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Research paper

Impact of interleukin-6 blockade with tocilizumab on SARS-CoV-2 viral kinetics and antibody responses in patients with COVID-19: A prospective cohort study

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ABSTRACT

Background: The virological and immunological effects of the immunomodulatory drugs used for COVID-19 remain unknown. We evaluated the impact of interleukin (IL)-6 blockade with tocilizumab on SARS-CoV-2 viral kinetics and the antibody response in patients with COVID-19.
Methods: Prospective cohort study in patients admitted with COVID-19. Serial nasopharyngeal and plasma samples were measured for SARS-CoV-2 RNA and S-IgG/N-IgG titers, respectively.
Findings: 138 patients with confirmed infection were included; 76 (55%) underwent IL-6 blockade. Median initial SOFA (p = 0•016) and SARS-CoV-2 viral load (p<0•001, Mann-Whitney-Wilcoxon test) were significantly higher among anti-IL-6 users. Patients under IL-6 blockade showed delayed viral clearance in the Kaplan-Meier curves (HR 0•35 [95%CI] [0•15−0•81], log-rank p = 0•014), but an adjusted propensity score matching model did not demonstrate a significant relationship of IL-6 blockade with viral clearance (HR 1•63 [0•35−7•7]). Cox regression showed an inverse association between SARS-CoV-2 RNA clearance and the initial viral load (HR 0•35 [0•11−0•89]). Patients under the IL-6 blocker showed shorter median time to seropositivity, higher peak antibody titers, and higher cumulative proportion of seropositivity in the Kaplan Meier curves (HR 3•1 [1•9−5] for S-IgG; and HR 3•0 [1•9−4•9] for N-IgG; log-rank p<0•001 for both). However, no significant differences between groups were found in either S-IgG (HR 1•56 [0•41−6•0]) nor N-IgG (HR 0•96 [0•26−3•5]) responses in an adjusted propensity score analysis.
Interpretation: Our results suggest that in patients infected with SARS-CoV-2, IL-6 blockade does not impair the viral specific antibody responses. Although a delayed viral clearance was observed, it was driven by a higher initial viral load. The study supports the safety of this therapy in patients with COVID-19.
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1. Introduction

The novel severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) disease (COVID-19) represents a major threat to human health worldwide. In contrast to other respiratory viruses, SARS-CoV-2 induces a hyper-inflammatory response associated with a disproportionate cytokine and chemokine release that leads to severe lung damage, multiorgan failure, and eventually death [1]. Consistently, therapeutic strategies against COVID-19 have not only involved antiviral agents, but also different immunomodulatory drugs in an attempt to block the inflammatory pathways activated by the virus. Among them, cytokine-targeted therapies have been commonly used in patients with COVID-19, and particularly tocilizumab, a humanized monoclonal antibody anti-interleukin 6 (IL-6) receptor [2,3]. Available data on the effects of IL-6 blockade in patients with COVID-19 come from observational studies, mostly in severely-ill patients, where it has been associated with clinical and radiological improvement [4–6]. However, the impact of IL-6 blockade on SARS-CoV-2 replication and on the immune response against the virus remain largely unknown. IL-6 is a multifunctional cytokine that regulates many aspects of innate and adaptive immunity [7]. In addition to inducing acute-phase protein production, this cytokine stimulates the differentiation and maturation of cytotoxic

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Research in context

Evidence before this study

The novel severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) disease (COVID-19) induces a hyper-inflammatory response associated with a disproportionate cytokine and chemokine release that leads to severe lung damage, multiorgan failure, and eventually death. Consistently, in addition to antiviral agents, therapeutic strategies have also included immunomodulatory drugs in an attempt to block the inflammatory pathways activated by the virus. Among them, cytokine-targeted therapies have been commonly used, and particularly tocilizumab, a humanized monoclonal antibody anti-interleukin 6 (IL-6) receptor. In the literature search, no published studies have assessed the impact of IL-6 blockade on SARS-CoV-2 replication and on the immune response against the virus.

Added value of this study

Our study is the first to show that IL-6 blockade does not impair the specific antibody response against SARS-CoV-2. Although viral clearance is delayed in patients receiving tocilizumab, this effect is mainly driven by the initial viral load.

Implications of all the available evidence

This study supports the safety of this anti-cytokine therapeutic strategy for COVID-19 from a virological and immunological perspective. Our results can also be extrapolated to patients receiving tocilizumab for rheumatologic diseases who acquire this infection, and potentially other acute viral infections, and warrant additional studies to confirm if the same effects occur with other anti-cytokine drugs.

T-lymphocytes, and macrophage/monocyte functions [8]. Consequently, therapy directed against IL-6 could interfere with viral clearance. Noteworthy, tocilizumab has been associated with severe viral infections caused by cytomegalovirus and varicella-zoster in patients with rheumatoid arthritis [9–11]. This would be particularly concerning due to the close correlation found between disease severity, IL-6 levels and SARS-CoV-2 viral load in patients with COVID-19 [12]. IL-6 also plays an important role in the differentiation of B-cells into antibody producing plasma cells and immunoglobulin secretion [13]. As a result, anti-IL-6 therapy might impair the antibody response against the virus, which could compromise viral clearance and future protection against reinfections.

In March 2020, the Spanish Agency of Medicines and Medical Devices granted an emergency-use authorization for using tocilizumab in the setting of COVID-19, and our center developed specific guidelines for treating patients requiring hospital admission. We investigated the longitudinal effects of IL-6 blockade on viral shedding and on the antibody response to SARS-CoV-2 in a cohort of patients admitted with COVID-19 and compared them with the non-anti-cytokine-treated patients.

2. Methods

2.1. Study design and patients

This prospective, observational study was carried out at the University Hospital of Elche, Spain. All patients admitted for COVID-19 between March 10th and April 17th, 2020, were included in the analysis. Patients had confirmed or probable COVID-19 according to European centre for Disease Prevention and Control criteria (https://www.ecdc.europa.eu/en/covid-19/surveillance/case-definition). Microbiological confirmation was performed through real-time polymerase chain reaction (RT-PCR), mostly from nasopharyngeal smear samples, and rarely from sputum, bronchial aspirate, or fecal samples.

Patients were managed according to a pre-defined protocol that included the diagnostic and therapeutic procedures during hospital stay. Blood samples for routine lab tests and biomarkers of cytokine release syndrome, serologic tests, and nasopharyngeal samples for SARS-CoV-2 were serially obtained at different time-points during hospital stay. Plasma samples for the measurement of the levels of antibodies to SARS-CoV-2 were collected and frozen at −80 °C.

Therapy for COVID-19 was given following institutional guidelines. Patients received antimicrobial and/or immunomodulatory therapy containing lopinavir/ritonavir, hydroxychloroquine, azithromycin, interferon-β−1b or remdesivir ± methylprednisolone. According to guidelines, tocilizumab was added to initial therapy on admission at a dose of 600 mg intravenously if the weight was ≥75 kg or 400 mg when the weight <75 kg if any of pre-established clinical (including oxygen saturation levels and respiratory frequency rate), radiological (presence of bilateral multilobar infiltrates) or inflammatory biomarkers (including lymphocyte count, interleukin-6, ferritin, D-dimer, fibrinogen and C-reactive protein) criteria were met (See supplementary (S)-Table 1). Patients were reevaluated on the following 24 h, and if no clinical response was achieved (S-Table 1), a second dose of tocilizumab (400 mg) or intravenous methylprednisolone were administered. The protocol was approved by the Ethical Committee of the Hospital General Universitario de Elche (Spain) as part of the COVID-19@Spain study. Informed consent was obtained from all subjects.

2.2. SARS-CoV-2 RNA measurement

For RNA extraction and RT-PCR analysis for SARS-CoV-2, nasal and oropharyngeal flock swabs were placed together into 3 mL transport medium (VICUM®, Deltalab, Rubí, Spain). Viral RNA was extracted from 350 μL of the medium using the RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and eluted in a final 50 μL nucleic acid elution sample. Eight μL RNA was used for detection of SARS-CoV-2 by RT-PCR, with a commercially available kit (AllplexTM 2019-nCoV Assay, Seegene, Seoul, Korea) which targeted the E, RdRP, and N genes. Viral load measurements of nasal/throat samples (log10 copies/sample) were performed with a standard curve of ten-fold serial dilutions from an in vitro RNA transcript (Macrogen, Seoul, Korea). The lower limit of detection was 64 copies/sample. Assay procedure was carried out in accordance with the manufacturer's protocol, in a CFX96 real-time thermocycler (Bio-Rad, California, USA). The success of RNA extraction and PCR were assessed by the internal control included in the kit and negative and positive controls were used in each assay.

2.3. Antibody measurement against SARS-CoV-2

IgG antibody plasma levels against the SARS-CoV-2 internal nucleocapsid (N) protein (N-IgG) (Anti-SARS-CoV-2-NCP IgG ELISA, Euroimmun, Lubeck, Germany) and surface S1 domain of the spike protein (S-IgG) (Anti-SARS-CoV-2 IgG ELISA, Euroimmun, Lubeck, Germany) were measured in EDTA plasma samples using commercial semi-quantitative EIA kits in an automated instrument (Dynex DS2® ELISA system) following the manufacturer instructions. Antibody levels were evaluated by calculating the ratio of the optical density (OD) of the patient sample over the OD of the calibrator (sample OD/calibrator OD = *S*/CO [absorbance/cut-off]). Results were interpreted according to the following criteria: ratio ≤1•1 was defined as negative and ratio >1•1 as positive.

2.4. Statistical analyses

Continuous variables are expressed as median ± 25th and 75th percentiles (Q1, Q3), and categorical variables as percentages. Wilcoxon or Student's *t*-test were used to compare continuous variables, and the chi-square or Fisher's exact test for categorical variables comparison among anti-IL6 therapy treated and untreated patients.

Kaplan Meier curves and the two-sided log-rank test were used to evaluate the differences in the duration of viral shedding and antibody positivity between patients receiving and not receiving anti-cytokine therapy. To balance treatment groups, a propensity score matching logistic regression model was fitted with a 1:1 ratio among groups. Covariates with a p-value <0•05 in the crude comparison between treatment groups were used for matching. To further adjust for the covariates that remained unbalanced between treatment groups after matching, Cox proportional hazard regression was run including the statistically different variables. Statistical analysis was performed using R-project version 3.6.2 (2019–12–12).

2.5. Role of the funding source

The funder of the study had no direct role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

3. Results

During the study period, 210 adult patients were admitted with COVID-19. Clinical data of the entire cohort are shown in S-Table 2. A total of 138 (65•7%) patients had confirmed SARS-CoV-2 infection with RT-PCR in a sample other than feces; 133 (96•4%) from nasopharyngeal samples (six patients also had positive results in sputum and four in bronchial aspirate), and 5 (3•6%) from sputum. Of them, 76 (55%) underwent IL-6 blockade. Demographic and clinical data from these patients are shown in Table 1. Patients undergoing IL-6 blockade were more frequently male, showed a higher severity of disease as supported by a higher median SOFA score at anti-cytokine therapy initiation, lower peripheral blood oxygen saturation/fraction of inspired oxygen rate on admission, higher frequency of pneumonia

Table 1
Clinical data of patients admitted with COVID-19 confirmed with real-time polymerase chain reaction.

Variable	Non Tocilizumab *N* = 62	Tocilizumab *N* = 76	Total*N* = 138	P
Sex, male	31 (50•0)	54 (71•1)	85 (61•6)	0•014
Age, years	68•5 (53•2–76•0)	62•0 (56•8–77•0)	64•0 (55•2–76•8)	0•961
Active smoking	35 (60•3)	42 (59•2)	77 (59•7)	1•000
Charlson comorbidity index	3•0 (1•0–5•8)	3•0 (1•0–5•0)	3•0 (1•0–5•0)	0•402
Comorbidities				
Diabetes	17 (27•4)	14 (18•4)	31 (22•5)	0•225
Congestive heart failure	5 (8•1)	4 (5•3)	9 (6•5)	0•731
Previous AMI	6 (9•7)	7 (9•2)	13 (9•4)	1•000
Stroke	8 (12•9)	1 (1•3)	9 (6•5)	0•011
Respiratory disease	10 (16•1)	13 (17•1)	23 (16•7)	1•000
Renal disease	8 (12•9)	8 (10•5)	16 (11•6)	0•791
Peripheral arterial disease	3 (4•8)	2 (2•6)	5 (3•6)	0•657
Clinical status				
Days from symptom onset to admission	6•0 (2•0–11•0)	7•0 (4•0–10•0)	7•0 (3•0–10•0)	0•321
SOFA score on admission	2•0 (1•2–2•8)	2•0 (2•0–3•0)	2•0 (2•0–3•0)	0•016
SOFA score at TCZ initiation*	2•0 (1•8–3•0)	3•0 (2•0–3•5)	2•0 (2•0–3•0)	0•017
SpO2/FIO2 on admission	354 (346–458)	346 (336–380)	350 (343–451)	0•010
Pneumonia	36 (59•0)	63 (82•9)	99 (72•3)	0•002
Bilateral lung infiltrates on X Ray	22 (35•5)	56 (75•7)	78 (67•8)	0•022
Microbiological data				
SARS-CoV-2 RNA, log10 copies/sample	1•98 (1•59–3•63)	3•77 (2•72–4•67)	3•11 (1•97–4•30)	<0•001
Cycle threshold <36	18 (32·1)	55 (73·3)	73 (55•7)	<0•001
Cycle threshold 36–38	11 (19·6)	12 (16·0)	23 (17•6)	
Cycle threshold >38	27 (48·2)	8 (10·7)	35 (26•7)	
Peak S-IgG, S/CO	2•7 (0•1–6•3)	6•4 (5•9–7•1)	6•0 (3•4–6•9)	<0•001
Peak N-IgG, S/CO	2•8 (0•1–4•7)	4•5 (3•7–5•0)	4•3 (2•5–4•9)	0•001
Biomarkers				
Interleukin-6, pg/mL	12•7 (5•4–30•5)	44•9 (18•0–105)	23•1 (11•4–80•4)	<0•001
Ferritin, ng/mL	221 (85•8- 384)	416 (257–611)	303 (142- 478)	<0•001
C-reactive protein, mg/L	34•5 (4•9–68•4)	60•1 (32•9–115)	50•0 (19•9 to 98•7)	0•001
Fibrinogen, mg/dL	452•0 (347–624)	637•0 (455–792)	552 (361–760)	0•011
Lymphocytes, x103/μL	1•4 (0•9–1•9)	1•1 (0•8–1•3)	1•2 (0•8–1•5)	<0•001
Neutrophil tolymphocyte ratio	4•9 (3•5–7•2)	4•3 (3•3–6•2)	4•6 (3•5–6•7)	0•122
D-dimer, μg/mL	0•8 (0•3–2•1)	0•7 (0•5–1•6)	0•7 (0•4–1•7)	0•950
NT-proBNP, pg/mL	73•5 (25•5–235)	85•0 (41•0–238)	81•0 (38•2–242)	0•699
Outcomes				
Death	8 (12•9)	2 (2•6)	10 (7•2)	0•043
ICU admission	8 (12•9)	9 (11•8)	17 (12•3)	1•0
Hospital stay, days	9•0 (6•0–13•0)	13•0 (11•0–20•8)	12.0 (9•0–17•0)	<0•001
Concomitant antimicrobial/ immunomodulatory drugs, no. (%)				
HCQ-based combinations	61 (98•4)	74 (97•4)	135 (97•8)	1•000
Azithromycin	54 (87•1)	73 (96•1)	127 (92•0)	0•064
Lopinavir/ritonavir	48 (77•4)	76 (100•0)	124 (89•9)	<0•001
Remdesivir	1 (1•6)		1 (0•7)	0•449
Interferon-β–1b	11 (17•7)	19 (25•0)	30 (21•7)	0•407
Methylprednisolone[&]	3 (4•8)	24 (31•6)	27 (19•6)	<0•001

Categorical variables are expressed as no. and (%), and continuous variables as median (Q1-Q3). Mann-Whitney-Wilcoxon test was used to compare continuous variables, and Fisher's exact test to compare categorical variables. AMI, acute myocardial infarction; SOFA, Sequential Organ Failure Assessment; TCZ, tociluzumab; SpO2/FIO2, peripheral blood oxygen saturation/fraction of inspired oxygen rate; S/CO, absorbance/cut-off; NT-proBNP, N-terminal pro b-type natriuretic peptide; ICU, Intensive Care Unit; HCQ, hydroxychloroquine. For the non-tocilizumab group, the number represents the median SOFA score of patients at anti-COVID therapy initiation. [&]Short course methylprednisolone 0•5–1 mg/kg/day divided in 2 intravenous doses for 3 days.

and bilateral lung infiltrates, and longer median hospital stay. The frequency of death was lower among patients on IL-6 blockade (2•6% vs 12•9%, p = 0•043, Fisher's exact test). Regarding concomitant treatment, patients receiving immunomodulatory therapy were more frequently treated with lopinavir/ritonavir, and received more frequently concomitant therapy with methylprednisolone (Table 1).

3.1. Viral dynamics of SARS-CoV-2

Patients undergoing IL-6 blockade showed significantly lower initial cycle threshold (Ct) values and higher median viral load (Table 1). At the last follow-up visit, after a median (Q1-Q3) of 39 (31–62) days and 31 (30–44) days from admission in patients with and without IL-6 blockade, respectively, 43 (57•3%) and 44 (78•6%), respectively, showed undetectable levels of SARS-CoV-2 RNA (p = 0•015, Fisher's exact test), and the number of patients with two consecutive negative results was 23 (30•7%) and 32 (57•1%), respectively (p = 0•004, Fisher's exact test). For patients who had achieved viral clearance, median (Q1-Q3) time to negative viral load was 39•0 (18•0 to 40•0) days vs 35•0 (10•8–45•0) days in patients with and without anti-cytokine therapy, respectively (p = 0•734, Mann-Whitney-Wilcoxon test). Kaplan Maier curves showed a greater probability of SARS-CoV-2 clearance during follow-up among non-anti-cytokine users: hazard ratio (HR) (95% confidence interval, CI) 0•35 (0•15–0•81), log-rank p = 0•01 for viral shedding (Fig. 1a). Cox proportional hazard regression model adjusted for the initial viral load and the SOFA score showed that the relationship of IL-6 blockade with viral clearance was significantly weakened (HR 0•60 [95% CI, 0•21–1•70], p = 0•34, Wald test). The model also showed that initial viral load levels (HR 0•56 [95% CI 0•36–0•87], p = 0•01, Wald test), but not the SOFA score (HR 1•28 [95% CI, 0•00–1•26], p = 0•20, Wald test) were associated with viral clearance. A propensity score matching model was fitted to further examine the effect of IL-6 blockade on viral shedding after balancing treatment groups. Covariates with a p-value <0•05 in the comparison between anti-cytokine treatment groups were included in adjustment. The model yielded a total of 58 participants (29 on each group) with remaining residual differences, so it was further adjusted for the covariates that remained unbalanced between groups with a Cox proportional hazard regression analysis, specifically the levels on admission of IL-6, ferritin, C-reactive-protein (CRP), lymphocytes, and viral load. Therapy with methylprednisolone was also included to assess its effect on viral clearance. In the adjusted model, the association of IL-6 blockade with viral shedding did not remain significant: HR 1•68 (0•36–7•81), p = 0•510, Wald test (Fig. 1b). In a non-inferiority analysis, treatment with tocilizumab showed to be non-inferior to not receiving tocilizumab for achieving viral clearance (p = 0.018) (normal approximation method using a Z test statistic), considering that treatment with tocilizumab was non-inferior if the HR of the adjusted model was greater than 0.33.

3.2. Antibody response to SARS-CoV-2

Of 181 patients with available serological samples, 120 with follow-up samples were finally included for analysis (see Supplementary Figure 1). Of them, 73 (60•8%) patients underwent IL-6 blockade. The proportion of patients receiving immunomodulatory therapy with positive S-IgG and N-IgG antibodies was 94•5% (n = 69) after a median (Q1-Q3) of 35 (28–60) days from the onset of symptoms vs 55•3% (n = 26) (p<0•001, Fisher test) after 31 (29–42•5) days in untreated patients. Median (Q1-Q3) time from the onset of symptoms to seropositivity for S-IgG in patients receiving anti-IL6 therapy was 14 (11–17) days vs 17 (15–24) days (p = 0•014, Mann-Whitney-Wilcoxon test) in untreated patients (Fig. 2a); and 12 (9–14) and 15 (11•2–20•2) days, respectively, for N-IgG (p = 0•017, Mann-Whitney-Wilcoxon test) (Fig. 2b). Peak S-IgG titers among patients with or without anti-cytokine therapy were 6•4 (5•9–7•1) vs 2•7 (0•1–6•3) S/CO (p<0•001, Mann-Whitney-Wilcoxon test) (Fig. 3a); and peak N-IgG titers were 4•5 (3•7–5•0) and 2•8 (0•1–4•7) S/CO, respectively (p<0•001, Mann-Whitney-Wilcoxon test) (Fig. 3b). When patients who had not achieved detectable antibody levels were excluded, peak S-IgG titers in the anti-IL-6 treated and untreated patients were 6•4 (5•8–7) vs 6•1 (5–6•6) S/CO (p = 0•037, Mann-Whitney-Wilcoxon test) (Fig. 3c); and peak N-IgG titers were 4•6 (4•0–5•0) vs 4•6 (4–5•0) S/CO, respectively (p = 0•877, Mann-Whitney-Wilcoxon test) (Fig. 3d).

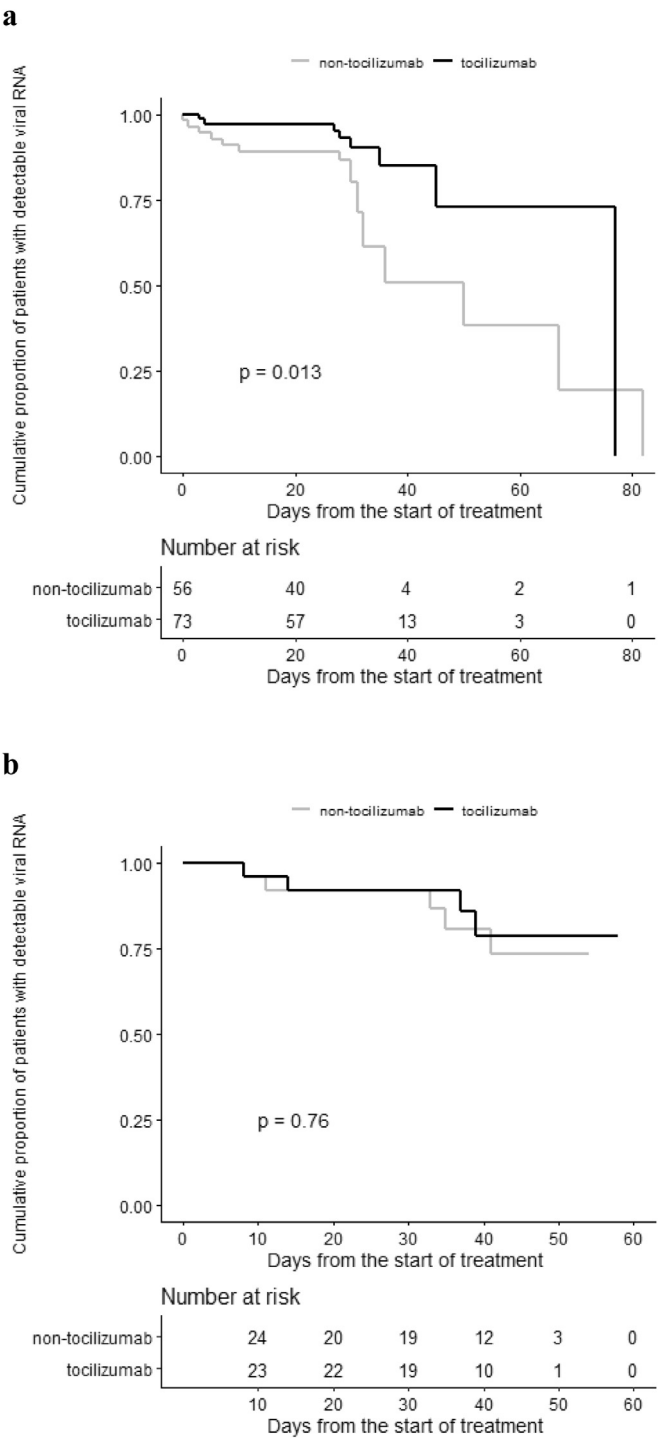

Fig. 1. Kaplan Meier curve to estimate the cumulative proportion of patients with detectable viral RNA according to therapy with tocilizumab. (a) Unadjusted. (b) Adjusted (Wald test).

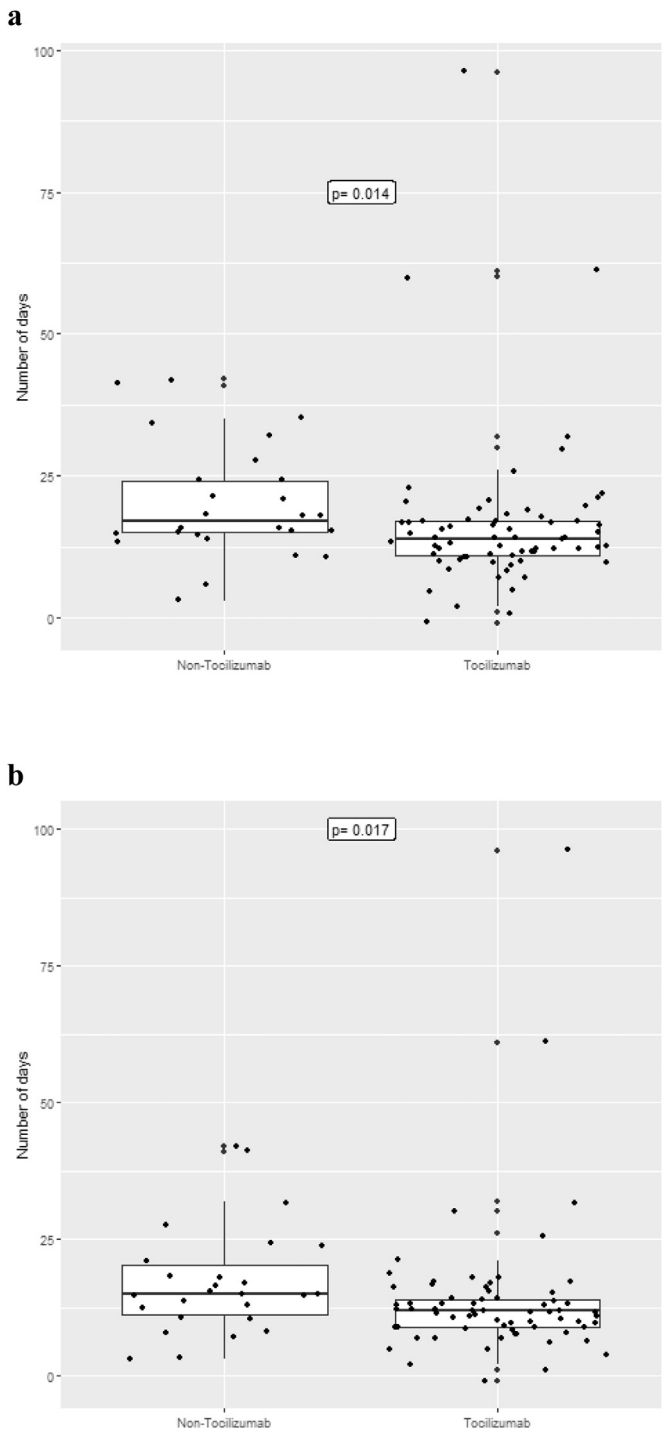

Fig. 2. Median time from the onset of symptoms to seropositivity according to therapy with tocilizumab (Mann-Whitney-Wilcoxon test). (a) S-IgG. (b) N-IgG.

Kaplan Meier curves showed a higher cumulative proportion of patients with detectable antibody levels among those under anti-cytokine therapy (HR [95% CI] 3•1 [1•9–5], $p<0•001$ for S-IgG and HR 3 [1•9–4•9], log-rank $p<0•001$ for N-IgG) (Figs. 4a and 4b). A propensity score matching model yielded 46 participants (23 on each group). In the adjusted final matched sample, where therapy with methylprednisolone was also included to assess its effect on antibody production, no significant association of IL-6 blockade with S-IgG or N-IgG response was found: HR (95% CI) for anti-IL-6 use was 1•66 (0•45–6•12) for S-IgG seropositivity (p = 0•444, log-rank test); and 1•05 (0•30–3•70) for N-IgG (p = 0•939, log-rank test) (Figs. 4c and 4d). In a non-inferiority analysis, treatment with tocilizumab showed to be non-inferior to not receiving tocilizumab for S-IgG (p = 0.007) and for N-IgG response (p = 0.049) (normal approximation method using a Z test statistic for both), considering that treatment with tocilizumab was non-inferior if the HR of the adjusted model was greater than 0.33.

3.3. Biomarkers of systemic release syndrome

The levels on admission and at immunomodulatory therapy initiation of IL-6, ferritin, CRP, fibrinogen, and the neutrophil-to-lymphocyte ratio (NLR) were significantly higher in patients receiving anti-IL6 therapy, and the lymphocyte and platelet counts were lower (Table 1). After anti-cytokine drug therapy, there was an early significant decrease at week-1 in the levels of CRP (median reduction [Q1 to Q3] - 69•9 [−84•1 to −55•8], $p<0•001$, Mann-Whitney-Wilcoxon test) and fibrinogen (median reduction −128•7 [−213•1 to −44•2 mg/dl], p = 0•003, Mann-Whitney-Wilcoxon test), and an increase in the lymphocyte (median increase 0•21 [0•09–0•32], $p<0•001$, Mann-Whitney-Wilcoxon test) and the platelet count (median increase 50•6 [33•6–67], $p<0•001$, Mann-Whitney-Wilcoxon test); at week-2, a decrease in the levels of IL-6 (−74•5 [−126•5 to −22•4], $p<0•001$, Mann-Whitney-Wilcoxon test), and NLR (−0•80 [−2•57 to 0•97], p = 0•028, Mann-Whitney-Wilcoxon test); and at week-4, a decrease of ferritin (−111•4 [−169•3 to −53•6], $p<0•001$, Mann-Whitney-Wilcoxon test). No significant changes were observed for these biomarkers in patients not receiving anti-cytokine therapy, with the exception of CRP. Peak decrease was observed at week-1 for CRP and fibrinogen (see above), at week-3 for the NLR (−3•48 [−7•61- 0•66], p = 0•005, Mann-Whitney-Wilcoxon test), at week-4 for ferritin (−111•4 [−169•3 to −53•56], $p<0•001$, Mann-Whitney-Wilcoxon test), and at week-5 for IL-6 (−154•7 [−282•3 to −27•09], p = 0•013, Mann-Whitney-Wilcoxon test).

4. Discussion

Cytokines regulate antiviral cellular and humoral responses [14]. We evaluated the virological and immunological impact of anti-cytokine therapy consisting on an IL-6 receptor blocker in patients with COVID-19. Our results suggest that IL-6 blockade does not impair the antibody response to SARS-CoV-2. Although viral shedding was longer in patients on anti-cytokine therapy, this effect was largely dependent on the initial viral load.

To the best of our knowledge, the effects of the immunomodulatory drugs used for SARS-CoV-2 infection on the viral kinetics and the viral-induced humoral immune response had not been previously assessed. Most of the clinical experience with tocilizumab comes from patients with chronic inflammatory disorders, mainly rheumatoid arthritis, where an increased risk of opportunistic and serious infections was reported in clinical trials [15]. In our cohort, patients with COVID-19 under IL-6 blockade exhibited prolonged viral shedding. Interleukin-6 plays a central role in the integrated innate and adaptive immune responses against pathogens. In experimental models, IL-6 deficiency was associated with increased susceptibility to certain viral infections, which was attributed to impaired activity of cytotoxic T-cells, and the T-helper-dependent virus neutralizing IgG response [14,16]. Clinical data about the risk of viral infections after IL-6 blockade are discordant, and although some cases of viral reactivation have been reported in patients with rheumatoid arthritis [9–11], no increase in the viral load of Epstein-Barr, cytomegalovirus, varicella zoster, or hepatitis C virus was evidenced during longitudinal follow-up [17,18]. Patients undergoing IL-6 blockade in our cohort had a higher initial viral load, along with clinical and biological data reflecting the greater disease severity of candidates to anti-

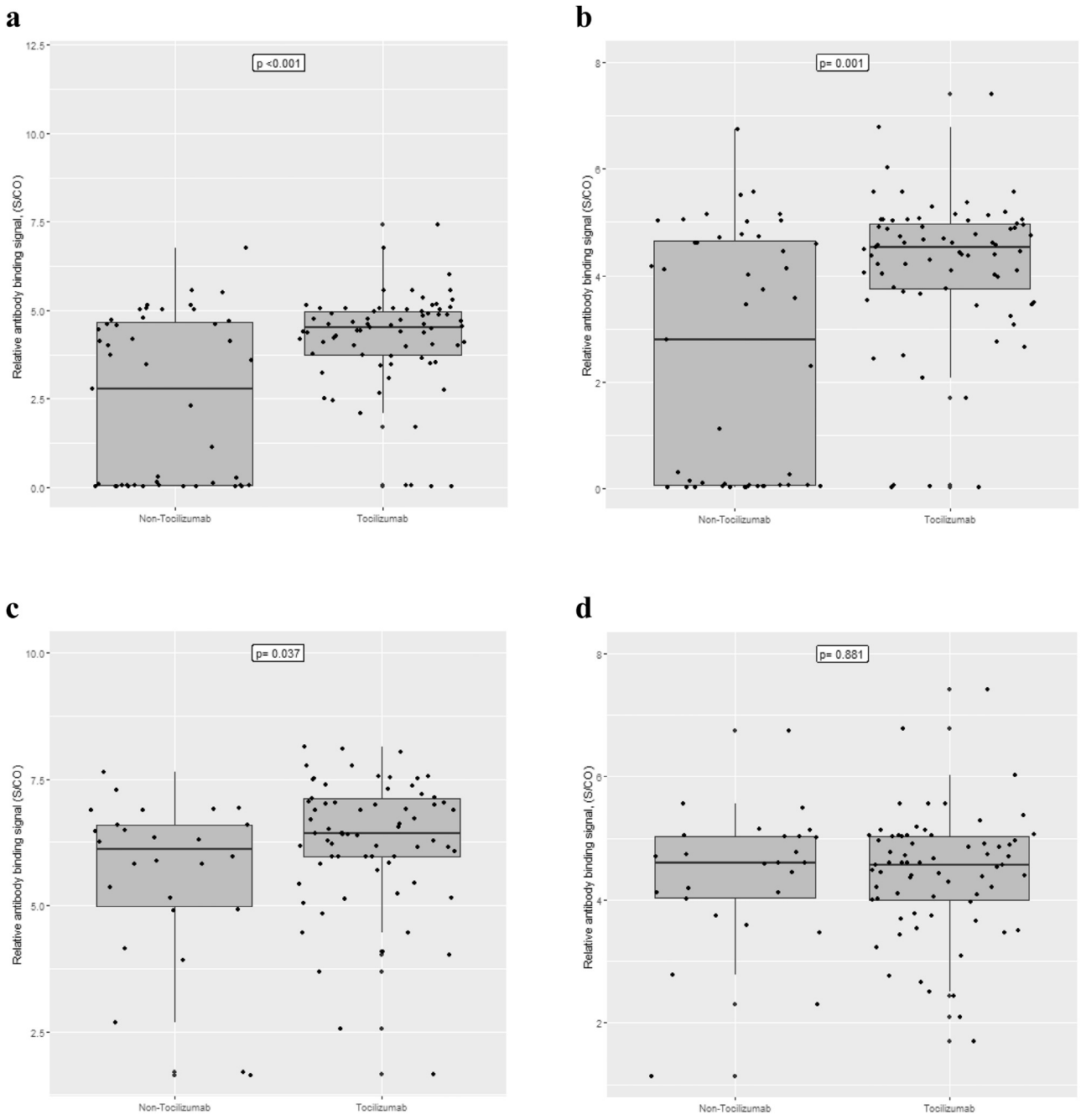

Fig. 3. IgG titers according to therapy with tocilizumab (Mann-Whitney-Wilcoxon test). (a) Peak S-IgG. (b) Peak N-IgG. (c) Peak S-IgG after excluding non seroconverters. (d) Peak N-IgG after excluding non seroconverters.

cytokine therapy. This is in agreement with the higher viral load and longer viral shedding described in severely-ill patients with COVID-19 compared with milder cases [19–22]. Adjustment for the viral load resulted in a significant attenuation of the relationship between IL-6 blockade and viral shedding, suggesting that viral load was a central factor implicated in viral persistence. The same effect was observed with adjustment for the variables related to severity status.

By contrast to duration of viral shedding, our results show that the blockage of IL-6 activity did not attenuate the antibody response to SARS-CoV-2. We found a rapid and robust humoral response in the group under IL-6 blockade, and neither the proportion of patients with positive antibody titers, the time to antibody production or the intensity of the antibody response were poorer than those observed in patients without anti-cytokine therapy. Our findings are consistent with the conserved immunogenicity after vaccination reported in patients with rheumatoid arthritis receiving tocilizumab [23–25]. One of the reasons argued for the apparently unaltered humoral response with anti-IL-6 therapy was that it does not induce depletion of B cells, and consequently other cytokines produced by T-helper cells could also stimulate lymphocytes B to induce the antibody production [24]. Early and strong antibody responses have been reported in severely-ill patients with COVID-19 [26], and an independent correlation between disease severity and higher levels of antibodies as well [26–28]. Patients under IL-6 blockade in our cohort had higher disease severity, and a higher initial viral load and longer viral shedding, which were likely involved in their enhanced humoral immune response. Actually, although patients under IL-6 blockade showed a higher frequency of detectable antibody titers, and in a faster time

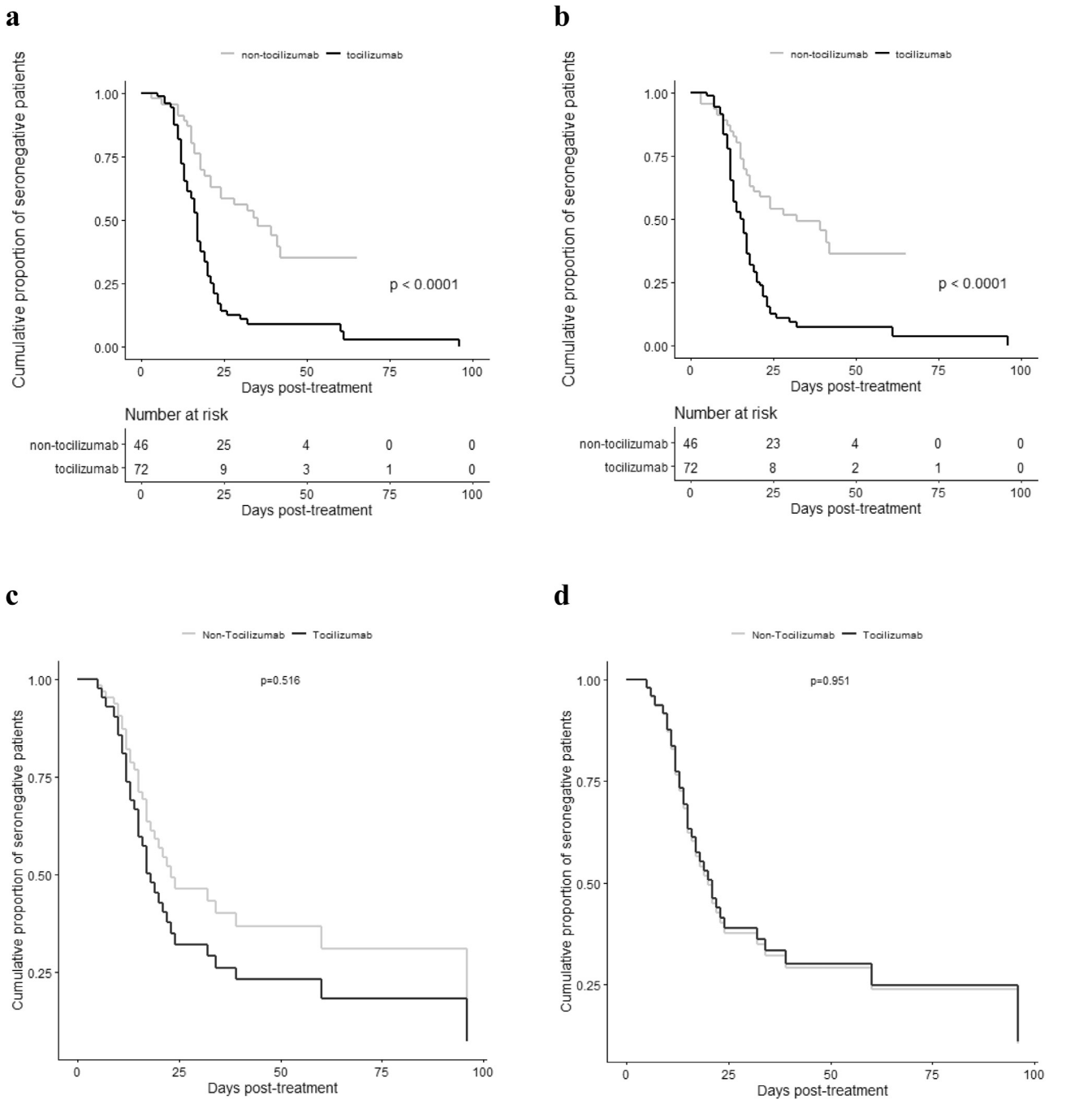

Fig. 4. Kaplan Meier curve to estimate the cumulative proportion of patients with negative titers of IgG according to therapy with tocilizumab. (a) S-IgG (unadjusted). (b) N-IgG (unadjusted). (c) S-IgG (adjusted) (log-rank test). (d) N-IgG (adjusted) (log-rank test).

than the non-anti-cytokine treatment group, no significant association between seropositivity and IL-6 blockade was found after adjustment for severity of disease and viral load levels. A high viral load might induce an immediate activation of extrafollicular B cells, leading to early and intense antibody production, although with less affinity than those induced by the antigen-specific B cells at the germinal center [28,29]. The strong and early antibody response observed in patients receiving anti-IL-6 therapy in our cohort included also high levels of the potentially neutralizing S-IgG antibodies [30], which might have contributed to their favorable outcome, despite this group contained a higher proportion of severely-ill patients.

IL-6 blockade was otherwise accompanied by the recognized positive impact on the biomarkers of cytokine release syndrome which, as expected, showed higher initial levels in the anti-cytokine treated group. The significant drop in the levels of IL-6 was delayed compared to the rapid decline observed in the concentrations of CRP, fibrinogen, or the lymphocytes after IL-6 blockade. This anti-inflammatory effect accompanied by the overall favorable outcome of the patients would argue in favor of a positive final balance of IL-6 blockade.

Limitations of the study include that SARS-CoV-2 RNA measurements were performed in upper respiratory tract samples, which may exhibit in some cases lower sensitivity than lower tract specimens. All patients also received combinations of antiviral agents with potential effects on viral dynamics, which differed between anti-cytokine treatment groups, especially regarding lopinavir/ritonavir and steroids. Because of the observational nature of the study,

patients receiving IL-6 blockade had a higher disease severity which might have confused some of the results in the comparisons with the non-treated groups. This is a single center study and the results may not therefore be generalizable to the wider population. There was a high proportion of patients with undetectable antibody titers among the non-anti-cytokine treated group. One of the potentially contributing factors could be the differences in the viral load and Ct values between the groups, although it would warrant further investigations. Strengths consist of the availability of serial measurements of respiratory and serum samples for the novel assessment of the effects of IL-6 blockade on viral dynamics and the antibody response in a high number of patients in real-life conditions.

Our data suggest that immunomodulatory therapy based on IL-6 blockade in patients with COVID-19 does not impair the specific antibody response. Although viral clearance was delayed, this effect was mainly driven by the initial viral load. Our findings contribute to expand the scientific knowledge about the effects of anti-cytokine therapy on SARS-CoV2 kinetics and on the humoral immune response to the virus, and support the safety of this strategy for the treatment of COVID-19. Further carefully designed studies are required to confirm that tocilizumab does not affect viral replication and immune response to SARS-CoV-2.

Contributors

MM and FG conceived the study and wrote the first draft; MF, PO and VA performed the laboratory analyses; SP, JGA, GT and LG participated in data acquisition; JAG analyzed the data; all authors critically revised and approved the final version of the manuscript.

Data sharing

The dataset generated and analysed during the current study are available from the corresponding author on reasonable request.

Members of the COVID19-Elx group

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Declaration of Competing Interests

Dr. Gutiérrez reports personal fees from Janssen-Cilag and from ViiV Health Care, outside the submitted work. The other authors have nothing to disclose.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ebiom.2020.102999.

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