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Tocilizumab in COVID-19 interstitial pneumonia

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Abstract. Pomponio G, Ferrarini A, Bonifazi M, Moretti M, Salvi A, Giacometti A, Tavio M, Titolo G, Morbidoni L, Frausini G, Onesta M, Amico D, Rocchi MLB, Menzo S, Zuccatosta L, Mei F, Menditto V, Svegliati S, Donati A, D'Errico MM, Pavani M, Gabrielli A (Ospedali Riuniti di Ancona; SOD Medicina di Laboratorio Ospedali Riuniti di Ancona; Ospedali Riuniti Marche Nord, Pesaro/Fano; Ospedale di Senigallia, Senigallia; Ospedale di Fabriano, Fabriano; Università di Urbino, Urbino; DISCLIMO, Università Politecnica delle Marche, Ancona, Italy). Tocilizumab in COVID-19 interstitial pneumonia (Brief Report). *J Intern Med* 2021;**289**: 738–746

Background. Published reports on tocilizumab in COVID-19 pneumonitis show conflicting results due to weak designs or heterogeneity in critical methodological issues.

Methods. This open-label trial, structured according to Simon's optimal design, aims to identify factors predicting which patients could benefit from anti-IL6 strategies and to enhance the design of unequivocal and reliable future randomized trials. A total of 46 patients with COVID-19 pneumonia needing of oxygen therapy to maintain SO2 > 93% and with recent worsening of lung function received a single infusion of tocilizumab. Clinical and biological markers were measured to test their predictive values. Primary end point was early and sustained clinical response.

Results. Twenty-one patients fulfilled pre-defined response criteria. Lower levels of IL-6 at 24 h after tocilizumab infusion ($P = 0.049$) and higher baseline values of PaO2/FiO2 ($P = 0.008$) predicted a favourable response.

Conclusions. Objective clinical response rate overcame the pre-defined threshold of 30%. Efficacy of tocilizumab to improve respiratory function in patients selected according to our inclusion criteria warrants investigations in randomized trials.

Keywords: COVID-19, IL-6, pneumonia, severe acute respiratory syndrome, tocilizumab.

Abbreviations: AF, atrial fibrillation; ALT, alanine aminotransferase; CP, CPAP; HRCT, high-resolution chest CT scan; HYQ, hydroxychloroquine; ICU, intensive care units; IL-6, interleukin 6; LMWH, low molecular weight heparin; P/F, ratio of PaO2 to FiO2; SO2, oxygen saturation; SP-D, surfactant protein D; TCZ, tocilizumab; TSS, Total Severity Score; UNL, upper normal level; V, Ventimask; vWF, von Willebrand Factor.

Introduction

Multifocal interstitial pneumonia represents the most common cause of admission in intensive care units (ICU) and death during SARS-CoV-2 infection. Available data highlight an intense 'cytokine storm' with a consequent inflammatory infiltrate of pulmonary interstitium and subsequent extended alveolar damage [1,2].

Trial registration: NCT 04315480

Tocilizumab, an anti-IL-6 receptor monoclonal antibody, has proved effective in rheumatoid arthritis, as well as in diseases characterized by an intense systemic inflammatory activation [3,4]. Moreover, small case series from China and Italy suggest that IL-6 blockade induces rapid clinical improvement in patients affected by COVID-19 interstitial pneumonia [5–8].

However, conflicting results have been recently reported, in particular in patients affected by more severe disease.

Indeed, favourable effect of the drug on mortality and risk of intubation [9] has not been confirmed in other studies [10,11]. In particular, in two recent press releases [12,13], divergent results from COVACTA and EMPACTA phase 3 trials have been reported. Moreover, almost simultaneously, multiple studies showing favourable effect of tocilizumab on clinical relevant end points appeared [14,15].

Different designs and heterogeneity in population and outcomes characteristics hamper a reliable comparison and interpretation of the results.

To investigate which patients could benefit from a single intravenous infusion of tocilizumab, we designed a trial with the goals:

1 to explore parameters useful to predict clinical response and select the population characterized by the higher benefit/risk ratio;

2 to acquire elements useful to design future unequivocal randomized trials.

Methods

Study design

The study was structured according to Simon's optimal two-stage design [16] to test the hypothesis that an early single intravenous tocilizumab administration can reverse worsening of lung function in patients affected by severe COVID-19 pneumonitis. Fourteen patients (variable: n1) entered the first stage of the trial. Enrolment would have been stopped and the drug rejected if < 10% of the patients (variable: P0) had met the primary end point 72 h after the administration of tocilizumab. Conversely, the study would have been continued until at least 46 patients were enrolled in total (variable: *n*). The drug would have been rejected in the case of a positive response rate < 30% (variable: *P*1) of the patients completing the study.

The values of variables *n*1, *n*, *P*0 and *P*1 were set to obtain a probability ≤ 0.01 of accepting a drug worse than *P*0 after the first stage and a probability ≤ 0.2 of rejecting a drug better than *P*1 at the end of the study.

Study population

Between 12 March and 26 March 2020, 46 consecutive patients were treated.

Patients have been enrolled and treated in the Department of Infectious Diseases (*n*°15), Department of Emergency Medicine (*n*°17) and Department of Respiratory Medicine (*n*°5) at Ospedali Riuniti-Ancona and in Department of Emergency and Internal Medicine (*n*°6) of Ospedali Riuniti-Marche Nord and in the Departments of Internal Medicine of Ospedale di Senigallia (*n*°2) and Ospedale di Fabriano (*n*°1), all in Italy.

Main selection criteria were as follows: patients 18–90 years of age; SARS-CoV-2 infection diagnosed by RT-PCR, multifocal interstitial pneumonia confirmed by Rx or chest CT-scan, need of oxygen therapy (FiO2 50%) to maintain peripheral oxygen saturation (SO2) >93%, recent (within the last 24 h) worsening of lung function, defined as a decrease of SO2 > 3% points and/or decrease of the ratio of PaO2 to FiO2 (P/F) >50% and/or P/F below 150 mm Hg^{-1}, capability to sign written informed consent.

Drug administration

Tocilizumab was administered as a single infusion of 8 mg kg^{-1}.

Concomitant therapies were allowed according to local protocols. No patient received systemic corticosteroids.

Assessment

Complete clinical evaluation, assessment of SO2, arterial blood gas analysis, blood count, ALT, D-dimer and creatinine, recording of both FiO2 and type of ventilation were performed at baseline, and after tocilizumab infusion at 24 and 72 h and day 7.

CT-scan

High-resolution chest CT scan (HRCT) was performed at baseline in 33 cases and at day +7 in 21 patients.

Two independent experienced pulmonologists evaluated the images blinded for sequence, and final decision was reached by consensus.

Assessment of radiological pattern and definition of the extent of parenchymal involvement were performed and scored as described [17].

The extent of parenchymal involvement at HRCT was first scored by visually evaluating the percentage of lesions' involvement at lobar basis according to a 5-point categorical scale. A Total Severity Score (TSS; range from 0 to 20) was obtained by summing the scores of the five lobes. A TSS ≥ 8 was assumed as threshold of severity.

Assays

Serum levels of IL-6 (Immulite 2000, Siemens Medical Solution-Diagnostic, Mt Olive, NJ USA), von Willenbrand Factor (vWF) (EHVWF, Human vWF ELISA Kit, Thermo Fisher Scientific Inc., Waltham, MA, USA), Surfactant protein D (SP-D) (RD194059101- Human Surfactant Protein D ELISA Kit, BioVendor Research and Diagnostic Products, Brno, Czech Republic) and Thrombomodulin (ab46508–Thrombomodulin [CD141] Human ELISA Kit, ABCAM, Abcam plc, Cambridge Biomedical Campus, Cambridge, UK) were determined with commercially available enzyme-linked immunosorbent assay following the manufacturers' recommendations.

Sera from 20 healthy sex- and age-matched individuals were obtained and tested as controls (Fig 1).

Response criteria

The primary outcome was the rate of responder patients. A patient was *a-priori* defined as responder if fulfilling either criteria 1 or 2 AND criteria 3 of the ones listed below:

1 Improvement of oxygen saturation by more than 3% points and/or increase in P/F by 50% and/or increase P/F above 150 mmHg 72 h after tocilizumab AND persistence of this improvement at day 7;

2 No worsening of respiratory function as defined in the inclusion criteria at 72 h AND improvement of oxygen saturation by more than 3% points and/or increase in P/F > 50% and/or increase P/F above 150 mmHg at day 7;

3 No need of endotracheal ventilation for all or CP for those not requiring it at baseline.

Secondary outcomes were as follows: (i) rate of admission to intensive care unit for endotracheal intubation or evidence of multiple organ dysfunction; (ii) death; and (iii) rate of severe adverse events.

Although multiple score systems have been proponed to assess activity and severity of COVID-19 patients, none of them has been validated so far [18,19]. Consequently, we preferred to avoid adopting a scoring system, in order to prevent possible misguiding conclusions.

Statistical analysis

Data were expressed as median (and range or interquartile range) unless otherwise stated. Comparisons were made using Mann–Whitney *U* test or chi-square test as appropriate. Two multivariate logistic regression analyses were performed using the primary outcome as dependent binary variable and the possible prognostic factors as independent variables. As possible prognostic variables, we considered age, sex, P/F at baseline, heparin and HYQ as co-treatments, and either IL-6 at baseline or IL-6 at 24 h. A significance level alpha = 0.05 will be used for all the statistical analyses.

Results

Characteristics of Patients

Since five out of the first 14 patients met the primary outcome, enrolment was completed up to a total of 46 cases. Demographic and clinical characteristics are summarized in Table 1.

All the subjects were affected by pneumonitis requiring high-flow oxygen therapy. Twenty-five (63%) patients had severe respiratory failure, characterized by a P/F ratio < 150 mmHg, and 30 (65%) were on C-PAP.

HRCT, available in 33 (72%) patients, revealed a diffuse pulmonary involvement, and 23 (69%) had a Total Severity Score (TSS) of eight or more, typical of the most severe patterns [17].

Treatment response

According to the *a priori* criteria, 72 h after tocilizumab 20 (43.5%) patients had an objective improvement and maintained the improvement in lung function at day 7. In 14 patients, improvement was already apparent 24 h after drug administration. One further patient was stable after 72 h and improved at day 7. Overall, 21 (45.6%) of the enrolled patients could be classified as responder.

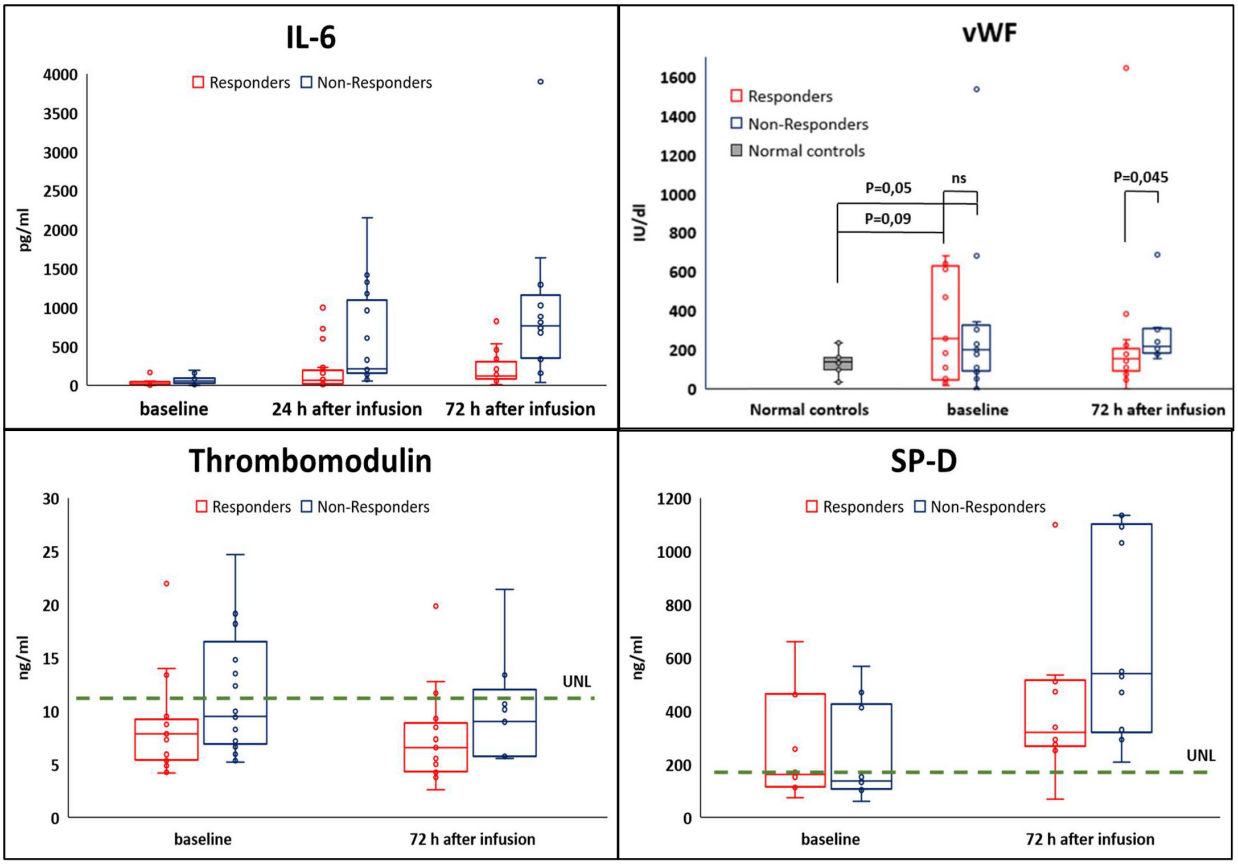

Fig. 1 *Upper normal limits of reference range at Ospedali Riuniti (Ancona) were IL-6: 5.2 pg mL^{-1}, thrombomodulin 11.2 ng mL^{-1} and SP-D 190 ng mL^{-1}; Using Mann–Whitney's* U *test;* P*-value Il-6 in responder vs non responder was 0.020, 0.001 and* <0.001 *at baseline, 24 and 72 h after infusion, respectively. Samples for IL-6 at baseline were available in 36 patients, 40 patients at 24 h, and 35 patients at 72 h; vWf and thrombomodulin were tested 30 patients and for vWF in 20 normal controls; S-PD was tested in 20 patients.*

None of the responder patients underwent endotracheal intubation, admission to ICU or died.

In this subgroup, the median P/F significantly increased at 72 h compared to baseline value (median; IQ range: 250; 197–362 vs 163; 136–241, $P = 0.009$).

At day 7, twenty-five patients were non-responder, although three of them had shown a transient improvement after 72 h. Eleven (44%) of 25 were intubated a median of 2 (range 0–9) days after treatment and four died. Of the remaining 14 patients, three died, and 11 were still classified as non-responder.

All responder patients were discharged after a median of 21 days (IQ range 17–27) after tocilizumab infusion, compared to 25.5 days (IQ range 20–34.25) of the non-responder patients.

Patients with P/F > 150 mmHg at baseline ($P = 0.003$) and lower CT-scan TSS ($P = 0.008$) were most likely to benefit from tocilizumab (Table 2).

Higher levels of IL-6 at baseline were higher in non-responders and showed a greater increase after 24 and 72 h (Fig 1).

No differences regarding to different CT-scan patterns, nor comorbidities number or type, emerged from the comparison between responders and non-responders.

We could compare chest CT scan taken at baseline and after 7 days in 21/46 patients.

Table 1. *Demographic, clinical, laboratory and radiological characteristics of patients enrolled in the experimental*

Median age (range) – years	67.5 (34–89)
Sex M/F (%)	33 (72)/13 (28)
Comorbidities *N* (%)	
Chronic heart failure	2 (4)
Hypertension	29 (63)
Diabetes	5 (11)
BPCO	0 (0)
Renal failure	1 (2)
Renal failure with renal transplantation	2 (4)
No comorbidity	21 (46)
1 comorbidity	17 (37)
2 comorbiditiesq	6 (13)
2+ comorbidities	2 (4)
Smoke *N* (%)	
Actual	0 (0)
Former	4 (10)
na	4 (9)
Time between onset of symptoms and TCZ infusion	
Days median (range)	9.5 (2–21)
na (%)	4 (9)
Time between onset of symptoms to hospital admission	
Days, median (range)	7 (0–14)
na (%)	4 (9)
Respiratory function at baseline *N* (%)	
Ventimask	16 (35)
C-PAP	30 (65)
P/F ratio > 150	15 (37.5)
P/F ratio < 150	25 (62.5)
na	6 (13)
Concomitant therapies *N* (%)	
Lopinavir-ritonavir or darunavir-cobicistat	35 (78)
Hydroxychloquine	41 (89)
Antibiotics	30 (67)
Prophylactic LMWH	18 (39)
Laboratory features at baseline	
IL-6 pg mL^{-1}	45.15
Median (25–75 IQ range)	(16.25–64.77)
na (%)	10 (22)
Lymphocyte $\times 10^9$/L	0.635
Median (25–75IQ range)	(522.5–790)
na (%)	4 (9)
ALT U/l	
Median (25–75IQ range)	30 (12–158)
na (%)	1 (2)
Extension of pulmonary involvement	
Chest CT-scan Total Severity Score	10
Median (25–75 IQ range)	(7–12)
na (%)	13

na, not available.

Changes in CT scan poorly correlate with clinical course.

Markers of endothelial and alveolar damage

Increased levels of vWF were detected in sera at baseline and 72 h after drug infusion, particularly in non-responder group. Thrombomodulin levels remained stable within the normal limits at baseline and after tocilizumab, in both groups.

The serum levels of SP-D, a glycoprotein secreted by type II pneumocytes and a potential surrogate marker of alveolar damage, were elevated at baseline in 35% of patients, and the concentrations rose overtime, particularly in non-responder patients (Fig 1).

In univariate analysis, baseline levels of the above-mentioned biomarkers did not correlate with outcome.

Multivariate analysis

In a multivariable model, lower IL-6 levels measured at 24 h ($P = 0.049$), and higher baseline values of P/F ($P = 0.008$) were associated with better response to the drug. In the alternative model, IL-6 levels at baseline did not emerge as independent variable.

Safety

Adverse events were reported in 29 (63%) of patients. Ten patients reported a 5-fold increase in alanine aminotransferase at day 7, with a subsequent normalization within 14 days. No case of hepatic failure was observed.

In three patients, significant neutropenia ($<1 \times 10^9$/L) appeared more than 7 days after

Table 2. *Clinical, biological and radiological characteristics of responder and non-responder patients*

	Responders	Non-Responders	*P*-value[a]
Number (%)	21 (46)	25 (54)	ns
Age median (range)	68 (37–89)	65 (34–89)	
Sex			
M	15 (45)	18 (55)	ns
F	6 (46)	7 (54)	
Number of comorbidities			
0	11 (52)	10 (48)	ns
1	9 (53)	8 (47)	
2 or 2+	1 (13)	7 (88)	
PaO2/FiO2 at baseline			
>150 mm Hg^{-1}	12	3	χ^2 (df1, N 40) = 8.64 P = 0.003
<150 mm Hg^{-1}	8	17	
mm Hg^{-1} median (IQ range; na)	163 (133–241; 1)	102 (88–142; 5)	nap
PaO2/FiO2 at 24 h median (IQ range; na)	211 (140–352; 5)	153 (121–177; 9)[b]	
PaO2/FiO2 at 72 h median (IQ range; na)	250 (197–362; 6)	138 (77–179; 9)[b]	
Prophylactic LMWH	6	13	ns
HYQ	18	23	ns
Extension of pulmonary involvement (Chest CT scan Total Severity Score)[c] median (IQ range)	8 (5.25–10)	11 (9.5–13)	U-MN = 61.5 P = 0.008
Discharged home (%)	21 (100)	16[d] (64)	nap
ICU admission requiring intubation	0	11	
Death	0	7	

nap, not applicable; ns, not significant.
[a]χ^2 = chi-square test, U-MN = Mann–Whitney U test.
[b]Intubated patients were excluded.
[c]CT scan images at baseline were available for 18 responder and 15 non-responder patients assessed as in references [11,12].
[d]Five patients have been discharged after having treating in ICU.

drug infusion. One of these was a non-responder and had a severe infection 1 month after tocilizumab infusion and despite normal neutrophil count.

Six bacterial infections were reported in six patients after admission to the ICU. The relationship with the experimental drug was considered uncertain by the investigators. Briefly, one patient was diagnosed with urinary infection caused by *Enterococcus Faecium* and *Candida albicans*. Three patients were found positive for methicillin-resistant *Staphylococcus aureus* in their bronchial aspirate culture. In a further case, a pulmonary infection caused by *Burkholderia gladioli* was diagnosed. Finally, a case of *Pseudomonas aeruginosa* pneumonia was reported.

Three patients, on hydroxychloroquine and lopinavir-ritonavir combination, suffered clinically relevant arrhythmias. One patient had a new episode of atrial fibrillation (AF) soon after tocilizumab infusion, with a spontaneous return to normal sinus rhythm within 24 h. A second patient developed AF 24 h after tocilizumab, and since this occurred with the worsening of respiratory function, he was transferred to the ICU and intubated. The third patient developed AF within 24 h after

infusion and underwent successful pharmacological cardioversion.

After the detection of pulmonary embolism 9 days after tocilizumab, one non-responder patient was anticoagulated and was discharged home once clinically improved.

No adverse reaction has been reported during or after parenteral administration of tocilizumab.

Discussion

The present study shows that 7 days after a single dose of tocilizumab administered within 24 h from evidence of clinical worsening, 21 patients with severe pneumonia fulfilled the responder criteria and none required admission to ICU or died. These patients were part of the 23 patients' subgroup who had improved at 72 h. The responder rate (46%) overcame the predefined threshold of 30%, and thus, the results justify future phase III randomized trials.

All 23 patients who did not improve at 72 h were also non-responder at day 7. All the patients who died were in the non-responder subgroup.

These data indicate that the response obtained 24–72 h after the administration of tocilizumab to patients with severe pneumonia foreshadows the likely prognosis.

At variance with what reported by others [20], high baseline values of IL-6 did not predict the clinical outcome of our patients, rather more informative were the serum levels at 24 h which better reflected the actual generation of IL-6 and thus the severity of the inflammation, as previously described in chronic inflammatory diseases [21]. The greater increase of the cytokine serum levels in patients who did not respond to tocilizumab supports the hypothesis that IL-6 plays a major role in the pathogenesis of organ damage in this infection, as also reported by others [22].

In addition, we investigated correlations between serum levels of three markers of endothelial or alveolar damage and clinical deterioration or response to the treatment.

In particular, in COVID-19 pneumonitis, increased plasma levels of thrombomodulin and vWF following endothelial apoptosis and necrosis lead to further endothelial damage [23].

Similarly, SP-D levels increase in respiratory distress syndromes triggered by septic shock as well as in patients affected by severe COVID-19 alveolar disruption [24].

In our experience, while the increasing levels of SP-D accurately illustrate the ongoing lung damage, vWF and thrombomodulin performed differently, the former higher in more severely affected patients at 72 h, the latter stable and within the normal limits at all time points. Since vWF is produced constitutively in endothelium, while thrombomodulin is an integral membrane protein expressed on the surface of endothelial cells, our findings suggest that at least an abnormal breakdown of vWF may be involved in the microvascular damage in COVID-19 patients.

Thus, the conclusions that can be drawn from these set of data are as follows: (i) a significant portion of severely ill and early deteriorating patients responded quickly to tocilizumab infusion; (ii) lower levels of IL-6, particularly 24 h after drug infusion and higher P/F ratio correlated with a better response; and (iii) patients not improving after 24–72 h, as well as those with unfavourable prognostic factors, should either receive a second administration of tocilizumab, or switched to other treatment regimen.

In our study, failure of tocilizumab in reducing mortality probably relies on its insufficient activity (at the scheduled dose) in the most severe cases. However, the rapid improvement observed in responsive patients is a very relevant end point, on a clinical and organizational ground, reducing the use of mechanical ventilation and making semi-intensive care beds more available for other patients.

Moreover, our data shed some light on conflicting results coming from randomized trials. Negative results of COVACTA trial, that is, could be attributed to the differences in many critical points, as severity of the disease, timing in tocilizumab administration and tardive end points' evaluation. According to our data, better results from EMPACTA trial could be linked to a less severe population at baseline and to the particular outcome chosen (cumulative proportion of participants requiring mechanical ventilation).

Several adverse events occurred in our patients. The three episodes of AF, which occurred within

24 h from tocilizumab, seem to point to a direct relationship with the medication. However, it is difficult to consider tocilizumab the sole culprit since: (i) arrhythmias are frequent in COVID-19 patients; (ii) tocilizumab seems to reduce the incidence of disorders of cardiac rhythm and cardiac events in inflammatory diseases [25]; and iii all three patients' co-treatments could have, at least, contributed to the rhythm abnormalities.

Our study has some weaknesses. First, it was a small, open-label trial without a control group. It cannot be ruled out that the improvement observed in some patients was due to a milder disease. However, in our opinion, the enrolment of patients with severe pneumonia and recent worsening of lung function in the previous 24 h, and appraising as responder only those who rapidly ameliorated after treatment, mitigated this problem. Second, due to the frantic days of the pandemic, there was quite a number of missing data, albeit equally distributed between the responder and non-responder subgroups. Complete reports were, however, available from two-thirds of the enrolled patients. Third, the small study group makes the multivariate analysis exposed to the risk of 'random effects'; thus, the data should be compared with those of ongoing prospective studies.

Conclusion

A prompt single infusion of 8 mg kg^{-1} of tocilizumab appears a promising strategy to rapidly improve respiratory function in patients with early severe and rapidly worsening COVID-19 lung disease, particularly with a P/F ratio > 150 mm kg^{-1} and lower generation of IL-6. This population should be considered the ideal target for future randomized trials.

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Not applicable.

Conflict of interest statement

The authors declare that they have no competing interests.

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Authors' contributions

AG, GP and AF contribute in study design, acquisition and interpretation of data and were major contributors in writing the manuscript. MR contributes to statistical analysis of data. MM, SS and MP perform biological X analysis. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was authorized on 12 March 2020 by the Internal Ethical Board of Ospedali Riuniti-Ancona (Italy).

Consent for publication

Not applicable.

Data availability statement

Not applicable.

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