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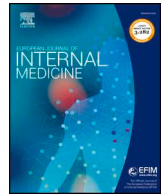
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Original article

Off-label use of tocilizumab for the treatment of SARS-CoV-2 pneumonia in Milan, Italy

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ABSTRACT

Background: Tocilizumab, a humanized monoclonal antibody, targets IL-6 receptors blocking downstream pro-inflammatory effects of IL-6. In preliminary reports it was suggested to be beneficial in patients with severe COVID-19.

Methods: In this open-label prospective study we describe clinical characteristics and outcome of 51 patients hospitalized with confirmed and severe COVID-19 pneumonia treated with tocilizumab intravenously. All patients had elevated IL-6 plasma level (>40 pg/mL) and oxygen saturation <93% in ambient air. Clinical outcomes, oxygen support, laboratory data and adverse events were collected over a follow-up of 30 days.

Results: Forty-five patients (88%) were on high-flow oxygen supplementation, six of whom with invasive ventilation. From baseline to day 7 after tocilizumab we observed a dramatic drop of body temperature and CRP value with a significant increase in lymphocyte count ($p < 0.001$). Over a median follow-up time of 34 days from tocilizumab, 34 patients (67%) showed an improvement in their clinical severity class; 31 were discharged; 17 (33%) showed a worsening of their clinical status, of these 14 died (27%). The mortality rate was significantly associated with mechanical ventilation at baseline (83.3% vs 20% of patients on non-invasive oxygen support; $p = 0.0001$). The most frequent side effects were an increase of hepatic enzymes (29%), thrombocytopenia (14%), and serious bacterial and fungal infections (27%).

Conclusion: Tocilizumab exerts a rapidly beneficial effect on fever and inflammatory markers, although no significant impact on the clinical outcome can be inferred by our results. Critically ill patients seem to have a high risk of serious infections with this drug.

1. Introduction

From December 2019 when the first outbreak of COVID-19 disease was reported in Wuhan, China, to April 19, 2020 a pandemic diffusion of a newly identified *Betacoronavirus* named SARS-CoV-2 occurred

worldwide reaching a global number exceeding 2,000,000 confirmed cases [1–3]. At the beginning of the epidemic in the western countries, Italy presented the highest number of diagnosed cases in Europe and is currently the third country in the world most hit by the epidemic with a total of 181,228 cases, mostly recorded in the Region of Lombardy

Abbreviations: COVID-19, CoronaVirus Disease (COVID)-19; SARS-CoV-2, Severe acute respiratory syndrome-Coronavirus-2; ARDS, Acute respiratory distress syndrome; IQR, interquartile rate; CPAP, Continuous positive airway pressure; ICU, intensive care unit; sHLH, haemophagocytic lymphohistiocytosis

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(37%) [3,4].

The case-fatality rate (CFR) of COVID-19 is approximately 6.8% [5–7] showing a wide range among countries (from 0.7 to 13.2), likely due to the differences in the estimate of the actual number of infected subjects. The significant morbidity and mortality of COVID-19 urgently requires effective and safe treatments.

In about 6–29% of infected patients a life-threatening pneumonia rapidly evolving to acute respiratory distress syndrome (ARDS) and requiring invasive ventilation is observed [8–11]. This severe clinical picture of COVID-19 has been associated with an hyperinflammatory state resembling a cytokine storm syndrome with release of high levels of proinflammatory cytokines including interleukin (IL) -6, tumour necrosis factor α (TNF- α), IL-12 granulocyte colony stimulating factor, interferon- γ inducible protein 10, monocyte chemoattractant protein 1, macrophage inflammatory protein 1- α [12,13].

Promising results of an immune-based approach to COVID-19 treatment were initially reported in China on small case series of patients with severe disease treated with tocilizumab [14,15], that is an IL-6 receptor blocker, licensed for rheumatoid arthritis [16], cytokine release syndrome [17,18] and idiopathic multicentric Castlemans disease [19]. Several multicentre, randomised controlled trials of tocilizumab are currently ongoing in patients with COVID-19 pneumonia and elevated IL-6 worldwide.

Pending the results of these studies we report here our experience with the off-label use of tocilizumab in 51 patients with severe COVID-19 infection hospitalized in Milan, Italy.

2. Patients and methods

From March 10 to March 23, 51 patients hospitalized at the Infection Disease ward (IDW) or intensive care unit (ICU) of Fatebenefratelli-Sacco Hospital in Milan with SARS-CoV-2 pneumonia were offered an off-label treatment access to tocilizumab. SARS-CoV-2 pneumonia was confirmed by nasopharyngeal swabs tested positive by real-time reverse-transcriptase-polymerase-chain-reaction (ELITE InGenius® system and the GeneFinder COVID-19 Plus RealAmp Kit assay; ELITechGroup, France) and by a chest X-ray showing the presence of interstitial alterations and/or consolidation(s). The protocol was approved in emergency by the Institutional Ethic Committee. A written informed consent was obtained from all the patients, except for those on mechanical ventilation in ICU, for whom we applied the urgency principle.

The inclusion criteria were: age \geq 18 years, respiratory rate \geq 30/minutes, SpO₂ < 93% while breathing room air, PaO₂/FiO₂ < 250 mmHg, IL-6 plasma level > 40 pg/mL. The exclusion criteria included: pregnancy, neutrophil count < 500 cells/ μ L, platelets count < 50,000/ μ L, concomitant immunosuppressive therapies, active tuberculosis, concomitant bacterial or fungal systemic infections.

According to the drug protocol established in our Hospital, patients who gave their consent received initially an off-label treatment with lopinavir-ritonavir (400 mg and 100 mg, respectively) BID plus hydroxychloroquine 200 mg BID.

Patients who satisfied the inclusion criteria received tocilizumab intravenously either at fixed dose of 400 mg at T0 followed by 400 mg after 12 hours or 8 mg/kg at T0 followed by 8 mg/kg after 12 hours (in patients with body weight \geq 60 Kg).

Following the Chinese Guidelines for the Diagnosis and Treatment of Novel Coronavirus (2019-nCoV) Infection, the severity of SARS-CoV-2 disease was classified into four grades: 1) mild, with slight clinical symptoms and no evidence of pneumonia; 2) moderate, with fever, respiratory symptoms and confirmed pneumonia; 3) severe, with any of the following: respiratory distress with RR > 30 times/minutes, oxygen saturation at rest < 93% or PaO₂/FiO₂ < 300 mmHg; 4) critically severe, with any of the following: respiratory failure needing mechanical ventilation, shock, or a combination of other organ failures requiring intensive care [20].

2.1. IL-6 test

Interleukin-6 serum concentrations were assessed on the fully automated immunochemistry platform COBAS e601 (Roche Diagnostics) by the proprietary electrochemiluminescent immunoassay (ref. 05109442190, lot 43676101).

2.2. Data collection

At enrolment the following data were collected: demographic data, concomitant diseases (and Charlson comorbidity index, CCI), concomitant medications, pre-treatment serum IL-6 levels. Moreover, clinical symptoms, fraction of inspired oxygen (FiO₂), peripheral oxygen saturations, ratio of the partial pressure of oxygen (PaO₂) to the fraction of inspired oxygen (P/F), white blood cell count, lymphocytes and platelets count, serum levels of d-dimer, lactate dehydrogenase (LDH), aspartate transaminase (AST), alanine transaminase (ALT), C-reactive protein (CRP) and creatinine were collected at baseline, day 1, day 3 and day 7 from the start of tocilizumab treatment.

Data were censored on April 19, 2020 and follow-up information was collected by telephone calls for patients who had been discharged. The primary endpoints were death or hospital discharge. Secondary endpoints included: 1) a change in the disease severity grade and the change of oxygen therapy support in a scale from the need of mechanical ventilation to oxygen-therapy weaning at different time-points from tocilizumab initiation. Finally, we analysed the changes in body temperature and blood examinations from baseline to day 7 after tocilizumab administration.

2.3. Statistical analysis

Descriptive analyses of the variables were expressed as median (interquartile range [IQR]), or number (%). Continuous variables were compared using a nonparametric test (Mann-Whitney test). For categorical variables the Fisher's exact test was used. Survival curves were calculated for overall survival according to Kaplan-Meier method and comparisons were made using the log rank test.

Competing risk survival analysis method was applied to estimate the cumulative incidence of improving over time from the tocilizumab administration. This method allows to handle death as competing event, avoiding overestimation and bias that occurs using the Kaplan-Meier method. Univariate and multivariable Cox proportional hazard regression was used to estimate hazard ratios for death after adjusting for age, gender, time between tocilizumab and onset of symptoms, basal IL-6 levels, invasive ventilation at the time of treatment.

The variations in body temperature, blood cells count, AST, ALT, LDH, CRP and D-dimer were assessed using a multivariable linear mixed effects regression model, and the SAS PROC MIXED procedure was used to correlate repeated measures. All the statistical analyses were made using SAS version 9.4 software, and differences with *p* values of < 0.05 were considered statistically significant.

3. Results

From March 10 to March 23, 51 patients received at least one dose of tocilizumab, including 18 (35%) who received a fixed dose of 400 mg for two doses 12 hours apart and 33 (65%) who initiated a schedule with 8 mg/Kg repeated after 12 hours. Two patients did not receive the second dose because of death and for the onset of rash, respectively. The baseline characteristics of patients are described in Table 1. Forty-five patients (88%) were on high-flow oxygen supplementation, six of whom with invasive ventilation, and only 6 (11.7%) had oxygen support by FiO₂ \leq 50% or by nasal cannula. Most of the patients at baseline had a disease classified as severe (84%), or critically severe (12%).

Almost 50% of patients showed a concomitant cardiovascular

Table 1
Characteristics of the study population.

Characteristic	Total (n = 51)
Gender, n (%) Male	40 (78.4)
Age, median years (IQR)	60 (50-70)
Age > 65 years, n (%)	19 (37.2%)
Ward of hospitalization	
Infectious Diseases ward	42 (82.4%)
Intensive care unit	9 (17.6%)
Signs/symptoms at hospital admission, n (%)	
Fever	38 (74.5)
Cough	32 (62.7)
Dyspnoea	28 (54.9)
Comorbidities, number, median (IQR)	1 (0-2)
Charlson Comorbidity Index, median (IQR)	2 (1-3)
Types of comorbidities, n (%)	
Cardiovascular diseases	25 (49.0)
Hypertension	15 (29.4)
Diabetes	6 (11.8)
Chronic lung diseases	5 (9.8)
Cancer*	3 (5.9)
Rheumatologic diseases	3 (5.9)
Others [§]	3 (5.9)
Time from hospitalization to baseline (days), median (IQR)	3 (2-6)
Time from illness onset to baseline (days), median (IQR)	12 (10-16)
Additional medications for COVID-19, n (%)	
Hydroxychloroquine	50 (98)
Lopinavir/ritonavir	42 (82)
Remdesivir	24 (42)
Antibiotics	39 (76)
Body temperature > 37.5°C, n (%)	39 (76)
Chest X-ray, n (%)	51 (100)
No alterations	1 (2.0)
Diffuse interstitial opacities**	43 (84)
Consolidation(s)	17 (33)
Pleural effusion	1 (2.0)
Oxygen support therapy, n (%)	
None	0 (0)
Nasal cannula	3 (5.9)
Venturi-type mask	20 (39.2)
Continuous positive airway pressure	22 (43.1)
Invasive ventilation	6 (11.8)
Disease severity [†] , n (%)	
Mild	0 (0)
Moderate	2 (3.9)
Severe	43 (84.3)
Critically severe	6 (11.8)
Laboratory examinations, median (IQR)	
WBC count × 10 ³ /μL	9.1 (6.5-10.9)
Lymphocyte count × 10 ³ /μL	0.8 (0.6-1.1)
Neutrophil count × 10 ³ /μL	7.3 (5.0-11.0)
Platelets × 10 ³ /μL	230 (169-337)
Lactate dehydrogenase (IU/L)	470 (400-561)
AST (IU/L)	48 (36-69)
ALT (IU/L)	39 (23-60)
C-reactive protein (mg/L)	189 (138-268)
D-dimer (μg/L)	1706 (860-5261)
Interleukin-6 (pg/mL)	116 (65-180)

IQR = interquartile range; WBC = white blood cell; CT = computed tomography; AST = aspartate aminotransferase; ALT = alanine aminotransferase.

* All in remission;
[§] HIV, chronic hepatitis C, previous tuberculosis;
 ** CT-scan was performed in 9 patients and showed Multiple ground-glass opacities †As classified by Wu et al. [11].

disease, with arterial hypertension being the most frequent comorbidity (60%). Besides tocilizumab, during the hospital stay most of the patients were treated with either hydroxychloroquine (98%) associated with lopinavir/ritonavir in 84% of these cases, or remdesivir (45%). Furthermore, 76% of patients also received antibiotic therapy. The timing of concomitant anti-CoV-2 drugs is reported in Fig. 1. The median interval time from the onset of symptoms to the start of treatment with tocilizumab was 12 days (IQR, 10-16).

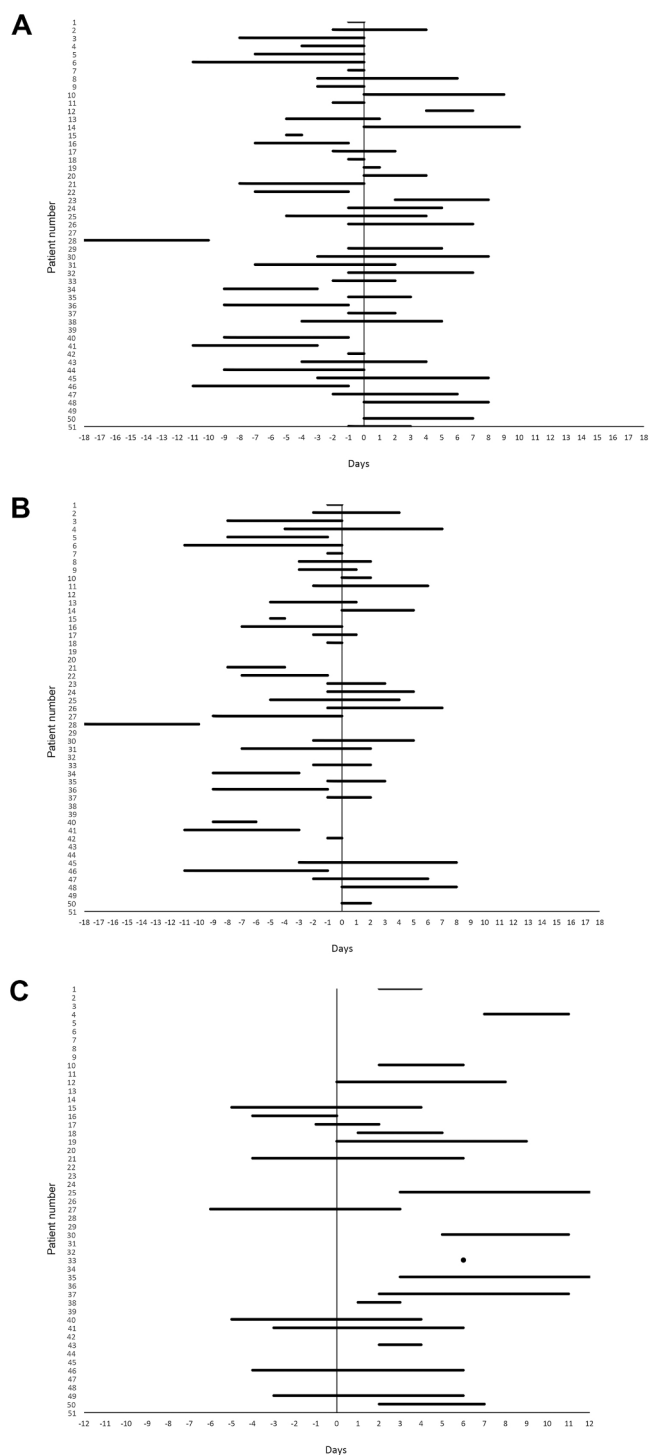


Fig. 1. Concomitant anti COVID-19 therapies administered during hospitalization for each patient. The point zero vertical bar represents the time of tocilizumab administration. The horizontal lines indicate the timing and duration of hydroxychloroquine (A), lopinavir-ritonavir (B) and remdesivir (C) administration for each patient.

3.1. Treatment outcomes

The patients were followed up for a median of 34 days (IQR 32-37) after receiving the first dose of tocilizumab; during this time 31 patients (61%) had been discharged, 14 (27%) had died and 6 (12%) were still hospitalized at censoring date.

The mortality rate over a 30-days observation period was 27%,

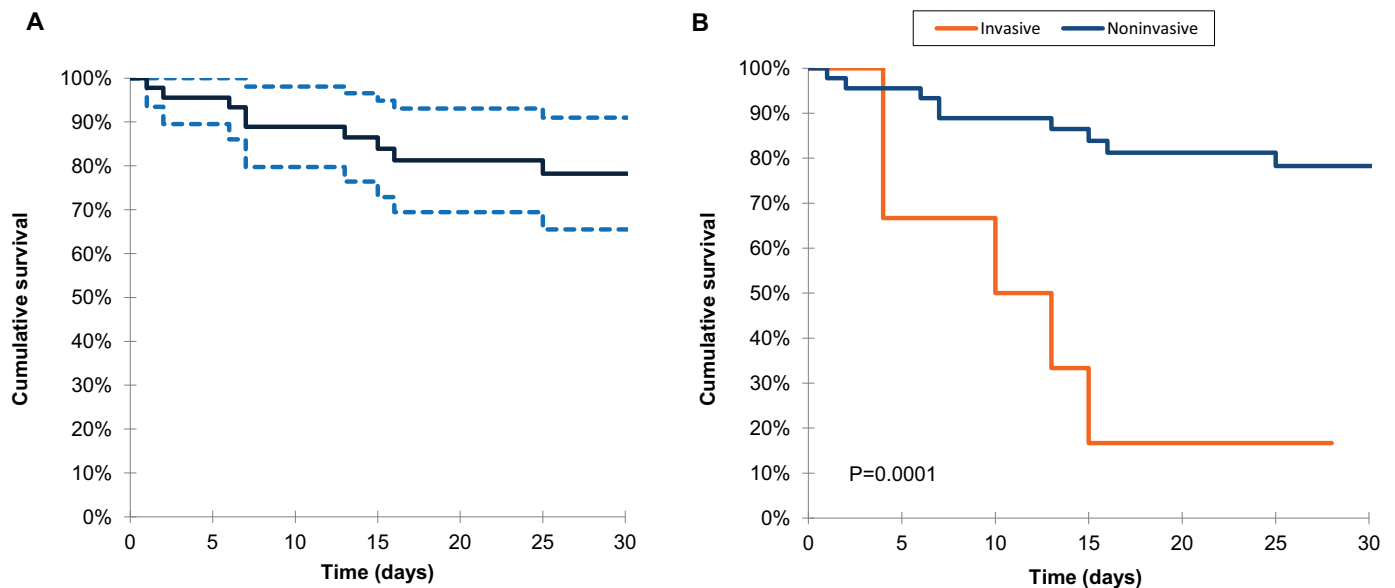


Fig. 2. A. Kaplan-Meier curves showing survival of participants with severe COVID-19 pneumonia treated with tocilizumab; B. Survival curves in the cohort stratified according to ventilation status at baseline.

including 5/6 patients (83%) who were receiving invasive ventilation at the time of tocilizumab treatment and 9 of 45 (20%) who were receiving noninvasive oxygen support. Fig. 2 shows the Kaplan Meier cumulative survival curve (A) and the survival curves stratified by invasive mechanical ventilation or non-invasive oxygen supplementation (B) ($p=0.0001$). Respiratory failure and ARDS was identified as the most frequent cause of death, although 4 patients had concomitant septic shock and multi-organ failure.

Results of the multivariable Cox proportional hazard model showed that only being on invasive mechanical ventilation at the time of treatment was independently associated with increased risk of death, adjusted HR 7.18 (CI 95% 2-25; $p=0.002$). Neither age and gender nor the interval between the onset of symptoms and tocilizumab administration resulted to significantly influence the clinical outcome.

3.2. Secondary outcomes

Over the follow-up time after tocilizumab administration, 34 patients (67%) showed an improvement in their clinical severity class; 17 (33%) showed a worsening of their clinical status, including the 14 patients who died. Fig. 3 describes the changes in oxygen-support at the different time points from tocilizumab administration. Overall, 34 of 51 (66,6%) patients reached a reduction in the intensity of oxygen therapy or were weaned off oxygen support. However, none of the 6 patients who were treated while receiving invasive mechanical ventilation have been extubated, and 16/51 (33%) patients did not change their status or needed an intensification of oxygen support, including 14 who eventually died.

The cumulative incidence of improving over time from the tocilizumab administration is shown by a competing risk survival analysis and death is handled as competing event. Dashed lines define the 95% confidence interval range.

The cumulative incidence of clinical improvement from baseline to day 30 of follow up, as defined by either a decrease of 1 or more points in the severity class category or discharge, was 67% (95% confidence interval [CI], 56-82) and is reported in Fig. 4.

As for the other secondary endpoints (body temperature, blood cells counts, AST, ALT, LDH, CRP and D-dimer), data were recorded at baseline and day 1, 3, and 7 after the first dose of tocilizumab. The baseline values are reported in Table 1. We observed a dramatic drop of

body temperature and CRP value, a significant increase in lymphocyte and platelet counts, and a significant increase in aminotransferases within day 7 (Fig. 5). Total white blood cells and neutrophils showed only a transitory decline at day 1 and 3 with no significant difference at day 7 (data not shown).

Total lymphocytes and CRP returned to normal values in 28 patients (55%), and 36 patients (71%), respectively. No change occurred for LDH and D-dimer values.

3.3. Safety

The most common adverse events were the increase of hepatic enzymes of at least 3 times above the normal values (29%), thrombocytopenia (14%), neutropenia (6%) and cutaneous rash (2%). Bacteremia emerged in 14 patients (27%), 86% of whom were hospitalized in ICU. Blood cultures gave a positive result in 31 episodes on 14 patients and the most frequently isolated pathogens were *Enterococcus* spp. (8), Carbapenemase-producing *Klebsiella pneumoniae* (5), extended spectrum beta-lactamase (ESBL)-producing *Klebsiella pneumoniae* (5), *Candida* spp. (3), Coagulase-negative Staphylococci (4), methicillin-resistant *Staphylococcus aureus* (3), *Pseudomonas aeruginosa* (1), ESBL producing *E. coli* (1), *Enterobacter aerogenes* (1). The median time from tocilizumab administration to the bacteremic event was 11 days (IQR 9-13).

4. Discussion

To the best of our knowledge, this is the largest cohort of patients with severe COVID-19 pneumonia treated in an off-label access with tocilizumab. It also analyzes the longest post-therapy follow-up so far.

Although at present the only recognized standard treatment of COVID-19 pneumonia is oxygen support and the management of ARDS, several therapeutic approaches have been proposed and tested including antivirals (lopinavir/ritonavir, hydroxychloroquine, remdesivir) [21-25] and anti-inflammatory drugs (corticosteroids, COX-2 inhibitors, (IL)-6 and (IL)-1 receptor antagonists) [26]. The use of tocilizumab, a recombinant humanized anti-human IL-6 receptor monoclonal antibody that binds both membrane bound IL-6 receptor (mIL6R) and soluble IL-6 receptor (sIL-6R), was inferred from the observation that patients with severe COVID-19 might develop a hyper-

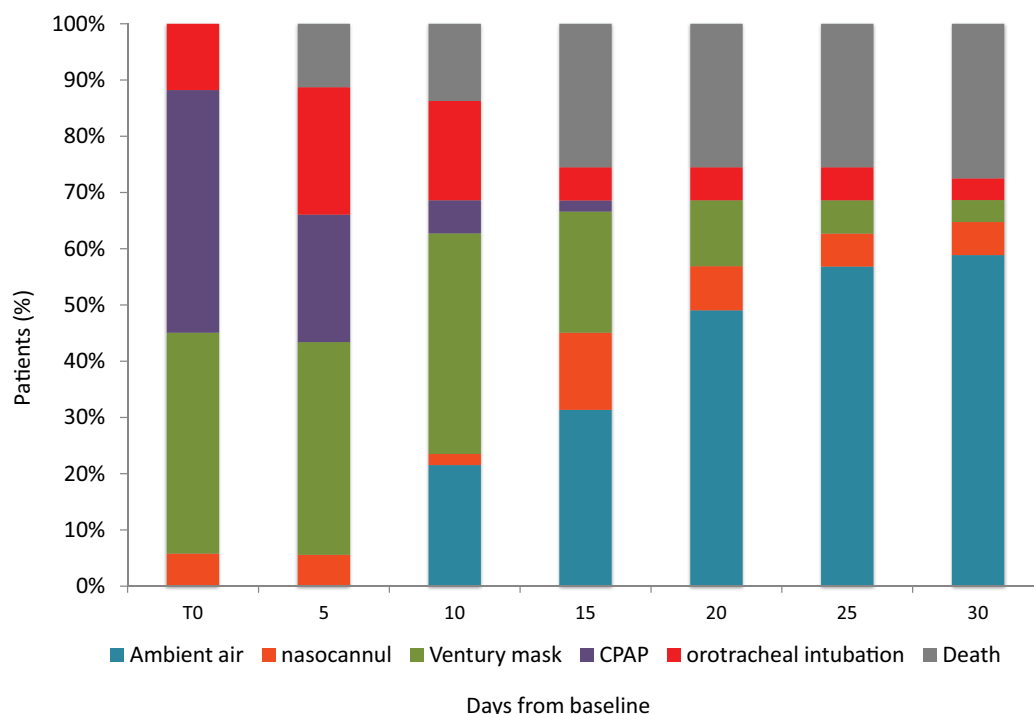


Fig. 3. Change in oxygen support, and deaths over the 30-days follow-up. T0 was the day on which tocilizumab was initiated. CPAP, Continuous positive airway pressure.

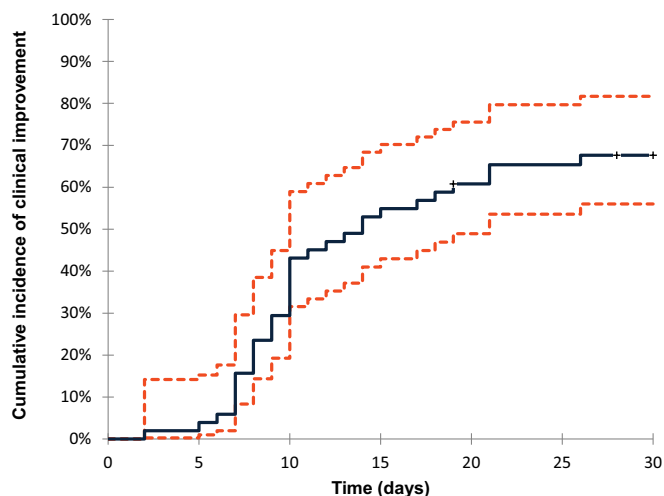


Fig. 4. Cumulative Incidence of clinical improvement from baseline to day 30. The cumulative incidence of improving over time from the tocilizumab administration is shown by a competing risk survival analysis and death is handled as competing event. Dashed lines define the 95% confidence interval range.

inflammatory state resembling the cytokine release syndrome observed in secondary haemophagocytic lymphohistiocytosis (sHLH) for which tocilizumab is registered [16,17]. sHLH is a hyperinflammatory syndrome that leads to fatal hypercytokinaemia and multiorgan failure and shows features of inflammation similar to those observed in COVID 19 [10,27]: fever, highly increased ferritin level, decreased platelet counts, high erythrocyte sedimentation rate and high circulating IL-6, IL-2, IL-7, IL-10, granulocyte-colony stimulating factor (G-CSF), interferon- γ -inducible protein (IP10), monocyte chemoattractant protein (MCP1), macrophage inflammatory protein 1 alpha (MIP1A), and TNF- α . [28].

Several phase 2 and 3 randomized clinical trials with the use of tocilizumab in severe COVID-19 infection are ongoing in China, Europe

and USA. To date, Xu et al. [14] reported encouraging preliminary results in 21 patients with severe COVID-19 pneumonia treated with tocilizumab in an open-label study conducted in China. Over a five-days follow-up, they observed an immediate improvement of all clinical symptoms: normalization of body temperature in all patients, reduction of oxygen requirements in 75% of cases, improvement of CT scan imaging in 90.5%, normalization of lymphocytes count in 52% of subjects, significant reduction of CRP values in 84%. No adverse events were reported and none of the patients died.

We report herein our experience regarding the off-label treatment with tocilizumab of the first 51 patients with severe and critical forms of COVID-19. Over a median follow-up of 34 days, 61% were discharged in good clinical conditions, 27% died and 12% are still hospitalized. A clinical improvement associated with reduced need of oxygen flows was observed in 67% of patients, while 33% worsened and the 30-days mortality was 27%, similar to that reported in Wuhan, China, on 201 patients hospitalized with severe disease where the overall a mortality was 22% and 66% in those on mechanical ventilation [11]. In agreement with Xu et al. a decrease in inflammatory symptoms and markers was rapidly seen in our patients associated with an increase in lymphocyte and platelet counts. Nevertheless, in the most critically ill patients and with a longer follow-up period, no clinical improvement or reduction of oxygen supplementation was seen and 5/6 patients receiving tocilizumab while mechanically ventilated died. Indeed, most of our deaths occurred between day 7 and day 21 of follow-up.

More recently, two small case series regarding patients with COVID-19 pneumonia treated with tocilizumab in China and Italy have been published [19,29]. Rapid improvement of respiratory conditions, fever and inflammation was observed in the Italian case report, although these three patients were moderately ill at dosing of tocilizumab. By contrast, a high mortality rate (40%) was shown in the series from China with the Authors concluding that tocilizumab did not modify the disease outcome in critically ill patients. However, these reports are not comparable mainly because of the different tocilizumab dosage used, the different concomitant medications and the characteristics of

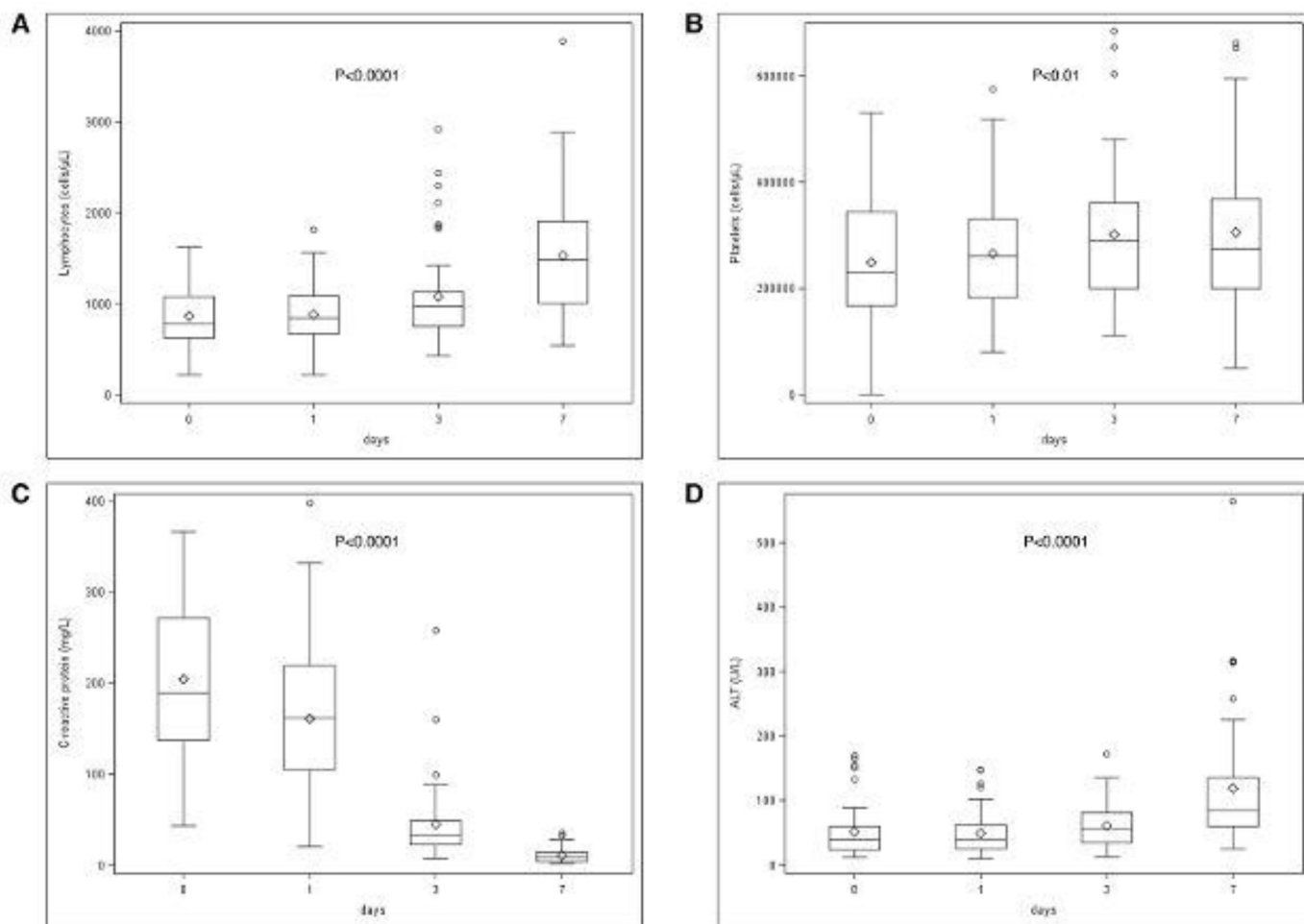


Fig. 5. Changes in the values of lymphocytes (A), platelets (B), CRP (C) and ALT (D) after treatment with tocilizumab. The time points considered are: T0, the day of tocilizumab administration, day 1, day 3 and day 7 after treatment.

patients studied.

Additionally, we observed a number of both early or late adverse events. Among the early potentially drug-related side effects the most frequently seen were hypertransaminasemia (15/51), thrombocytopenia (7/51) and neutropenia (3/51), although the role of SARS-CoV-2 *per se* or of concomitant medications cannot be excluded. The late events were serious bacterial and fungal infections of the bloodstream. The latter occurred in 12/14 patients while in ICU. In particular, the three episodes of candidemia are concerning because of their life threatening nature and may indicate a severe underlying immunosuppression. Indeed, the use of tocilizumab in patients with rheumatoid arthritis is known to be associated with an increased risk of serious bacterial and fungal infections [30, 31] as a result of the blockade of IL-6 that may impair B-cell proliferation and T-cell differentiation and cytotoxicity, essential for immune control of infections [32]. This is particularly true for patients hospitalized in ICU, who are known to be at higher risk of bloodstream infections [33]. Indeed, we found mechanical ventilation at the time of tocilizumab therapy to be independently associated with the risk of death at multivariable analysis. A possible explanation of the different incidence of side effects, in particular infective events, between our study and previous reports might be the longer follow up of our observation, given the half-life of tocilizumab and the persistence of active drug levels for weeks after the infusion [34].

Our study has several limitations. First and foremost, the lack of a randomized control group, does not allow us to draw definitive conclusions. In addition, all our patients were treated with

hydroxychloroquine, lopinavir/ritonavir or remdesivir, alone or in combination. The variety of treatment schedules used accounted for the absence of defined treatment protocols that reflects the first few weeks of the COVID-19 epidemic and represents an evident confounding factor in the analysis of potential therapeutic efficacy. However, in our cohort the high mortality rate was observed over a longer follow-up period than in other published, uncontrolled experiences with tocilizumab. In addition, this elevated mortality rate did not differ from what is reported in other cohorts of severely ill patients in whom different treatments other than tocilizumab were used [11]. Only randomized, controlled trials will clarify the efficacy of tocilizumab in the different severity categories of patients and the best timing of its administration and will identify the subgroup of patients for whom immunosuppression could be harmful.

In conclusion, tocilizumab seems to exert a rapidly beneficial effect on fever and inflammation, although no significant impact on the clinical outcome can be inferred by our results. Furthermore, special attention must be paid when treating critically ill patients with COVID-19, because of the high risk of life-threatening secondary infections we have documented in this fragile population.

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Declaration of Competing Interests

None.

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