Princesa. Instituto de Investigación Sanitaria Princesa (IIS-IP).

c/Diego de León 62, 28006 - Madrid. Spain.

e-mail: isidoro.ga@ser.es

phone: (+34) 650 17 94 41

AUTHOR'S CONTRIBUTION

JMG-R: Conceptualization, Methodology design, Data collection, Data analysis, Manuscript submission, Writing and editing all the drafts. SCR-G: Conceptualization, Methodology design, Data collection, Statistical analysis, Graphs and figures, Writing and editing all the drafts. ER-V: Conceptualization, Methodology design, Data collection, Data analysis, Writing and editing all the drafts. AM-J: Data collection, Investigation and laboratory work, Writing the original draft. SS-A: Data collection, Investigation and laboratory work, Writing the original draft. CF-D: Methodology design, Data collection, Writing and editing the original draft. AA-S: Data collection, Investigation and laboratory work, Writing and editing the original draft. TM-A: Data collection, Investigation and laboratory work, Writing and editing the original draft. PR-C: Conceptualizaction, Data collection, Methodology design. IS-C: Data collection, Investigation and laboratory work. LE: Data collection, Investigation and laboratorywork. PM-F: Data collection, Investigation and laboratory work. CL-S: Datacollection, Investigation and laboratory work. LG: Data collection, Investigation andlaboratory work. LdCG: Data collection, Investigation and laboratory work. CS: Methodology design, Review & editing the final draft. JA: Methodology design, Review &

editing the final draft. AC: Methodology design, Review & editing the final draft. PA: Data collection, Review & editing the final draft. DAR-S: Data collection, Review & editing the final draft. JMA: Data collection, Review & editing the final draft. CA: Methodology design, Review & editing the final draft. IS: Methodology design, Review & editing the final draft. LG-F: Data collection, Review & editing the final draft. RC: Methodology design, Review & editing the final draft. JMS: Data collection, Review & editing the final draft. ER: Methodology design, Review & editing the final draft. TA: Data collection, Review & editing the final draft. PL: Data collection, Review & editing the final draft. JS: Methodology design, Review & editing the final draft. EMG: Conceptualization, Methodology design, Investigation and laboratory work, Writing and editing all the drafts. AFT: Data collection, Review & editing the final draft. NDZC: Data collection, Review & editing the final draft. RG-V: Conceptualization, Methodology design, Data interpretation, Writing and editing all the drafts. LC: Methodology design, Data analysis, Writing and editing all the drafts. FS-M: Conceptualization, Methodology design, Data interpretation, Writing and editing all the drafts. AA: Conceptualization, Methodology design, Investigation and laboratory work, Data interpretation Writing and editing all the drafts. CM: Conceptualization, Methodology design, Investigation and laboratory work, Data interpretation, Writing and editing all the drafts. IG-A: Conceptualization, Methodology design, Data collection, Statistical analysis, Graphs and figures, Writing and editing all the drafts.

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None of these sponsors have had any role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

CONFLICTS OF INTEREST

IG-A reports grants from Instituto de Salud Carlos III, during the course of the study; personal fees from Lilly and Sanofi; personal fees and non-financial support from BMS; personal fees and non-financial support from Abbvie; research support, personal fees and non-financial support from Roche Laboratories; non-financial support from MSD, Pfizer and Novartis, not related to the submitted work.

RdC reports personal fees from MSD, ASTELLAS, Clinigen, Janssen, Roche and IQONE Health Care outside the submitted work.

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CF-D reports personal fees from BMS, non-financial support from Novartis, outside the submitted work.

DR-S reports personal fees from MSD, outside the submitted work.

CM-C reports competitive grants from ISCIII during the conduct of the study.

The remaining authors report no competing interests with the submitted work.

RUNNING TITLE

IL-6 levels predict response to TCZ in COVID-19

ABSTRACT

Background

COVID-19 patients can develop a cytokine release syndrome that eventually leads to acute respiratory distress syndrome (ARDS) requiring invasive mechanical ventilation (IMV). Since interleukin-6 (IL-6) is a relevant cytokine in ARDS, the blockade of its receptor with Tocilizumab (TCZ) could reduce mortality and/or morbidity in severe COVID-19.

Objective

To determine whether baseline IL-6 serum levels can predict the need for IMV and the response to TCZ.

Methods

Retrospective observational study performed in hospitalized patients diagnosed of COVID-19. Clinical information and laboratory findings, including IL-6 levels, were collected approximately 3 and 9 days after admission to be matched with pre- and post-administration of TCZ. Multivariable logistic and linear regressions, and survival analysis were performed depending on outcomes: need for IMV, evolution of arterial oxygen tension/fraction of inspired oxygen ratio (PaO_2/FiO_2) or mortality.

Results

One hundred and forty-six patients were studied, predominantly male (66%); median age was 63 years. Forty-four patients (30%) required IMV, and 58 patients (40%) received treatment with TCZ. IL-6 levels>30 pg/ml was the best predictor for IMV (OR:7.1; p<0.001). Early administration of TCZ was associated with improvement of oxygenation (PaO_2/FiO_2) in patients with high IL-6 (p=0.048). Patients with high IL-6 not treated with TCZ showed high mortality (HR: 4.6; p=0.003), as well as those with low IL-6 treated with TCZ (HR: 3.6; p=0.016). No relevant serious adverse events were observed in TCZ-treated patients.

Conclusion

Baseline IL-6>30 pg/ml predicts IMV requirement in patients with COVID-19 and contributes to establish an adequate indication for TCZ administration.

CLINICAL IMPLICATIONS

Elevated levels of circulating IL-6 predict the need for IMV in severe COVID-19 patients and may contribute to establish the indication for timely administration of TCZ, possibly reducing ICU demand.

KEYWORDS

COVID-19, Interleukin-6, Tocilizumab, Invasive Mechanical Ventilation

ABBREVIATIONS

AEMPS: Spanish Agency for Drugs and Health Devices

ARDS: acute respiratory distress syndrome

AUC: Area under curve

CAR: chimeric antigen receptor

COPD: chronic obstructive pulmonary disease

COVID-19: coronavirus disease 2019

CRP: C-reactive protein

CRS: cytokine release syndrome

IL: interleukin

IMV: invasive mechanical ventilation

IQR: interquartile range

LR+: positive likelihood ratio; LR- negative likelihood ratio

PaO_2: arterial oxygen tension

PaO_2/FiO_2: arterial oxygen tension/fraction of inspired oxygen ratio

PCT: procalcitonin

ROC: receiver operating characteristic

$SatO_2$: mean oxygen saturation

SD: standard deviation

STROBE: Strengthening the Reporting of Observational Studies in Epidemiology

TCZ: Tocilizumab

TNF: tumor necrosis factor

CAPSULE SUMMARY

Baseline IL-6 serum levels>30 pg/ml identify severe COVID-19 patients and ~~can~~ should be used to guide the intervention with IL-6R inhibitors, aiming to improve their use in an uncertain and evolving therapeutic scenario.

INTRODUCTION

The recent exponential increase in cases of severe acute respiratory syndrome caused by coronavirus 2 (SARS-CoV-2) has led the World Health Organization to declare a pandemic. The disease, known as coronavirus disease 2019 (COVID-19), has pushed national health systems to the brink of collapse, prompting national governments to impose complete population lockdowns in an attempt to slow down the dynamics of infection (1, 2)

The spectrum of COVID-19 clinical manifestations varies widely, from mild to severe cases of atypical pneumonia, some of them developing acute respiratory distress syndrome (ARDS), which often requires invasive mechanical ventilation (IMV) and is the leading cause of death. It is suggested that the severity of the respiratory disease caused by SARS-CoV-2 is largely due to an exacerbated immune response against the virus (3, 4). This response has been observed in previous respiratory virus outbreaks and can be also seen in patients treated with chimeric antigen receptor (CAR) T cell therapy (5-7). Pro-inflammatory cytokines such as interleukin (IL) 1 and IL-6 are crucial mediators of this process (3, 6). In this regard, recent studies have indicated the usefulness of IL-6 serum levels, lymphocyte count, fibrinogen or D-dimer to evaluate the development of ARDS and its mortality (8-11). However, further evidence is required to support these observations (12).

Tocilizumab (TCZ), an anti-IL-6 receptor (IL-6R) antibody, is the only drug currently licensed for the treatment of the cytokine release syndrome (CRS) associated with CAR-T cell therapy (13). Due to the virulence of the current outbreak, its use has been advised in severe cases of COVID-19 (14-16). The rationale is to curb the deleterious effects of inflammation, thereby limiting lung damage. Early promising results have prompted ongoing randomized clinical trials (RCT) (17). We aimed to explore the ability of IL-6 serum levels at baseline to predict the need for IMV and the response to TCZ in severe COVID-19 patients.

METHODS

Study design and population

This is a retrospective observational study including 146 consecutive patients with confirmed detection of SARS-CoV-2 RNA, baseline IL-6 serum level measurement and admitted to the Hospital Universitario La Princesa (HUP) with severe to critical COVID-19 (18), from February 24th to March 23rd 2020 (Flow chart in Suppl. Fig. 1).

This time frame was decided based on the fact that after March 23rd, clinicians involved in the treatment of these patients were aware of the possibility of measuring IL-6 serum levels to decide treatment initiation with TCZ (RoActemra, Roche). Furthermore, around that date Spanish facilities capable of providing IMV were overrun (Suppl. Fig. 2), thus the decision for IMV application was based on availability rather than the patients' needs. Notwithstanding, during the study period, patients were prompted to IMV when they presented a marked worsening of oxygenation (arterial oxygen tension/fraction of inspired oxygen ratio [PaO_2/FiO_2]<200), provided that their baseline conditions did not contraindicate it.

Data collection

Clinical, laboratory and therapeutic data were collected from electronic clinical records and included in an anonymized database. Baseline evaluation was performed around the third day of admission (median=3 days, interquartile range [IQR] 2 to 5). The second evaluation was obtained around the ninth day of admission (median=9 days, IQR 7-12). No significant differences at both evaluation time points were observed between patients treated and not treated with TCZ.

In addition, twenty-three serum samples from healthy donors obtained before the pandemic onset were used to determine the variability of IL-6 serum levels in baseline conditions.

SARS-CoV-2 RNA detection

Samples from nasopharyngeal and throat exudates were obtained with specific swabs as previously described (19). Then, we performed real-time RT-PCR assay targeting the E gene of SARS-CoV-2 as the first-line screening tool, with Real Time ready RNA Virus Master (Roche), followed by confirmatory testing with the assay TaqPath™ COVID-19 CE-IVD Kit RT-PCR (Applied Biosystems™), including three assays that target SARS-CoV-2 genes (Orf1ab, S gene, N gene) and one positive control assay that targets the human RNase P RPPH1 gene (20). All the procedures were performed on Applied Biosystems TM Quant Studio-5 Real-Time PCR System.

IL-6 serum level measurement

Surplus sera from laboratory routine determinations were used to assess IL-6 levels, which were retrospectively quantified in duplicate with the Human IL-6 Quantikine high sensitivity enzyme-immune assay from R&D Systems Europe Ltd. (Abingdon, UK). The intra-assay and inter-assay variability were 3% and 5%, respectively.

Tocilizumab treatment

The rationale to treat severe COVID-19 patients with TCZ was based on its previously approved indication for treating the CRS associated with CAR-T cell therapy, as well as unpublished experience in COVID-19 patients from China and Italy. Treatment required

approval by the HUP COVID-19 Committee, following the recommendations of the Spanish Agency for Drugs and Health Devices (AEMPS) (21):

- Interstitial pneumonia with severe respiratory failure (score = 2);
- Rapid respiratory worsening requiring non-invasive or invasive ventilation (score ≥ 3 on the COVID respiratory severity scale);
- Presence of extrapulmonary organ failure (shock or score ≥ 3 on the SOFA scale);
- Criteria for severe systemic inflammatory response. In adults: elevated levels of IL-6 (> 40 pg / ml); alternatively, increased levels of D-dimer (> 1500 ng / ml) or progressively increasing D-dimer.
- Patients who, according to their baseline clinical condition, would be IMV subsidiary.

Therefore, the decision to treat with TCZ was based on the AEMPS criteria, excluding IL-6>40 pg/ml, since no information about IL-6 results was available to physicians during the study period. After a preliminary analysis of data by the end of March, IL-6 measurement was included in the baseline assessment of COVID-19 patients.

The administration schedule of TCZ at the time of the study was a first intravenous infusion of 8 mg/kg (maximum 800 mg) followed by a second one after 12 hours.

Variables

To analyze whether IL-6 levels can predict disease severity, two main outcomes were considered: need for IMV and all-cause mortality.

To determine the effect of TCZ we analyzed the evolution of PaO_2/FiO_2 between both evaluation times. In 160 out of 267 evaluations arterial oxygen tension (PaO_2) was

unavailable. So, in order to avoid missing data in these relevant outcomes, this parameter was estimated from mean oxygen saturation ($SatO_2$) as proposed elsewhere (22).

IL-6 serum levels showed a heterogeneous distribution in patients and in healthy donors (Suppl. Fig. 3A). To improve its representation in figures, this variable was normalized through natural logarithmic transformation (Suppl. Fig. 3B). The procedure to determine the cut-off for high baseline IL-6 is described below.

Statistical analysis

Statistical analyses were performed using Stata 14 for Windows (Stata Corp LP, College Station, TX, USA). Quantitative variables following a non-normal distribution were represented as median and IQR and the Mann Whitney test was used to assess significant differences. Variables with a normal distribution were described by mean±standard deviation (SD) and differences between groups were assessed with Student's t-test. Qualitative variables were described as counts and proportions and $\chi 2$ or Fisher´s exact test was used for comparisons. Correlation between quantitative variables was analyzed using the Pearson correlation test. To estimate the 95% confidence interval of correlation coefficients we used the *ci2* command of Stata.

To determine whether IL-6 serum levels were able to discriminate between: i) COVID-19 patients vs. healthy donors; ii) patients requiring IMV vs. those that did not; or iii) patients treated with TCZ vs. not treated, receiver operating characteristic (ROC) analysis was performed using the roctab command. Each cut-off point was selected based on the best trade-off values between sensitivity, specificity and the percentage of patients correctly classified. Positive and negative likelihood ratios and ROC curves were also obtained.

To determine the variables associated with the need for IMV, we performed a multivariable logistic regression analysis that was first modeled by adding all the variables with a p value lower than 0.15 in the bivariable analysis, namely total lymphocyte count, D-dimer, LDH, PaO_2/FiO_2, COPD, obesity, hypertension, C-reactive protein, and IL-6 (high vs low). The final model was reached with backward stepwise removal of variables with p-value higher than 0.15, and using Wald tests to demonstrate that each model was better than its previous iteration.

Next, we performed a multivariable analysis using generalized linear models nested by patient and visit (*xtgee* command) in which the dependent variable was PaO_2/FiO_2. This approach allowed us to identify which variables influenced the evolution of PaO_2/FiO_2. The first model included all variables with a p value <0.15 in the bivariable analysis, namely hypertension, baseline radiological pattern, LDH, total lymphocyte count, baseline C-reactive protein, IMV. After that, through backward stepwise approach, we obtained the best model as described above. Then, to assess the role of IL-6 as predictor of TCZ effect on PaO_2/FiO_2, the composite variable IL-6/TCZ (low IL-6/no TCZ, low IL-6/Early TCZ, low IL-6/Late TCZ, high IL-6/no TCZ, high IL-6/Early TCZ and high IL-6/Late TCZ) was forced in the model.

Survival time was analyzed by Kaplan-Meier method with the *sts* command of Stata. Date of admission was considered the date of entry and for exit date we considered the exitus date. For those patients without the event, the last revision of the database (electronic chart or telephone call) on May 21st was used to censor their follow-up. Differences in time to death by different variables were analyzed by log-rank test.

Ethics

This study was approved by the local Research Ethics Committee (register number 4070) and

it was carried out following the ethical principles established in the Declaration of Helsinki. All included patients (or their representatives) were informed about the study and gave an oral informed consent as proposed by AEMPS due to COVID-19 emergency.

This article was written following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines taking into consideration the difficulties to obtain all needed information in the setting of the COVID-19 pandemic.

RESULTS

Demographic and clinical characteristics of the study population

One hundred and forty-six patients were included; their main demographic and clinical characteristics are shown in Table 1. Median age was 63 (IQR [54-71]; range, 30 to 86), 97 (66%) were men and 100 (69%) presented comorbidities. The most frequent were hypertension, 55 (38%); obesity, 23 (16%); diabetes mellitus, 26 (18%); and chronic obstructive pulmonary disease (COPD) 9 (6%); 19 (13%) patients had a history of malignancy. Median duration of symptoms before admission was 6 days (IQR 4-7); 36 (25%) arrived at the emergency room presenting fever (≥38ºC), with a $SatO_2$ of 91%±5%. Most individuals (121 [83%]) were admitted to the internal Medicine or Pneumology wards; however, 16 (11%) patients admitted directly to the Intensive Care Unit because of IMV requirement, and 9 (6%) to the Hematology ward because of pre-existing conditions. Additional details of patient baseline features can be found in supplementary table 1.

IL-6 serum levels and disease severity

IL-6 serum levels above 10 pg/ml discriminated COVID-19 patients from healthy donors with low accuracy (Area under ROC curve [AUC] 0.695; sensitivity [Se] 84%, specificity [Sp] 46%, positive likelihood ratio [LR+] 1.5, LR- 0.4; Suppl. Fig. 3C), probably due to their intrinsic heterogeneity (Suppl. Fig. 3A and B).

No significant correlation was found between PaO_2 and IL-6 serum levels at baseline (r= -0.09 [95% CI: -0.270 to 0.085]; p=0.299; Figure 1A), likely due to a higher oxygen supply in the most severe cases; in fact, serum IL-6 levels showed a significant negative correlation with

PaO_2/FiO_2 (Figure 1B; r= -0.38 [95% CI: -0.526 to -0.218]; p<0.001), meaning that higher levels of IL-6 at baseline were associated with lower PaO_2/FiO_2.

In this regard, forty-four patients (30%) required IMV at some point during their hospitalization. As expected, these patients showed significantly worse PaO_2/FiO_2 levels than those not requiring IMV (p<0.001; Table 2). In addition, they showed increased leukocytes, total lymphocytes, IL-6, C-reactive protein (CRP), and procalcitonin (PCT) showing a higher inflammatory status than those not requiring IMV (p≤0.001 except p=0.003 for CRP and p=0.029 for lymphocyte count; Table 2).

Furthermore, a baseline IL-6 above 30 pg/ml (henceforth high IL-6) discriminated patients requiring IMV with 68% Se and 73% Sp. AUC was 0.725 (Figure 1C; LR+ 2.5, LR- 0.4). A logistic regression model, adjusted for COPD and baseline white blood cell count, also showed that high baseline IL-6 was a predictive biomarker for IMV (OR:7.1; 95% CI: 3.0 to 16.6; Supp Table 2).

Response to Tocilizumab

Fifty-eight patients (40%) received treatment with TCZ. No significant differences between groups were observed in most sociodemographic and therapeutic variables, except for patients not treated with TCZ, which were more often obese and COPD (p=0.023 and p=0.071 respectively, Supplementary Table 3). Importantly, patients in the TCZ-treated group presented several baseline findings indicating that they suffered more severe COVID-19 disease, such as lower PaO_2/FiO_2 (p<0.001; Table 3), higher levels of serum Lactate Dehydrogenase (p<0.001), CRP (p=0.005), IL-6 levels at baseline (p=0.007), and total lymphocyte count (p=0.001). Other elevated markers in this group included Aspartate amino-transferase, ferritin, and procalcitonin (p<0.05 for all comparisons. Table 3).

Even before physicians were aware of the potential value of IL-6 serum levels as a predictor of severe disease, those patients with high IL-6 were more frequently treated with TCZ (Figure 1D; AUC 0.634; 30 pg/ml as cut-off showed Se 57%, Sp 69%, LR+ 1.9, LR- 0.7), although with less accuracy than for IMV. The median time from the beginning of symptoms to TCZ treatment was 11 days (IQR: 8-12.5). Therefore, we considered early TCZ when the treatment was applied before 11 days of disease duration and late TCZ after this cut-off.

As a consequence of IL-6R blockade with TCZ, a significant trend toward higher IL-6 serum levels after administration of the drug was observed (Figure 2A; p=0.005). However, IL-6R blockade induced a fast and significant down-regulation of CRP (Figure 2B; p<0.001) and PCT (Figure 2C; p=0.026) and a non-significant decrease of ferritin (Figure 2D) and LDH (Figure 2E). Conversely, it was associated with a significant increase of D-Dimer levels (Figure 2F; p<0.001). After an average 6 days of TCZ treatment, despite this improvement of inflammatory parameters, PaO_2/FiO_2 did not show a significant improvement in the whole population (Data not shown). Only patients with high IL-6 that underwent early TCZ treatment showed a significant PaO_2/FiO_2 increase (Figure 3A mid boxes; p=0.048). Patients with low IL-6 did not improve their PaO_2/FiO_2 after treatment with TCZ (Figure 3B). Those patients not treated with TCZ showed a heterogeneous behavior in their PaO_2/FiO_2 with a trend to improve in patients with low IL-6 (Figure 3A and B two left boxes of each panel).

Since relevant differences were observed between patients treated and not treated with TCZ, we used a multivariable analysis to determine which variables influenced the evolution of PaO_2/FiO_2 at the short-term. Baseline PaO_2/FiO_2 and radiological pattern, HTA, LDH and CRP levels and total lymphocyte blood count significantly explained variation in PaO_2/FiO_2 (Suppl Table 4). Adjusted by these confounders, the best PaO_2/FiO_2 evolution was achieved in

patients with low IL-6 not requiring TCZ treatment (Figure 3C first dot on the left; reference group in analysis showed in Suppl Table 4), likely because they were the less severe patients. Patients with low IL-6 that due to their bad evolution were prescribed with TCZ showed a significant worsening of PaO_2/FiO_2 (Figure 3C, 2nd and 3rd dots; Suppl Table 4). A similar evolution was observed in patients with high IL-6 and late TCZ treatment, whereas those with high IL-6 not treated or treated early with TCZ showed no significant differences compared with the reference group (Figure 3C; Suppl Table 4).

Mortality

Next, we were interested in the long-term evolution of patients depending on baseline IL-6 and treatment with TCZ. After a median follow-up of 61 days (IQR: 58-64) we observed 30 deaths in our sample (21%). The survival curves according to the baseline level of IL-6 and TCZ treatment are shown in Figure 4. Six patients out of 59 (10%) died in the reference group (low IL-6/no TCZ), 9 out of 28 (32%) in the low IL-6/TCZ treated group (HR 3.6 [CI 95%: 1.3 – 10.0], p=0.016), 10 out of 28 (36%) in the high IL-6/no TCZ group (HR 4.6 [CI 95%: 1.7 – 12.7], p=0.003) and 5 out of 31 (16%) in the high IL-6/TCZ treated group (HR 1.6 [CI 95%: 0.5 – 5.4], p=0.411).

Safety of Tocilizumab treatment

Regarding safety, no relevant cytopenia, hypertransaminasemia, bowel perforation or secondary bacterial infections were observed during or after treatment with TCZ for the time of the study. Ten (7%) patients had positive blood cultures; most of them in the non-TCZ treated group (7 vs 3; p=0.03).

DISCUSSION

To our knowledge, this is the first study showing that high baseline IL-6 levels predict both the need for IMV and the response to TCZ in severe patients hospitalized with COVID-19. Our results confirm the hypothesis that respiratory failure in the advanced phase of severe COVID-19 is mainly due to an exacerbated inflammatory response. These findings are in accordance with cytokine storms described in previous experiences with H5N1 influenzae virus (5) and previous coronaviruses SARS-CoV and MERS (7), as well as CAR T cell therapy (23, 24). Together, these data suggest a key role for inflammation of the small distal airways in the severity of this condition (25). Hence, approximately one-third of patients with ARDS display elevated levels of inflammatory mediators (IL-6, IL-8 and soluble TNF receptor 1, among others), increasing the prevalence of shock and mortality (26). Accordingly, our data show that IL-6 levels higher than 30 pg/ml predict the need for IMV, and correlated with other severity data. A similar threshold has been described to discriminate between mild and severe COVID-19 in Chinese patients (27).

The association of high IL-6 with a more severe disease in our population supports the use of TCZ to treat COVID-19 patients. TCZ is a humanized antibody that blocks both soluble and membrane-bound forms of IL-6 receptor. Thus, TCZ prevents ligand binding, which likely explains why IL-6 serum levels significantly increased after treatment (28). However, the TCZ-mediated blockade of IL-6R signaling led to the observed relevant decrease in circulating inflammatory mediators. Severe high IL-6 COVID-19 patients treated with TCZ showed an early respiratory improvement, represented by a moderate but significantly increased PaO_2/FiO_2 when TCZ was prescribed before 11^{th} day of symptoms and lower overall mortality, independently of other treatments or clinical factors. Furthermore, patients with severe COVID-19 and high IL-6 levels that were not treated with TCZ displayed a higher mortality.

On the other hand and of outstanding interest, patients with low IL-6 that were treated with TCZ due to a severe or critical COVID-19 did not improve and showed significantly higher mortality. These observations pose the question whether it is possible~~It is likely~~ that severe patients with low IL-6 included in phase 3 trials with Tocilizumab and Sarilumab account for the failure to meet their primary endpoints (29, 30). Therefore, our results support the measurement of baseline IL-6 levels in hospitalized COVID-19 patients, since ~~that~~ in th~~e~~ose severe or critical patients with low IL-6 levels, other cytokines ~~(~~such as IL-1 or tumor necrosis factor ~~[~~(TNF~~]~~) could be ~~responsible for~~driving the exacerbated inflammatory response in lungs (25)~~., and~~ ~~p~~Probably, this specific group could benefit ~~they should be treated with~~from receiving other anti-inflammatory agents such as IL-1 or TNF blockers. In this regard, the need for biomarkers of response to TCZ has been recently highlighted (31) and neither COVACTA study (Tocilizumab trial; NCT04320615) nor ex-US Sarilumab clinical trial (NCT04327388) listed increased baseline IL-6 serum levels within the inclusion criteria. Nevertheless, both clinical trials reported a decrease in duration of hospital stay in the active arm, that was statistically significant in COVACTA (29, 30). ~~.~~

In agreement with our data, other case series with few patients have shown that tocilizumab can improve the outcomes of COVID-19 patients with ARDS (6, 8, 31, 32); in some of these reports described the use of, lower doses of TCZ were used. In this regard, the protocol in our hospital, after these preliminary data, has evolved towards lower doses of TCZ (a single dose of TCZ 400 mg if<80 kg and 600 mg if >80 kg) administered earlier with similar efficacy (unpublished observation).

Additionally, during our study the use of TCZ was safe and it did not increase the number of serious bacterial infections. These findings are consistent with the results of both the COVACTA and Sarilumab trials (29, 30). The only unexpected observation was the rise in D-

dimer levels at the second evaluation. Possible explanations are either: i) IL-6 does not play a role in the regulation of D-dimer production; or ii) more likely, D-dimer production has a slower kinetics than CRP or other acute phase reactants, since in patients not treated with TCZ a similar rise in D-dimer levels was observed (data not shown).

Apart from the novelty and the immediate clinical utility of these findings, a drawback of our study is its retrospective and observational nature involving mainly very severe cases in the group of treatment with TCZ. A stricter selection of a control group through a propensity score strategy was unfeasible, since once the physicians were aware of IL-6 measurement they focused their efforts on treating with TCZ those patients with the highest IL-6 levels. Therefore, prospective studies should be carried out to confirm our observations.

In addition, there is some controversy about the reliability of PaO_2/FiO_2 as an outcome for improvement of lung damage, especially in patients with IMV. However, our population was a mix of patients with IMV and non-IMV, so despite the many factors that could interfere with PaO_2/FiO_2, we considered it the most objective outcome for patients' assessment.

Finally, our findings are of relevance for clinical decision making in the ongoing COVID-19 pandemic. Increased levels of circulating IL-6 predict IMV requirement in patients with severe disease, and ~~may~~ can contribute to establish the indication for timely TCZ administration. Furthermore, the improvement of respiratory parameters achieved upon treatment with TCZ may reduce IMV demand in these patients. On the other hand, in our population there was a small group of patients with severe COVID-19 and low IL-6 serum levels, which probably should have been treated with blockade of IL1 or TNF-α since their evolution with TCZ was inadequate.

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REINMUN-COVID group includes:

- Cardiology: Teresa Alvarado, Pablo Martínez, Francisco Javier de la Cuerda Llorente
- Emergency Service: Carmen del Arco, Juan Mariano Aguilar, Natalia Villalba, Mónica Negro, Elvira Contreras, Ana del Rey, Cristina Santiago, Manuel Junquera, Raquel Caminero, Francisco Javier Val, Sonia González, Marta Caño, Isabel López, Andrés von Wernitz, Bárbara Retana, Iñigo Guerra, Jorge Sorando, Lydia Chao, María José Cárdenas, Verónica Espiga, Pablo Chicharro, Pedro Rodríguez.
- Endocrinology: Iñigo Hernando Alday, Miguel Sampedro
- ENT: Jorge Prada
- Gastroenterology: Eukene Rojo Aldama, Yolanda Real, María Caldas, Sergio Casabona, Aitor Lanas-Gimeno.
- Hematology Service: Rafael de la Camara, Angela Figuera Alvárez, Beatriz Aguado.
- Hospital Pharmacy: Alberto Morell, Esther Ramírez, Amparo Ibáñez Zurriaga, María Pérez Abanades, Silvia Ruiz García, Tomás Gallego Aranda, María Ruiz, Concepción Martínez Nieto, José María Serra.
- Immunology: Francisco Sánchez-Madrid, Cecilia Muñoz-Calleja, Arantzazu Alfranca, Javier Aspa, Ana Marcos-Jiménez, Santiago Sánchez-Alonso, Ana Alcaraz-Serna, Tamara Mateu-Albero, Ildefonso Sánchez-Cerrillo, Laura Esparcia, Pedro Martínez-Fleta, Celia López-Sanz, Ligia Gabrie, Luciana del Campo Guerola, Elena Fernández, Mª José Calzada, Reyes Tejedor.

- Intensive Care Unit: Alfonso Canabal, Patricia Albert, Diego A. Rodriguez-Serrano, Judit Iglesias, Fernando Suarez, Juan Antonio Sánchez, Beatriz Abad.
- Internal Medicine-Infectious Diseases: Carmen Suarez, Ignacio de los Santos, José María Galván-Roman, Emilia Roy, Pablo Rodríguez-Cortes, Lucio García-Fraile, Jesus Sanz, Eduardo Sanchez, Fernando Moldenhauer, Pedro Casado, Jose Curbelo, Angela Gutierrez, Azucena Bautista, Nuria Ruiz Giménez, Angelica Fernandez, Pedro Parra, Berta Moyano, Ana Barrios, Diego Real de Asua, Beatriz Sanchez, Carmen Saez, Marianela Ciudad.
- Medical Biology: Desiré Navas
- Microbiology: Laura Cardeñoso Domingo, María del Carmen Cuevas Torresano, Diego Domingo García, Teresa Alarcón Cavero, Alicia García Blanco, Alexandra Martín Ramírez, María Auxiliadora Semiglia Chong, Ainhoa Gutiérrez Cobos, Nelly Daniela Zurita Cruz, Arturo Manuel Fraile Torres.
- Nephrology: Carmen Sanchez-Gonzalez, Antonio Fernádez Perpén
- Neurology: Carolina Díaz Pérez
- Pneumology: Julio Ancochea, Tamara Alonso, Pedro Landete, Joan Soriano, Carolina Cisneros, Elena García Castillo, Francisco Javier García Pérez, Rosa María Girón, Celeste Marcos, Enrique Zamora.
- Radiology: Patricia García García
- Rheumatology: Santos Castañeda, Rosario García-Vicuña, Isidoro González-Álvaro, Sebastián Rodríguez-García, Carlos Fernández-Díaz, Irene Llorente Cubas, Eva G. Tomero, Noelia García Castañeda, Ana Mª Ortiz, Cristina Valero, Miren Uriarte, Nuria Montes.

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652

653

654 Table 1. Baseline clinical characteristics and laboratory findings of the study population

	Study Population (n=146)
Age	63 (54-71)
Male sex	97 (66)
Comorbidities	100(69)
Duration of symptoms at admission (days)	6 (4-7)
Baseline PaO2/FiO2	215 (112-310)
Treatment during hospitalization	
Hydroxychloroquine	137 (96)
Lopinavir/Ritonavir	119 (83)
Azithromycin	82 (57)
Interferon-β	7 (5)
Glucocorticoids	85 (59)
Methylprednisolone bolus	61 (42)
Laboratory Findings	
White Blood Count (10^3/mm^3)	7.64 (5.25–10.68)
Lymphocyte Count (10^3/mm^3)	0.83 (0.60–11.7)
Creatinine. mg/dl	0.86 (0.70-1.10)
LDH (U/L)	341 (256-461)
CK (U/L)	72 (48-155)
Serum IL-6 (pg/ml)	21.36 (7.53-54.21)
Ferritin (ng/ml)	1598 (830-2305)
CRP (mg/dL)	11.55 (5.16-22.53)
PCT (ng/ml)	0.15 (0.10-0.35)
D-dimer (mg/ml)	0.75 (0.48–1.48)

655 All categorical variables are expressed as number (%) and quantitative variables as
656 median (p25-p75). PaO_2/FiO_2: arterial oxygen tension - fraction of inspired oxygen
657 ratio; LDH: Lactate Dehydrogenase; IL-6: Interleukin-6; CRP: C-reactive protein; PCT:
658 Procalcitonin.
659

Table 2. Baseline clinical characteristics of groups requiring vs not requiring Invasive Mechanical Ventilation.

	Invasive Mechanical Ventilation		
	Required (n=44)	Not-required (n=102)	p value
Age	63.5 (56.5-72)	62 (54-71)	0.517
Male sex	32 (73)	65 (64)	0.291
Comorbidities	30 (68)	70 (69)	0.893
Duration of symptoms at admission (days)	5 (5-7)	7 (4-8)	0.265
Baseline PaO_2/FiO_2	125.5 (75-207)	247 (172-348)	<0.001
Treatment during hospitalization			
Hydroxychloroquine	38 (86)	99 (100)	<0.001
Lopinavir/Ritonavir	38 (86)	81 (82)	0.502
Azithromycin	24 (55)	58 (59)	0.652
Interferon-β	3 (7)	4 (4)	0.676
Glucocorticoids	27 (61)	58 (59)	0.755
Methylprednisolone bolus	21 (48)	40 (40)	0.414
Laboratory Findings			
White Blood Count ($10^3/mm^3$)	9.39 (6.59-13.31)	6.93 (5.13-8.78)	<0.001
Lymphocyte Count ($10^3/mm^3$)	0.74 (0.58-1.08)	0.87 (0.62-1.26)	0.029
Creatinine (mg/dl)	0.99 (0.71-1.20)	0.85 (0.72-1.1)	0.398
LDH (U/L)	413 (315-496)	302 (224-443)	0.001
CK (U/L)	67 (39.50-167.50)	94 (59-140)	0.617
Serum IL-6 (pg/ml)	49.20 (17.28-103.57)	16.08 (6.09-42.03)	<0.001
Ferritin (ng/ml)	1665 (602-2765)	1573 (1012-2300)	0.832
CRP (mg/dL)	17.09 (7.69-28.98)	10.13 (4.83-18.48)	0.003
PCT (ng/ml)	0.29 (0.14-0.46)	0.13 (0.08-0.26)	0.001
D-dimer (mg/ml)	0.92 (0.56-2.31)	0.71 (0.48-1.19)	0.058

All categorical variables are expressed as number (%) and quantitative variables as median (p25-p75). PaO_2/FiO_2: arterial oxygen tension - fraction of inspired oxygen ratio; LDH: Lactate Dehydrogenase; IL-6: Interleukin-6; CRP: C-reactive protein; PCT: Procalcitonin.

667 **Table 3.** Baseline clinical characteristics of groups treated vs not treated with Tocilizumab.

	Tocilizumab		
	Treated (n=58)	Not treated (n=88)	p value
Age	61 (54-70)	64 (54-72)	0.288
Male sex	40 (69)	57 (65)	0.600
Comorbidities	35 (61)	64 (73)	0.124
Duration of symptoms at admission (days)	6 (5-7)	7 (4-8)	0.612
Baseline PaO_2/FiO_2	137 (88-232)	248 (183-348)	<0.001
Treatment during hospitalization			
Hydroxychloroquine	53 (93)	84 (98)	0.171
Lopinavir/Ritonavir	51 (89)	68 (79)	0.103
Azithromycin	33 (58)	49 (57)	0.913
Interferon-β	2 (4)	5 (6)	0.532
Glucocorticoids	38 (67)	47 (55)	0.152
Methylprednisolone bolus	31 (54)	30 (35)	0.018
Laboratory Findings			
White Blood Count ($10^3/mm^3$)	7.99 (5.17-11.85)	7.52 (5.4–10.36)	0.527
Lymphocyte Count ($10^3/mm^3$)	0.74 (0.52–0.997)	0.93 (0.66–1.47)	0.001
Creatinine (mg/dl)	0.83 (0.70-1.05)	0.90 (0.72-1.14)	0.177
LDH (U/L)	425 (302-510)	293.5 (221-388)	<0.001
CK (U/L)	69 (38-270)	75.5 (49-125)	0.785
Serum IL-6 (pg/ml)	41.85 (12.37-71.95)	16.25 (6.27-44.95)	0.007
Ferritin (ng/ml)	1888 (1152-2844)	1461 (471-1861)	0.038
CRP (mg/dL)	13.73 (8.75-27.08)	9.09 (4.78-19.31)	0.005
PCT (ng/ml)	0.25 (0.13-0.36)	0.14 (0.1-0.3)	0.045
D-dimer (mg/ml)	0.75 (0.48–1.48)	0.71 (0.53–1.22)	0.491

668 All categorical variables are expressed as number (%) and quantitative variables as median (p25-p75).
669 PaO_2/FiO_2: arterial oxygen tension - fraction of inspired oxygen ratio; LDH: Lactate Dehydrogenase; IL-
670 6: Interleukin-6; CRP: C-reactive protein; PCT: Procalcitonin.
671

672 FIGURE LEGENDS

673 **Figure 1.** IL-6 serum levels predict disease severity and tocilizumab (TCZ) use. (A) Correlation
674 between log-transformed IL-6 serum levels and arterial oxygen tension (PaO2). (B) Correlation
675 between log-transformed IL-6 serum levels and arterial oxygen tension - fraction of inspired
676 oxygen ratio (PaO2/FiO2). Data in panels A and B are shown as dot-plot and their fitted linear
677 prediction with 95% confidence interval (transparent grey shadow) estimated using the
678 twoway command of Stata with the lfitci option. (C) ROC curve showing the ability of log-
679 transformed IL-6 serum levels to discriminate between patients requiring vs not-requiring
680 invasive mechanical ventilation (IMV). (D) ROC curve for the ability of log-transformed IL-6
681 serum levels to discriminate between TCZ treated and non-treated patients. The best cut-off
682 for discrimination of patients requiring IMV (panel C) or TCZ treatment (panel D) was 30
683 pg/ml.

684 **Figure 2**. Response of laboratory parameters to Tocilizumab (TCZ) treatment. (A) Differences
685 in log-transformed IL-6 serum levels, (B) C-Reactive Protein and (C) Procalcitonin, (D) Ferritin,
686 (E) Lactate Dehydrogenase (LDH), and (F) D-Dimer. Data are presented as the interquartile
687 range (p75 upper edge, p25 lower edge, p50 midline), p95 (line above the box), and p5 (line
688 below the box) of levels for each parameter before (grey boxes) and after (white boxes)
689 treatment with TCZ.

690 **Figure 3**. Change of PaO_2/FiO_2 in COVID-19 patients treated early (before 11 day of symptoms
691 onset) or late with Tocilizumab and not-treated. (A) Patients with high baseline IL-6 (cut-off
692 30 pg/ml). (B) Subjects with low baseline IL-6 serum levels. Data in A and B are shown as the
693 interquartile range (p75 upper edge, p25 lower edge, p50 midline), p95 (line above the box),
694 and p5 (line below the box) before (grey boxes) and after (white boxes) treatment with TCZ. In
695 non-treated patients PRE and POST mean first and second evaluation, respectively. Statistical

696 significance was determined with the Mann-Whitney test. (C) The graph represents the
697 predicted mean (dots) with 95% confidence intervals (bars) of PaO_2/FiO_2 according to baseline
698 IL-6 levels and early or late TCZ treatment. Data were obtained with the command
699 marginsplot of Stata, after adjustment by baseline PaO_2/FiO_2 and radiological pattern,
700 Hypertension, Lactic dehidrogenase and C-reactive protein levels, lymphocyte blood count
701 and need for IMV, according to the multivariable analysis displayed in Supplementary Table 2
702 (see Methods for further information).
703 **Figure 4**. Survival curves of COVID-19 patients grouped according to baseline IL-6 levels and
704 TCZ treatment. Statistical significance was established with log-rank test.
705
706
707

TITLE

IL-6 serum levels predict severity and response to Tocilizumab in COVID-19: an observational study

AUTHORS

José María Galván-Román[1*] MD, Sebastián C. Rodríguez-García[2*] MD, Emilia Roy-Vallejo[1] MD, Ana Marcos-Jiménez[3] MSc, Santiago Sánchez-Alonso[3] MSc, Carlos Fernández-Díaz[2]MD, Ana Alcaraz-Serna[3] MD, Tamara Mateu-Albero[3] MSc, Pablo Rodríguez-Cortes[1] MD, Ildefonso Sánchez-Cerrillo[3] MD, Laura Esparcia[3] BSc, Pedro Martínez-Fleta[3] MSc, Celia López-Sanz[3] BSc, Ligia Gabrie[3] MD, Luciana del Campo Guerola[3] MD, Carmen Suarez[1] MD Prof, Julio Ancochea[4] MD Prof, Alfonso Canabal[5] MD/PhD, Patricia Albert[5] MD/PhD, Diego A. Rodríguez-Serrano[5] MD/PhD, Juan Mariano Aguilar[6] MD/PhD, Carmen del Arco[6] MD/PhD, Ignacio de los Santos[1] MD/PhD, Lucio García-Fraile[1] MD, Rafael de la Camara[7] MD/PhD, José María Serra[8] Pharm, Esther Ramírez[8] PharmD, Tamara Alonso[4] MD, Pedro Landete[4] MD/PhD, Joan B Soriano[4] MD/PhD, Enrique Martín-Gayo[3] PhD, Arturo Fraile Torres[9] Pharm, Nelly Daniela Zurita Cruz[9] Pharm, Rosario García-Vicuña[2] MD/PhD, Laura Cardeñoso[9]MD/PhD, Francisco Sánchez-Madrid[3] Prof, Arantzazu Alfranca[3$] MD/PhD, Cecilia Muñoz-Calleja[3$] Prof, Isidoro González-Álvaro[2$] MD/PhD on behalf of REINMUN-COVID group (see Acknowledments).

[1]Internal Medicine Service; [2]Rheumatology Service; [3]Immunology Service; [4]Pneumology Service; [5]Intensive Care Unit; [6]Emergency Service; [7]Hematology Service, [8]Hospital Pharmacy Service, [9]Microbiology Service. Hospital Universitario de la Princesa. Universidad Autónoma Madrid. Instituto de Investigación Sanitaria Princesa (IIS-IP). [10]CIBER CV.

* These authors have contributed equally

$ These authors share senior authorship

AUTHOR FOR CORRESPONDENCE

Isidoro González-Alvaro.

Rheumatology Service. Hospital Universitario de la Princesa. Instituto de Investigación Sanitaria Princesa (IIS-IP).

c/Diego de León 62, 28006 - Madrid. Spain.

e-mail: isidoro.ga@ser.es

phone: (+34) 650 17 94 41

AUTHOR'S CONTRIBUTION

JMG-R: Conceptualization, Methodology design, Data collection, Data analysis, Manuscript submission, Writing and editing all the drafts. SCR-G: Conceptualization, Methodology design, Data collection, Statistical analysis, Graphs and figures, Writing and editing all the drafts. ER-V: Conceptualization, Methodology design, Data collection, Data analysis, Writing and editing all the drafts. AM-J: Data collection, Investigation and laboratory work, Writing the original draft. SS-A: Data collection, Investigation and laboratory work, Writing the original draft. CF-D: Methodology design, Data collection, Writing and editing the original draft. AA-S: Data collection, Investigation and laboratory work, Writing and editing the original draft. TM-A: Data collection, Investigation and laboratory work, Writing and editing the original draft. PR-C: Conceptualizaction, Data collection, Methodology design. IS-C: Data collection, Investigation and laboratory work. LE: Data collection, Investigation and laboratorywork. PM-F: Data collection, Investigation and laboratory work. CL-S: Datacollection, Investigation and laboratory work. LG: Data collection, Investigation andlaboratory work. LdCG: Data collection, Investigation and laboratory work. CS: Methodology design, Review & editing the final draft. JA: Methodology design, Review &

editing the final draft. AC: Methodology design, Review & editing the final draft. PA: Data collection, Review & editing the final draft. DAR-S: Data collection, Review & editing the final draft. JMA: Data collection, Review & editing the final draft. CA: Methodology design, Review & editing the final draft. IS: Methodology design, Review & editing the final draft. LG-F: Data collection, Review & editing the final draft. RC: Methodology design, Review & editing the final draft. JMS: Data collection, Review & editing the final draft. ER: Methodology design, Review & editing the final draft. TA: Data collection, Review & editing the final draft. PL: Data collection, Review & editing the final draft. JS: Methodology design, Review & editing the final draft. EMG: Conceptualization, Methodology design, Investigation and laboratory work, Writing and editing all the drafts. AFT: Data collection, Review & editing the final draft. NDZC: Data collection, Review & editing the final draft. RG-V: Conceptualization, Methodology design, Data interpretation, Writing and editing all the drafts. LC: Methodology design, Data analysis, Writing and editing all the drafts. FS-M: Conceptualization, Methodology design, Data interpretation, Writing and editing all the drafts. AA: Conceptualization, Methodology design, Investigation and laboratory work, Data interpretation Writing and editing all the drafts. CM: Conceptualization, Methodology design, Investigation and laboratory work, Data interpretation, Writing and editing all the drafts. IG-A: Conceptualization, Methodology design, Data collection, Statistical analysis, Graphs and figures, Writing and editing all the drafts.

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RUNNING TITLE

IL-6 levels predict response to TCZ in COVID-19

ABSTRACT

Background

COVID-19 patients can develop a cytokine release syndrome that eventually leads to acute respiratory distress syndrome (ARDS) requiring invasive mechanical ventilation (IMV). Since interleukin-6 (IL-6) is a relevant cytokine in ARDS, the blockade of its receptor with Tocilizumab (TCZ) could reduce mortality and/or morbidity in severe COVID-19.

Objective

To determine whether baseline IL-6 serum levels can predict the need for IMV and the response to TCZ.

Methods

Retrospective observational study performed in hospitalized patients diagnosed of COVID-19. Clinical information and laboratory findings, including IL-6 levels, were collected approximately 3 and 9 days after admission to be matched with pre- and post-administration of TCZ. Multivariable logistic and linear regressions, and survival analysis were performed depending on outcomes: need for IMV, evolution of arterial oxygen tension/fraction of inspired oxygen ratio (PaO_2/FiO_2) or mortality.

Results

One hundred and forty-six patients were studied, predominantly male (66%); median age was 63 years. Forty-four patients (30%) required IMV, and 58 patients (40%) received treatment with TCZ. IL-6 levels>30 pg/ml was the best predictor for IMV (OR:7.1; p<0.001). Early administration of TCZ was associated with improvement of oxygenation (PaO_2/FiO_2) in patients with high IL-6 (p=0.048). Patients with high IL-6 not treated with TCZ showed high mortality (HR: 4.6; p=0.003), as well as those with low IL-6 treated with TCZ (HR: 3.6; p=0.016). No relevant serious adverse events were observed in TCZ-treated patients.

Conclusion

Baseline IL-6>30 pg/ml predicts IMV requirement in patients with COVID-19 and contributes to establish an adequate indication for TCZ administration.

CLINICAL IMPLICATIONS

Elevated levels of circulating IL-6 predict the need for IMV in severe COVID-19 patients and may contribute to establish the indication for timely administration of TCZ, possibly reducing ICU demand.

KEYWORDS

COVID-19, Interleukin-6, Tocilizumab, Invasive Mechanical Ventilation

ABBREVIATIONS

AEMPS: Spanish Agency for Drugs and Health Devices

ARDS: acute respiratory distress syndrome

AUC: Area under curve

CAR: chimeric antigen receptor

COPD: chronic obstructive pulmonary disease

COVID-19: coronavirus disease 2019

CRP: C-reactive protein

CRS: cytokine release syndrome

IL: interleukin

IMV: invasive mechanical ventilation

IQR: interquartile range

LR+: positive likelihood ratio; LR- negative likelihood ratio

PaO_2: arterial oxygen tension

PaO_2/FiO_2: arterial oxygen tension/fraction of inspired oxygen ratio

PCT: procalcitonin

ROC: receiver operating characteristic

$SatO_2$: mean oxygen saturation

SD: standard deviation

STROBE: Strengthening the Reporting of Observational Studies in Epidemiology

TCZ: Tocilizumab

TNF: tumor necrosis factor

CAPSULE SUMMARY

Baseline IL-6 serum levels>30 pg/ml identify severe COVID-19 patients and should be used to guide the intervention with IL-6R inhibitors, aiming to improve their use in an uncertain and evolving therapeutic scenario.

INTRODUCTION

The recent exponential increase in cases of severe acute respiratory syndrome caused by coronavirus 2 (SARS-CoV-2) has led the World Health Organization to declare a pandemic. The disease, known as coronavirus disease 2019 (COVID-19), has pushed national health systems to the brink of collapse, prompting national governments to impose complete population lockdowns in an attempt to slow down the dynamics of infection (1, 2)

The spectrum of COVID-19 clinical manifestations varies widely, from mild to severe cases of atypical pneumonia, some of them developing acute respiratory distress syndrome (ARDS), which often requires invasive mechanical ventilation (IMV) and is the leading cause of death. It is suggested that the severity of the respiratory disease caused by SARS-CoV-2 is largely due to an exacerbated immune response against the virus (3, 4). This response has been observed in previous respiratory virus outbreaks and can be also seen in patients treated with chimeric antigen receptor (CAR) T cell therapy (5-7). Pro-inflammatory cytokines such as interleukin (IL) 1 and IL-6 are crucial mediators of this process (3, 6). In this regard, recent studies have indicated the usefulness of IL-6 serum levels, lymphocyte count, fibrinogen or D-dimer to evaluate the development of ARDS and its mortality (8-11). However, further evidence is required to support these observations (12).

Tocilizumab (TCZ), an anti-IL-6 receptor (IL-6R) antibody, is the only drug currently licensed for the treatment of the cytokine release syndrome (CRS) associated with CAR-T cell therapy (13). Due to the virulence of the current outbreak, its use has been advised in severe cases of COVID-19 (14-16). The rationale is to curb the deleterious effects of inflammation, thereby limiting lung damage. Early promising results have prompted ongoing randomized clinical trials (RCT) (17). We aimed to explore the ability of IL-6 serum levels at baseline to predict the need for IMV and the response to TCZ in severe COVID-19 patients.

METHODS

Study design and population

This is a retrospective observational study including 146 consecutive patients with confirmed detection of SARS-CoV-2 RNA, baseline IL-6 serum level measurement and admitted to the Hospital Universitario La Princesa (HUP) with severe to critical COVID-19 (18), from February 24th to March 23rd 2020 (Flow chart in Suppl. Fig. 1).

This time frame was decided based on the fact that after March 23rd, clinicians involved in the treatment of these patients were aware of the possibility of measuring IL-6 serum levels to decide treatment initiation with TCZ (RoActemra, Roche). Furthermore, around that date Spanish facilities capable of providing IMV were overrun (Suppl. Fig. 2), thus the decision for IMV application was based on availability rather than the patients' needs. Notwithstanding, during the study period, patients were prompted to IMV when they presented a marked worsening of oxygenation (arterial oxygen tension/fraction of inspired oxygen ratio [PaO_2/FiO_2]<200), provided that their baseline conditions did not contraindicate it.

Data collection

Clinical, laboratory and therapeutic data were collected from electronic clinical records and included in an anonymized database. Baseline evaluation was performed around the third day of admission (median=3 days, interquartile range [IQR] 2 to 5). The second evaluation was obtained around the ninth day of admission (median=9 days, IQR 7-12). No significant differences at both evaluation time points were observed between patients treated and not treated with TCZ.

In addition, twenty-three serum samples from healthy donors obtained before the pandemic onset were used to determine the variability of IL-6 serum levels in baseline conditions.

SARS-CoV-2 RNA detection

Samples from nasopharyngeal and throat exudates were obtained with specific swabs as previously described (19). Then, we performed real-time RT-PCR assay targeting the E gene of SARS-CoV-2 as the first-line screening tool, with Real Time ready RNA Virus Master (Roche), followed by confirmatory testing with the assay TaqPath™ COVID-19 CE-IVD Kit RT-PCR (Applied Biosystems™), including three assays that target SARS-CoV-2 genes (Orf1ab, S gene, N gene) and one positive control assay that targets the human RNase P RPPH1 gene (20). All the procedures were performed on Applied Biosystems TM Quant Studio-5 Real-Time PCR System.

IL-6 serum level measurement

Surplus sera from laboratory routine determinations were used to assess IL-6 levels, which were retrospectively quantified in duplicate with the Human IL-6 Quantikine high sensitivity enzyme-immune assay from R&D Systems Europe Ltd. (Abingdon, UK). The intra-assay and inter-assay variability were 3% and 5%, respectively.

Tocilizumab treatment

The rationale to treat severe COVID-19 patients with TCZ was based on its previously approved indication for treating the CRS associated with CAR-T cell therapy, as well as unpublished experience in COVID-19 patients from China and Italy. Treatment required

approval by the HUP COVID-19 Committee, following the recommendations of the Spanish Agency for Drugs and Health Devices (AEMPS) (21):

- Interstitial pneumonia with severe respiratory failure (score = 2);
- Rapid respiratory worsening requiring non-invasive or invasive ventilation (score ≥ 3 on the COVID respiratory severity scale);
- Presence of extrapulmonary organ failure (shock or score ≥ 3 on the SOFA scale);
- Criteria for severe systemic inflammatory response. In adults: elevated levels of IL-6 (> 40 pg / ml); alternatively, increased levels of D-dimer (> 1500 ng / ml) or progressively increasing D-dimer.
- Patients who, according to their baseline clinical condition, would be IMV subsidiary.

Therefore, the decision to treat with TCZ was based on the AEMPS criteria, excluding IL-6>40 pg/ml, since no information about IL-6 results was available to physicians during the study period. After a preliminary analysis of data by the end of March, IL-6 measurement was included in the baseline assessment of COVID-19 patients.

The administration schedule of TCZ at the time of the study was a first intravenous infusion of 8 mg/kg (maximum 800 mg) followed by a second one after 12 hours.

Variables

To analyze whether IL-6 levels can predict disease severity, two main outcomes were considered: need for IMV and all-cause mortality.

To determine the effect of TCZ we analyzed the evolution of PaO_2/FiO_2 between both evaluation times. In 160 out of 267 evaluations arterial oxygen tension (PaO_2) was

unavailable. So, in order to avoid missing data in these relevant outcomes, this parameter was estimated from mean oxygen saturation ($SatO_2$) as proposed elsewhere (22).

IL-6 serum levels showed a heterogeneous distribution in patients and in healthy donors (Suppl. Fig. 3A). To improve its representation in figures, this variable was normalized through natural logarithmic transformation (Suppl. Fig. 3B). The procedure to determine the cut-off for high baseline IL-6 is described below.

Statistical analysis

Statistical analyses were performed using Stata 14 for Windows (Stata Corp LP, College Station, TX, USA). Quantitative variables following a non-normal distribution were represented as median and IQR and the Mann Whitney test was used to assess significant differences. Variables with a normal distribution were described by mean±standard deviation (SD) and differences between groups were assessed with Student's t-test. Qualitative variables were described as counts and proportions and $\chi 2$ or Fisher´s exact test was used for comparisons. Correlation between quantitative variables was analyzed using the Pearson correlation test. To estimate the 95% confidence interval of correlation coefficients we used the *ci2* command of Stata.

To determine whether IL-6 serum levels were able to discriminate between: i) COVID-19 patients vs. healthy donors; ii) patients requiring IMV vs. those that did not; or iii) patients treated with TCZ vs. not treated, receiver operating characteristic (ROC) analysis was performed using the roctab command. Each cut-off point was selected based on the best trade-off values between sensitivity, specificity and the percentage of patients correctly classified. Positive and negative likelihood ratios and ROC curves were also obtained.

To determine the variables associated with the need for IMV, we performed a multivariable logistic regression analysis that was first modeled by adding all the variables with a p value lower than 0.15 in the bivariable analysis, namely total lymphocyte count, D-dimer, LDH, PaO_2/FiO_2, COPD, obesity, hypertension, C-reactive protein, and IL-6 (high vs low). The final model was reached with backward stepwise removal of variables with p-value higher than 0.15, and using Wald tests to demonstrate that each model was better than its previous iteration.

Next, we performed a multivariable analysis using generalized linear models nested by patient and visit (*xtgee* command) in which the dependent variable was PaO_2/FiO_2. This approach allowed us to identify which variables influenced the evolution of PaO_2/FiO_2. The first model included all variables with a p value <0.15 in the bivariable analysis, namely hypertension, baseline radiological pattern, LDH, total lymphocyte count, baseline C-reactive protein, IMV. After that, through backward stepwise approach, we obtained the best model as described above. Then, to assess the role of IL-6 as predictor of TCZ effect on PaO_2/FiO_2, the composite variable IL-6/TCZ (low IL-6/no TCZ, low IL-6/Early TCZ, low IL-6/Late TCZ, high IL-6/no TCZ, high IL-6/Early TCZ and high IL-6/Late TCZ) was forced in the model.

Survival time was analyzed by Kaplan-Meier method with the *sts* command of Stata. Date of admission was considered the date of entry and for exit date we considered the exitus date. For those patients without the event, the last revision of the database (electronic chart or telephone call) on May 21st was used to censor their follow-up. Differences in time to death by different variables were analyzed by log-rank test.

Ethics

This study was approved by the local Research Ethics Committee (register number 4070) and

it was carried out following the ethical principles established in the Declaration of Helsinki. All included patients (or their representatives) were informed about the study and gave an oral informed consent as proposed by AEMPS due to COVID-19 emergency.

This article was written following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines taking into consideration the difficulties to obtain all needed information in the setting of the COVID-19 pandemic.

RESULTS

Demographic and clinical characteristics of the study population

One hundred and forty-six patients were included; their main demographic and clinical characteristics are shown in Table 1. Median age was 63 (IQR [54-71]; range, 30 to 86), 97 (66%) were men and 100 (69%) presented comorbidities. The most frequent were hypertension, 55 (38%); obesity, 23 (16%); diabetes mellitus, 26 (18%); and chronic obstructive pulmonary disease (COPD) 9 (6%); 19 (13%) patients had a history of malignancy. Median duration of symptoms before admission was 6 days (IQR 4-7); 36 (25%) arrived at the emergency room presenting fever (≥38ºC), with a $SatO_2$ of 91%±5%. Most individuals (121 [83%]) were admitted to the internal Medicine or Pneumology wards; however, 16 (11%) patients admitted directly to the Intensive Care Unit because of IMV requirement, and 9 (6%) to the Hematology ward because of pre-existing conditions. Additional details of patient baseline features can be found in supplementary table 1.

IL-6 serum levels and disease severity

IL-6 serum levels above 10 pg/ml discriminated COVID-19 patients from healthy donors with low accuracy (Area under ROC curve [AUC] 0.695; sensitivity [Se] 84%, specificity [Sp] 46%, positive likelihood ratio [LR+] 1.5, LR- 0.4; Suppl. Fig. 3C), probably due to their intrinsic heterogeneity (Suppl. Fig. 3A and B).

No significant correlation was found between PaO_2 and IL-6 serum levels at baseline (r= -0.09 [95% CI: -0.270 to 0.085]; p=0.299; Figure 1A), likely due to a higher oxygen supply in the most severe cases; in fact, serum IL-6 levels showed a significant negative correlation with

PaO_2/FiO_2 (Figure 1B; r= -0.38 [95% CI: -0.526 to -0.218]; p<0.001), meaning that higher levels of IL-6 at baseline were associated with lower PaO_2/FiO_2.

In this regard, forty-four patients (30%) required IMV at some point during their hospitalization. As expected, these patients showed significantly worse PaO_2/FiO_2 levels than those not requiring IMV (p<0.001; Table 2). In addition, they showed increased leukocytes, total lymphocytes, IL-6, C-reactive protein (CRP), and procalcitonin (PCT) showing a higher inflammatory status than those not requiring IMV (p≤0.001 except p=0.003 for CRP and p=0.029 for lymphocyte count; Table 2).

Furthermore, a baseline IL-6 above 30 pg/ml (henceforth high IL-6) discriminated patients requiring IMV with 68% Se and 73% Sp. AUC was 0.725 (Figure 1C; LR+ 2.5, LR- 0.4). A logistic regression model, adjusted for COPD and baseline white blood cell count, also showed that high baseline IL-6 was a predictive biomarker for IMV (OR:7.1; 95% CI: 3.0 to 16.6; Supp Table 2).

Response to Tocilizumab

Fifty-eight patients (40%) received treatment with TCZ. No significant differences between groups were observed in most sociodemographic and therapeutic variables, except for patients not treated with TCZ, which were more often obese and COPD (p=0.023 and p=0.071 respectively, Supplementary Table 3). Importantly, patients in the TCZ-treated group presented several baseline findings indicating that they suffered more severe COVID-19 disease, such as lower PaO_2/FiO_2 (p<0.001; Table 3), higher levels of serum Lactate Dehydrogenase (p<0.001), CRP (p=0.005), IL-6 levels at baseline (p=0.007), and total lymphocyte count (p=0.001). Other elevated markers in this group included Aspartate amino-transferase, ferritin, and procalcitonin (p<0.05 for all comparisons. Table 3).

Even before physicians were aware of the potential value of IL-6 serum levels as a predictor of severe disease, those patients with high IL-6 were more frequently treated with TCZ (Figure 1D; AUC 0.634; 30 pg/ml as cut-off showed Se 57%, Sp 69%, LR+ 1.9, LR- 0.7), although with less accuracy than for IMV. The median time from the beginning of symptoms to TCZ treatment was 11 days (IQR: 8-12.5). Therefore, we considered early TCZ when the treatment was applied before 11 days of disease duration and late TCZ after this cut-off.

As a consequence of IL-6R blockade with TCZ, a significant trend toward higher IL-6 serum levels after administration of the drug was observed (Figure 2A; p=0.005). However, IL-6R blockade induced a fast and significant down-regulation of CRP (Figure 2B; p<0.001) and PCT (Figure 2C; p=0.026) and a non-significant decrease of ferritin (Figure 2D) and LDH (Figure 2E). Conversely, it was associated with a significant increase of D-Dimer levels (Figure 2F; p<0.001). After an average 6 days of TCZ treatment, despite this improvement of inflammatory parameters, PaO_2/FiO_2 did not show a significant improvement in the whole population (Data not shown). Only patients with high IL-6 that underwent early TCZ treatment showed a significant PaO_2/FiO_2 increase (Figure 3A mid boxes; p=0.048). Patients with low IL-6 did not improve their PaO_2/FiO_2 after treatment with TCZ (Figure 3B). Those patients not treated with TCZ showed a heterogeneous behavior in their PaO_2/FiO_2 with a trend to improve in patients with low IL-6 (Figure 3A and B two left boxes of each panel).

Since relevant differences were observed between patients treated and not treated with TCZ, we used a multivariable analysis to determine which variables influenced the evolution of PaO_2/FiO_2 at the short-term. Baseline PaO_2/FiO_2 and radiological pattern, HTA, LDH and CRP levels and total lymphocyte blood count significantly explained variation in PaO_2/FiO_2 (Suppl Table 4). Adjusted by these confounders, the best PaO_2/FiO_2 evolution was achieved in

patients with low IL-6 not requiring TCZ treatment (Figure 3C first dot on the left; reference group in analysis showed in Suppl Table 4), likely because they were the less severe patients. Patients with low IL-6 that due to their bad evolution were prescribed with TCZ showed a significant worsening of PaO_2/FiO_2 (Figure 3C, 2nd and 3rd dots; Suppl Table 4). A similar evolution was observed in patients with high IL-6 and late TCZ treatment, whereas those with high IL-6 not treated or treated early with TCZ showed no significant differences compared with the reference group (Figure 3C; Suppl Table 4).

Mortality

Next, we were interested in the long-term evolution of patients depending on baseline IL-6 and treatment with TCZ. After a median follow-up of 61 days (IQR: 58-64) we observed 30 deaths in our sample (21%). The survival curves according to the baseline level of IL-6 and TCZ treatment are shown in Figure 4. Six patients out of 59 (10%) died in the reference group (low IL-6/no TCZ), 9 out of 28 (32%) in the low IL-6/TCZ treated group (HR 3.6 [CI 95%: 1.3 – 10.0], p=0.016), 10 out of 28 (36%) in the high IL-6/no TCZ group (HR 4.6 [CI 95%: 1.7 – 12.7], p=0.003) and 5 out of 31 (16%) in the high IL-6/TCZ treated group (HR 1.6 [CI 95%: 0.5 – 5.4], p=0.411).

Safety of Tocilizumab treatment

Regarding safety, no relevant cytopenia, hypertransaminasemia, bowel perforation or secondary bacterial infections were observed during or after treatment with TCZ for the time of the study. Ten (7%) patients had positive blood cultures; most of them in the non-TCZ treated group (7 vs 3; p=0.03).

DISCUSSION

To our knowledge, this is the first study showing that high baseline IL-6 levels predict both the need for IMV and the response to TCZ in severe patients hospitalized with COVID-19. Our results confirm the hypothesis that respiratory failure in the advanced phase of severe COVID-19 is mainly due to an exacerbated inflammatory response. These findings are in accordance with cytokine storms described in previous experiences with H5N1 influenzae virus (5) and previous coronaviruses SARS-CoV and MERS (7), as well as CAR T cell therapy (23, 24). Together, these data suggest a key role for inflammation of the small distal airways in the severity of this condition (25). Hence, approximately one-third of patients with ARDS display elevated levels of inflammatory mediators (IL-6, IL-8 and soluble TNF receptor 1, among others), increasing the prevalence of shock and mortality (26). Accordingly, our data show that IL-6 levels higher than 30 pg/ml predict the need for IMV, and correlated with other severity data. A similar threshold has been described to discriminate between mild and severe COVID-19 in Chinese patients (27).

The association of high IL-6 with a more severe disease in our population supports the use of TCZ to treat COVID-19 patients. TCZ is a humanized antibody that blocks both soluble and membrane-bound forms of IL-6 receptor. Thus, TCZ prevents ligand binding, which likely explains why IL-6 serum levels significantly increased after treatment (28). However, the TCZ-mediated blockade of IL-6R signaling led to the observed relevant decrease in circulating inflammatory mediators. Severe high IL-6 COVID-19 patients treated with TCZ showed an early respiratory improvement, represented by a moderate but significantly increased PaO_2/FiO_2 when TCZ was prescribed before 11^{th} day of symptoms and lower overall mortality, independently of other treatments or clinical factors. Furthermore, patients with severe COVID-19 and high IL-6 levels that were not treated with TCZ displayed a higher mortality.

On the other hand and of outstanding interest, patients with low IL-6 that were treated with TCZ due to a severe or critical COVID-19 did not improve and showed significantly higher mortality. These observations pose the question whether it is possible that severe patients with low IL-6 included in phase 3 trials with Tocilizumab and Sarilumab account for the failure to meet their primary endpoints (29, 30). Therefore, our results support the measurement of baseline IL-6 levels in hospitalized COVID-19 patients, since in those severe or critical patients with low IL-6 levels, other cytokines such as IL-1 or tumor necrosis factor (TNF) could be driving the exacerbated inflammatory response in lungs (25). Probably, this specific group could benefit from receiving other anti-inflammatory agents such as IL-1 or TNF blockers. In this regard, the need for biomarkers of response to TCZ has been recently highlighted (31) and neither COVACTA study (Tocilizumab trial; NCT04320615) nor ex-US Sarilumab clinical trial (NCT04327388) listed increased baseline IL-6 serum levels within the inclusion criteria. Nevertheless, both clinical trials reported a decrease in duration of hospital stay in the active arm, that was statistically significant in COVACTA (29, 30).

In agreement with our data, other case series with few patients have shown that tocilizumab can improve the outcomes of COVID-19 patients with ARDS (6, 8, 31, 32); in some of these reports described the use of, lower doses of TCZ were used. In this regard, the protocol in our hospital, after these preliminary data, has evolved towards lower doses of TCZ (a single dose of TCZ 400 mg if<80 kg and 600 mg if >80 kg) administered earlier with similar efficacy (unpublished observation).

Additionally, during our study the use of TCZ was safe and it did not increase the number of serious bacterial infections. These findings are consistent with the results of both the COVACTA and Sarilumab trials (29, 30). The only unexpected observation was the rise in D-dimer levels at the second evaluation. Possible explanations are either: i) IL-6 does not play a

role in the regulation of D-dimer production; or ii) more likely, D-dimer production has a slower kinetics than CRP or other acute phase reactants, since in patients not treated with TCZ a similar rise in D-dimer levels was observed (data not shown).

Apart from the novelty and the immediate clinical utility of these findings, a drawback of our study is its retrospective and observational nature involving mainly very severe cases in the group of treatment with TCZ. A stricter selection of a control group through a propensity score strategy was unfeasible, since once the physicians were aware of IL-6 measurement they focused their efforts on treating with TCZ those patients with the highest IL-6 levels. Therefore, prospective studies should be carried out to confirm our observations.

In addition, there is some controversy about the reliability of PaO_2/FiO_2 as an outcome for improvement of lung damage, especially in patients with IMV. However, our population was a mix of patients with IMV and non-IMV, so despite the many factors that could interfere with PaO_2/FiO_2, we considered it the most objective outcome for patients' assessment.

Finally, our findings are of relevance for clinical decision making in the ongoing COVID-19 pandemic. Increased levels of circulating IL-6 predict IMV requirement in patients with severe disease,and can contribute to establish the indication for timely TCZ administration. Furthermore, the improvement of respiratory parameters achieved upon treatment with TCZ may reduce IMV demand in these patients. On the other hand, in our population there was a small group of patients with severe COVID-19 and low IL-6 serum levels, which probably should have been treated with blockade of IL1 or TNF-α since their evolution with TCZ was inadequate.

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REINMUN-COVID group includes:

- Cardiology: Teresa Alvarado, Pablo Martínez, Francisco Javier de la Cuerda Llorente
- Emergency Service: Carmen del Arco, Juan Mariano Aguilar, Natalia Villalba, Mónica Negro, Elvira Contreras, Ana del Rey, Cristina Santiago, Manuel Junquera, Raquel Caminero, Francisco Javier Val, Sonia González, Marta Caño, Isabel López, Andrés von Wernitz, Bárbara Retana, Iñigo Guerra, Jorge Sorando, Lydia Chao, María José Cárdenas, Verónica Espiga, Pablo Chicharro, Pedro Rodríguez.
- Endocrinology: Iñigo Hernando Alday, Miguel Sampedro
- ENT: Jorge Prada
- Gastroenterology: Eukene Rojo Aldama, Yolanda Real, María Caldas, Sergio Casabona, Aitor Lanas-Gimeno.
- Hematology Service: Rafael de la Camara, Angela Figuera Alvárez, Beatriz Aguado.
- Hospital Pharmacy: Alberto Morell, Esther Ramírez, Amparo Ibáñez Zurriaga, María Pérez Abanades, Silvia Ruiz García, Tomás Gallego Aranda, María Ruiz, Concepción Martínez Nieto, José María Serra.
- Immunology: Francisco Sánchez-Madrid, Cecilia Muñoz-Calleja, Arantzazu Alfranca, Javier Aspa, Ana Marcos-Jiménez, Santiago Sánchez-Alonso, Ana Alcaraz-Serna, Tamara Mateu-Albero, Ildefonso Sánchez-Cerrillo, Laura Esparcia, Pedro Martínez-Fleta, Celia López-Sanz, Ligia Gabrie, Luciana del Campo Guerola, Elena Fernández, Mª José Calzada, Reyes Tejedor.

- Intensive Care Unit: Alfonso Canabal, Patricia Albert, Diego A. Rodriguez-Serrano, Judit Iglesias, Fernando Suarez, Juan Antonio Sánchez, Beatriz Abad.
- Internal Medicine-Infectious Diseases: Carmen Suarez, Ignacio de los Santos, José María Galván-Roman, Emilia Roy, Pablo Rodríguez-Cortes, Lucio García-Fraile, Jesus Sanz, Eduardo Sanchez, Fernando Moldenhauer, Pedro Casado, Jose Curbelo, Angela Gutierrez, Azucena Bautista, Nuria Ruiz Giménez, Angelica Fernandez, Pedro Parra, Berta Moyano, Ana Barrios, Diego Real de Asua, Beatriz Sanchez, Carmen Saez, Marianela Ciudad.
- Medical Biology: Desiré Navas
- Microbiology: Laura Cardeñoso Domingo, María del Carmen Cuevas Torresano, Diego Domingo García, Teresa Alarcón Cavero, Alicia García Blanco, Alexandra Martín Ramírez, María Auxiliadora Semiglia Chong, Ainhoa Gutiérrez Cobos, Nelly Daniela Zurita Cruz, Arturo Manuel Fraile Torres.
- Nephrology: Carmen Sanchez-Gonzalez, Antonio Fernádez Perpén
- Neurology: Carolina Díaz Pérez
- Pneumology: Julio Ancochea, Tamara Alonso, Pedro Landete, Joan Soriano, Carolina Cisneros, Elena García Castillo, Francisco Javier García Pérez, Rosa María Girón, Celeste Marcos, Enrique Zamora.
- Radiology: Patricia García García
- Rheumatology: Santos Castañeda, Rosario García-Vicuña, Isidoro González-Álvaro, Sebastián Rodríguez-García, Carlos Fernández-Díaz, Irene Llorente Cubas, Eva G. Tomero, Noelia García Castañeda, Ana Mª Ortiz, Cristina Valero, Miren Uriarte, Nuria Montes.

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Table 1. Baseline clinical characteristics and laboratory findings of the study population

	Study Population (n=146)
Age	63 (54-71)
Male sex	97 (66)
Comorbidities	100(69)
Duration of symptoms at admission (days)	6 (4-7)
Baseline PaO2/FiO2	215 (112-310)
Treatment during hospitalization	
Hydroxychloroquine	137 (96)
Lopinavir/Ritonavir	119 (83)
Azithromycin	82 (57)
Interferon-β	7 (5)
Glucocorticoids	85 (59)
Methylprednisolone bolus	61 (42)
Laboratory Findings	
White Blood Count (10^3/mm^3)	7.64 (5.25–10.68)
Lymphocyte Count (10^3/mm^3)	0.83 (0.60–11.7)
Creatinine. mg/dl	0.86 (0.70-1.10)
LDH (U/L)	341 (256-461)
CK (U/L)	72 (48-155)
Serum IL-6 (pg/ml)	21.36 (7.53-54.21)
Ferritin (ng/ml)	1598 (830-2305)
CRP (mg/dL)	11.55 (5.16-22.53)
PCT (ng/ml)	0.15 (0.10-0.35)
D-dimer (mg/ml)	0.75 (0.48–1.48)

All categorical variables are expressed as number (%) and quantitative variables as median (p25-p75). PaO_2/FiO_2: arterial oxygen tension - fraction of inspired oxygen ratio; LDH: Lactate Dehydrogenase; IL-6: Interleukin-6; CRP: C-reactive protein; PCT: Procalcitonin.

Table 2. Baseline clinical characteristics of groups requiring vs not requiring Invasive Mechanical Ventilation.

	Invasive Mechanical Ventilation		
	Required (n=44)	Not-required (n=102)	p value
Age	63.5 (56.5-72)	62 (54-71)	0.517
Male sex	32 (73)	65 (64)	0.291
Comorbidities	30 (68)	70 (69)	0.893
Duration of symptoms at admission (days)	5 (5-7)	7 (4-8)	0.265
Baseline PaO_2/FiO_2	125.5 (75-207)	247 (172-348)	<0.001
Treatment during hospitalization			
Hydroxychloroquine	38 (86)	99 (100)	<0.001
Lopinavir/Ritonavir	38 (86)	81 (82)	0.502
Azithromycin	24 (55)	58 (59)	0.652
Interferon-β	3 (7)	4 (4)	0.676
Glucocorticoids	27 (61)	58 (59)	0.755
Methylprednisolone bolus	21 (48)	40 (40)	0.414
Laboratory Findings			
White Blood Count ($10^3/mm^3$)	9.39 (6.59-13.31)	6.93 (5.13-8.78)	<0.001
Lymphocyte Count ($10^3/mm^3$)	0.74 (0.58-1.08)	0.87 (0.62-1.26)	0.029
Creatinine (mg/dl)	0.99 (0.71-1.20)	0.85 (0.72-1.1)	0.398
LDH (U/L)	413 (315-496)	302 (224-443)	0.001
CK (U/L)	67 (39.50-167.50)	94 (59-140)	0.617
Serum IL-6 (pg/ml)	49.20 (17.28-103.57)	16.08 (6.09-42.03)	<0.001
Ferritin (ng/ml)	1665 (602-2765)	1573 (1012-2300)	0.832
CRP (mg/dL)	17.09 (7.69-28.98)	10.13 (4.83-18.48)	0.003
PCT (ng/ml)	0.29 (0.14-0.46)	0.13 (0.08-0.26)	0.001
D-dimer (mg/ml)	0.92 (0.56-2.31)	0.71 (0.48-1.19)	0.058

All categorical variables are expressed as number (%) and quantitative variables as median (p25-p75). PaO_2/FiO_2: arterial oxygen tension - fraction of inspired oxygen ratio; LDH: Lactate Dehydrogenase; IL-6: Interleukin-6; CRP: C-reactive protein; PCT: Procalcitonin.

667 **Table 3.** Baseline clinical characteristics of groups treated vs not treated with Tocilizumab.

	Tocilizumab		
	Treated (n=58)	Not treated (n=88)	p value
Age	61 (54-70)	64 (54-72)	0.288
Male sex	40 (69)	57 (65)	0.600
Comorbidities	35 (61)	64 (73)	0.124
Duration of symptoms at admission (days)	6 (5-7)	7 (4-8)	0.612
Baseline PaO_2/FiO_2	137 (88-232)	248 (183-348)	<0.001
Treatment during hospitalization			
Hydroxychloroquine	53 (93)	84 (98)	0.171
Lopinavir/Ritonavir	51 (89)	68 (79)	0.103
Azithromycin	33 (58)	49 (57)	0.913
Interferon-β	2 (4)	5 (6)	0.532
Glucocorticoids	38 (67)	47 (55)	0.152
Methylprednisolone bolus	31 (54)	30 (35)	0.018
Laboratory Findings			
White Blood Count ($10^3/mm^3$)	7.99 (5.17-11.85)	7.52 (5.4–10.36)	0.527
Lymphocyte Count ($10^3/mm^3$)	0.74 (0.52–0.997)	0.93 (0.66–1.47)	0.001
Creatinine (mg/dl)	0.83 (0.70-1.05)	0.90 (0.72-1.14)	0.177
LDH (U/L)	425 (302-510)	293.5 (221-388)	<0.001
CK (U/L)	69 (38-270)	75.5 (49-125)	0.785
Serum IL-6 (pg/ml)	41.85 (12.37-71.95)	16.25 (6.27-44.95)	0.007
Ferritin (ng/ml)	1888 (1152-2844)	1461 (471-1861)	0.038
CRP (mg/dL)	13.73 (8.75-27.08)	9.09 (4.78-19.31)	0.005
PCT (ng/ml)	0.25 (0.13-0.36)	0.14 (0.1-0.3)	0.045
D-dimer (mg/ml)	0.75 (0.48–1.48)	0.71 (0.53–1.22)	0.491

668 All categorical variables are expressed as number (%) and quantitative variables as median (p25-p75).
669 PaO_2/FiO_2: arterial oxygen tension - fraction of inspired oxygen ratio; LDH: Lactate Dehydrogenase; IL-
670 6: Interleukin-6; CRP: C-reactive protein; PCT: Procalcitonin.
671

672 FIGURE LEGENDS

673 **Figure 1.** IL-6 serum levels predict disease severity and tocilizumab (TCZ) use. (A) Correlation
674 between log-transformed IL-6 serum levels and arterial oxygen tension (PaO2). (B) Correlation
675 between log-transformed IL-6 serum levels and arterial oxygen tension - fraction of inspired
676 oxygen ratio (PaO2/FiO2). Data in panels A and B are shown as dot-plot and their fitted linear
677 prediction with 95% confidence interval (transparent grey shadow) estimated using the
678 twoway command of Stata with the lfitci option. (C) ROC curve showing the ability of log-
679 transformed IL-6 serum levels to discriminate between patients requiring vs not-requiring
680 invasive mechanical ventilation (IMV). (D) ROC curve for the ability of log-transformed IL-6
681 serum levels to discriminate between TCZ treated and non-treated patients. The best cut-off
682 for discrimination of patients requiring IMV (panel C) or TCZ treatment (panel D) was 30
683 pg/ml.

684 **Figure 2**. Response of laboratory parameters to Tocilizumab (TCZ) treatment. (A) Differences
685 in log-transformed IL-6 serum levels, (B) C-Reactive Protein and (C) Procalcitonin, (D) Ferritin,
686 (E) Lactate Dehydrogenase (LDH), and (F) D-Dimer. Data are presented as the interquartile
687 range (p75 upper edge, p25 lower edge, p50 midline), p95 (line above the box), and p5 (line
688 below the box) of levels for each parameter before (grey boxes) and after (white boxes)
689 treatment with TCZ.

690 **Figure 3**. Change of PaO_2/FiO_2 in COVID-19 patients treated early (before 11 day of symptoms
691 onset) or late with Tocilizumab and not-treated. (A) Patients with high baseline IL-6 (cut-off
692 30 pg/ml). (B) Subjects with low baseline IL-6 serum levels. Data in A and B are shown as the
693 interquartile range (p75 upper edge, p25 lower edge, p50 midline), p95 (line above the box),
694 and p5 (line below the box) before (grey boxes) and after (white boxes) treatment with TCZ. In
695 non-treated patients PRE and POST mean first and second evaluation, respectively. Statistical

696 significance was determined with the Mann-Whitney test. (C) The graph represents the
697 predicted mean (dots) with 95% confidence intervals (bars) of PaO_2/FiO_2 according to baseline
698 IL-6 levels and early or late TCZ treatment. Data were obtained with the command
699 marginsplot of Stata, after adjustment by baseline PaO_2/FiO_2 and radiological pattern,
700 Hypertension, Lactic dehidrogenase and C-reactive protein levels, lymphocyte blood count
701 and need for IMV, according to the multivariable analysis displayed in Supplementary Table 2
702 (see Methods for further information).
703 **Figure 4**. Survival curves of COVID-19 patients grouped according to baseline IL-6 levels and
704 TCZ treatment. Statistical significance was established with log-rank test.
705
706
707

Figure No.1

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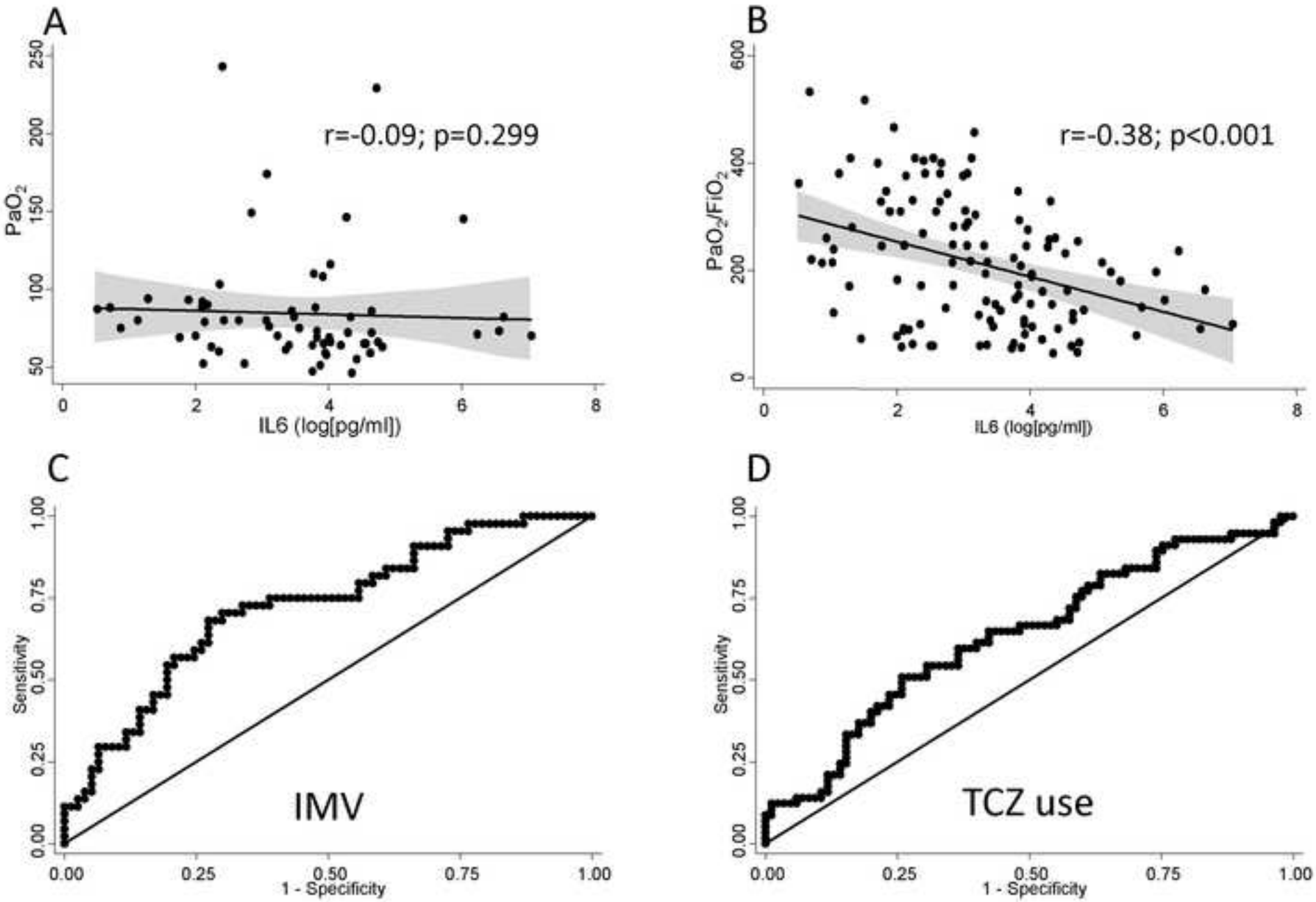

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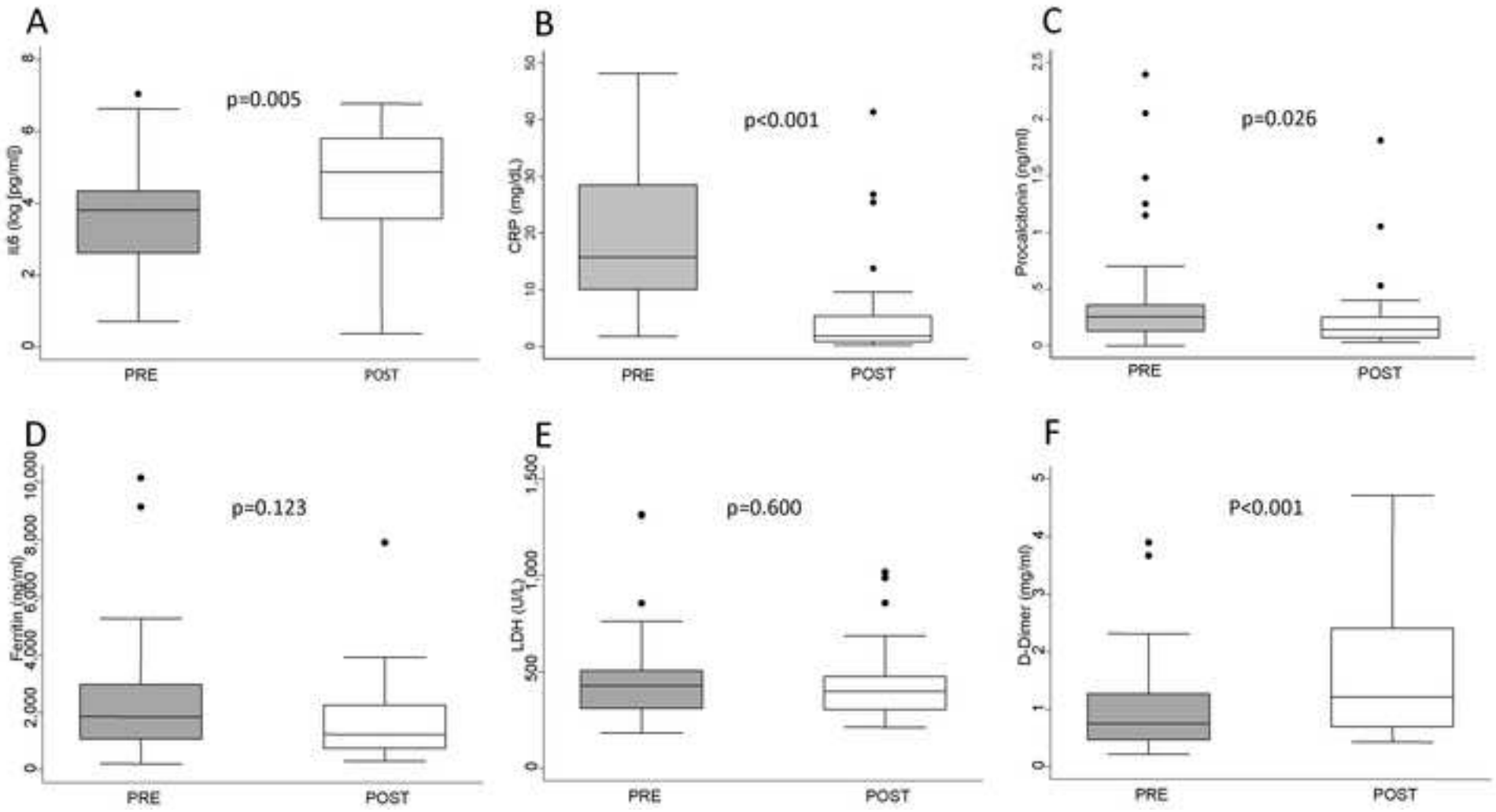

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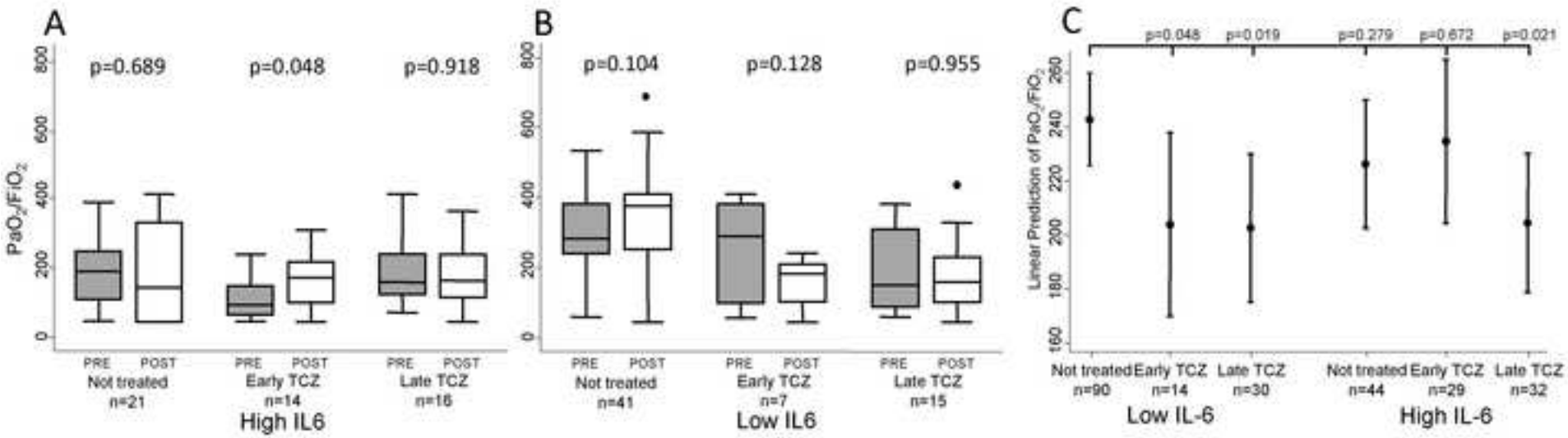

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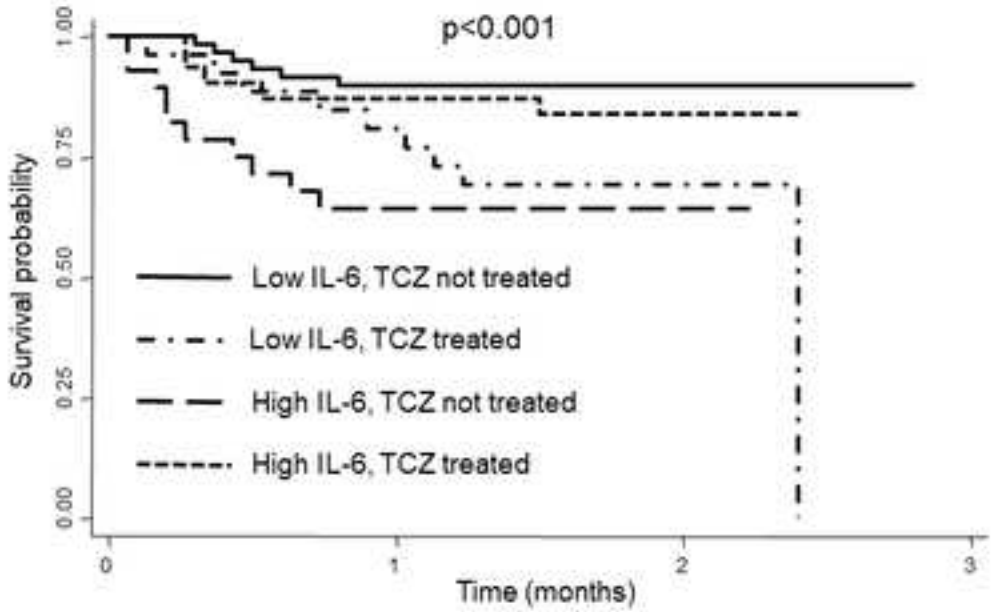


SUPPLEMENTARY DATA

Supplementary Table1: Baseline clinical characteristics of the study population and groups requiring vs not requiring Invasive Mechanical Ventilation.

	Study Population (n=146)	Invasive Mechanical Ventilation		
		Required (n=44)	Not-required (n=102)	p value
Age	63 (54-71)	63.5 (56.5-72)	62 (54-71)	0.517
Male sex	97 (66)	32 (73)	65 (64)	0.291
Comorbidities (1, 0.68%)	100(69)	30 (68)	70 (69)	0.893
Hypertension (1, 0.68%)	55 (38)	20 (45)	35 (35)	0.218
Obesity (1, 0.68%)	23 (16)	11 (25)	12 (12)	0.047
Diabetes Mellitus (1, 0.68%)	26 (18)	8 (18)	18 (18)	0.883
COPD (1, 0.68%)	9 (6)	5 (11)	4 (4)	0.089
Immune-mediated Disease† (1, 0.68%)	8 (6)	3 (7)	5 (5)	0.702
History of Malignancy‡ (1, 0.68%)	19 (13)	5 (11)	14 (14)	0.792
Others§ (1, 0.68%)	76 (52)	25 (57)	51 (52)	0.558
Duration of symptoms at admission (days) (1, 0.68%)	6 (4-7)	5 (5-7)	7 (4-8)	0.265
Fever at admission (≥38ºC) (15, 10%)	36 (27)	14 (32)	21 (25)	0.389
Baseline PaO2/FiO2 (5, 3%)	215 (112-310)	125.5 (75-207)	247 (172-348)	<0.001
Treatment during hospitalization				
Hydroxychloroquine (2, 1.35%)	137 (96)	38 (86)	99 (100)	<0.001
Lopinavir/Ritonavir (2, 1.35%)	119 (83)	38 (86)	81 (82)	0.502
Azithromycin (2, 1.35%)	82 (57)	24 (55)	58 (59)	0.652
Interferon-β (2, 1.35%)	7 (5)	3 (7)	4 (4)	0.676
Glucocorticoids (2, 1.35%)	85 (59)	27 (61)	58 (59)	0.755
Methylprednisolone bolus (1, 0.68%)	61 (42)	21 (48)	40 (40)	0.414
Laboratory Findings				
White Blood Count (10^3/mm^3) (9, 6%)	7.64 (5.25–10.68)	9.39 (6.59-13.31)	6.93 (5.13-8.78)	<0.001
Lymphocyte Count (10^3/mm^3) (10, 6.76%)	0.83 (0.60–11.7)	0.74 (0.58-1.08)	0.87 (0.62-1.26)	0.029
Creatinine. mg/dl (7, 4.73%)	0.86 (0.70-1.10)	0.99 (0.71-1.20)	0.85 (0.72-1.1)	0.398
Bilirubin. mg/dl (9, 6.08%)	0.55 (0.41-0.87)	0.62 (0.44-1.15)	0.53 (0.39-0.78)	0.057
AST. U/L (9, 6.08%)	41 (28-64)	43 (30-60)	39 (27-70)	0.526
ALT. U/L (9, 6.08%)	37 (24-68)	30 (22-63)	39 (24-71)	0.287
GGT. U/L (26, 17.57%)	73 (36-159)	81.5 (41-152)	67 (36-159)	0.623
LDH. U/L (14, 9.46%)	341 (256-461)	413 (315-496)	302 (224-443)	0.001
CK. U/L (102, 69%)	72 (48-155)	67 (39.50-167.50)	94 (59-140)	0.617
Serum IL-6. pg/ml (7, 4.73%)	21.36 (7.53-54.21)	49.20 (17.28-103.57)	16.08 (6.09-42.03)	<0.001
Ferritin. ng/ml (96, 64.86%)	1598 (830-2305)	1665 (602-2765)	1573 (1012-2300)	0.832
CRP. mg/dL (23, 15.54%)	11.55 (5.16-22.53)	17.09 (7.69-28.98)	10.13 (4.83-18.48)	0.003
PCT. ng/ml (52, 35.14%)	0.15 (0.10-0.35)	0.29 (0.14-0.46)	0.13 (0.08-0.26)	0.001
D-dimer. mg/ml (25, 16.89%)	0.75 (0.48–1.48)	0.92 (0.56-2.31)	0.71 (0.48-1.19)	0.058
Radiologic findings (1, 0.68%)				0.209
Clear	11 (8)	4 (9)	8 (7)	
Diffuse ground glass opacities	50 (35)	13 (30)	37 (37)	
Unilateral alveolar pattern	20 (14)	3 (7)	17 (17)	
Bilateral alveolar pattern	63 (43)	24 (55)	37 (37)	

All categorical variables are expressed as number (%) and quantitative variables as median (p25-p75). Missing data in each clinical characteristic are expressed as: (number, %). Variables not disclosing it do not present any missing values. COPD: Chronic obstructive pulmonary disease; PaO_2/FiO_2: arterial oxygen tension - fraction of inspired oxygen ratio; AST: Aspartate amino-transferase; ALT: Alanine amino- transferase; GGT: Gamma-glutamyl transferase; LDH: Lactate Dehydrogenase; CK: Creatine-kinase; IL-6: Interleukin-6; CRP: C-reactive protein; PCT: Procalcitonin.
†Includes Rheumatoid Arthritis, Systemic Lupus Erythematosus, Ulcerative Colitis, etc
‡Includes solid organ and hematologic malignancies
§Includes obstructive sleep apnea syndrome, asthma, hypothyroidism, Ischemic cardiomyopathy, etc

Supplementary Table 2. Logistic regression model for invasive mechanical ventilation.

	OR	p	95%CI
COPD	5.407	0.030	1.172 to 24.938
White Blood Count (10^3)	1.050	0.116	0.998 to 1.115
High IL-6 baseline serum levels	7.087	<0.001	3.022 to 16.617

OR: Odds Ratio; 95%CI: 95% Confidence Interval ;COPD: Chronic Obstructive Pulmonary Disease; IL-6: interleukin 6; High IL-6 was considered if >30 pg/ml.

Supplementary Table 3: Baseline clinical characteristics of groups treated vs not treated with Tocilizumab.

	Tocilizumab (TCZ)		
	Treated (n=58)	Not-treated (n=88)	p value
Age	61 (54-70)	64 (54-72)	0.288
Male sex	40 (69)	57 (65)	0.600
Comorbidities (1, 0.68%)	35 (61)	64 (73)	0.124
Hypertension (1, 0.68%)	17 (30)	37 (42)	0.124
Obesity (1, 0.68%)	14 (25)	9 (10)	0.023
Diabetes Mellitus (1, 0.68%)	9 (16)	16 (18)	0.687
COPD (1, 0.68%)	1 (2)	8 (9)	0.071
Immune-mediated Disease† (1, 0.68%)	5 (9)	3 (3)	0.181
History of Malignancy# (1, 0.68%)	7 (12)	12 (14)	0.763
Others‡ (1, 0.68%)	30 (52)	46 (52)	0.892
Duration of symptoms at admission (days) (1, 0.68%)	6 (5-7)	7 (4-8)	0.612
Fever at admission (≥38ºC) (15, 10%)	17 (31)	19 (25)	0.483
Baseline PaO2/FiO2 (5, 3%)	137 (88-232)	248 (183-348)	<0.001
Treatment during hospitalization			
Hydroxychloroquine (2, 1.35%)	53 (93)	84 (98)	0.171
Lopinavir/Ritonavir (2, 1.35%)	51 (89)	68 (79)	0.103
Azithromycin (2, 1.35%)	33 (58)	49 (57)	0.913
Interferon-β (2, 1.35%)	2 (4)	5 (6)	0.532
Glucocorticoids (2, 1.35%)	38 (67)	47 (55)	0.152
Methylprednisolone bolus (1, 0.68%)	31 (54)	30 (35)	0.018
Laboratory Findings			
White Blood Count (10^3/mm^3) (9, 6%)	7.99 (5.17-11.85)	7.52 (5.4–10.36)	0.527
Lymphocyte Count (10^3/mm^3) (10, 6.76%)	0.74 (0.52–0.997)	0.93 (0.66–1.47)	0.001
Creatinine. mg/dl (7, 4.73%)	0.83 (0.70-1.05)	0.90 (0.72-1.14)	0.177
Bilirubin. mg/dl (9, 6.08%)	0.62 (0.46-1.04)	0.52 (0.38-0.78)	0.070
AST. U/L (9, 6.08%)	47 (29-77.50)	34.5 (28-53)	0.047
ALT. U/L (9, 6.08%)	37.5 (25-77)	36 (22.5-64.5)	0.588
GGT. U/L (26, 17.57%)	73 (41-186)	71 (35-145)	0.374
LDH. U/L (14, 9.46%)	425 (302-510)	293.50 (221.50-388.50)	<0.001
CK. U/L (102, 69%)	69 (38-270)	75.5 (49-125)	0.785
Serum IL-6. pg/ml (7, 4.73%)	41.85 (12.37-71.95)	16.25 (6.27-44.95)	0.007
Ferritin. ng/ml (96, 64.86%)	1888 (1152-2844)	1461 (471-1861)	0.038
CRP. mg/dL (23, 15.54%)	13.73 (8.75-27.08)	9.09 (4.78-19.31)	0.005
PCT. ng/ml (52, 35.14%)	0.25 (0.13-0.36)	0.14 (0.1-0.3)	0.045
D-dimer. mg/ml (25, 16.89%)	0.75 (0.48–1.48)	0.71 (0.53–1.22)	0.491
Radiologic findings (1, 0.68%)			0.818
Clear	4 (7)	8 (8)	
Diffuse ground glass opacities	18 (32)	32 (37)	
Unilateral alveolar pattern	10 (18)	10 (11)	
Bilateral alveolar pattern	25 (44)	37 (43)	

All categorical variables are expressed as number (%) and quantitative variables as median (p25-p75). Missing data are expressed as number (%). Variables not disclosing it do not present any missing values.

COPD: Chronic obstructive pulmonary disease; PaO_2/FiO_2: arterial oxygen tension - fraction of inspired oxygen ratio; AST: Aspartate amino-transferase; ALT: Alanine amino- transferase; GGT: Gamma-glutamyl transferase; LDH: Lactate Dehydrogenase; CK: Creatine-kinase; IL-6: Interleukin-6; CRP: C-reactive protein; PCT: Procalcitonin.

†Includes Rheumatoid Arthritis, Systemic Lupus Erythematosus, Ulcerative Colitis, etc

‡Includes solid organ and hematologic malignancies

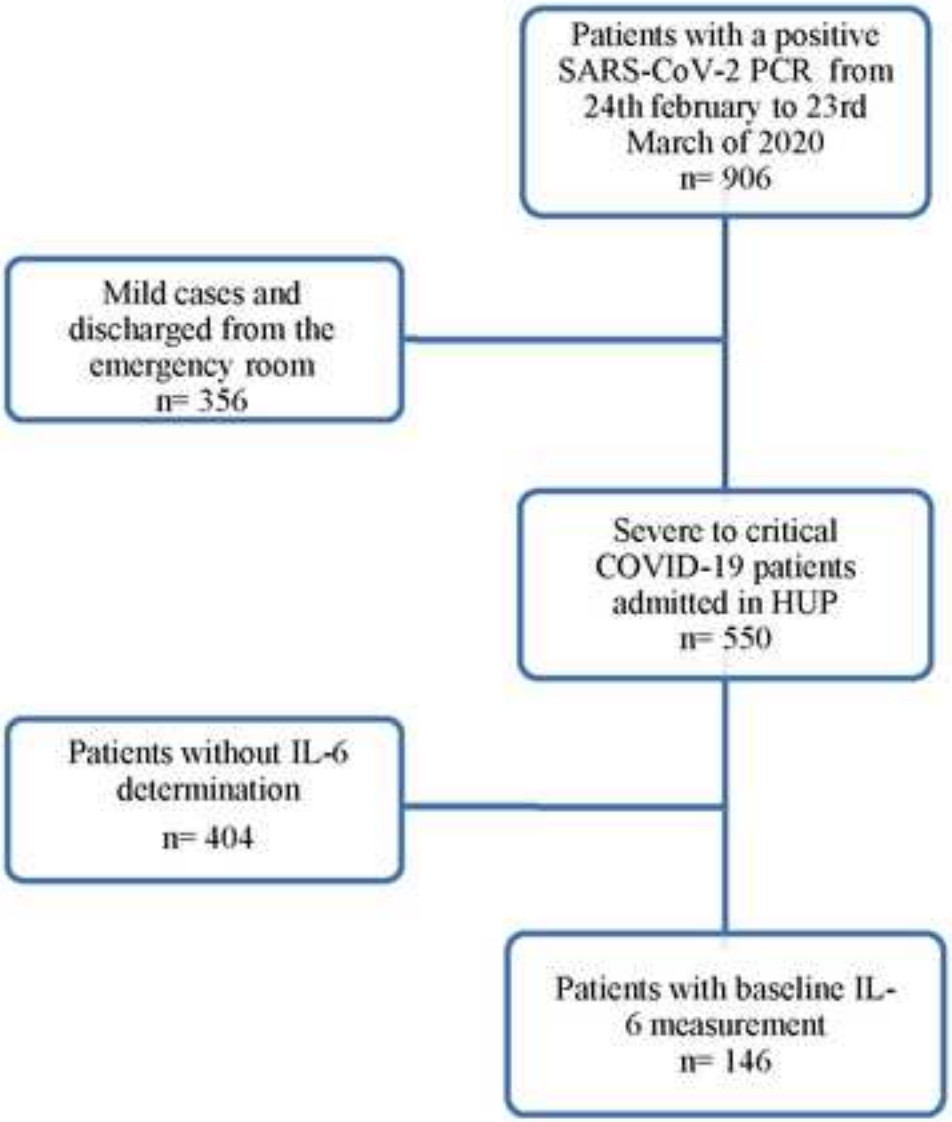
§Includes obstructive sleep apnea syndrome, asthma, hypothyroidism, Ischemic cardiomyopathy, etc

Supplementary Table 4. Variables explaining evolution of PaO_2/FiO_2

	ß coeff.	p	95%CI
Baseline PaO_2/FiO_2	0.713	<0.001	0.567 to 0.753
Lymphocyte Count	0.033	<0.001	0.015 to 0.052
Hypertension	-19.059	0.067	-39.432 to 1.314
LDH	- 0.101	0.001	-0.163 to -0.039
CRP	- 1.188	0.038	-2.311 to 0.065
Radiologic Findings			
Clear	ref		
Diffuse ground glass opacities	49.256	0.006	13.785 to 84.727
Unilateral alveolar pattern	31.638	0.131	-9.378 to 72.654
Bilateral alveolar pattern	60.708	0.001	25.986 to 95.431
Others	-123.818	0.113	-276.853 to 29.217
IL-6 serum / TCZ*			
Low IL-6, no TCZ	ref		
Low IL-6, early TCZ	-38.854	0.048	-77.390 to -0.319
Low IL-6, late TCZ	-40.150	0.019	-73.765 to -6.535
High IL-6, no TCZ	-16.513	0.279	-46.440 to 13.413
High IL-6, early TCZ	-8.134	0.672	-45.831 to 29.562
High IL-6, late TCZ	-38.359	0.021	-70.837 to -5.881

PaO_2/FiO_2: arterial oxygen tension - fraction of inspired oxygen ratio; ß coeff.: Beta-coefficient; 95%CI: 95% Confidence Interval; LDH: Lactate Dehydrogenase; CRP: C-reactive protein; IMV: Invasive Mechanical Ventilation; ref: reference; IL-6: interleukin 6; TCZ: Tocilizumab

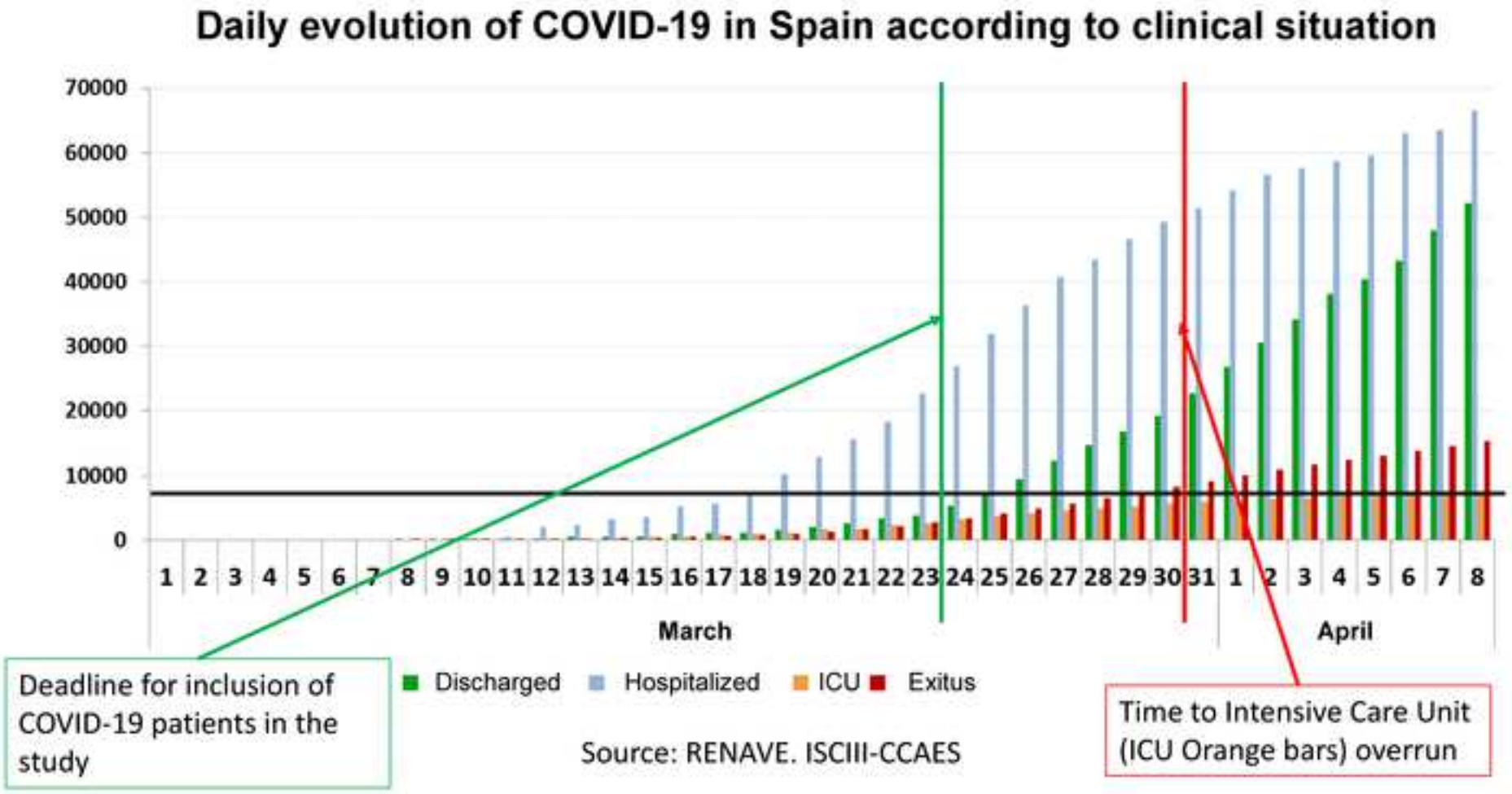
* IL-6 serum / TCZ: refers to the interaction between both high or low IL-6 (cut-off 30 pg/ml) and TCZ (No treatment, Early or late treatment; cut-off 11 days since symptoms onset) as an independent predictor within the model.

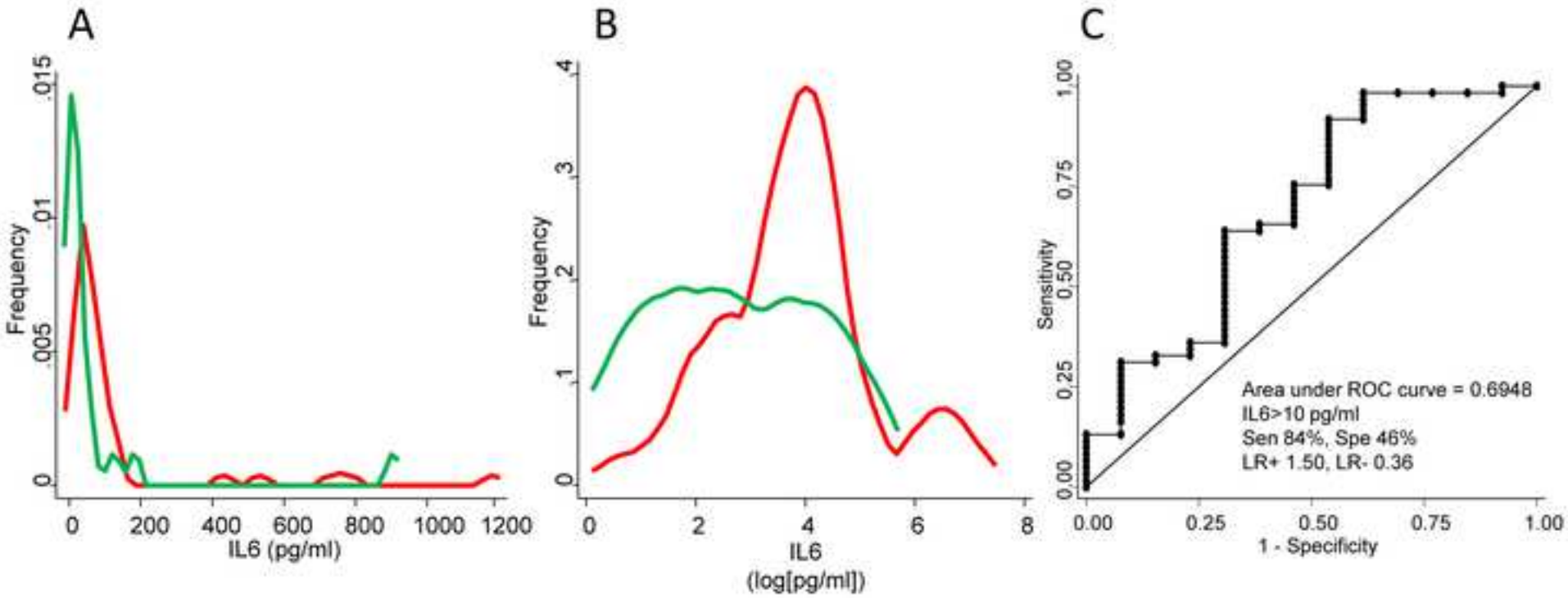


Sociodemographic characteristics of patients hospitalized due to COVID-19 until 24th March 2020.

Sociodemographic Characteristics	All COVID-19 hospitalized population (n=550)	Patients with IL-6 measurement		
		Yes (n=146)	No (n=404)	p value
Age	66.6 [55.0 – 77.9]	63.0 [54.0 – 72]	68.9 [55.2 – 80.0]	0.0001
Male sex	329 (59.8)	97 (66.4)	232 (57.4)	0.057
Mortality during hospitalization	115 (20.9)	30 (20.6)	85 (21.0)	0.900

Categorical variables are expressed as number (%) and quantitative variables as median [interquartile range]





LEGENDS FOR THE SUPPLEMENTARY FIGURES

Supplementary Figure 1. Flow chart of patients included in the study.

Supplementary Figure 2. Histogram showing the daily evolution of COVID-19 cases in Spain according to their clinical situation.
Source: https://covid19.isciii.es/resources/CURVASTATUS.png. Accessed on April 9th 2020.

Supplementary Figure 3. (A) Plots showing the distribution of raw IL-6 serum levels in patients (red) vs healthy donors (green). (B) Distribution of log-transformed serum IL-6 levels in the former groups. (C) ROC curve showing the ability of IL-6 serum levels to classify COVID-19 patients vs healthy donors. (A) Plots showing the distribution of raw IL-6 serum levels in patients (red) vs healthy donors (green). (B) Distribution of log-transformed serum IL-6 levels in the former groups. (C) ROC curve showing the ability of IL-6 serum levels to classify COVID-19 patients vs healthy donors.