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Compassionate use of anti-IL6 receptor antibodies in critically ill patients with acute respiratory distress syndrome due to SARS-COV-2

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ABSTRACT

BACKGROUND

COVID-19 patients developing the acute respiratory distress syndrome (ARDS) show increased production of pro-inflammatory cytokines, including interleukin-6 (IL-6). The use of humanized monoclonal antibody against interleukin-6 receptor (IL-6R) may represent a potential treatment strategy. We analyzed the effects of compassionate use of Tocilizumab and Sarilumab on clinical outcome of patients affected by ARDS due COVID-19.

METHODS

This single-center, observational, exploratory study was performed during the acute phase of COVID-19 outbreak, between March 7th and April 21st, 2020 in a University Hospital in Rome, Italy. All consecutive adult patients admitted to the intensive care unit with laboratory-confirmed COVID-19 and fulfilling ARDS criteria were enrolled. Patients who were treated with anti-IL-6R therapy were compared to those who were not, as per clinical decision. Inverse probability weights were applied to weight individual's contribution to survival curves and in the multivariate regression model.

RESULTS

Among 105 ARDS patients, 65 received compassionate treatment with anti-IL-6R therapy [43 (66%) Tocilizumab and 22 (34%) Sarilumab, respectively], with oxygenation improvement. In the multivariable Cox proportional regression hazards model with propensity score inverse probability weighting, patients who received anti-IL-6R treatment had lower risk of death compared to those who did not, with a hazard ratio of 0.34 [95% confidence interval 0.17-0.74], $p=0.001$.

CONCLUSION

Our data suggest that immune modulator therapy based on anti-human IL-6 receptor monoclonal antibodies might lead to improved outcome in patients with ARDS due to COVID-19. These data support the need for confirmatory randomized trials to assess the effect of immune modulator therapies on mortality.

Keywords: SARS-Cov-2; ARDS; ICU-mortality; immune modulator therapy; personalized medicine; interleukin-6 receptor; humanized monoclonal antibody; inverse probability weighting;

Abbreviation list: SARS-CoV-2 (severe acute respiratory syndrome coronavirus), COVID-19 (coronavirus disease 2019), ICU (intensive care unit), IL-6 (interleukin-6), IL-6R (interleukin-6 receptor), AIFA (Italian Pharmacological Agency), ARDS (acute respiratory distress syndrome), WHO (World Health Organization), RT-PCR (real-time reverse transcriptase-polymerase), $\text{PaO}_2/\text{FiO}_2$ (ratio of arterial oxygen tension to inspired fraction of oxygen), mmHg (millimetre of mercury), AKI (acute kidney injury), SAPS II (Simplified Acute Physiologic Score Two), SOFA (Sequential Organ Failure Assessment), IQR (interquartile range), CRP (C-reactive protein), IPTW (Inverse probability of treatment weights), RCTs (randomized, double-blind, placebo-controlled trials), NIH (National Institute of Health)

BACKGROUND

Patients with SARS-CoV-2 infection can develop coronavirus disease 2019 (COVID-19) with high rates of intensive care unit (ICU) admission and short-term mortality rates ranging from 35% to as high as 50% to 62%^(1, 2, 3, 4).

Covid-19 may be associated with an aberrant host immune response and hyper-inflammation resulting in an excessive cytokine release with a dysfunction of the alveolar-capillary membrane and impaired oxygen diffusion⁽⁵⁾.

Although a recent study⁽⁶⁾ suggests that the primary pathophysiologic process is an immunosuppression rather than hyperinflammation, the patients with COVID-19 included in that study had an increase in plasma IL-6 within the first 24 hours from ICU admission in accordance with the observations shown by other reports⁽⁷⁾. Besides, elevated IL-6 plasmatic levels have been found to be predictive of the likelihood of mechanical ventilation⁽⁸⁾.

Consequently, the combined therapeutic approaches including both antiviral and blockage of inflammatory pathways⁽⁹⁾ have been advocated for an optimal disease control⁽¹⁰⁾. A previous trial showed that the antiviral agent Remdesivir⁽¹¹⁾ reduced the length of recovery but did not reduce the number of patients needing mechanical ventilation, nor the death rate. The RECOVERY collaborative group demonstrated that dexamethasone decreased the 28-day mortality among patients receiving mechanical ventilation.

Also, the use of humanized monoclonal antibodies against the interleukin-6 receptor (IL-6R), seem a promising immune modulation therapy in critically ill patients with COVID-19⁽¹²⁾. Consequently, these drugs were chosen by the Covid-19 Task Force of our Hospital to treat patients with severe SARS-CoV-2 pneumonia.

In this single-center, exploratory study, we evaluated the effect of compassionate use of Tocilizumab and Sarilumab on clinical outcome of patients with ARDS due to SARS-CoV-2 treated in the ICUs of the Fondazione Policlinico Universitario Agostino Gemelli, IRCCS.

METHODS

This single-center, exploratory, prospective observational study was performed during the first acute phase of COVID-19 outbreak in Italy, between March 7th and April 21st, 2020. The methodology adheres to the STROBE statement for reporting observational studies⁽¹³⁾.

The study was approved by the Ethical Committee of the Policlinico Gemelli (approval number: 3146). Written informed consent was obtained from each patient or from the patient's legally authorized representative if the patient was unable to provide consent. All data were prospectively recorded on electronic worksheet and have been anonymized.

All consecutive adult patients admitted in our ICUs with laboratory-confirmed SARS-CoV-2 infection and fulfilling ARDS criteria were enrolled⁽¹⁴⁾.

According to the WHO guidance⁽¹⁵⁾, laboratory confirmation for SARS-CoV-2 was defined as a positive result of real-time reverse transcriptase-polymerase chain reaction (RT-PCR) assay of nasal and pharyngeal swabs. This guidance was implemented locally with the addition of using RT-PCR assay from lower respiratory tract specimens.

Two monoclonal antibodies against the IL-6R were used: Tocilizumab and Sarilumab, both for compassionate use.

The shortage of Tocilizumab together with the increasing number of patients with progressive respiratory symptoms led the multidisciplinary team of our Institution to use Sarilumab, according to an internal collaborative clinical-pharmacological protocol (approval number: 926)

Patients received Tocilizumab administration at the dosage of 8 mg/kg, with a second dose administered 12 hours after the first and a possible third dose after further 24-36 hours, according to clinical response.

Sarilumab treatment was scheduled as follows: 400 mg intravenously on day 1 (final injectable solution was obtained combining with 2 Sarilumab 200 mg prefilled syringes mixed in 100 ml 0.9% sodium chloride solution for intravenous use). In case of clinical worsening or unchanged status, a repeated dose of Sarilumab 400 mg i.v. was administered.

The decision to administer anti-IL-6R therapy or not was left to the clinical judgment of the physician in charge.

Concomitant standard therapies were set for all patients at the time of confirmed positive naso-pharyngeal swab for SARS-CoV-2 upon entering the Hospital, unless differently required by clinical situation, as follow: Lopinavir/Ritonavir 400/100 mg BID or Darunavir/Ritonavir 800/100 mg QD, orally); Hydroxychloroquine (400 mg BID for the first day, followed by 200 mg BID); Azithromycin combined at the dose of 500 mg orally or intravenously. Moreover, prophylactic dose heparin subcutaneously (4000-6000 UI QD) was added. None of the patients in overall study population received treatment with systemic corticosteroids.

Patients met the discharge criteria from ICU if they significantly improved respiratory function and had negative SARS-CoV-2 result of RT-PCR assay of nasal and pharyngeal swabs twice in succession. All patients received a daily follow-up from visit after ICU discharge up to hospital discharge or death.

Statistical analysis

The aim of this study was to report the clinical outcomes of critically ill patients with ARDS due to SARS-CoV-2 treated with monoclonal antibodies against the IL-6R during the first acute phase of COVID-19 outbreak. Therefore, there were no formal hypotheses being implemented to drive the sample size calculation and we included the maximum number of patients who met the inclusion criteria.

Continuous variables are described as median (interquartile range [IQR]). Categorical variables are described as frequency. Propensity score weighting analysis was carried out to address the risk of selection bias given by non-randomized treatment allocation. Age, sex, PaO₂/FiO₂ at ICU admission, SAPS II score, SOFA score at ICU admission

and comorbidities were considered potential confounders, i.e. information which could have influenced treatment assignment. Balance of all potential confounders between treatment groups was evaluated with t-test or Wilcoxon sum-rank test for continuous variables and with Chi-square test or Fisher test for categorical variables, as appropriate. Unbalancing was established when $p < 0.05$. Propensity scores were calculated by a multivariable logistic regression model with treatment as the binary outcome and all unbalanced confounders as covariates. Inverse probability of treatment weights (IPTW) were then computed as $1/(\text{propensity score})$ for anti-IL-6R treated patients and $1/(1 - \text{propensity score})$ for patients treated with standard therapy. Inverse probability weights were used to weight each individual's contribution to the survival curves and to the Cox regression model.

Survival curves for both treatment groups were estimated with Kaplan-Meier method and compared with the log-rank test. To explore the risk factors associated with ICU-mortality, multivariable Cox proportional regression hazards models were used. Conditional stepwise variable selection was performed with 0.1 as the critical p value for entry into the model. Interactions and correlations between the explanatory variables and the validity of the proportional hazards assumption, were carefully checked. All analyses were conducted using inverse probability of treatment weighting.

In order to minimize the risk that patients with a very early event (death) were more likely assigned to the untreated group, a sensitivity analysis was also carried out excluding patients who died within 3 days following the start of the follow-up.

Tests were two-sided with significance set at α less than 0.05. Imputation of missing data was not performed. All statistical analyses were performed using SAS 9.4 software and R software version 4.0.2.

RESULTS

GENERAL POPULATION

From March 7 to April 21, 2020, 145 critically ill patients were admitted to the ICUs with confirmed COVID-19. Of these, 105 fulfilled ARDS and were included in the analysis. Demographics and clinical characteristics in survivors and non-survivors are displayed in Table 1^(16,17,18).

During the study period, 72 (69%) patients were discharged alive from the ICU, while 33 (31%) died. The 72 patients discharged alive from ICU were younger (median [IQR], 66 [56-73] vs. 78 [70-80] years; $P < 0.0001$) and had a lower SAPS II score (median [IQR], 32 [22-37] vs. 48 [38-65]; $P < 0.0001$) in comparison to the 33 who died.

Overall, 84 individuals were male, similarly distributed among survivors and non-survivors. The most common underlying diseases, were arterial hypertension, affecting 53 (50%) patients and the presence of cardiovascular diseases that regarded 20 patients (19%). Only 8 patients (8%) had a history of chronic obstructive pulmonary disease. The underlying diseases were similar between survivors and non-survivors. Forty-four patients (42%) developed AKI during ICU stay. Of them, 27 out of 44 patients (61%) died, compared to 6 out of the 61 patients (10%) without AKI ($P < 0.0001$) (Table 1^(16,17,18)).

ANTI-IL-6R TREATMENT AND STANDARD TREATMENT GROUPS

Of the 105 patients, 40 (38%) received standard therapy and 65 (62%) received anti-IL-6R therapy within 3 days after ICU admission with a median time of 24 hours (IQR 24-84). Among them, 43 (66%) received Tocilizumab and 22 (34%) patients were treated with Sarilumab.

Characteristics at ICU admission

The 65 patients under anti-IL-6R therapy were younger than those with standard treatment (median [IQR], 65 [62-69] vs. 77 [70-80] $P < 0.001$) and 38 (59%) had arterial hypertension. The patients receiving anti-IL-6R treatment had a lower rate of mild ARDS and a higher rate of moderate ARDS in comparison with those without anti-IL-6R therapy (5 vs. 13 and 59 vs. 25, respectively; $P < 0.001$). The rate of non-invasive and invasive mechanical support was similar between these two groups (Table 2^(16,17,18)).

Primary outcome

Compared with patients treated with standard therapy, patients receiving anti-IL-6R therapy had a lower rate of ICU mortality (Table 2^(16,17,18)). Among groups, the most cause of ICU mortality was refractory hypoxemia (Supplementary Table 1)

Logistic regression multivariable model shown that $\text{PaO}_2/\text{FiO}_2$ at ICU admission, age and hypertension were covariates unbalanced between groups. After applying the weights derived from the inverse probability weighting, the groups were well balanced (Supplementary Table 2).

IPTW Kaplan-Meier analysis indicated a significant survival benefit for patients receiving the anti-IL-6R treatment (Figure 1). IPTW multivariable Cox proportional hazards model demonstrated that patients receiving anti-IL-6R treatment had a lower hazard of death compared with those with standard therapy (Table 3). As a sensitivity analysis, we performed the comparison between anti-IL-6R- treated patients and those without anti IL-6R drugs excluding the 6 patients who died within 3 days from ICU admission. The results of the sensitivity analysis were consistent with those of the primary analysis (Supplementary Table 3).

Secondary outcomes

The median days free of respiratory support at 28 and at 90 days were similar between the 65 patients receiving the anti-IL-6R therapy and the 40 patients receiving the standard treatment. Prone position was more frequently implemented in the anti-IL-6R group compared with standard therapy group ($P < 0.001$).

The number of patients who developed secondary microbiologically documented infections between those who received anti-IL-6R and those who did not was similar. Rate of patients without AKI (65%) was higher in anti-IL6R therapy group. Compared with patients treated with standard therapy, patients receiving anti-IL6R therapy had similar median days free of ICU stay (Table 2^(16,17,18)).

Inflammatory biomarkers

For temporary laboratory shortage of the reagent, the IL-6 serum level was tested in 66 among 105 enrolled patients. Of them, 52 received anti-IL-6R therapy and 14 had standard therapy. Among the 72 survivors, samples of IL-6 plasmatic levels were obtained in 54 patients compared to 12 patients among 33 non-survivors.

At ICU admission, the patients receiving anti-IL-6R therapy had a similar IL-6 plasmatic level respect to those treated with standard therapy (Table 2 ^(16,17,18)).

Within the 72 hours following anti-IL-6R administration body temperature declined from 37.2 ± 1.2 C° to 36.4 ± 0.8 C°. PaO₂/FiO₂ ratio improved significantly over time in who received anti-IL-6R treatment respect to those did not (Figure 2). Compared with patients treated with standard therapy, patients receiving anti-IL-6R therapy developed a significant improvement of serum levels of CRP (Supplementary Figure 1) and of Fibrinogen (Supplementary Figure 2) over time. Differently, plasmatic D-dimers levels were similar in both groups over time (Supplementary Figure 3).

Follow up

The date of final follow-up was June 21, 2020. Among 72 patients discharged from ICU, 62 out of 72 (86%) were discharged from the hospital. Of the 10 patients discharge from ICU, 8 out of 10 (80%) died in the hospital and 2 out of 10 (20%) were still admitted at the last follow-up visit. The in-hospital mortality between those who received anti-IL-6R and those who did not was similar (Supplementary Figure 4).

DISCUSSION

This observational, exploratory study describes the clinical outcomes in a cohort of critically ill patients with ARDS due to Covid-19 who were treated with Tocilizumab and Sarilumab during the first acute phase of COVID-19 outbreak in Italy. We found that the anti-IL-6R therapy, in combination with antiviral and standard supportive therapy, might improve the clinical outcome of critically ill patients with ARDS due to COVID 19, ameliorating the PaO₂/FiO₂, reducing fever and inflammatory markers as the CRP and fibrinogen, possibly improving survival. Furthermore, ICU acquired infections did not increased in patients treated with anti-IL-6R therapy compared to standard therapy group.

The rationale of using the anti-IL-6R antibodies is based on the key role played by IL-6 in patients infected with SARS-CoV-2 and the experience with Tocilizumab in the treatment of cytokine release syndrome caused by Chimeric Antigen Receptors T-cell (CAR-T) therapy⁽¹⁹⁾. Sarilumab is a IL-6R inhibitor approved by FDA for Rheumatoid Arthritis and proposed in alternative to Tocilizumab for SARS-CoV-2⁽²⁰⁾.

Several observational studies^(21,22,23) have shown that among critically ill patients with mechanical ventilation the risk of mortality was lower in patients treated with Tocilizumab in the first days of ICU admission compared with patients whose treatment did not include early use of this drug^(24,25); in accordance with this finding, a recent genetic study showed that genetic variants in the IL-6 inflammatory pathway are associated with the life-threatening disease⁽²⁶⁾.

Recently, RCTs reported no clear evidence of IL-6R blockade for the treatment of less severely ill patients without respiratory support^(27,28,29). In the COVACTA trial, in which 38% patients received mechanical ventilation, no

significant difference was observed between the Tocilizumab and placebo group respect to clinical status and risk of death although the rate of patients discharged at 28 day was higher in the Tocilizumab group. Also, the higher rate of patients treated with glucocorticoids in the control group might have reduced the difference between Tocilizumab group and placebo. Compared with our study, patients enrolled in this trial were most heterogeneous showing a wide range of values for IL-6 at baseline and a lower grade of the acute respiratory failure⁽³⁰⁾. Other clinical benefit were reported in the RECOVERY trial, where the number of patients that received a systemic corticosteroid was the same in the two groups under study and an additional treatment with Tocilizumab improved clinical outcomes, including survival, respect to standard therapy group⁽³¹⁾. In agreement with these findings, the EMPACTA trial has shown that the rate of patients receiving mechanical ventilation or who had died at 28 day was lower in Tocilizumab group than in placebo⁽³²⁾. Interestingly, in the REMAP-CAP trial, in which were enrolled 895 critically ill patients with COVID-19 and among them 613 (68.5%) with mechanical ventilation, it was observed that patients receiving Tocilizumab and Sarilumab needed a shorter time for clinical improvement and a lower mortality than control group. In this group, only 12.4% out of patients received a systemic corticosteroid and the patients who did not receive it were similar with our study population regarding PaO₂/FiO₂ at baseline⁽³³⁾. In agreement these findings, a RCT has reported a lower risk of death in critically ill patients treated with Sarilumab⁽³⁴⁾.

Despite the potential benefits of anti-IL6R antibodies in critically ill patients with COVID-19, it is important to weigh its administration against potential adverse events associated with the drug. No adverse drug reactions were registered during the follow-up of our patients according to previously published data⁽²²⁾.

A key feature of our study was that patients fulfilling ARDS criteria were enrolled and a higher rate of moderate ARDS together with the placing in prone position were observed in patients receiving anti-IL-6R therapy. Second, compared with other RCTs, in our population none of the patients received a systemic corticosteroid. Therefore, these findings confirm that anti-IL6R therapy together with corticosteroids offers a more clinical benefit in the most severe cases who have a significant inflammatory response with a higher risk for death. Finally, our data support a personalized medicine approach where the IL-6 serum level should be determined in severely ill patients with ARDS to select those fit for anti-IL6R therapy at the right time.

Our study has some limitations.

First of all, the observational nature of our investigation together with the fact that anti-IL-6R monoclonal antibodies did not adhere to WHO guidelines published at the time of the study. However, this study describes what happened in a real clinical situation dictated by the emergency with high mortality rate and accompanied by a substantial lack of a real effective therapy during the first acute phase of COVID-19 outbreak. Of note that, the guidelines of the NIH updated at 21st April, 2021 recommended the use of Tocilizumab together with dexamethasone in patients with severe COVID-19 requiring mechanical ventilation⁽³⁵⁾.

Second, the lack of a randomized control group that limits the interpretation of the drug-specific effect and warrants caution until more rigorous data are available. To address the risk of selection bias given by non-randomized treatment allocation, we used the model's estimated probabilities to calculate stabilized inverse probability weights, which were then used to weight each individual's contribution to the survival curves and to the Cox regression model. Finally, we performed a sensitivity analysis to prevent immortal time bias and reduce the risk that the treatment strategy depended by the patient's disease course.

Third, the relatively small number of patients studied. We decided to stop the enrollment at 21st April 2020 in the waning phase of the pandemic. Therefore, during that time we included the maximum number of patients who met the inclusion criteria. However, the study was conducted using comprehensive data prospectively collected from a consecutive critically ill patients with ARDS and laboratory-confirmed COVID-19 minimizing selection and/or surveillance bias.

Fourth, the IL-6 serum levels was tested in only 66 patients due to temporary laboratory shortage of the reagents. Anyway, sampling IL-6 plasmatic levels at ICU admission did not affect the administration of the anti-IL6R therapy, that was left to clinical judgment of the physician in charge. Its elevated plasmatic level, together with a high level of CRP were considered as exploratory variables to better identify a hyper-inflammatory state.

Although these limitations preclude definitive conclusions, our data suggest that immune modulator therapy based on anti-human IL-6R monoclonal antibodies might lead to better patients outcomes, and support the need for confirmatory randomized clinical trials to assess the role of immune modulator therapies in patients with ARDS due to COVID-19.

KEY MESSAGES

What is known

SARS-CoV-2 induces a hyper-inflammatory response associated with a disproportionate cytokine and chemokine release that leads to severe lung damage, multi-organ failure, and eventually death. It has been observed that elevated levels of IL-6 have been positively correlated with severe cases and have been found to be predictive of the likelihood of mechanical ventilation. In addition to antiviral medications such as remdesivir, treatments modulating the host immune-response to infection have been proposed to potentially diminish inflammation and improve outcomes in a subgroup of patients with more severe disease.

What is new

Our study enrolled fulfilling ARDS criteria patients with COVID-19 receiving mechanical ventilation. We showed that immune modulator therapy based on anti-human IL-6 receptor monoclonal antibodies might lead to clinical benefit, including ICU survival. Therefore, our data support a personalized medicine approach where the IL-6 serum level should be determined in severely ill patients with ARDS to early select those fit for anti-IL6R therapy at the right time.

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DECLARATIONS

Ethics approval

The study was approved by the Ethical Committee of the Fondazione Policlinico Gemelli (approval number: 3146).

Consent to participate

Not applicable

Consent to publication

Not applicable

Availability of data and material

After publication, the data will be made available to others on reasonable requests to the corresponding author. A proposal with detailed description of study objectives and statistical analysis plan will be needed for evaluation of the reasonability of requests. De-identified participant data will be provided after approval from the corresponding author and the Ethical Committee of the “Fondazione Policlinico Gemelli”.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

L Montini, G De Pascale and M Antonelli contributed to study concept and design. They take responsibility for the integrity of the data and the accuracy of the data analysis.

E D'Arcangelo and L Montini performed the statistical analysis.

G Grasselli, A Pesenti, V. Ranieri, M Cecconi and E Gremese, contributed in critical revision of the article for important intellectual content.

G Bello and DL Grieco contributed to acquisition and interpretation of data.

All authors have participated to drafting the manuscript, M Antonelli revised it critically. All authors read and approved the final version of the manuscript.

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Table 1 Clinical characteristics and outcomes in survivors and non-survivors with ARDS due to SARS-CoV-2

Variables	Survivors (n=72)	Non survivors (n=33)	p-value
Age, years, median (IQR)	66 (56-73)	78 (70-80)	<0.0001
Males N (%)	59 (82)	22 (67)	0.46
BMI (Kg/m ²), median (IQR)	26 (24.5-28.5)	28 (25-29.3)	0.37
SAPS II score, median (IQR)	32 (22-37)	48 (38-65)	<0.0001
Comorbidities, N (%)			
None	26 (36)	5 (15)	0.03
Hypertension	34 (47)	19 (57)	0.324
Cardiovascular disease	11 (15)	9 (27)	0.15
Hypercholesterolemia	3 (4)	1(3)	0.78
COPD	4 (5)	4 (12)	0.24
Chronic Kidney disease	3 (4)	1 (3)	0.78
Immunodepression ^(†)	7 (10)	3 (10)	0.92
Diabetes	12(17)	3(10)	0.3
Other ^(††)	4(5)	3(10)	0.67
At ICU admission			
SOFA score, median (IQR)	3 (3-5)	5 (4-10)	0.0003
PaO ₂ /FiO ₂ , median (IQR)	167 (146-188)	148 (124-179)	0.04
Interleukin-6 serum levels (ng/l), median (IQR) ^(†††)	179 (69-1070)	394 (94-2688)	0.53
ARDS categories, N (%)			
Mild	12 (17)	6 (19)	0.84
Moderate	58 (81)	26 (79)	0.83
Severe	2 (3)	1 (3)	1
Respiratory support, N (%)			
NIV	65 (90)	22 (67)	0.003
IMV	7 (10)	11 (33)	
Outcome throughout the ICU stay			
Respiratory support-free days, median (IQR)			
28 d	21 (15-25)	0 (0-0)	<0.0001
90 d	83 (77-87)	0 (0-0)	<0.0001
IMV, N (%)	40	33	<0.001
Prone position, N (%)	23 (32)	15 (45)	0.18
ECMO, N(%)	1(1)	1(3)	0.53
Tracheostomy, N (%)	2 (3)	0 (0)	0.34
ICU acquired infection, N (%)	10 (14)	5 (15)	0.86
Septic shock, N (%) ^(‡)	0(0)	1(3)	0.31
Acute Kidney injury, N (%) ^(††††)	17 (24)	27 (82)	<0.0001
Renal replacement therapy, N (%)	4 (5)	8 (24)	0.008
ICU stay-free days, median (IQR)			
28 d	17 (5-22)	0 (0-0)	<0.0001
90 d	79 (67-84)	0 (0-0)	<0.0001

Severity of illness was assessed by Simplified Acute Physiologic Score Two (SAPS II) based on the worst variables recorded during the first 24 hours of the ICU admission. Organ dysfunction was assessed by Sequential Organ Failure Assessment (SOFA).

^(†) Immunodepression was defined as non-AIDS-related immune deficiency with hematologic malignancy or solid tumor (active or in remission for less than 5 years, including recipients of autologous or allogeneic stem cell transplantation), solid organ transplant, long-term (> 30 days) or high-dose (> 1 mg/kg/day) steroids, or any immunosuppressive drug for more than 30 days⁽¹⁶⁾.

^(††) Other includes: epilepsy, endocrine disorders, neurologic disorders.

^(†††) Interleukin-6 serum level was tested only in 66 patients for temporary laboratory shortage of the reagent. IL-6 plasma levels were assessed using ELISA assay (Multi-cytokine test for ELLA-Bio-Techne, Minneapolis). Imputation of missing data was not performed.

^(‡) Septic shock was defined according to International criteria⁽¹⁷⁾.

BMI = body mass index; ARDS = acute respiratory distress syndrome; COPD = chronic obstructive pulmonary disease; ECMO = extracorporeal membrane oxygenation; FiO₂ = Fraction of inspired oxygen; ICU = intensive care unit; PaO₂ = arterial partial pressure of oxygen; NIV = non-invasive mechanical ventilation; IMV = invasive mechanical ventilation.

(††††) Acute kidney injury was defined according to the operational decision⁽¹⁸⁾.

IQR = interquartile range; N = number of patients; d =day. *P*-values were calculated by Mann-Whitney test, Chi-square test and Fisher exact test, as appropriate.

Table 2 Clinical characteristics and outcomes in critically ill patients with ARDS due to SARS-CoV-2 according to therapy group

Variables	Anti-IL-6R therapy group (n=65)	Standard therapy group (n=40)	<i>p</i> -value
Age, years, median(IQR)	65 (62-69)	77 (70-80)	< 0.01
Males, N (%)	56(86)	28(70)	0.05
BMI (Kg/m ²), median (IQR)	27.7 (24.7-29.5)	26 (24.5-27.7)	0.07
SAPS II score, median (IQR)	36 (26-41)	40 (31-61)	0.06
Comorbidities, N (%)			
None	18 (28)	13 (32)	0.60
Hypertension	38 (59)	15 (38)	0.04
Cardiovascular disease	10 (15)	10 (25)	0.22
Hypercholesterolemia	2 (3)	2 (5)	0.61
COPD	5 (8)	3 (7)	0.1
Chronic Kidney disease	2 (3)	2 (5)	0.62
Immunodepression ^(†)	7 (11)	3 (7)	0.6
Diabetes	11 (17)	4 (10)	0.33
Other ^(††)	4 (6)	3 (7)	0.8
Primary outcome			
ICU mortality, N (%)	12(18)	21(52)	< 0.01
Secondary outcomes			
Respiratory support-free days, median (IQR)			
28 d	17 (3-23)	0 (0-24)	0.32
90 d	79 (65-85)	0 (0-86)	0.23
IMV, N (%)	45 (69)	28 (70)	0.9
Prone position, N (%)	30 (46)	8 (20)	< 0.01
ECMO, N(%)	2 (3)	1 (2)	1
Tracheostomy, N (%)	2 (3)	0 (0)	0.1
ICU acquired infection, N (%)	9 (14)	6 (15)	0.87
Septic shock, ^(†††) N (%)	1 (1)	0 (0)	0.43
Acute Kidney injury, N (%)	23 (35.3)	21 (52.5)	0.08
Renal replacement therapy, N (%)	9 (14)	3 (5)	0.32
ICU stay-free days, median (IQR)			
28 d	12 (0-20)	0 (0-20)	0.68
90 d	74 (33-82)	0 (0-82)	0.13
At ICU admission			
SOFA score, median (IQR)	4 (3-6)	4 (2-11)	0.4
PaO ₂ /FiO ₂ , median (IQR)	155 (144-182)	169 (135-217)	0.12
Interleukin-6 serum levels (ng/l) median (IQR) ^(††††)	221.3 (75-1854)	123.6 (95-465.7)	0.37
ARDS categories, N (%)			
Mild	5 (8)	13 (32)	< 0.01
Moderate	59 (91)	25 (62)	< 0.01
Severe	1 (1)	2 (5)	0.55
Respiratory support, N (%)			
NIV	57 (88)	30 (75)	0.1
IMV	8 (12)	10 (25)	

Severity of illness was assessed by Simplified Acute Physiologic Score Two (SAPS II) based on the worst variables recorded during the first 24 hours of the ICU admission. Organ dysfunction was assessed by Sequential Organ Failure Assessment (SOFA).

^(†)Immunodepression was defined as non-AIDS-related immune deficiency with hematologic malignancy or solid tumor (active or in remission for less than 5 years, including recipients of autologous or allogeneic stem cell transplantation), solid organ transplant, long-term (> 30 days) or high-dose (> 1 mg/kg/day) steroids, or any immunosuppressive drug for more than 30 days⁽¹⁶⁾

^(††) Other includes: epilepsy, endocrine disorders, neurologic disorders

^(†††) Septic shock was defined according to International criteria⁽¹⁷⁾

^(††††) Acute kidney injury was defined according to the operational decision⁽¹⁸⁾

(††††) Interleukin-6 serum level was tested only in 66 patients for temporary laboratory shortage of the reagent. IL-6 plasma levels were assessed using ELISA assay (Multi-cytokine test for ELLA-Bio-Techne, Minneapolis). Imputation of missing data was not performed.

BMI = body mass index; ARDS = acute respiratory distress syndrome; COPD = chronic obstructive pulmonary disease; ECMO = extracorporeal membrane oxygenation; FiO₂ = Fraction of inspired oxygen; ICU = intensive care unit; PaO₂ = arterial partial pressure of oxygen; NIV = non-invasive mechanical ventilation; IMV = invasive mechanical ventilation; IL-6R = interleukin-6 receptor.

IQR = interquartile range; N = number of patients; d = day

P-values were calculated by Mann-Whitney test, Chi-square test and Fisher exact test, as appropriate.

Table 3 Inverse probability of treatment weighting-adjusted multivariable Cox proportional hazards model on ICU mortality in overall study population (n=105)

Variable	HR	95% HR confidence limit	p-value
Anti IL-6R group vs. standard treatment group	0.34	0.17 – 0.64	0.001
Age (years)	1.16	1.04 - 1.31	0.009
SAPS II score at admission	1.07	1.04 - 1.10	<0.001
Hypertension	2.35	0.962 - 5.74	0.061
AKI	6.89	2.22 - 21.42	0.001

The following variables: “ARDS categories at ICU admission”, “invasive mechanical ventilation throughout the ICU stay” and “prone position” were not included in the final model based on the conditional stepwise variable selection. The interactions between therapy strategy and covariates included in the model were not statistically significant.

n = number of patients; vs. = versus; IL-6R = interleukin-6 receptor; SAPS II score = Simplified Acute Physiologic Score Two; AKI = acute kidney injury.

Fig. 1 Kaplan Meir curves of survival on overall study population (n=105)

Legend. Kaplan Meir curves of survival on overall study population, according to study group (n=105)

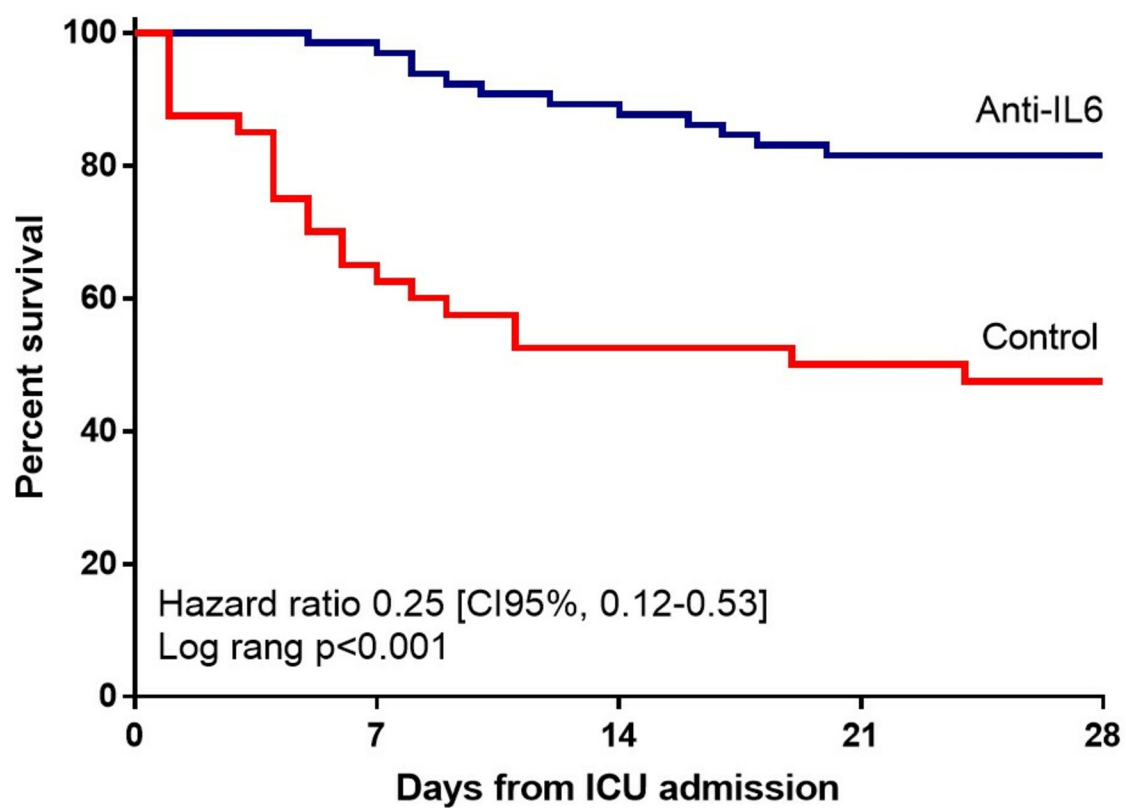
Fig. 2 Changing in $\text{PaO}_2/\text{FiO}_2$ ratio in study population stratified by treatment over time

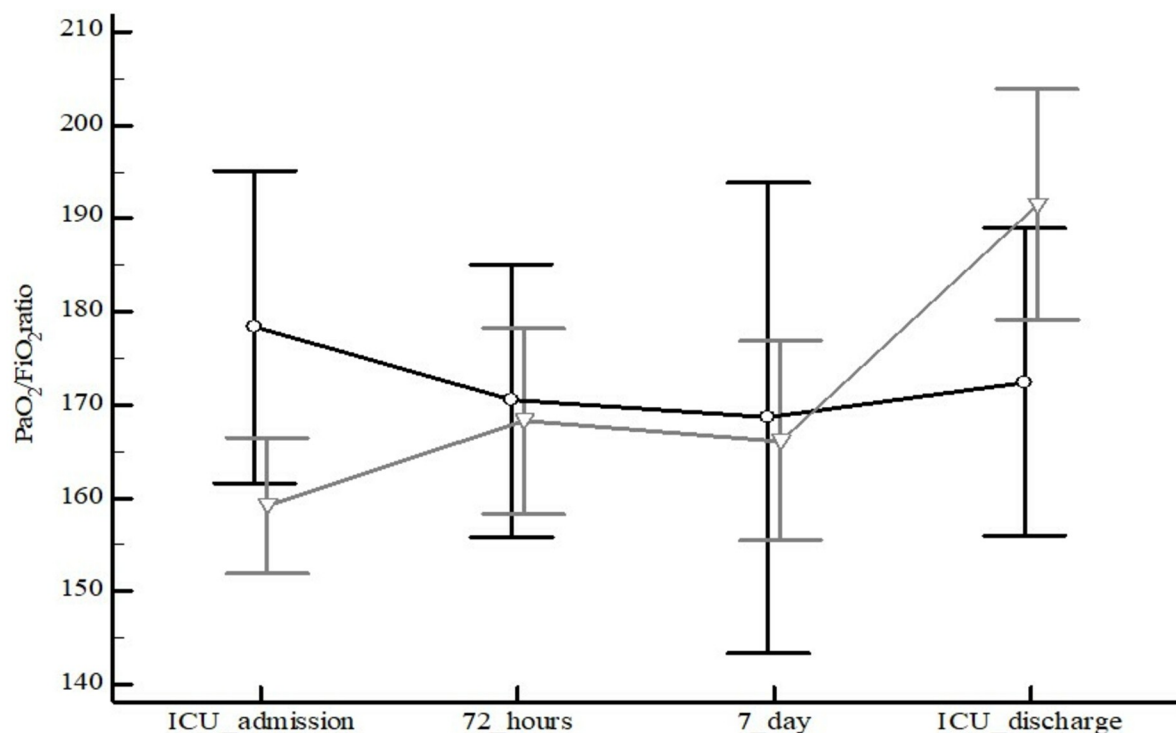
Legend. Figure shows temporal changes in $\text{PaO}_2/\text{FiO}_2$ ratio from ICU admission to ICU discharge. Data are mean with 95% confidence interval for mean. Changing in $\text{PaO}_2/\text{FiO}_2$ ratio were assessed with a repeated measures analysis of variance on log-transformed data. There were significant differences between the measurements over time in the two groups ($p=0.03$). The interaction was statistically significant ($p=0.01$).

FiO_2 = Fraction of inspired oxygen; PaO_2 = arterial partial pressure of oxygen.

Gray line = anti-IL-6R group; black line = Standard treatment group.

N is the total number of patients with available data





PaO ₂ /FiO ₂ ratio	Anti-IL-6R therapy group	N	Standard therapy group	N
At ICU admission	159 (152-166)	65	174 (159-190)	40
At 72 hours	168 (158-178)	65	169 (156-182)	40
At 7 day	166 (155-177)	65	168 (143-194)	40
At ICU discharge	191 (179-204)	65	172 (157-187)	40

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