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Influence of anti-Spike protein antibody levels on tocilizumab efficacy in hospitalized patients with severe COVID-19 pneumonia: a post-hoc analysis of the COVACTA trial

José Aguareles[1,2,3], Carles Forné[4,5], Ana García-Casas[1,3], Guillermo Santamaría-Corral[1,3], Daniel Carnevali-Ruiz[1,2], Gabriel Sotres-Fernández[1,2], Javier T Solera[1,2] and Pablo Guisado-Vasco[1,2*]

Abstract

Background Our aim in this work is to find biomarkers to optimize therapy with IL-6 inhibitors, as not all clinical trials have shown clear benefits on mortality or mechanical ventilation progression. Given the link between delayed seroconversion and higher complication risks, we aim to test if evaluating SARS-CoV-2 spike protein antibody status before treatment could enhance IL-6 inhibitor therapy effectiveness in COVID-19 patients.

Methods We conducted a post hoc analysis of the COVACTA study, a phase 3, randomized, double-blind, placebo-controlled trial of the efficacy and safety of tocilizumab in hospitalized patients with severe COVID-19. Cox and logistic regression analysis were used to assess the tocilizumab's efficacy in severe COVID-19 patients on survival and ICU stay at day 28, based on SARS-CoV-2 S-spike and neutralizing antibody levels.

Results Tocilizumab reduced 28-day mortality over placebo in patients with low S-spike antibody titers (20% vs. 29%). No benefit was observed for higher antibody levels. Patients with low S-spike antibody levels treated with tocilizumab exhibited a lower probability of ICU stay at day 28 compared to those treated with placebo (63% vs. 82%). No significant differences were noted in mortality and ICU stay based on whole neutralizing antibody titers.

Conclusions Our findings suggest that using IL-6 inhibitors in severe COVID-19 patients with low S-spike antibody titers may improve clinical outcomes.

Clinical trial Not applicable.

Keywords Tocilizumab, SARS-CoV-2, COVID-19, Antibodies, Serostatus, IL-6 inhibitors

*Correspondence:
Pablo Guisado-Vasco
pablo.guisado@quironsalud.es
[1]Internal Medicine Department, Hospital Universitario Quirónsalud Madrid, Madrid, Spain
[2]Department of Medicine, Faculty of Medicine, Health and Sports, European University of Madrid, Madrid, Spain
[3]Research and Clinical Trials Unit, Hospital Universitario Quirónsalud Madrid, Madrid, Spain
[4]Heorfy Consulting, Lleida, Spain
[5]Department of Basic Medical Sciences, University of Lleida, Lleida, Spain

Introduction

The emergence of COVID-19 has spurred extensive research into immunomodulation for individuals with severe symptoms. The immunopathogenesis of COVID-19 is primarily driven by the innate immune system, mainly triggered by proinflammatory cytokines such as type I interferon or complement proteins, among others. Consequently, numerous clinical trials have tested various molecules to enhance outcomes in cases that progress to severe respiratory failure. As a result, randomized open-label platform trials found that therapy with dexamethasone and IL-6 inhibitors (mainly tocilizumab) reduces mortality in severe COVID-19 [1, 2]. However, not all IL-6 inhibitor clinical trials have uniformly shown benefits in terms of mortality or progression to mechanical ventilation [3, 4]. Hence, several questions arise regarding their appropriate timing, administration, and patient selection.

Conversely, some research groups pointed out that patients with delayed seroconversion to the SARS-CoV-2 spike protein (S-spike) face an elevated risk of complications beyond the second week of the disease [5, 6]. Considering these previous findings, we suggest that assessing patient serostatus before initiating treatment could enhance the efficacy of IL-6 inhibitors in managing COVID-19. In this context, we hypothesize that patients with delayed seroconversion may derive greater benefit from IL-6 inhibitors compared to those with faster seroconversion post-SARS-CoV-2 infection and subsequent respiratory failure [7].

Materials/Methods

This post hoc study analyzed data from the modified intention-to-treat (mITT) population of the COVACTA trial [4, 8]. The COVACTA trial was a global, randomized, double-blind, placebo-controlled phase 3 study comparing tocilizumab with placebo in patients hospitalized with severe COVID-19 pneumonia. The inclusion criteria required confirmed SARS-CoV-2 infection via PCR and either a blood oxygen saturation of 93% or lower, or a partial pressure of oxygen/fraction of inspired oxygen ratio below 300 mm Hg. The study population consisted of patients from Europe and North America. Patient recruitment took place from April 2020 to May 2020 [4, 8]. The study included 438 patients (294 receiving tocilizumab and 144 receiving placebo), with raw data including SARS-CoV-2 S-spike and neutralizing antibody (Ab) titers. Neutralizing Ab levels provide a comprehensive measure of various Abs that neutralize SARS-CoV-2, determined through the plaque reduction neutralization test (PRNT80) [9, 10]. In contrast, S-spike Abs, which specifically target the receptor binding domain (RBD) of the spike protein, are measured using an immunoassay. This assay allows for precise detection of these antibodies, utilizing a validated in vitro diagnostic method (Roche Cobas; Roche Diagnostics, Indianapolis, IN), based on a double-antigen sandwich assay with a recombinant S antigen receptor binding domain. Both Ab titer were measured at enrollment day.

Multivariable regression analyses were conducted to evaluate how SARS-CoV-2 S-spike and neutralizing Ab levels, in relation to treatment (tocilizumab or placebo), impacted on predefined clinical outcomes: time to death up to day 28 (using Cox regression models), and intensive care unit (ICU) stay at day 28 (using logistic regression models). The likelihood ratio test (LRT) was used to assess the statistical significance of interaction term between the biomarkers and the study groups. Two modeling approaches for biomarkers association were employed: restricted cubic splines to allow for more flexible non-linear relationships, and dichotomization based on the empirical median value due to limited existing literature on immunomodulation and COVID-19. The models were adjusted for sex, days from symptoms onset, and age with restricted cubic splines. Cox models were also adjusted for baseline ICU stay. Kaplan-Meier curves were obtained for death, improvement in Ordinal Clinical Status and hospital discharge, according to study arm and groups of S-spike Ab levels and compared by means of the log-rank test.

Results

Baseline characteristics were well-balanced across both trial groups (see detailed results in Table 1), with approximately 70% male participants. The median age was 62 years (interquartile range (IQR): 51, 70) in the tocilizumab group and 61 years (IQR: 53.5, 70) in the placebo group. The median time from the first SARS-CoV-2 symptom to randomization was 11 days (IQR:7.5, 15) in the tocilizumab group and 10 days (IQR: 7, 14) in the placebo group. About 56% of patients were admitted to the ICU at baseline.

Following a pre-specified follow-up period, the 28-day mortality rates were 19.7% ($n = 58$) in the tocilizumab group and 19.4% ($n = 28$) in the placebo group. ICU stay at day 28 was 66% ($n = 194$) for tocilizumab-treated patients and 71.5% ($n = 103$) for those in the placebo group (Supplementary Table 1).

Two types of Abs were considered for assessing their association with the 28-day mortality and ICU stay: total neutralizing Abs and S-spike protein Abs, the latter being the primary neutralizing Ab against SARS-CoV-2. Both Abs showed a positive correlation, with a Spearman coefficient (95% CI) of 0.822 (0.777 to 0.858), as expected given that S-spike protein Abs are one of the main neutralizing Abs against SARS-Cov-2 (Supplementary Fig. S1).

Table 1 Demographics and clinical characteristics of population at baseline

	Tocilizumab	Placebo
Male sex, n (%)	205 (69.7)	101 (70.1)
Age [yr], Median (P25, P75)	62 (51, 70)	61 (53.5, 70)
Mechanical Ventilation at baseline = Yes, n (%)	110 (37.4)	55 (38.2)
Extracorporeal membrane oxygenation (ECMO) at baseline = Yes, n (%)	10 (3.4)	8 (5.6)
Intensive Care Unit (ICU) Stay at baseline = Yes, n (%)	167 (56.8)	80 (55.6)
Days from first COVID-19 symptom at baseline, Median (P25, P75)	11 (7.5, 15)	10 (7, 14)
Antiviral Treatment at baseline = Yes, n (%)	71 (24.1)	42 (29.2)
Coexisting illness, n (%)		
≥ 1 Diagnosis	231 (78.6)	124 (86.1)
Obesity	63 (21.4)	27 (18.8)
Diabetes	105 (35.7)	62 (43.1)
Cardiovascular impairment	88 (29.9)	35 (24.3)
Hypertension	178 (60.5)	94 (65.3)
Hepatic impairment	6 (2.0)	2 (1.4)
Chronic lung disease	49 (16.7)	22 (15.3)

Antiviral treatment corresponds to treatment with remdesivir, which was the only available treatment at that time. The coexisting illnesses are sourced from the COVACTA clinical trial article [4]

Analyzing 28-day mortality based on the S-spike Ab levels revealed a trend of poorer response to tocilizumab among patients with higher levels of this marker. In contrast, the placebo group exhibited the opposite trend (Fig. 1a). A cutoff point at the median S-spike Ab level (57.66 U/mL) was established to identify associations with treatment response (see levels of SARS-CoV-2 Abs by group in Supplementary Table 2). Low S-spike Ab levels showed statistically significant differences in 28-day mortality between treatment groups (LRT p-value = 0.049). Tocilizumab demonstrated favorable outcomes over placebo in patients with low S-spike Ab titers, with a 28-day cumulative probability of death (95% CI) of 0.20 (0.08 to 0.31) compared to 0.29 (0.10 to 0.45) in the placebo group. Conversely, patients with higher S-spike Ab levels did not benefit from tocilizumab compared to the placebo: 0.27 (0.12 to 0.38) vs. 0.13 (0.00 to 0.24), respectively (Fig. 1b).

Similarly, S-spike Ab levels showed a comparable effect on the probability of ICU stay at day 28. Tocilizumab treatment did not confer benefits in patients with high S-spike Ab titers (Fig. 1c). On the other hand, patients with low S-spike Ab levels treated with tocilizumab exhibited a lower probability of ICU stay at day 28 compared to those treated with placebo (LRT p-value = 0.022): 0.63 (0.50 to 0.74) and 0.82 (0.68 to 0.91), respectively (Fig. 1d).

In contrast, no differential effects were observed between groups based on whole neutralizing Ab titers, neither on 28-day mortality (Fig. 2a-b) nor on ICU stay at day 28 (Fig. 2c-d). Similarly, there were also no differences in either 28-day mortality (Fig. 2b) or ICU stay at day 28 (Fig. 2d) when neutralizing Ab titers were dichotomized using the empirical median (< 83 vs. 83+).

Figure 3 shows the patients' clinical status as assessed on the seven-category ordinal scale at day 28, according to the category at baseline. On the one hand, better clinical status at day 28 is observed among tocilizumab-treated patients with low S-spike Ab levels, compared to the placebo arm, especially for those with grade 4 at baseline, i.e., patients requiring non-invasive ventilation or high-flow oxygen (Fig. 3a). On the other hand, slightly worse clinical status evolution is observed among patients with higher S-spike Ab levels treated with tocilizumab, compared to those in the placebo group, particularly in patients with higher grades at baseline (Fig. 3b).

Kaplan-Meier curves for death, improvement in Ordinal Clinical Status and hospital discharge showed no statistically significant differences between groups defined on the study arm and S-spike Ab levels (Supplementary Fig. 2).

To see if these populations presented a differential immune signature, we analyzed different markers of inflammation and the immune system, without finding statistically significant differences neither in IL-6 nor CRP levels (Supplementary Table 3). Statistically significant differences were observed in leukocyte count but were not clinically relevant. Interestingly, days from the first symptoms of COVID-19 showed differences between both populations. Overall, the group with the best response (characterized by low levels of Abs) exhibited a shorter disease course, indicated by fewer days with symptoms. Additionally, it is noteworthy that this population demonstrated a higher viral load (Supplementary Table 3).

Discussion

This post-hoc analysis demonstrated that tocilizumab reduced the probability of mortality on or before day 28, as well as the duration of ICU stay in severe COVID-19 patients with low S-spike Ab levels during the early stages of the disease, compared to those patients with higher Ab titers. These findings may contribute to our understanding of various COVID-19 trials using IL-6 inhibitors, which have shown heterogeneous results. This study provides the first evidence highlighting a positive impact of IL-6 inhibition in COVID-19, particularly among patients experiencing delayed Ab seroconversion [11, 12]. In this manner, our findings are a complementary approach in the use of IL-6 inhibitors in COVID-19, adding new clinical data to improve the selection of cases based on the previously reported recommendations of the RECOVERY trial [1], specifically aimed at patients

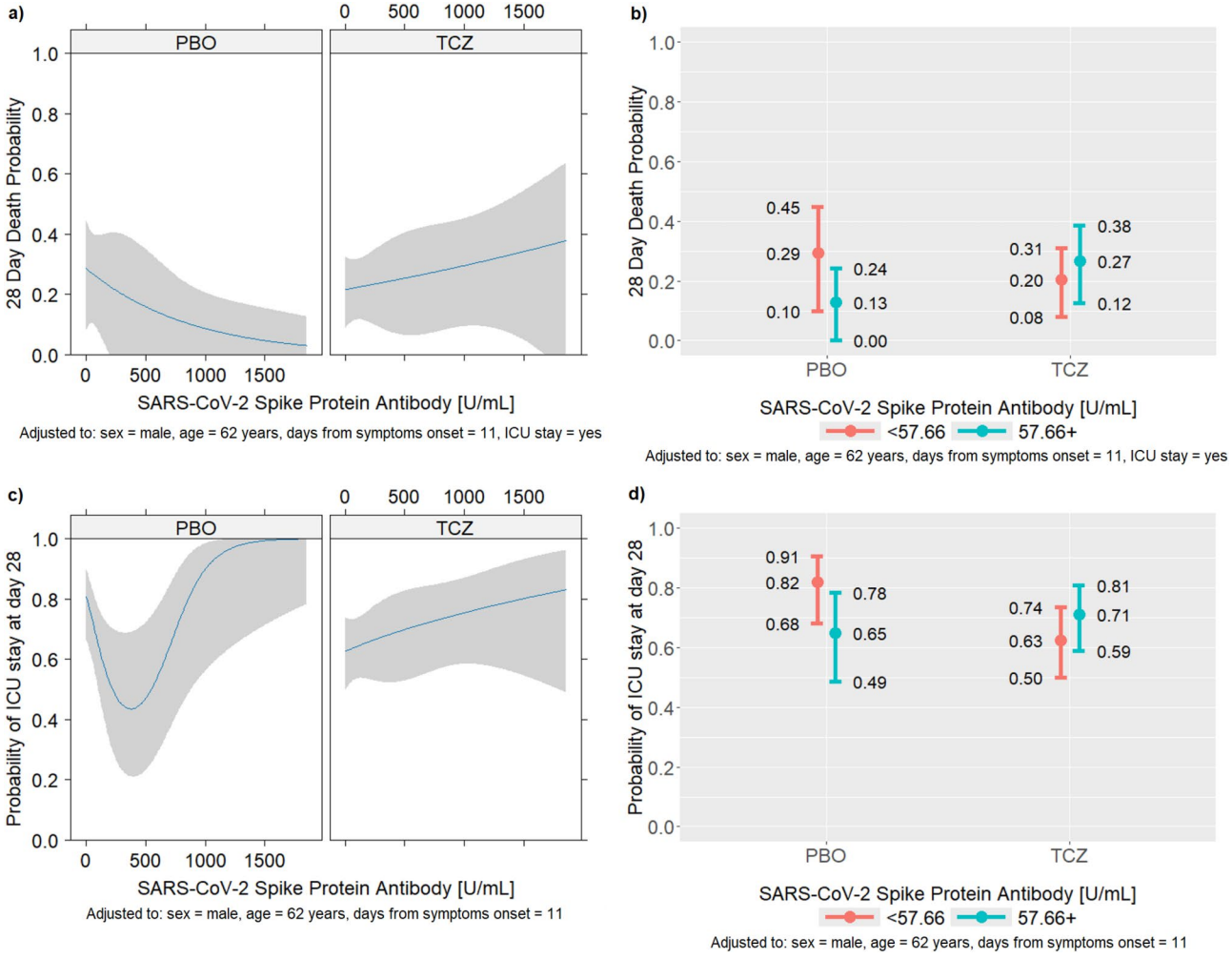


Fig. 1 Outcomes of the population from COVACTA trial based on treatment and S-spike Ab levels Probability of mortality at day 28 and 95% CI by **(a)** study arm and S-spike Ab levels adjusted for sex, age, days from symptoms, and baseline ICU stay, or by **(b)** study arm and spike protein dichotomized (median = 57.66 U/mL) adjusted for sex, age, days from symptoms, and baseline ICU stay. Mean was 0.20 (0.08 to 0.31) in the tocilizumab group with low levels of S-spike Ab patients and 0.29 (0.10 to 0.45) in the placebo group with low levels of S-spike Ab (LRT p-value = 0.049). Probability of ICU stay at day 28 and 95% CI by **(c)** study arm and S-spike Ab levels adjusted for sex, age, and days from symptoms, or by **(d)** study arm and spike protein dichotomized (median = 57.66 U/mL) adjusted for sex, age, and days from symptoms. Mean was 0.63 (0.50 to 0.74) in the tocilizumab group with low levels of S-spike Ab patients and 0.82 (0.68 to 0.91) in the placebo group with low levels of S-spike Ab (LRT p-value = 0.022). PBO = placebo, TCZ = tocilizumab

with ongoing oxygen therapy support and C-reactive protein levels above 75 mg/L.

In our study, we analyzed the outcomes of COVACTA patients (i.e., the probability of death and ICU stay at day 28) based on Ab levels, distinguishing between neutralizing and S-spike Abs. In the first case, the total neutralizing Abs present in serum are measured, whereas S-spike Abs are a subset of these neutralizing Abs and are the primary neutralizing Ab against SARS-CoV-2 [9].

Our findings underscore the importance of S-spike Ab levels, which are increasingly recognized, alongside neutralizing Abs, as flexible and indirect markers of immune dysregulation. Currently, ongoing research aims to establish precise thresholds linked to waning immunity against SARS-CoV-2 variants of concern (VOC), known for evading host immunity [1]. Concerns also persist regarding the vulnerability of the immunosuppressed population to severe COVID-19. Previously reported results indicated that patients with low S-spike Ab levels faced worse clinical outcomes [12], highlighting a population that stands to gain the most from immunomodulatory treatment, as supported by this study.

This study demonstrated significant impacts of the S-spike Abs, unlike whole neutralizing Ab titers, which showed no distinction. The observed results may be attributed to technical factors in the procedures used to detect neutralizing Abs and S-spike Abs [9]. The PRNT80 assay, employed to detect neutralizing Ab titers, evaluates the ability of Abs in serum or plasma samples to inhibit SARS-CoV-2 replication in cell cultures,

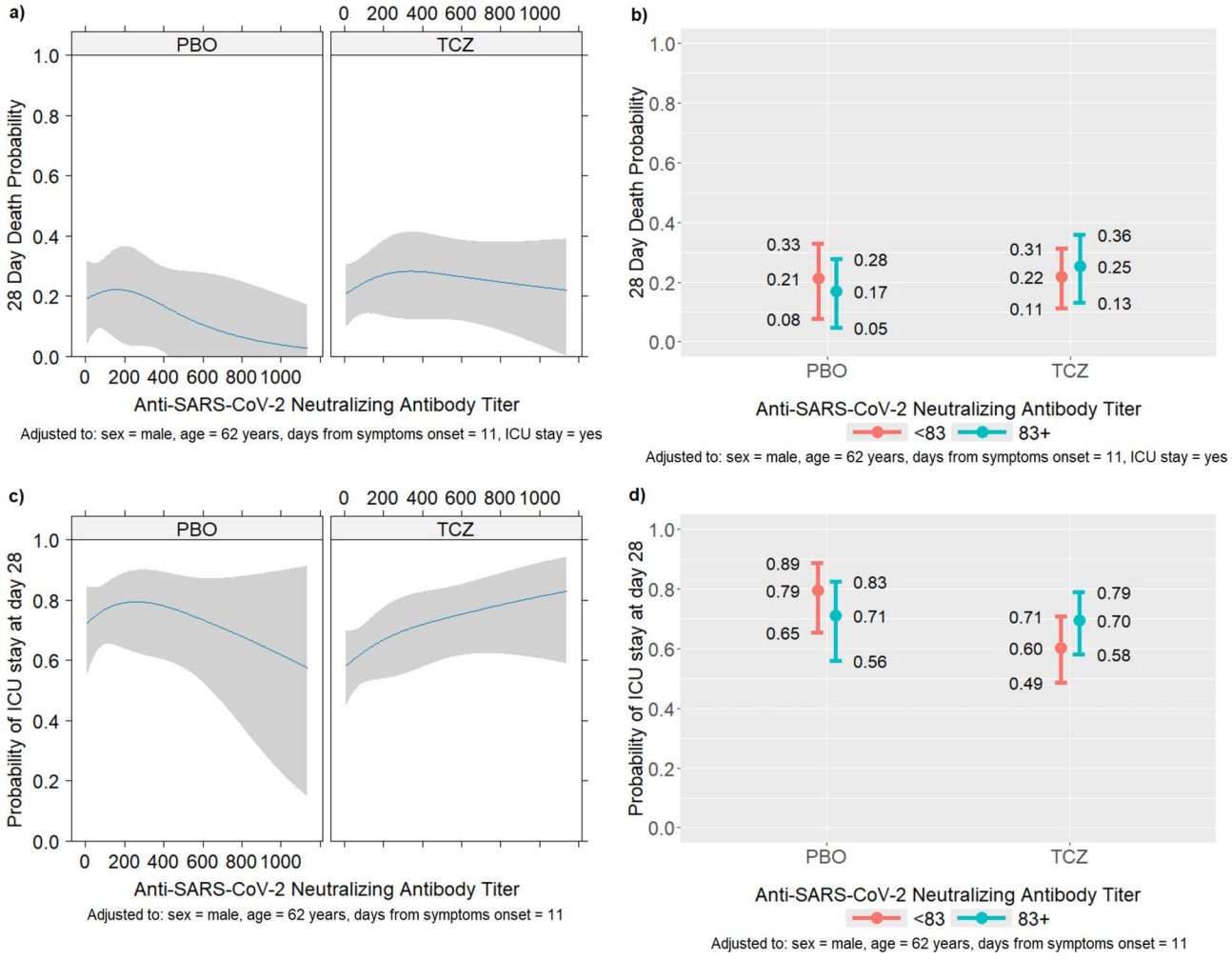
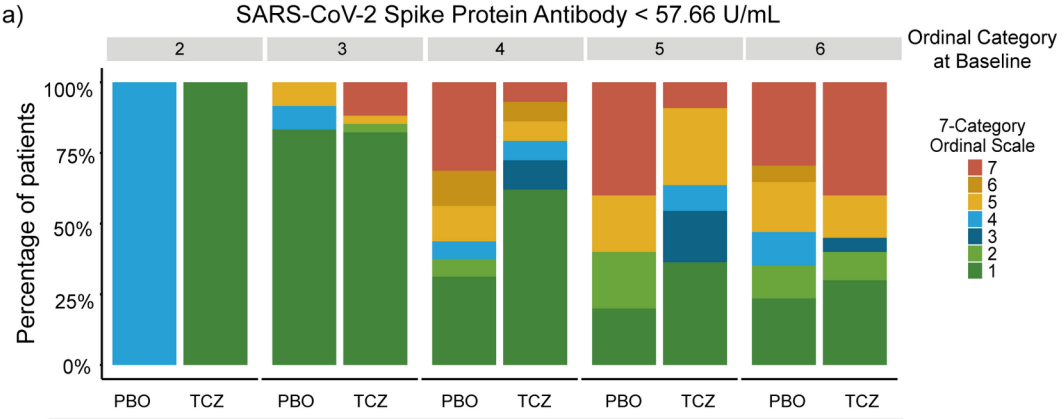


Fig. 2 Outcomes of the population from the COVACTA trial based on treatment and neutralizing Ab titers Probability of mortality at day 28 and 95% CI by **(a)** study arm and neutralizing Ab titers adjusted for sex, age, days from symptoms, and baseline ICU stay, or by **(b)** study arm and neutralizing Ab titers dichotomized (median = 83) adjusted for sex, age, days from symptoms, and baseline ICU stay. Mean was 0.22 (0.11 to 0.31) in the tocilizumab group with low titers of neutralizing Ab and 0.21 (0.08 to 0.33) in the placebo group with low levels of neutralizing Ab (LRT p-value = 0.427). Probability of ICU stay at day 28 and 95% CI by **(c)** study arm and neutralizing Ab titers adjusted for sex, age, and days from symptoms or by **(d)** study arm and neutralizing Ab titers dichotomized (median = 83) adjusted for sex, age, and days from symptoms. Mean was 0.60 (0.49 to 0.71) in the tocilizumab group with low levels of neutralizing Ab titers and 0.79 (0.65 to 0.89) in the placebo group with low levels of neutralizing Ab titers (LRT p-value = 0.111). PBO = placebo, TCZ = tocilizumab

thereby measuring all neutralizing antibodies present in the serum. Conversely, the immunoassay technique specifically measures the presence of a single type of Ab, in this case, S-spike Ab [9]. It is crucial to highlight these differences in detection techniques, as they elucidate why we found a significant predictive value for S-spike Abs compared to neutralizing Abs, despite observing a similar effect for S-spike Abs without significant differences. Furthermore, the predictive value of S-spike Ab levels offers practical advantages over neutralizing Abs due to the higher cost and complexity of analysing the latter, which requires laborious, costly PRNT techniques and biosafety level 3 laboratory settings [9, 13], which standard laboratories are not equipped with. The immunoassay technique used for S-spike Abs is a widespread method present in most laboratories, can be automated, offers quick turnaround times, and is widely available in routine laboratories.

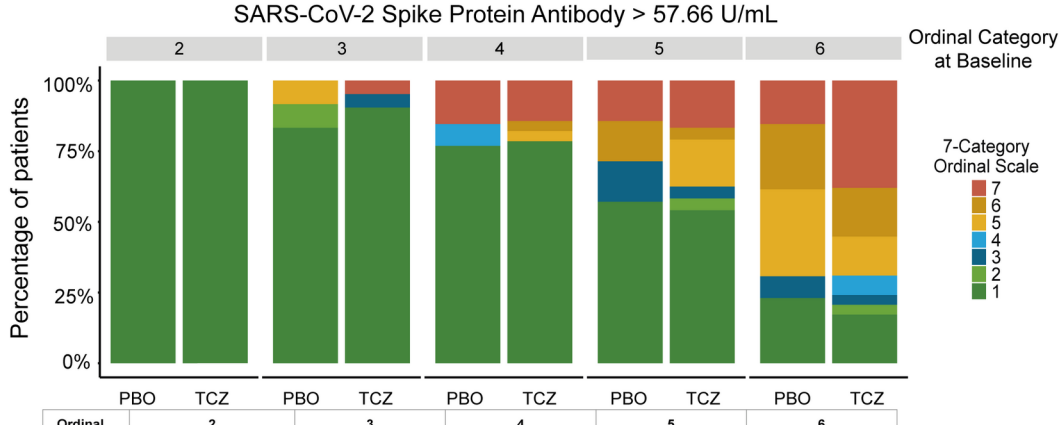
Focusing on S-spike Abs, tocilizumab shows a better response in patients with low Ab levels, as reflected in the evolution of the ordinal clinical scale used in COVACTA. A higher percentage of patients in the tocilizumab group are found in lower grades of the scale, especially those initially at grade 4 (non-invasive ventilation and high-flow oxygen). Additionally, patients with low S-spike Ab levels had fewer days from the onset of COVID-19 symptoms. These two findings underscore the importance of the timing of this treatment, particularly regarding

a)



Ordinal Category	2		3		4		5		6	
	PBO	TCZ	PBO	TCZ	PBO	TCZ	PBO	TCZ	PBO	TCZ
7	0	0	0	4 (11.8%)	5 (31.2%)	2 (6.9%)	2 (40.0%)	1 (9.1%)	5 (29.4%)	8 (40.0%)
6	0	0	0	0	2 (12.5%)	2 (6.9%)	0	0	1 (5.9%)	0
5	0	0	1 (8.3%)	1 (2.9%)	2 (12.5%)	2 (6.9%)	1 (20.0%)	3 (27.3%)	3 (17.6%)	3 (15.0%)
4	1 (100.0%)	0	1 (8.3%)	0	1 (6.2%)	2 (6.9%)	0	1 (9.1%)	2 (11.8%)	0
3	0	0	0	0	0	3 (10.3%)	0	2 (18.2%)	0	1 (5.0%)
2	0	0	0	1 (2.9%)	1 (6.2%)	0	1 (20.0%)	0	2 (11.8%)	2 (10.0%)
1	0	4 (100.0%)	10 (83.3%)	28 (82.4%)	5 (31.2%)	18 (62.1%)	1 (20.0%)	4 (36.4%)	4 (23.5%)	6 (30.0%)

b)



Ordinal Category	2		3		4		5		6	
	PBO	TCZ	PBO	TCZ	PBO	TCZ	PBO	TCZ	PBO	TCZ
7	0	0	0	1 (4.8%)	2 (15.4%)	4 (14.3%)	1 (14.3%)	4 (16.7%)	2 (15.4%)	11 (37.9%)
6	0	0	0	0	0	1 (3.6%)	1 (14.3%)	1 (4.2%)	3 (23.1%)	5 (17.2%)
5	0	0	1 (8.3%)	0	0	1 (3.6%)	0	4 (16.7%)	4 (30.8%)	4 (13.8%)
4	0	0	0	0	1 (7.7%)	0	0	0	0	2 (6.9%)
3	0	0	0	1 (4.8%)	0	0	1 (14.3%)	1 (4.2%)	1 (7.7%)	1 (3.4%)
2	0	0	1 (8.3%)	0	0	0	0	1 (4.2%)	0	1 (3.4%)
1	2 (100.0%)	1 (100.0%)	10 (83.3%)	19 (90.5%)	10 (76.9%)	22 (78.6%)	4 (57.1%)	13 (54.2%)	3 (23.1%)	5 (17.2%)

Fig. 3 (See legend on next page.)

(See figure on previous page.)

Fig. 3 7-Category ordinal scale at day 28, according to the category at baseline, stratified by S-spike Ab. Percentage of patients in each category of the ordinal scale at day 28 in each arm of treatment and grade at baseline in patients with **(a)** low levels of SARS-CoV-2 Spike Protein Abs (levels below 57.66 U/mL) and **(b)** high levels of SARS-CoV-2 Spike Protein Abs (levels above 57.66 U/mL). Below each bar plot, the contingency table of each graph is represented. The 7 categories are as follows: (1) discharged/ready for discharge, (2) non-ICU hospital ward/ready for hospital ward, not requiring supplemental oxygen, (3) non-ICU hospital ward/ready for hospital ward, requiring supplemental oxygen, (4) ICU or non-ICU hospital ward, requiring noninvasive ventilation or high-flow oxygen, (5) ICU, requiring intubation and mechanical ventilation, (6) ICU, requiring extracorporeal membrane oxygenation or mechanical ventilation and additional organ support, (7) death. PBO = placebo, TCZ = tocilizumab

oxygen support and symptom duration. Other clinical trials, such as RECOVERY and REMAP-CAP, have also shown tocilizumab's effectiveness in patients with rapid onset of respiratory failure, those admitted to ICU within 24 h, and those receiving noninvasive ventilation, including high-flow nasal oxygen [1, 2].

In this line, our results highlight the potential risks of indiscriminate use of tocilizumab, especially in patients with high levels of S-spike Ab. When Ab titers exceed the suggested median value, tocilizumab administration may be associated with poorer outcomes compared to placebo in the current cohort, especially regarding the 28-day death probability. The ambiguous effects of tocilizumab have been documented in various studies [14–16]. One plausible explanation for the lack of benefit in patients with high antibody levels is that these individuals may have already developed adaptive immunity and progressed beyond the phase of immune dysregulation and pro-inflammatory response [6]. Consequently, the anti-inflammatory benefits of tocilizumab are not observed, and its null or potentially harmful effects become more pronounced. Another reason may be the aberrant production of Abs which could trigger an exacerbated cellular immune response, leading to worse outcomes. It has been reported that patients with severe COVID-19 exhibit a specific Ab profile (afucosylated IgG Abs targeting the S-spike protein and the N protein) that induces potent inflammatory responses. This involves the activation of primary monocytes and natural killer cells, among others, thereby worsening the patient's condition [17], and thus invalidating the therapeutic effect of tocilizumab.

Our research has several limitations. Firstly, despite conducting the analysis using data from a randomized clinical trial, some outcomes may be influenced by a relatively small number of events and require validation in larger cohorts. Secondly, the trial had a higher proportion of ICU-admitted cases, limiting data from general ward patients. Therefore, further examination of the effects of additional therapies (e.g., antiviral drugs) in patients with low S-spike Ab levels is warranted [10]. Finally, our results mainly apply to an unvaccinated population, as patients were enrolled in the COVACTA trial before COVID-19 vaccines were available (first semester of 2020) [6]. SARS-CoV-2 vaccines induce the generation of Abs, including S-spike Ab, which often reach levels above the proposed cutoff point shown in our article [18]. Although these spike-binding Abs decay over time, their levels remain above the proposed cutoff point [19]. Moreover, previous SARS-CoV-2 infections confer high levels of neutralizing Abs that can persist for 4 to 7 months [20], adding another difference to the COVACTA population, who were not previously infected. Therefore, the use of S-spike Ab levels as a predictor of tocilizumab efficacy should primarily be considered in unvaccinated populations or individuals with immune deficiencies, who exhibit delayed or absent adaptive immune responses. These patients particularly need such therapies.

In summary, our findings emphasize a critical window for effective tocilizumab response, specifically for individuals with low S-spike Ab levels. This is particularly relevant for vulnerable individuals with declining immunity, who are at risk of severe COVID-19. Additionally, this marker can be easily detected in blood, providing results within 24 h of hospital admission, enabling the identification of patients most likely to benefit from these therapies.

Conclusions

This study has shown that tocilizumab, compared to placebo, significantly improved 28-day mortality and reduced ICU stay in severe COVID-19 patients with low S-spike antibody levels.

Abbreviations

Ab	Antibody
ICU	Intensive care unit
LRT	Likelihood ratio test
mITT	Modified intention-to-treat
PRNT	Plaque reduction neutralization test
RBD	Receptor binding domain
VOC	Variants of concern

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-025-11001-6.

Supplementary Material 1

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Author contributions
All authors made significant contributions to this manuscript and reviewed the final version. JA was responsible for conceptualization, data interpretation, writing, and critical revision. CF handled data curation, statistical analysis, data interpretation, writing, and critical review. AGC focused on data curation, data interpretation, writing, and critical review. GSC contributed to data interpretation, writing, and critical review. DCR, GSF, and JTSR were involved in the critical review of the manuscript. PGV was in charge of conceptualization, data interpretation, writing, critical revision, supervision, and project administration.

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Data availability
The research data that support the findings of this study belongs to the COVACTA trial and are available from Vivli, Inc., a global data-sharing platform for clinical research. Access to these data will be granted following the guidance outlined in the article from the REMAP-CAP investigators in the New England Journal of Medicine (N Engl J Med. 2021;384(16):1503-1516).

Declarations

Ethics approval and consent to participate
Ethical approval was not required as the work consists of a post hoc analysis of published data.

Consent for publication
Not applicable.

Competing interests
José Aguareles received meeting grant from PharmaMar SA and served as a scientific consultant. Carles Forné (Heorfy Consulting) has received fees from Hospital Universitario Quirónsalud Madrid for statistical analysis. Pablo Guisado-Vasco received speaker fees from FLS Science, PharmaMar SA (Madrid, Spain) and GlaxoSmithKline (Spain); consulting fees from Angelini Pharma and PharmaMar SA; served as an advisory board member for Berlin Cures GmbH and PharmaMar SA; and meeting grants from GlaxoSmithKline and PharmaMar SA. All remaining authors have declared no conflicts of interest.

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