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Tocilizumab in patients with severe COVID-19: a retrospective cohort study

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Summary

Background No therapy is approved for COVID-19 pneumonia. The aim of this study was to assess the role of tocilizumab in reducing the risk of invasive mechanical ventilation and death in patients with severe COVID-19 pneumonia who received standard of care treatment.

Methods This retrospective, observational cohort study included adults (≥18 years) with severe COVID-19 pneumonia who were admitted to tertiary care centres in Bologna and Reggio Emilia, Italy, between Feb 21 and March 24, 2020, and a tertiary care centre in Modena, Italy, between Feb 21 and April 30, 2020. All patients were treated with the standard of care (ie, supplemental oxygen, hydroxychloroquine, azithromycin, antiretrovirals, and low molecular weight heparin), and a non-randomly selected subset of patients also received tocilizumab. Tocilizumab was given either intravenously at 8 mg/kg bodyweight (up to a maximum of 800 mg) in two infusions, 12 h apart, or subcutaneously at 162 mg administered in two simultaneous doses, one in each thigh (ie, 324 mg in total), when the intravenous formulation was unavailable. The primary endpoint was a composite of invasive mechanical ventilation or death. Treatment groups were compared using Kaplan-Meier curves and Cox regression analysis after adjusting for sex, age, recruiting centre, duration of symptoms, and baseline Sequential Organ Failure Assessment (SOFA) score.

Findings Of 1351 patients admitted, 544 (40%) had severe COVID-19 pneumonia and were included in the study. 57 (16%) of 365 patients in the standard care group needed mechanical ventilation, compared with 33 (18%) of 179 patients treated with tocilizumab (p=0·41; 16 [18%] of 88 patients treated intravenously and 17 [19%] of 91 patients treated subcutaneously). 73 (20%) patients in the standard care group died, compared with 13 (7%; p<0·0001) patients treated with tocilizumab (six [7%] treated intravenously and seven [8%] treated subcutaneously). After adjustment for sex, age, recruiting centre, duration of symptoms, and SOFA score, tocilizumab treatment was associated with a reduced risk of invasive mechanical ventilation or death (adjusted hazard ratio 0·61, 95% CI 0·40–0·92; p=0·020). 24 (13%) of 179 patients treated with tocilizumab were diagnosed with new infections, versus 14 (4%) of 365 patients treated with standard of care alone (p<0·0001).

Interpretation Treatment with tocilizumab, whether administered intravenously or subcutaneously, might reduce the risk of invasive mechanical ventilation or death in patients with severe COVID-19 pneumonia.

Introduction

Since December, 2019, COVID-19 spread rapidly in Wuhan and throughout the Hubei province of China, and more recently in Europe and worldwide. Although a comparison of crude fatality rates across countries is made difficult by different testing policies, data from February and March, 2020, suggest that the fatality rate in Italy has been higher than that in China. The clinical presentation of COVID-19 is highly heterogeneous, ranging from asymptomatic to severe pneumonia with respiratory failure that could lead to invasive mechanical ventilation or death. The disease is characterised by an initial phase of viral replication that can be followed by a second phase driven by the host inflammatory response. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection might cause a hyperimmune response that is associated with acute respiratory distress syndrome, as suggested by typical radiological findings. The most critical patients can develop a so-called cytokine storm, characterised by increased production of many cytokines that produce long-term damage and lung tissue fibrosis.

No therapy has been approved for COVID-19 pneumonia, but current clinical approaches consider the combination of antiviral drugs and immunomodulatory drugs. Although lopinavir/ritonavir showed no benefit beyond the standard of care in an initial study, clinical trials on antivirals are ongoing. Drawing on a wider
immunomodulatory drugs, including tocilizumab, a recombinant humanised monoclonal antibody against the interleukin-6 receptor. Literature research was done through PubMed, Embase, Cochrane Review, ISI Web of Science, and SCOPUS up to May 31, 2020. This research revealed an increasing interest in tocilizumab use in COVID-19 pneumonia, but no randomised clinical trial has been published so far. In a single centre study from Wuhan, China, that included 15 patients with COVID-19 pneumonia at risk for cytokine storm, treatment with tocilizumab appeared to have clinical benefit, although the doses were variable, ranging from 80 mg to 600 mg. A small, retrospective, case-control study from France found that death, intensive care unit admission, or both was higher in patients without tocilizumab than in the tocilizumab group (72% v 25%; p=0.002). A randomised ongoing clinical study (CORIMUNO) anticipates a beneficial effect of tocilizumab compared with standard of care.

In our multicentre, retrospective study of 544 patients with severe COVID-19 pneumonia, the use of tocilizumab administered either intravenously or subcutaneously was associated with reduced risk of mechanical ventilation and death (adjusted hazard ratio 0.61, 95% CI 0.40–0.92, p=0.020). We also found a strong association between the use of tocilizumab and reduced risk of death (adjusted hazard ratio 0.38, 0.17–0.83; p=0.015).

**Evidence before this study**

No therapy is approved for COVID-19 pneumonia, but current clinical approaches consider the combination of antiviral and immunomodulative drugs, including tocilizumab, a recombinant humanised monoclonal antibody against the interleukin-6 receptor. Literature research was done through PubMed, Embase, Cochrane Review, ISI Web of Science, and SCOPUS up to May 31, 2020. This research revealed an increasing interest in tocilizumab use in COVID-19 pneumonia, but no randomised clinical trial has been published so far. In a single centre study from Wuhan, China, that included 15 patients with COVID-19 pneumonia at risk for cytokine storm, treatment with tocilizumab appeared to have clinical benefit, although the doses were variable, ranging from 80 mg to 600 mg. A small, retrospective, case-control study from France found that death, intensive care unit admission, or both was higher in patients without tocilizumab than in the tocilizumab group (72% v 25%; p=0.002). A randomised ongoing clinical study (CORIMUNO) anticipates a beneficial effect of tocilizumab compared with standard of care.

**Methods**

**Study design and participants**

The Tocilizumab in Patients with Severe COVID-19 Pneumonia (TESEO) Study is a retrospective, observational cohort study done in three tertiary care centres in the Emilia-Romagna region, Italy, on patients with severe COVID-19 pneumonia. All centres contributed data on tocilizumab and standard of care treatment (appendix p 1).

In the Modena cohort, we collected data on baseline signs, symptoms, comorbidities, blood count, and biochemical markers.

The study population was adults (≥18 years) with COVID-19, confirmed by PCR on nasopharyngeal swab, who were admitted to the centres in Bologna and Reggio Emilia between Feb 21 and March 24, 2020, and to the centre in Modena between Feb 21 and April 30, 2020 (after reviewers requested a follow-up extension, which was only possible at the Modena centre). Eligible patients had severe pneumonia, defined as at least one of the following: presence of a respiratory rate of 30 or more breaths per minute, peripheral blood oxygen saturation (SaO2) of less than 93% in room air, a ratio of arterial oxygen partial pressure (PaO2) to fractional inspired oxygen (FiO2) of less than 300 mm Hg in room air, and lung infiltrates of more than 50% within 24–48 h, according to Chinese management guidelines for COVID-19 (version 6.0).1,2

Exclusion criteria for the use of tocilizumab were coexistent infection other than COVID-19; a PaO2/FiO2 ratio greater than 300 mm Hg; chronic or current glucocorticoid use; history of severe allergic reactions to monoclonal antibodies; less than 500 per µL neutrophils or less than 50×10⁹ platelets; active diverticulitis, inflammatory bowel disease, or another symptomatic gastrointestinal tract condition that might predispose patients to bowel perforation; severe haematological, renal, or liver function impairment.

The study was approved by the Regional Ethical Committee of Emilia Romagna. All patients who received tocilizumab provided verbal, not written, informed consent because of isolation precautions.

**Procedures**

All patients received standard of care treatment at the time of hospital admission according to the regional
COVID-19 guidelines of Emilia Romagna and updated data on treatment of COVID-19. Standard of care treatment included oxygen supply to target SaO2 reaching at least 90%, hydroxychloroquine (400 mg twice on day 1, followed by 200 mg twice per day on days 2–5, eventually adjusted for creatinine clearance estimated by a chronic kidney disease algorithm), azithromycin (500 mg once per day for 5 days) at the physician’s discretion when suspecting a bacterial respiratory super-infection, lopinavir–ritonavir (400/100 mg twice per day) or darunavir–cobicistat (800/150 mg once per day) for 14 days, and low molecular weight heparin for prophylaxis of deep vein thrombosis according to bodyweight and renal function.

In addition to receiving the standard of care treatment, a non-randomly selected subset of patients also received tocilizumab treatment. Patients were considered eligible for tocilizumab treatment if they showed SaO2 of less than 93% and a PaO2/FiO2 ratio of less than 300 mm Hg in room air or a more than 30% decrease in their PaO2/FiO2 ratio in the previous 24 h during hospitalisation. Tocilizumab was administered by the intravenous or subcutaneous route depending on the availability of specific formulation at time of treatment. It should be mentioned that during the observation period, the high national requirements created an intermittent shortage of the intravenous formulation, at a dose of 162 mg administered in two simultaneous doses, one in each thigh (ie, 324 mg in total). This approach was used to mimic, as much as possible, the pharmacokinetic activity of the intravenous formulation to achieve similar levels of drug exposure. Because the site and depth of subcutaneous injection can influence absorption and distribution, the rate of absorption might vary markedly between dosing sites. The peak plasma concentration might take a few days to be reached after a single subcutaneous dose because of slow absorption through the lymphatic system into the systemic circulation. Because the monoclonal antibody might undergo proteolytic degradation by the cells of the reticuloendothelial system, higher doses than usual subcutaneous dosing for other indications were provided through separate injections. This decision was supported by a comparative pharmacokinetic and pharmacodynamic study of subcutaneous versus intravenous tocilizumab, which showed that after a single 162 mg dose in healthy subjects, subcutaneous bioavailability was 48.8%, whereas the pharmacodynamic activity of subcutaneous and intravenous tocilizumab against a soluble IL-6 receptor was similar during 1 week.

The patients’ full medical history, chronic comorbidities (including the Charlson Comorbidity Index), demographic and epidemiological data, and baseline PaO2/FiO2 ratio were obtained at hospital admission. Other treatments were recorded, including glucocorticoids for acute respiratory distress syndrome. The risk of multorgan failure and mortality was assessed with a standardised Subsequent Organ Failure Assessment (SOFA) score. Clinical data, including symptoms, complete blood count, coagulation, inflammatory, and biochemical markers were routinely registered in the electronic patient charts for the Modena cohort only.

### Outcomes

The primary outcome of the study was a composite of death or invasive mechanical ventilation. The indications for mechanical ventilation were neurological failure (ie, altered consciousness with a Glasgow Coma Scale score of <10), cardiovascular failure (ie, vasopressor requirement or major electrocardiogram changes, including arrhythmia or changes in repolarisation phase), and respiratory failure, defined by the presence of at least two of the following criteria: respiratory rate of 30 or more breaths per minute, respiratory distress with activation of accessory respiratory muscles, the need for FiO2 at 80% or more to maintain a SaO2 level of 90%, or a PaO2/FiO2 ratio of less than 100 mm Hg.

### Statistical analysis

We compared the baseline characteristics of the participants treated with standard of care with tocilizumab and those treated with standard of care alone, including signs...
and symptoms, existing comorbidities, and blood count markers. Continuous variables were expressed as median (IQR) and compared by the Mann-Whitney U test for two groups and Kruskal Wallis test for three groups. Categorical variables were expressed as numbers (%) and compared by the χ² test or Fisher’s exact test across the groups. In a secondary analysis, the group treated with standard of care and tocilizumab was further split into those who received subcutaneous or intravenous formulations of tocilizumab.

We did a standard survival analysis, following participants from the date of entry into clinics until initiation of invasive mechanical ventilation or death. We compared the time to invasive mechanical ventilation or death by treatment group using unweighted Kaplan-Meier curves and univariable and multivariable Cox regression analysis with baseline fixed covariates. The effect of treatment was shown using an unadjusted and adjusted hazard ratio (HR) with 95% CI. Key confounders were identified as sex, age, recruiting centre, duration of symptoms, and baseline SOFA score, which were the most probable causes of both treatment assignment and outcome risk.

To control for potential additional sources of time-fixed and time-varying confounding, we did various additional analyses. First, we adjusted the analysis for the baseline level of inflammation and coagulation in a subset of participants with available C-reactive protein and D-dimer values. The effect of treatment was shown using an unadjusted and adjusted hazard ratio (HR) with 95% CI. Key confounders were identified as sex, age, recruiting centre, duration of symptoms, and baseline SOFA score, which were the most probable causes of both treatment assignment and outcome risk.

We also did a similar stratification analysis using age (18–64 years vs ≥65 years) to further investigate the possible confounding or effect modification caused by age.

In the subset of participants from the Modena cohort, we compared the mean trajectories of IL-6 (on a log10 scale) and of aspartate aminotransferase (raw scale) over time between tocilizumab and standard of care, using a linear mixed model with random intercept and slope. We considered a two-sided p value test of less than 0.05 to be statistically significant. Statistical analyses were performed using the SAS software, version 9.4 (Cary, NC, USA).

| Table 1: Characteristics of patients from all centres combined | Tocilizumab plus standard care group (n=179) | Standard care group (n=365) | p value | All patients (n=544) |
|---|---|---|---|---|
| **Baseline characteristics** | Subcutaneous (n=91) | Intravenous (n=88) | Overall (n=179) | |
| Age (years) | 67 (55–73) | 63 (54–72) | 64 (54–72) | 69 (57–78) | 0.0064 | 67 (56–77) |
| Sex | - | - | - | - | 0.088 | - |
| Female | 28 (31%) | 24 (27%) | 52 (29%) | 133 (36%) | - | 185 (34%) |
| Male | 63 (69%) | 64 (73%) | 127 (71%) | 232 (64%) | - | 359 (66%) |
| Baseline PaO2/FiO2 (mm Hg) | 199 (123–262) | 145 (102–229) | 169 (106–246) | 277 (191–345) | <0.0001 | 239 (139–306) |
| Baseline SOFA score | 2 (1–3) | 3 (2–4) | 3 (2–4) | 2 (0–3) | 0.0004 | 2 (1–4) |
| Duration of symptoms (days from symptom onset) | 8 (5–10) | 4 (3–8) | 7 (4–10) | 5 (2–9) | 0.0017 | 6 (3–9) |
| **Outcomes** | Follow-up (days) | 12 (6–17) | 13 (7–18) | 12 (6–17) | 8 (4–14) | <0.0001 | 9 (4–15) |
| Events | Mechanical ventilation | 17 (19%) | 16 (18%) | 33 (18%) | 57 (16%) | 0.41 | 90 (17%) |
| Deaths after mechanical ventilation* | 2 (12%) | 3 (19%) | 5 (15%) | 14 (25%) | 0.51 | 19 (21%) |
| Death | 7 (8%) | 6 (7%) | 13 (7%) | 73 (20%) | 0.0007 | 86 (16%) |

Data are median (IQR) or n (%) unless otherwise indicated. The p values refer to differences between overall tocilizumab and standard of care and were calculated using the χ² test or Kruskal-Wallis test as appropriate. PaO2/FiO2 = ratio of arterial oxygen partial pressure to fractional inspired oxygen. SOFA = Subsequent Organ Failure Assessment.

*Percentages show the proportion of those who were mechanically ventilated.
**Table 2:** Baseline signs, symptoms, and comorbidities for patients at the Modena centre only

Data are median (IQR) or n (%) unless otherwise indicated. *p* values were calculated using the *χ²* test as appropriate.

| Comorbidities               | All patients (n=354) | Tocilizumab plus standard care group (n=132) | Standard care group (n=222) | p value |
|----------------------------|----------------------|-----------------------------------------------|-----------------------------|---------|
| Age (years)                | 67 (55–76)           | 61 (52–74)                                    | 67 (55–78)                  | 0·34    |
| Sex                        |                      |                                               |                             |         |
| Female                     | 26 (31%)             | 15 (31%)                                      | 68 (32%)                    | 0·0001  |
| Male                       | 58 (69%)             | 33 (69%)                                      | 154 (69%)                   |         |
| Any comorbidity            | 39 (46%)             | 24 (50%)                                      | 36 (16%)                    |         |
| Comorbidities              |                      |                                               |                             |         |
| Cancer                     | 2 (2%)               | 0                                              | 8 (4%)                      | 0·045   |
| Cardiovascular disease     | 9 (11%)              | 6 (13%)                                       | 12 (5%)                     | 0·12    |
| Chronic renal insufficiency| 2 (2%)               | 5 (10%)                                       | 7 (3%)                      | 0·045   |
| Diabetes                   | 11 (13%)             | 6 (13%)                                       | 7 (3%)                      | 0·0008  |
| Hypertension               | 37 (44%)             | 22 (46%)                                      | 30 (14%)                    | <0·0001 |
| Fever (°C)                 | 37 (36–37)           | 37 (36–37)                                    | 5·54                        |         |
| Headache                   | 5 (6%)               | 7 (10%)                                       | 7 (3%)                      | 0·059   |
| Myalgia                    | 5 (6%)               | 4 (2%)                                        | 10 (5%)                     | 0·059   |
| Myalgia                    | 5 (6%)               | 4 (2%)                                        | 10 (5%)                     | 0·49    |
| Sputum                     | 5 (6%)               | 4 (2%)                                        | 10 (5%)                     | 0·059   |
| Systolic pressure (mm Hg) | 124 (110–135)        | 124 (110–135)                                 | 124 (110–135)               |         |
| Days from symptoms onset to hospitalisation | 8 (6–11) | 5 (3–9)                                      | 5 (2–9)                     | 0·0016  |
| Days from hospitalisation to intubation | 3 (0–5) | 2 (1–4)                                      | 2 (0–3)                     | 0·49    |

Data are median (IQR) or n (%) unless otherwise indicated. *p* values were calculated using the *χ²* test or Kruskal-Wallis test as appropriate.

**Results**

Of 1351 patients admitted to the recruiting centres, 544 (40%) patients with severe pneumonia were included in our analysis (figure 1). Overall, 359 (66%) of the patients were male, with a median age of 67 years (IQR 56–77; table 1). All patients showed clinical deterioration, with a median SOFA score of 2 (IQR 1–4), mainly driven by respiratory failure and requirement of oxygen support. The SOFA scores and PaO₂/FiO₂ ratios at baseline differed substantially across centres, with patients in Modena being the most compromised (appendix p 1).

365 (67%) patients received standard care treatment alone and 179 (33%) received treatment with tocilizumab in addition to standard of care (88 [16%] received intravenous and 91 [17%] subcutaneous tocilizumab; table 1). The standard of care group included older patients with less severe disease, and the group treated with intravenous tocilizumab included the most compromised patients, as shown by their PaO₂/FiO₂ ratios and SOFA scores (table 1). After baseline, 53 (30%) of 179 patients treated with tocilizumab started glucocorticoids versus 61 (17%) of 365 patients in the standard of care group.

Data for comorbidities, signs, and symptoms were available for the patients from the Modena cohort only, which accounted for 354 (65%) of all patients (table 2). Among these patients, those treated with tocilizumab had a higher burden of hypertension and diabetes, and more had symptoms such as headache and cough (table 2). Biochemical markers were available for 304 (86%) of the patients in Modena, and showed that patients treated with tocilizumab had higher lactate dehydrogenase and worse inflammatory profiles at baseline, with higher C-reactive protein and IL-6 concentrations (table 3).

Overall, invasive mechanical ventilation was started in 90 (17%) of 544 patients, including 57 (16%) of 365 patients in the standard care group versus 33 (18%) of 179 patients treated with tocilizumab (*p*<0·041; table 1). 86 (16%) of 544 patients died, including 73 (20%) patients in the standard care group versus 13 (7%) (*p*<0·0007) patients treated with tocilizumab (table 1). The percentage of patients who were ventilated differed by centre (*p*<0·028), but risk of mortality did not (*p*<0·049; appendix p 1).

19 further deaths occurred after the date of initiation of mechanical ventilation (two in the subcutaneous tocilizumab group, three in the intravenous tocilizumab group, and 14 in the standard of care group), giving a total of 105 deaths, which we analysed using a competing risk approach.

At 14 days from hospital admission, the cumulative probabilities estimated with Kaplan-Meier analyses for all groups were 36·1% (95% CI 31·2–40·9) for the primary composite endpoint of invasive mechanical ventilation or death, 18·8% (15·1–22·5) for mechanical ventilation, and 21·1% (16·3–25·8) for death (appendix p 7; figure 2).

Unweighted Kaplan-Meier estimates showed the beneficial effect of treatment with tocilizumab compared with standard of care only (figure 2). At day 14 after hospital admission, the proportion of patients with the composite outcome was 22·6% (95% CI 16·2–29·0) for the tocilizumab group versus 36·5% (30·7–42·2) for the standard of care only group (log rank *p*=0·0023; figure 2A; appendix p 7). The difference was larger and the association stronger for the mortality endpoint, both with the cause-specific hazard approach (log rank *p*=0·0001; figure 2C) and the competing risk approach (*p*<0·0001).

After splitting the tocilizumab group by administration route, both groups showed a benefit compared with standard of care, with no marked difference between the intravenous and subcutaneous groups (log rank *p*=0·0091 [figure 2B]; log rank *p*=0·0001 [figure 2D]).

Patients who received tocilizumab showed a significant reduction in risk of invasive mechanical ventilation or death when compared with those receiving standard of care only, as estimated from the unadjusted Cox regression model (HR 0·60, 95% CI 0·43–0·84; *p*=0·0030; table 4). After controlling for the key identified confounders of sex, age, SOFA score, recruiting centre, and duration of symptoms, the treatment effect was even larger (adjusted HR [aHR] 0·61, 0·40–0·92; *p*=0·020; table 4). These results
were supported by various analyses that aimed to control for further sources of confounding, namely after adjusting for baseline C-reactive protein values (aHR 0·57, 95% CI 0·31–0·98; p=0·042; table 5). For the composite endpoint, we found no evidence for a differential benefit of tocilizumab by baseline PaO2/FiO2 ratio (interaction p=0·12; table 5), but the reduction in risk of death was found for tocilizumab treatment compared with standard of care treatment alone after controlling for SOFA score, recruiting centre, and duration of symptoms (aHR 0·38, 95% CI 0·19–0·74; p=0·015; table 5). We found no statistical evidence for a differential benefit of tocilizumab by baseline PaO2/FiO2 value (interaction p=0·12; table 5), but the reduction in risk of death was stronger in people with a baseline PaO2/FiO2 ratio of less than 150 mm Hg.

We also repeated this analysis after controlling for the Charlson Comorbidity Index instead of SOFA, and the results were similar (aHR 0·36, 95% CI 0·19–0·65; p=0·0008). Finally, after including the additional 19 deaths that occurred after the date of initiation of invasive ventilation, the results from the competing risk analysis were similar to those of the main analysis (aHR 0·27, 95% CI 0·16–0·47; p<0·0001).

The mixed linear models showed that IL-6 plasma levels were slightly higher at study entry in the tocilizumab group than in the standard of care group (2·46 log10 pg/mL vs 2·25 log10 pg/mL; p=0·091; appendix p 3). Over time, IL-6 was stable in the tocilizumab group and decreased in the standard of care group with a difference in slope of −0·02 log10 pg/mL (95% CI −0·03 to −0·00; p=0·0042; appendix pp 3–4).

Adverse events were carefully monitored during the study period. In the tocilizumab group, one (<1%) patient had an episode of injection site reaction, with spontaneous resolution in a few hours. One (<1%) episode of severe neutropenia required granulocyte-colony...
Kaplan-Meier estimates of the cumulative probability of mechanical ventilation or death (A, B) and death (C, D) by treatment group.

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**Figure 2:** Kaplan-Meier estimates of the cumulative probability of mechanical ventilation or death (A, B) and death (C, D) by treatment group.

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stimulating factor administration. Finally, there was no evidence for a difference in the rate of increase of aspartate aminotransferase between treatment groups (appendix pp 5–6). There was only one (<1%) patient in the standard of care group whose aspartate aminotransferase concentrations increased from 19 U/L before treatment level to 139 U/L at 6 days after treatment, and none in the tocilizumab group.

We paid careful attention to new episodes of infections in the tocilizumab and standard of care groups, which included bloodstream infections (three vs four), bacterial pneumonia (eight vs six), candidemia (two vs two), urinary tract infection (one vs one), *Pneumocystis jirovecii* pneumonia (one vs one), invasive aspergillosis, (four vs none), hepatitis B virus reactivation (one vs none), and herpes simplex virus 1 reactivation (four vs none). Of note, one (<1%) severe adverse event occurred in the tocilizumab group 12 days after subcutaneous injection, consisting of severe liver failure due to herpes simplex virus 1 reactivation, leading to death. This patient received high-dose glucocorticoids after the administration of tocilizumab. Overall, 24 (13%) of 179 patients treated with tocilizumab were diagnosed with new infections, versus 14 (4%) of 365 patients treated with standard of care alone (p<0.0001).

**Discussion**

In the real-life setting of the TESEO cohort, we found a significant reduction in risk of invasive mechanical ventilation or death in patients with severe COVID-19 pneumonia who were treated with either intravenous or subcutaneous tocilizumab and standard of care, compared with those treated with standard of care only. The association with the use of tocilizumab was stronger when overall mortality risk was analysed alone.

Our results are consistent with those of a smaller, retrospective, case-controlled French study by Klopfenstein and...
colleagues,\textsuperscript{20} in which death or intensive care unit (ICU) admissions were higher in patients who did not receive tocilizumab than those who did (72\% vs 25\%; p=0.002). The CORIMUNO randomised clinical trial, from which some data are already available, also anticipates a beneficial effect of tocilizumab when compared with standard of care.\textsuperscript{21}

The natural history of severe COVID-19 pneumonia is thought to be driven by a so-called cytokine storm.\textsuperscript{14} Nevertheless, current recommendations do not include any immunologically active drug in routine clinical practice, and use of glucocorticoids is controversial.\textsuperscript{35,36} Tocilizumab, administered intravenously or subcutaneously, can be considered together with anakinra as one of the immunomodulatory drugs that have been tested in clinical care for the treatment of severe COVID-19 pneumonia.\textsuperscript{19–21,37} In the discussion for the combination of immunomodulatory drugs (ie, monoclonal antibodies) with anti-inflammatory drugs (ie, glucocorticoids and non-steroid anti-inflammatory drugs). Importantly, very similar results were obtained regardless of the route of tocilizumab administration. Nevertheless, we cannot rule out the presence of other time-varying confounders that were affected by the chosen treatment strategy and were not accounted for in the analysis. Of note, antiviral drugs (protease inhibitors such as lopinavir–ritonavir and darunavir–cobicistat) were used in both groups and were never started after baseline in the tocilizumab group.

The real-life setting, including three different hospitals, accounted for the heterogeneity in clinical characteristics and disease severity across intervention groups. As expected, the comparator group showed a higher baseline PaO$_2$/FiO$_2$ value than did the intervention group. Thus, in the unadjusted analysis the magnitude of the beneficial effect associated with the use of tocilizumab could have been underestimated. We attempted to control for this confounding bias by adjusting for SOFA score, which includes baseline PaO$_2$/FiO$_2$, and the difference was indeed larger after adjustment. In addition, the effect of tocilizumab was at least two times higher in people with a baseline PaO$_2$/FiO$_2$ ratio of less than 150 mm Hg, implying that the benefit of tocilizumab could be greater in patients with a greater risk of death or mechanical ventilation. Further studies are needed to evaluate the optimal timing of tocilizumab initiation on the basis of PaO$_2$/FiO$_2$ values and severity of disease stage.

Our results were similar after further adjusting for post-baseline use of glucocorticoids. This analysis opens the discussion for the combination of immunomodulatory drugs (ie, monoclonal antibodies) with anti-inflammatory drugs (ie, glucocorticoids and non-steroid anti-inflammatory drugs). Importantly, very similar results were obtained regardless of the route of tocilizumab administration. Nevertheless, we cannot rule out the presence of other time-varying confounders that were affected by the chosen treatment strategy and were not accounted for in the analysis. Of note, antiviral drugs (protease inhibitors such as lopinavir–ritonavir and darunavir–cobicistat) were used in both groups and were never started after baseline in the tocilizumab group.
A major concern is adverse events. We observed a significantly higher prevalence of infection in the tocilizumab group than in the standard of care only group. The study design and short follow-up period do not allow us to make conclusions regarding the early and long-term side-effects of receiving tocilizumab followed by glucocorticoids; data from ongoing randomised clinical trials are required. Nevertheless, the case of severe herpes simplex virus 1 hepatitis in the tocilizumab group suggests the importance of screening for herpes virus reactivation, especially if glucocorticoids are added.

We chose a composite outcome including both invasive mechanical ventilation and all-cause mortality. The crude fatality rate in our cohort was 16% (86 deaths before mechanical ventilation and 105 [19%] deaths in total among 544 patients diagnosed with severe pneumonia). A large multicentre cohort study from China showed a fatality rate of 28% among hospitalised patients, despite patients being younger by a median of 15 years compared with our study. However, the largest effect of treatment was for SOFA, which controls for respiratory function. This high baseline SOFA score among our patients may explain the difference in the fatality rate. In the European setting, a recent large study with 1591 patients admitted to ICUs in the Lombardy region of Italy showed that 1150 (88%) received mechanical ventilation, 137 (11%) received non-invasive ventilation, and the fatality rate was 26%. However, this analysis did not exclude patients who received tocilizumab in addition to standard care treatment were mainly selected based on the usual assumptions about the model being correctly specified (ie, that the assumed underlying causal structure is correct and we have adjusted for all sources of measured confounding). The participants who received standard of care only were older and therefore at higher baseline risk for mortality, and rates of mechanical ventilation alone made little contribution to the difference observed.

Our study has some limitations. First, it is not a randomised comparison, and therefore unmeasured confounding cannot be ruled out. In addition, the results rely on the usual assumptions about the model being correctly specified (ie, that the assumed underlying causal structure is correct and we have adjusted for all sources of measured confounding). The participants who received standard of care only were older and therefore at higher baseline risk for invasive ventilation and death. However, these patients were also more likely to be women, and female sex has been shown to be associated with better outcomes. The patients who received tocilizumab in addition to standard care treatment were mainly selected based on the availability of the drug (which was intermittent over the recruitment phase because of shortages), and they were more compromised patients with lower PaO2/FiO2 ratios and higher SOFA scores compared with those treated with standard of care alone. We adjusted the analysis for SOFA, which controls for respiratory function (baseline PaO2/FiO2 ratio) and separately for the Charlson Comorbidity Index (which controls for the extent of...
comorbidities present at admission). In the tocilizumab group, there were two patients with cancer and two patients with renal insufficiency, and in the standard of care group, there were eight patients with cancer and seven with chronic renal insufficiency. Therefore, we cannot rule out residual confounding that cannot be controlled by regression interpolation.

Another limitation is that although the key confounder measurements (for sex, age, SOFA score, duration of symptoms, and Charlson Comorbidity Index) were available for all participants, this was not true for some of the biomarkers of inflammation and coagulation, which were available for only the participants in Modena. However, the results were similar when we repeated the analysis using the Modena centre dataset alone. Importantly, when we used a marginal structural model instead of the standard estimate of the hazard ratio that is conditioned on covariates, which additionally controlled for glucocorticoid use after baseline, the difference in risk between treatment strategies was even larger. This is a key result, given the wide use of these methods, the time-varying structure of the data, and our attempt to emulate the results of randomised comparisons. 11

The study was also open label, so that staff involved knew which patients were receiving tocilizumab. This knowledge might have led to variability in the decision of when to move a patient to invasive ventilation (ie, a quicker decision for those receiving standard of care only). Moreover, it should be acknowledged that the indication for mechanical ventilation, even if suggested by guidelines, still relies on clinical judgment, which might vary according to experience and the availability of resources. The ICU staff involved in the study shared similar protocols and resources. Finally, because of the short follow-up period, we were not able to assess long-term safety and adverse effects. Further studies are needed to define appropriate dosing and minimise the side-effects.

Our study has also strengths. First, it was a large study that included patients from a real-life hospital setting. Second, key confounding factors were collected daily in a standardised way for a minimum of 14 days and were linked to the electronic charts of blood counts and clinical data. Second, key confounding factors were collected daily in a standardised way for a minimum of 14 days and were linked to the electronic charts of blood counts and clinical data.

Many questions remain open. The generalisability of the results must be considered in relation to different epidemiological settings, particularly regarding the tocilizumab dose and use at the appropriate time point of the disease course. Other drugs that act directly in the inflammatory response pathway triggered in COVID-19 might be capable of reducing the risk of invasive mechanical ventilation or death in patients with severe COVID-19 pneumonia. Although these results are encouraging, they should be confirmed in ongoing randomised studies.

Contributors
GG, MMes, AC-L, JM, RT, CS, MMas, PIV, and CM conceptualised and designed the study. GG, MMes, AC-L, JM, RT, and CM wrote and revised the manuscript. GG, MMes, AC-L, JM, and CM supervised the final version of the manuscript. AC-L did the statistical analysis. GG, ST, and GD were in charge of the database of the three centres. MMen did the figures. All authors contributed to data collection, clinical management of the patients, and data interpretation.

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Declaration of interests
We declare no competing interests.

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