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

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Abstract:	Background There is no approved therapy for COVID-19 pneumonia. The aim of this multicentre cohort study was to assess the role of tocilizumab in reducing the risk of invasive mechanical ventilation and/or death in patients with severe COVID-19 pneumonia who received standard of care (SoC) treatment. Methods The TESEO Cohort Study is a retrospective, multicentre observational cohort study of patients with COVID-19 severe pneumonia treated with SoC with or without tocilizumab using intravenous (IV) or subcutaneous (SC) formulations, identifying respectively treated and comparator groups. Survival analysis was performed with participants' follow-up accruing from the date of entry into clinics until initiation of invasive mechanical ventilation or death, used as a composite outcome. Treatment groups were compared using Kaplan-Meier curves and Cox regression analysis after adjusting for gender, age and baseline Sequential Organ Failure Assessment (SOFA) score. Findings Of 544 patients included, 179 patients were treated with tocilizumab: 88 with the IV (16.1%) and 91 with SC formulation (16.7%). Mortality was significantly higher in the comparator group (20%) as opposed to tocilizumab IV (6.8%) and tocilizumab SC (7.7%) ($p<0.001$). A reduced risk of invasive mechanical ventilation/death was shown for participants treated with tocilizumab from fitting a Cox regression analysis adjusted for gender, age and SOFA score (aHR=0.61, 95% CI:0.40-0.92; p=0.02). We found no evidence for a difference between IV and SC administration route of tocilizumab. With regards to the mortality endpoint alone, a reduced risk was observed comparing tocilizumab with the comparator group (aHR=0.38 95% CI:0.17-0.83, p=0.02) . Interpretation Tocilizumab, regardless of IV or SC administration may be capable of reducing invasive mechanical ventilation or death in severe COVID-19 pneumonia. Our observations should be confirmed in randomised studies. Funding This study was not funded.

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

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RESEARCH IN CONTEXT

Evidence before this study

There is no approved therapy for COVID-19 pneumonia, but current clinical approaches consider the combination of antiviral agents and immuno-active drugs, including tocilizumab, a recombinant humanized monoclonal antibody against interleukin-6 receptors. In a single centre study from Wuhan, China, including 15 patients with COVID-19 pneumonia at risk for cytokine storm, treatment with tocilizumab, although with variable doses ranging from 80 to 600mg, appeared to have a clinical benefit.

Added value of this study

In our multicentre, retrospective study of 544 patients with severe COVID-19 pneumonia, use of tocilizumab administered either intravenously or subcutaneously was related to reduced risk of mechanical ventilation and death (aHR=0.61, 95% CI:0.40-0.92; p=0.02). We also found a strong association between use of tocilizumab and the risk of death (aHR=0.38 95% CI:0.17-0.83, p=0.02 .

Implications of all the available evidence

Tocilizumab, regardless of IV or SC administration may be capable of reducing invasive mechanical ventilation or death in severe COVID-19 pneumonia.

Abstract

Background

There is no approved therapy for COVID-19 pneumonia. The aim of this multicentre cohort study was to assess the role of tocilizumab in reducing the risk of invasive mechanical ventilation and/or death in patients with severe COVID-19 pneumonia who received standard of care (SoC) treatment.

Methods

The TESEO Cohort Study is a retrospective, multicentre observational cohort study of patients with COVID-19 severe pneumonia treated with SoC with or without tocilizumab using intravenous (IV) or subcutaneous (SC) formulations, identifying respectively treated and comparator groups. Survival analysis was performed with participants' follow-up accruing from the date of entry into clinics until initiation of invasive mechanical ventilation or death, used as a composite outcome. Treatment groups were compared using Kaplan-Meier curves and Cox regression analysis after adjusting for gender, age and baseline Sequential Organ Failure Assessment (SOFA) score.

Findings

Of 544 patients included, 179 patients were treated with tocilizumab: 88 with the IV (16.1%) and 91 with SC formulation (16.7%). Mortality was significantly higher in the comparator group (20%) as opposed to tocilizumab IV (6.8%) and tocilizumab SC (7.7%) ($p<0.001$). A reduced risk of invasive mechanical ventilation/death was shown for participants treated with tocilizumab from fitting a Cox regression analysis adjusted for gender, age and SOFA score (aHR=0.61, 95% CI:0.40-0.92; p=0.02). We found no evidence for a difference between IV and SC administration route of tocilizumab. With regards to the mortality endpoint alone, a reduced risk was observed comparing tocilizumab with the comparator group (aHR=0.38 95% CI:0.17-0.83, p=0.02) .

Interpretation

Tocilizumab, regardless of IV or SC administration may be capable of reducing invasive mechanical ventilation or death in severe COVID-19 pneumonia. Our observations should be confirmed in randomised studies.

Funding

This study was not funded.

Background

Since December 2019, Coronavirus disease-19 (COVID-19) has rapidly spread in Wuhan and throughout the Hubei province of China and more recently in Europe and worldwide. Although it is difficult to compare crude fatality rates across countries, due to different testing policies, the fatality rate in Italy is higher than that reported in China [1,2].

The clinical presentation of COVID-19 is extremely heterogeneous, ranging from asymptomatic cases to severe pneumonia with respiratory failure that could lead to invasive mechanical ventilation and/or death [3–5]. The time course of the disease is characterized by an initial phase of viral replication that may be followed by a second phase driven by the host inflammatory response [6]. Severe Acute Respiratory Syndrome – Coronavirus-2 (SARS-CoV-2) infection may cause an host hyperimmune response that is associated with an acute respiratory distress syndrome (ARDS) characterized by typical radiological findings [7]. The most critical patients may develop a so-called "cytokine storm", characterized by the increase of many cytokines that produce long-term damage and lung tissue fibrosis [8].

There is no approved therapy for COVID-19 pneumonia, but current clinical approaches consider the combination of antiviral agents and immuno-active drugs. Although antiviral agents showed no benefit beyond the standard of care (SoC) in an initial study [9], clinical trials are still ongoing. From a wider immunological perspective, derived from rheumatology [10], immunomodulatory agents, e.g. selective cytokine blockade, leading to inhibition of either the ligand or the receptor of a cytokine have been considered [11].

Tocilizumab is a recombinant humanized monoclonal antibody (mAb), of the IgG1 class, directed against both the soluble and the membrane bound IL-6 receptor [12,13]. It is recommended for the treatment of severe rheumatoid arthritis, systemic juvenile idiopathic arthritis, giant cell arteritis and life-threatening cytokine release syndrome induced by the chimeric antigen receptor T-cell [14–16]. In a single centre study from Wuhan, China, including 15 patients with COVID-19 pneumonia at risk for cytokine storm, treatment with tocilizumab, although with variable doses ranging from 80 to 600mg, appeared to have a clinical benefit [17]. This study and other anecdotal observations [18,19] raised the chance in Italy of off-label use of tocilizumab to treat patients with COVID-19

severe pneumonia. More recently, an increasing number of studies are reporting use of tocilizumab in COVID-19 [20,21].

The aim of this multicentre cohort study was to assess the role of tocilizumab in reducing the risk of invasive mechanical ventilation and/or death in a cohort of patients with severe COVID-19 pneumonia who received SoC treatment.

Methods

Study Setting

The TESEO Study (Tocilizumab in patients with severe COVID-19 pneumonia) is a retrospective, multicenter observational cohort study carried out in the Emilia-Romagna region, Italy, among patients with COVID-19 severe pneumonia. Centres participating in the study were Modena, Bologna and Reggio Emilia and all contributed either to tocilizumab or SoC. The contribution of each recruiting centre is specified in Supplementary Table 1. Modena cohort collected data in a rich data set obtained by electronic chart and allowed a more detailed description of the TESEO cohort.

The study was approved by *Regional ethical committee* of Emilia Romagna.

Patients and case definition

The target population was the universe of COVID-19 cases with confirmed PCR nasopharyngeal swab admitted to the participating centres between 21 February and 24 March while for Modena follow up was extended to 30 April 2020. The TESEO cohort considered all of the consecutive adult patients (≥18 years) with severe pneumonia defined as the concomitant presence of a respiratory rate (RR) ≥ 30 breaths per minute (bpm), peripheral blood oxygen saturation (SaO_2) < 93% in room air, a PaO_2/FiO_2 ratio < 300 mmHg in room air and lung infiltrates >50% within 24-48 hours, according to Chinese management guidelines for COVID-19 (version 6.0) [3,22]. The flowchart describes patients included in the analyses.

Standard of care (SoC) treatment

All patients received SoC treatment at the time of hospital admission. This was in agreement with the Regional COVID-19 Guidelines of Emilia Romagna [23] and with the updated data on treatment of COVID-19 [24] and it included:

- Oxygen supply to target $SaO_2 \geq 90\%$;
- Hydroxychloroquine (400 mg BID on day 1 followed by 200 mg BID on days 2 to 5 eventually adjusted for creatinine clearance estimated by a CKD algorithm);
- Azithromycin (500 mg QD for 5 days) at physician's discretion when suspecting a bacterial respiratory superinfection;
- Lopinavir/ritonavir (400/100 mg BID) or Darunavir/cobicistat (800/150 mg QD) for 14 days;
- Low molecular weight heparin for prophylaxis of deep vein thrombosis according to body weight and renal function.

Tocilizumab treatment

A non-randomly selected subset of patients received tocilizumab treatment in addition to the SoC. Patients were considered eligible for tocilizumab treatment in presence of $SaO_2<93\%$ and a $PaO_2/FiO_2<300$ mmHg in room air or a decrease in $PaO_2/FiO_2 > 30\%$ in the previous 24 hours during hospitalization. All tocilizumab-treated patients provided verbal, not written, informed consent due to isolation precautions. Tocilizumab was administered by the intravenous (IV) or the subcutaneous (SC) route depending on the hospital availability of the type of formulation at time of treatment. It should be mentioned that during the observation period, the high national request created an intermittent shortage of both formulations of the drug. IV tocilizumab was administered at the dose of 8 mg/kg of body weight (up to a maximum dosage of 800 mg) repeated twice, 12 hours apart. The second dose was given because PK data were suggesting that adequate plasma levels of the drug could be obtained only after two doses. The rationale for the second dose was based on the results of pharmacokinetic models for severe or life-threatening chimeric antigen receptor T cell-induced cytokine storm in adults and in paediatric patients [25].

The SC formulation was used in the shortage of the IV formulation at the dose of 162 mg administered twice simultaneously, one per each thigh. This approach was focused at mimicking, as much as possible, the pharmacokinetic activity of the IV formulation in order to achieve similar levels of drug exposure of those achieved using the IV administration. The site and depth of injection can influence the absorption and distribution [26]; the rate of absorption may vary markedly between dosing sites [26]; the peak plasma concentration may take a few days to be reached after

a single SC dose [27] and the mAb may undergo proteolytic degradation by the cells of the reticuloendothelial system [28].

Exclusion criteria for tocilizumab use were as follows:

- Coexistent infection other than COVID-19;
- Chronic or current glucocorticoid use;
- History of severe allergic reactions to monoclonal antibodies;
- Neutrophils < 500/mmc or platelets <50.000/mmc;
- Active diverticulitis, inflammatory bowel disease, or another symptomatic gastro-intestinal tract condition that might predispose to bowel perforation;
- Severe hematologic, renal or liver function impairment.

Covariates

The patients' full medical history, chronic co-morbidities including Charlson Comorbidity index [29], demographic and epidemiological data and baseline PaO_2/FiO_2 were obtained at the hospital admission. Eventual other treatments, including glucocorticoids for ARDS were recorded. The risk of multiorgan failure and mortality was assessed with standardized Subsequent Organ Failure Assessment (SOFA) score [30]. Clinical data with signs and symptoms, and complete blood count, coagulation, inflammatory and biochemical markers and were routinely registered in the electronic patient charts for the Modena cohort, only.

Outcome measures

The primary outcome of the study was the composite of death or invasive mechanical ventilation. Indication for mechanical ventilation were neurologic failure (i.e. altered consciousness with a Glasgow Coma Scale score <10), cardiovascular failure (i.e. vasopressor requirement or major ECG changes including arrythmia or changes in repolarization phase) and respiratory failure defined by the presence of at least 2 of the following criteria: respiratory rate > 30 bpm, respiratory distress with activation of accessory respiratory muscles, need for FiO_2 at 80% or more to maintain a SaO_2 level at 90%, or a PaO_2/FiO_2 < 100 mm Hg [31,32].

Statistical analysis

Baseline characteristics of the participants treated with SoC with tocilizumab and SoC alone were compared. These included signs and symptoms, existing co-morbidities and blood count markers. Continuous variables were expressed as median (IQR) and compared by Kruskal Wallis test. Categorical variables were expressed as numbers (%) and compared by χ^2 test or Fisher's exact test across the SoC with tocilizumab and the SoC groups. In a secondary analysis, the group treated with SoC and tocilizumab was further split into those who received tocilizumab in its SC or IV formulation.

Standard survival analysis was performed. Participants' follow-up accrued from the date of entry into clinics until initiation of invasive mechanical ventilation or death. Time to invasive mechanical ventilation or death by treatment groups was compared using unweighted Kaplan-Meier curves and univariable and multivariable Cox regression analysis with baseline fixed covariates. The effect of treatment was shown by means of unadjusted and adjusted hazard ratio (HR) with 95% CI. Key confounders were identified as gender, age, recruiting centre, duration of symptoms and baseline SOFA score as the most likely causes of both treatment assignment and outcome risk. A number of additional analyses have been performed to control for potential additional sources of time-fixed and time-varying confounding.

First of all, the analysis was adjusted for baseline level of inflammation and coagulation in a subset of participants with available C-reactive protein and D-dimer values. Secondly, SOFA score was replaced by alternative measures of the extent of concomitant morbidities at baseline such as a binary indicator (≥1 of the comorbidities among: diabetes, hypertension, cardiovascular disease, chronic renal insufficiency or cancer) as well as the Charlson Comorbidity Index. Thirdly, with the aim of emulating a randomized trial with similar characteristics as well as appropriately controlling for the time varying confounder of glucocorticoids use, we also fitted a marginal structural Cox regression models with stabilized inverse probability weights constructed using gender, age, SOFA score, recruiting centre, duration of symptoms and time varying use of glucocorticoids and inverse probability of censoring [33]. A secondary analysis with endpoint death alone used both the cause-specific hazard approach, assuming non-informative censoring and a competing risk approach in which deaths, which occurred after initiation of invasive mechanical ventilation, have been included as events.

Finally, in order to test the hypothesis that the difference between treatment groups might vary according to the baseline PaO_2/FiO_2 value, we formally included an interaction term in the Cox regression model. Results were shown after categorizing the population in two strata using a clinical threshold of PaO_2/FiO_2 = 150 mmHg. A similar stratification analysis has been also performed using age strata (18-64 vs. 65+ years) to further investigate the possible confounding/ effect modification due to age.

In the subset of participants from the Modena cohort alone, mean trajectories of IL-6 (in the log10 scale) and of AST (raw scale) over time were compared between tocilizumab and SoC using a linear mixed model with random intercept and slope.

A two-sided test of less than 0.05 was considered statistically significant. Statistical analyses were performed using the SAS software, version 9.4 (Carey USA), unless otherwise indicated.

Results

A subset of 544 patients with severe pneumonia were included in this analysis. Epidemiological and respiratory characteristics are shown in Table 1. The vast majority of patients were males (66%) with a median age of 67 years. All patients showed clinical deterioration with a median SOFA score of 2 (95% CI: 1-4), mainly driven by respiratory failure with a median PaO_2/FiO_2 <250 mmHg requiring oxygen support. SOFA score and PaO_2/FiO_2 at baseline differed substantially across centres, with patients in Modena being the most compromised (Supplementary Table 1).

Concerning treatment, 365 patients received SoC alone (67.2%), and 179 received additional treatment with tocilizumab (32.8%; in detail 16.1% by IV and 16.7% by SC) (Table 1). The groups had different characteristics (Table 1). The comparator group included older patients with a less severe disease, and the group treated with IV tocilizumab included the most compromised patients as depicted by PaO_2/FiO_2 ratio and SOFA score. Post baseline, 53 out of 179 (29.6%) treated with tocilizumab started glucocorticoids vs. 61 out of 365 (16.7%) in the SoC group.

Comorbidities, signs and symptoms were available for the 354 patients from the Modena cohort (Table 2a). Tocilizumab treated patients had a higher burden of hypertension and of symptoms such as headache and cough. Among these patients, biochemical markers were available for a subset of

304 patients (Table 2b). Tocilizumab treated patients had a higher lactate dehydrogenase and worse baseline inflammatory profile with higher C-reactive protein and IL6 values.
Overall, invasive mechanical ventilation was started in 90 out of 544 patients (16.5%), but the percentage varied significantly across the centres (p=0.028). Eighty-six patients died (15.8%) and the risk of mortality did not differ significantly across centres (p=0.49) (Supplementary Table 1a). There were further 19 deaths which occurred after the date of initiation of mechanical ventilation for a total of 105 deaths which have been analysed using a competing risk approach. With regards to study outcomes, mortality was significantly higher in the SoC group (20%) compared with both IV and SC tocilizumab groups (6.8% and 7.7%, respectively, p<0.001) (Table 1).

At 14 days from hospital admission, the overall cumulative Kaplan-Meier estimated probabilities amounted to 36.1% (95% CI:31.2-40.9%) for the primary composite endpoint of invasive mechanical ventilation or death, and 21.1% (95% CI: 16.3-25.8%) for death (Supplementary Table 4, Figure 1).
Unweighted Kaplan-Meier estimates showed the beneficial effect of the use of tocilizumab compared to SoC (Figure 1). At day 14 from hospital admission the proportion of patients experiencing the composite outcome was 27.0% (95% CI: 19.6-34.4%) for the tocilizumab group vs. 41.5% (95% CI: 35.1-47.9%) for SoC (log rank p=0.0023, Figure 1a; Supplementary Table 4). The difference was even larger and the association stronger when using the mortality endpoint both with cause-specific hazard approach (log rank p<0.0001, Figure 1b) and competing risk approach (p<0.0001). After splitting the tocilizumab group by administration route, both groups showed a benefit as compared to SoC with no marked difference between the IV and the SC group (log-rank p<0.001, Figure 1c, 1d).

A significant reduction in risk of invasive mechanical ventilation or death was shown comparing patients receiving tocilizumab with those receiving SoC, as estimated by hazard ratio (HR) from the unadjusted Cox regression model (HR= 0.60; 95% CI: 0.43-0.84, p<0.003, Table 3).

After controlling for the key identified confounders of gender, age and SOFA score, the treatment effect was even larger (aHR=0.61, 95% CI:0.40-0.92, p<0.02, Table 3). These results were confirmed in a number of analyses aiming to control for further sources of confounding, namely after adjusting for i) baseline CRP values (aHR=0.57, 95% CI:0.38-0.84, p=0.005); ii) baseline d-dimer levels (aHR=0.66, 95% CI:0.42-1.05, p=0.08), iii) after replacing the SOFA score with the Charlson

Comorbidity Index (aHR=0.64, 95% CI:0.46-0.91, p=0.01) and iv) after controlling for time-varying confounding of using glucocorticoids and informative censoring (aHR=0.44, 95% CI:0.29-0,65, p<0.001) (Table 3).

The largest difference was found when comparing IV tocilizumab with SoC.. After adjusting for the same set of identified confounders we estimated the following reduction in risk of invasive ventilation/death (aHR=0.55, 95% CI: 0.31-0.98, p=0.042, Table 3). Still comparing the risk for the composite endpoint and regarding the potential difference by administration route, we found no evidence for a difference between SC and IV (aHR=1.50 (95% CI:0.36; 6.24, p=0.58), Table 3).

Finally, the main results for this end point were similar after restricting the analysis to people enrolled in Modena, only (aHR=0.65, 95% CI: 0.43-0.99, p=0.04).

The formal test for interaction and the stratified analyses showed evidence that this difference varied by baseline PaO_2/FiO_2 value (p=0.011). In particular, the effect of tocilizumab was two-fold higher in people with baseline PaO_2/FiO_2 value<150 (aHR=0.19, 95% CI:0.08;0.44) (Table 3). No difference in the results was found after controlling for age using stratification (18-65 vs. 65+ years, data not shown).

Analysing the mortality endpoint, a significant reduction in risk of death was found comparing tocilizumab with SoC after controlling for gender, age and SOFA score (aHR= 0.38, 95% CI:0.17;0.83, p=0.015, Table 4). Although there was little statistical evidence for a differential benefit of tocilizumab by baseline PaO_2/FiO_2 value (interaction p=0.12, Table 4), again the effect was much stronger in people with baseline PaO_2/FiO_2 <150.

We also repeated this analysis after controlling for the Charlson Comorbidity Index instead of SOFA and results were similar (aHR=0.36, 95% CI:0.19-0.65, p=0.008; data not shown). Finally, after including the additional 19 deaths which occurred after the date of initiation of invasive ventilation, results from the competing risk analysis were again similar to those of the main analysis (aHR=0.27, 95% CI (0.16; 0.47, p<0.001).

The mixed linear models showed that IL-6 plasma levels were slightly higher at study entry in the intervention group vs. comparator (2.46 vs. 2.25 log10 mg/l, p=0.09) (Supplementary Table 2). Over

time, there was tendency for IL-6 to slowly increase in the intervention group and decrease in the comparator group with a significant difference in slope (-0.02 log10 mg/l; 95% CI:-0.03;-0:00, p=0.004) (Supplementary Figure 1).

Serious adverse events were carefully monitored during the study period. In the tocilizumab group a single episode of injection site reaction occurred with spontaneous resolution in a few hours. Moreover, one episode of severe neutropenia required Granulocyte-Colony Stimulating Factor administration. Finally, there was no evidence for a difference in the rate of increase of AST between treatment groups (Supplementary table 3 and Supplementary figure 2). Aside from, the case of acute liver failure (mentioned below), there was only one person in the SoC group whose AST increased from 19 U/I pre-treatment level to 139 six days post treatment, none in the tocilizumab group.

A great attention was given to new episodes of infections occurring both in tocilizumab and comparator group. They included bloodstream infections (3 vs. 4), bacterial pneumonia (8 vs. 6), candidemia (2 vs. 2), urinary tract infection (1 vs. 1), *Pneumocistis jirovecii* pneumonia (1 vs. 1), invasive aspergillosis. (4 vs. 0). *Hepatitis B virus* reactivation (1 vs. 0), *Herpes simplex virus 1* (HSV1) reactivation (4 vs. 0). Of note, one episode of severe adverse event occurred in the tocilizumab group at 12 days after SC injection consisting of severe liver failure due to HSV1 reactivation, leading to death. This patient received high dose glucocorticoids after the administration of tocilizumab. To summarize, 24 infections were diagnosed in 179 tocilizumab patients (13%) vs 14 out of 365 SoC patients (4%) (p<0.001).

Discussion

In the real-life setting of the TESEO cohort, we reported a significant reduction in risk of invasive mechanical ventilation or death in patients with severe COVID-19 pneumonia treated with either IV or SC tocilizumab, compared to SoC. The association with the use of tocilizumab was even stronger when overall mortality risk was analysed alone.

Our results are consistent with those of a smaller retrospective case-controlled French study by Klopfesntein et al [20], in which death and/or ICU admissions were higher in patients without tocilizumab than in the tocilizumab group (72% vs 25%, p=0.002) vs 41.5 vs 27.0 % by 14 days in our

study. A press release of the CORIMUNO randomized clinical trial also anticipated a beneficial effect of tocilizumab when compared to SoC [34].

Natural history of severe COVID-19 pneumonia is thought to be driven by a “cytokine storm” [14]. Nevertheless, current recommendations do not comprise any immunologically active agent in routine clinical practice, while glucocorticoids use is still controversial [35,36]. Tocilizumab, administered both intravenously or subcutaneously, can be considered together with anakinra as one of the immune-active agents which have been tested in clinical care for the treatment of severe COVID-19 pneumonia [19–21,37]. IL-6 levels increased after tocilizumab administration, compared to people receiving SoC. This is an expected finding since tocilizumab competitively blocks IL-6 receptors and leaves free IL-6 in plasma. Longer follow-up and larger sample are needed to better understand the prognostic role of IL-6 in patients with COVID-19 pneumonia treated with tocilizumab.

The real-life setting, including three different hospitals, accounted for the heterogenicity in clinical characteristics and disease severity across intervention groups. In particular, as expected, the comparator group showed a higher baseline PaO_2/FiO_2 value than the intervention group. Thus, in the unadjusted analysis the magnitude of the beneficial effect associated with the use of tocilizumab could have been even underestimated. We attempted to control for this confounding bias by adjusting for SOFA score, which comprises baseline PaO_2/FiO_2 and indeed, the difference was larger after adjustment. In particular, the effect of tocilizumab was at least two-fold higher in people with baseline PaO_2/FiO_2 value<150, implying that the benefit of tocilizumab could be even higher 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 glucocorticoid use. This analysis reinforces our findings and open the discussion for combination of immunomodulatory agents (i.e. monoclonal antibodies) with anti-inflammatory drugs (i.e. glucocorticoids and non-steroid anti-inflammatory drugs). Importantly, very similar results were obtained regardless of the route of administration. Nevertheless, we cannot rule out the presence of other time-varying confounders affected by the chosen treatment strategy that have been not accounted for in the

analysis. Of note, antiretroviral drugs (protease inhibitors, i.e. lopinavir/ritonavir and darunavir/cobicistat) were used both in SoC or tocilizumab group and were never started post baseline in the tocilizumab group.

A major concern are adverse events. We observed a significant higher prevalence of infection in the tocilizumab vs SoC. The design and the short follow-up period of this study does not allow us to drive conclusions regarding early and long-term side effects of tocilizumab, eventually followed by glucocorticoids. This signal needs to be confirmed by the ongoing randomised clinical trials. Nevertheless, the case of a severe HSV1 hepatitis occurring in the tocilizumab group suggests screening for herpes virus reactivation especially if glucocorticoids are added.

We chose a composite outcome including both invasive mechanical ventilation and all-cause mortality. Crude fatality rate in our cohort was 16.8% (86 deaths over 544 diagnosed with severe pneumonia). A large multicentre cohort study from China, showed a fatality rate of 28% among hospitalized patients [3]. The reduction in mortality shown here in people receiving tocilizumab is particularly relevant because our patients were older by a median of 15 years than those included in the Chinese study. Moreover, in a study conducted in Wuhan, 84 out of 201 patients (41.8%) developed ARDS and 44 of them (52.4%) died [1]. In the European setting, a recent large study conducted in 1,591 patients admitted to ICUs in the Lombardy region, Italy, showed that 88% received mechanical ventilation and 11% non-invasive ventilation, while the fatality rate was 26% [38]. However, a latter analysis did not exclude patients still hospitalized and did not evaluate patients outside ICU, therefore it is not fully comparable to our findings.

Our composite end point allowed us to describe not only the most critical clinical event, but also the most burdensome issue for health care systems that need to rapidly increase their ICU resources availability. It is also important to note that many countries are facing the shortage of mechanical ventilators. This could lead to very difficult clinical choices about patients to be prioritized for treatment. As a consequence, a treatment that reduces ICU admission is highly relevant not only for ameliorating the prognosis of the hospitalized patients, but also to give more patients the opportunity to receive intensive care when needed. However, the largest effect was detected for mortality with little contribution to the difference provided by the rates of mechanical ventilation alone.

Due to a shortage of IV formulation, we were challenged to administer SC tocilizumab in a schedule trying to emulate the pharmacodynamic activity of the IV formulation. The SC route takes a few days to reach the peak plasma concentration after a single dose. This is the case because of the slow absorption through the lymphatic system into the systemic circulation [27]. To overcome all these limitations, higher doses for SC administration were provided through separate injections [27]. This choice was supported from the findings of a comparative pharmacokinetic/pharmacodynamic study of SC vs. IV tocilizumab. The study showed that, after a single 162 mg dose in healthy subjects, SC bioavailability amounted to 48.8%, while the pharmacodynamic activity of SC and IV tocilizumab against soluble IL-6 receptor was very similar over 1 week (AUC SC/IV ratio 1.09 at 162 mg) [28].

Our study suffers from a few limitations. To begin with, it is not a randomized comparison, therefore unmeasured confounding cannot be ruled out. In addition, results rely on the usual assumptions about the model being correctly specified (i.e. we have adjusted for all sources of measured confounding). Participants in SoC were older, therefore at higher baseline risk of invasive ventilation and death. On the other hand they were also more likely to be females and female gender has been shown to be associated with better outcomes. Patients allocated to tocilizumab were mainly selected for availability of the drug (which was intermittent over the recruitment phase due to shortages) and they were more compromised patients with a lower PaO_2/FiO_2 ratio and higher SOFA score. The analysis was controlled for SOFA (which controls for both respiratory function, namely baseline PaO_2/FiO_2 ratio) and for the Charlson Comorbidity Index (which controls for the extent of comorbidities present at admission). Of course, because only one patient with cancer and two with renal insufficiency were allocated to tocilizumab, we cannot rule out residual confounding that cannot be controlled by regression interpolation.

Secondly, although the key measured confounders (gender, age, SOFA score and Charlson Comorbidity Index) were available for all participants in the cohort, this was not true for some of the biomarkers of inflammation and coagulation which were available only for a subset of the participants. However, results were similar when we repeated the analysis using the Modena centre dataset alone. Importantly, when using a marginal structural model instead of the standard estimate of the hazard ratio conditioned on covariates, which additionally controlled for glucocorticoids use post baseline the difference in risk between treatment strategies was even larger. This is a key

result, given the wide use of these methods in situation of complicated time-varying structure of the data and while attempting to emulate the results of randomised comparisons [33].

Another weakness of the study is the fact that it was open label so that staff involved knew which patients were receiving tocilizumab or not. Indeed, this knowledge might have led to variability in the decision of when to move a patient to invasive ventilation (faster for those who were receiving SoC). Moreover, it should be acknowledged that indication for mechanical ventilation, even if suggested by guidelines, still relies on clinical judgment that may vary according to centre experience and resources availability. Notwithstanding, ICU staffs involved in the study shared similar protocols and resources.

Last but not least, due to short period of follow-up, we were not able to assess long-term safety and adverse effects. Further studies are needed to define appropriate dosage of therapeutic effect and minimize the side effects.

Our study has also many strengths. First of all, this was a large study which included patients from a real-life hospital setting. Secondly, data were extremely rich with key confounding factors collected in a standardized way daily for a minimum of 14 days and linked to the electronic charts of blood counts and clinical data.

Many questions still remain open. Generalizability of the results must be discussed in the light of different epidemiological settings with particular regards to tocilizumab dosage and use at the appropriate time point of the disease course. Other immune-active agents directly acting in the inflammatory response pathway triggered by SARS-CoV-2 are being tested. Tocilizumab use in severe COVID-19 pneumonia is still in its infancy and best strategies have yet to be developed. For instance, our experience also described SC tocilizumab, which paves the way to future studies of immune-active therapy in out-patient settings.

In conclusion, tocilizumab, regardless of IV or SC administration may be capable of reducing invasive mechanical ventilation or death in severe COVID-19 pneumonia. Although these results are encouraging, they should be confirmed in the large number of currently ongoing randomised studies.

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Authors' contribution

CM, GG, MaMes, ACL, JM, RT, CS, MaMass and PLV conceptualized and designed the study. CM, GG, MaMes, ACL, JM, RT wrote and revised the manuscript. CM, GG, MaMes, ACL and JM did the supervision of the final version of the manuscript. ACL did the statistical analysis. GG, GD and ST were in charge of the database of three centres. MarMen did the figures. All the authors contributed to data collection, clinical management of the patients and data interpretation.

Conflict of interest

None to declare.

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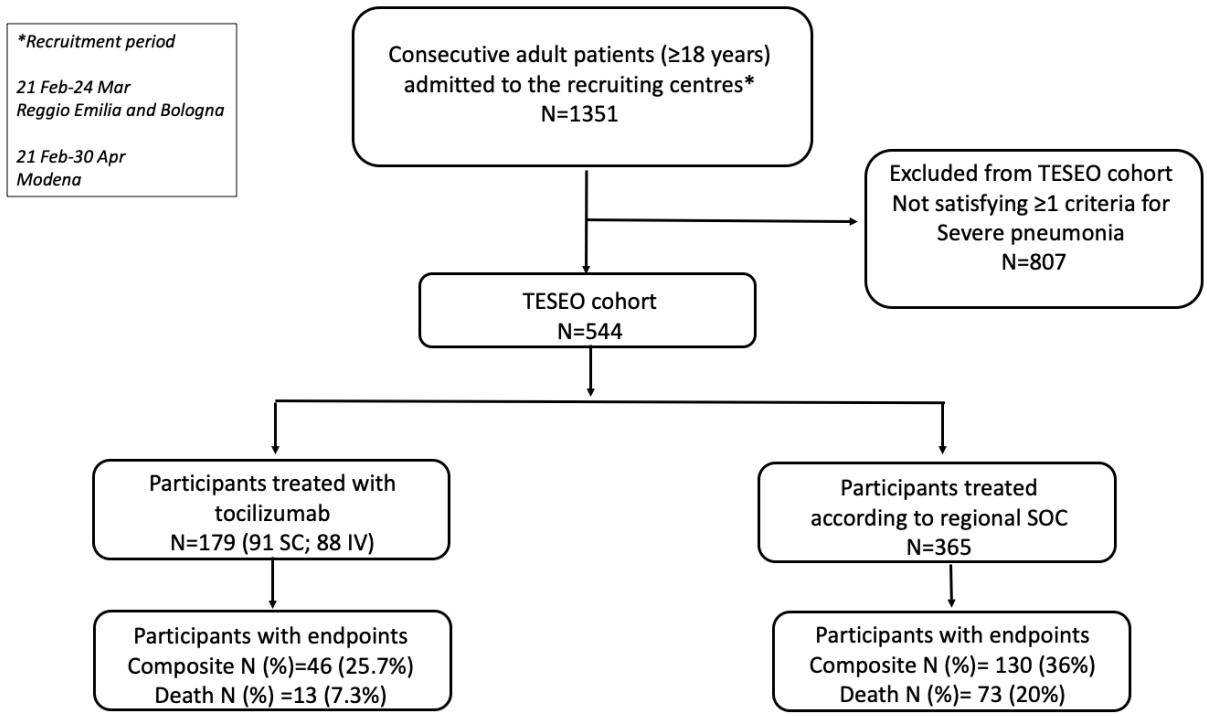
Flowchart


*Recruitment period
21 Feb-24 Mar
Reggio Emilia and Bologna
21 Feb-30 Apr
Modena
Consecutive adult patients (≥18 years) admitted to the recruiting centres*
N=1351
Excluded from TESEO cohort
Not satisfying ≥1 criteria for Severe pneumonia
N=807
TESEO cohort
N=544
Participants treated with tocilizumab
N=179 (91 SC; 88 IV)
Participants treated according to regional SOC
N=365
Participants with endpoints
Composite N (%)=46 (25.7%)
Death N (%) =13 (7.3%)
Participants with endpoints
Composite N (%)= 130 (36%)
Death N (%)= 73 (20%)

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

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RESEARCH IN CONTEXT

Evidence before this study

There is no approved therapy for COVID-19 pneumonia, but current clinical approaches consider the combination of antiviral agents and immuno-active drugs, including tocilizumab, a recombinant humanized monoclonal antibody against interleukin-6 receptors. In a single centre study from Wuhan, China, including 15 patients with COVID-19 pneumonia at risk for cytokine storm, treatment with tocilizumab, although with variable doses ranging from 80 to 600mg, appeared to have a clinical benefit.

Added value of this study

In our multicentre, retrospective study of 544 patients with severe COVID-19 pneumonia, ~~a 48% reduction in risk of invasive mechanical ventilation/death was observed, comparing people treated with~~use of tocilizumab administered either intravenously or subcutaneously was related to reduced risk of mechanical ventilation and death –(aHR=0.61, 95% CI:0.40-0.92; p=0.02)~~with controls receiving SoC~~. ~~The all-cause mortality was reduced by 75%.~~ We also found a stroing association between use of tocilizumab and the risk of death (aHR=0.38 95% CI:0.17-0.83, p=0.02 ~~a~~.

Implications of all the available evidence

Tocilizumab, regardless of IV or SC administration may be capable of reducing invasive mechanical ventilation or death in severe COVID-19 pneumonia.

Abstract

Background

There is no approved therapy for COVID-19 pneumonia. The aim of this multicentre cohort study was to assess the role of tocilizumab in reducing the risk of invasive mechanical ventilation and/or death in patients with severe COVID-19 pneumonia who received standard of care (SoC) treatment.

Methods

The TESEO Cohort Study is a retrospective, multicentre observational cohort study of patients with COVID-19 severe pneumonia treated with ~~standard of care (~~SoC~~)~~ with or without tocilizumab using intravenous (IV) or subcutaneous (SC) formulations, identifying respectively treated and ~~control~~comparator groups. Survival analysis was performed with participants' follow-up accruing from the date of entry into clinics until initiation of invasive mechanical ventilation or death, used as a composite outcome. Treatment ~~arms~~groups were compared using Kaplan-Meier curves and Cox regression analysis after adjusting for gender, age and baseline Sequential Organ Failure Assessment (-SOFA) score.

Findings

Of 544 patients included, 179 patients were treated with tocilizumab: 88 with the IV (16.1%) and 91 with SC formulation (16.7%). Mortality was significantly higher in the ~~control~~comparator group (20%) as opposed to tocilizumab IV (6.8%) and tocilizumab SC (7.7%) (p<0.001). A ~~48% reduction in risk~~reduced risk of invasive mechanical ventilation/death was shown for participants treated with tocilizumab from fitting a Cox regression analysis adjusted for gender, age and ~~total~~ SOFA score (aHR=0.61, 95% CI:0.40-0.92; p=0.02). We found no evidence for a difference between IV and SC administration route of tocilizumab ~~(aHR= 0.94, 95% CI:0.31-2.85), p=0.89 comparing SC vs. IV)~~. With regards to the mortality endpoint alone, a ~~75% reduction was found~~reduced risk was observed comparing tocilizumab with ~~control~~ the comparator group~~s~~ (aHR=0.38 95% CI:0.17-0.83, p=0.02)~~, 0.25, 95% CI:0.14-0.47; p<0.01~~.

Interpretation

Tocilizumab, regardless of IV or SC administration may be capable of reducing invasive mechanical ventilation or death in severe COVID-19 pneumonia. Our observations should be confirmed in randomised studies.

Funding

This study was not funded.

Background

Since December 2019, Coronavirus disease-19 (COVID-19) has rapidly spread in Wuhan and throughout the Hubei province of China and more recently in Europe and worldwide. Although it is difficult to compare crude fatality rates across countries, due to different testing policies, the fatality rate in Italy is higher than that reported in China [1,2].

The clinical presentation of COVID-19 is extremely heterogeneous, ranging from asymptomatic cases to severe pneumonia with respiratory failure that could lead to invasive mechanical ventilation and/or death [3–5]. The time course of the disease is characterized by an initial phase of viral replication that may be followed by a second phase driven by the host inflammatory response [6] ~~(ref)~~. Severe Acute Respiratory Syndrome – Coronavirus-2 (SARS-CoV-2) infection may cause an host hyperimmune response that is associated with an acute respiratory distress syndrome (ARDS) characterized by typical radiological findings [7]. The most critical patients may develop a so-called "cytokine storm", characterized by the increase of many cytokines that produce long-term damage and lung tissue fibrosis [8].

There is no approved therapy for COVID-19 pneumonia, but current clinical approaches consider the combination of antiviral agents and immuno-active drugs. Although antiviral agents showed no benefit beyond the standard of care (SoC) in an initial study [9], clinical trials are still ongoing. From a wider immunological perspective, derived from rheumatology [10], immunomodulatory agents, e.g. selective cytokine blockade, leading to inhibition of either the ligand or the receptor of a cytokine have been considered [11].

Tocilizumab is a recombinant humanized monoclonal antibody (mAb), of the IgG1 class, directed against both the soluble and the membrane bound IL-6 receptor [12,13]. It is recommended for the treatment of severe rheumatoid arthritis, systemic juvenile idiopathic arthritis, ~~and~~ giant cell arteritis and life-threatening cytokine release syndrome induced by the chimeric antigen receptor T-cell [14–16]. In a single centre study from Wuhan, China, including 15 patients with COVID-19 pneumonia at risk for cytokine storm, treatment with tocilizumab, although with variable doses ranging from 80 to 600mg, appeared to have a clinical benefit [17]. This study and other anecdotal observations [18,19] raised the chance in Italy of off-label use of tocilizumab to treat patients with

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COVID-19 severe pneumonia. More recently, an increasing number ofd studies are reporting use of tocilizumab in COVID-19 [20,21] (ref).

The aim of this multicentre cohort study was to assess the role of tocilizumab in reducing the risk of invasive mechanical ventilation and/or death in a cohort of patients with severe COVID-19 pneumonia who received SoC treatment.

Methods

Study Setting

The TESEO Study (Tocilizumab in patients with severe COVID-19 pneumonia) is a retrospective, multicenter observational cohort study carried out in the Emilia-Romagna region, Italy, among patients with COVID-19 severe pneumonia. Centres participating in the study were Modena, Bologna and Reggio Emilia and all contributed either to tocilizumab or controlSoCs. The contribution of each recruiting centercentre is specified in (Supplementary Ttable 1). Modena cohort collected data in a rich data set obtained by electronic chart and allowed a more detailed description of the TESEO cohort.

The study was approved by *R*r*egional ethical committee* of Emilia Romagna.

Patients and case definition

The target population was the universe of COVID-19 cases with confirmed PCR nasopharyngeal swab admitted to the participating centres between 21 February and 24 March while for Modena follow up was extended to 30 April 2020. The TESEO cohort considered all of the consecutive adult patients (≥18 years) with severe pneumonia defined as the concomitant presence of a respiratory rate (RR) ≥ 30 breaths per minute (bpm), peripheral blood oxygen saturation (SaO_2) < 93% in room air, a PaO_2/FiO_2 ratio < 300 mmHg in room air and lung infiltrates >50% within 24-48 hours, according to Chinese management guidelines for COVID-19 (version 6.0) [3,22]. The flowchart describes patients included in the analyses.

Standard of care (Standard of careSoC) treatment

All patients received standard of care (SoC) treatment at the time of hospital admission. This was in agreement with the Regional COVID-19 Guidelines of Emilia Romagna [23] and with the updated

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data on treatment of COVID-19 [24] and it included:

- Oxygen supply to target $SaO_2 \geq 90\%$;
- Hydroxychloroquine (400 mg BID on day 1 followed by 200 mg BID on days 2 to 5 eventually adjusted for creatinine clearance estimated by a CKD algorithm);
- Azithromycin (500 mg QD for 5 days) at physician's discretion when suspecting a bacterial respiratory superinfection;
- Lopinavir/ritonavir (400/100 mg BID) or Darunavir/cobicistat (800/150 mg QD) for 14 days;
- Low molecular weight heparin for prophylaxis of deep vein thrombosis according to body weight and renal function.

Tocilizumab treatment

A non-randomly selected subset of patients received tocilizumab treatment in addition to the SoC. Patients were considered eligible for tocilizumab treatment in presence of SaO_2<93% and a PaO_2/FiO_2<300 mmHg in room air or a decrease in PaO_2/FiO_2 > 30% in the previous 24 hours during hospitalization. All tocilizumab-treated patients provided verbal, not written, informed consent due to isolation precautions. Tocilizumab was administered by the intravenous (IV) or the subcutaneous (SC) route depending on the hospital availability of the type of formulation at time of treatment. It should be mentioned that during the observation period, the high national request created an intermittent shortage of both formulations of the drug. IV tocilizumab was administered at the dose of 8 mg/kg of body weight (up to a maximum dosage of 800 mg) repeated twice, 12 hours apart. The second dose was given because PK data ~~The second dose of Tocilizumab was chosen joining the real-life experience which indicated difficult to assess benefit in 12 hours and according to PK data which suggest plasma level is obtained in two subsequent doses.~~ ~~The second dose was given because, from clinical experience when treating the first patients there was reduced benefit after 12 hours of the single dose and also because PK data~~ were suggesting that adequate plasma levels of the drug could be obtained only after two doses. The rationale for the second dose was based on the results of pharmacokinetic models for severe or life-threatening chimeric antigen receptor T cell-induced cytokine storm in adults and in paediatric patients [25].

The SC formulation was used in the shortage of the IV formulation at the dose of 162 mg administered twice simultaneously, one per each thigh. This approach was focused at mimicking, as

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much as possible, the pharmacokinetic activity of the IV formulation in order to achieve similar levels of drug exposure of those achieved using the IV administration. The site and depth of injection can influence the absorption and distribution [26]; the rate of absorption may vary markedly between dosing sites [26]; the peak plasma concentration may take a few days to be reached after a single SC dose [27] and the mAb may undergo proteolytic degradation by the cells of the reticuloendothelial system [28].

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Exclusion criteria for tocilizumab use were as follows:

- Coexistent infection other than COVID-19;
- Chronic or current glucocorticoid use;
- History of severe allergic reactions to monoclonal antibodies;
- Neutrophils < 500/mmc or platelets <50.000/mmc;
- Active diverticulitis, inflammatory bowel disease, or another symptomatic gastro-intestinal tract condition that might predispose to bowel perforation;
- Severe hematologic, renal or liver function impairment.

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Covariates

The patients' full medical history, chronic co-morbidities including Charlson Comorbidity index [29], demographic and epidemiological data and baseline PaO_2/FiO_2 were obtained at the hospital admission. Eventual other treatments, including glucocorticoids for ARDS were recorded. The risk of multiorgan failure and mortality was assessed with standardized Subsequent Organ Failure Assessment (SOFA) score [30]. Clinical data with signs and symptoms, and complete blood count, coagulation, inflammatory and biochemical markers and were routinely registered in the electronic patient charts for the Modena cohort, only.

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Outcome measures

The primary outcome of the study was the composite of death or invasive mechanical ventilation. Indication for mechanical ventilation were neurologic failure (i.e. altered consciousness with a Glasgow Coma Scale score <10), cardiovascular failure (i.e. vasopressor requirement or major ECG changes including arrythmia or changes in repolarization phase) and respiratory failure defined by

the presence of at least 2 of the following criteria: respiratory rate > 30 bpm, respiratory distress with activation of accessory respiratory muscles,- need for FiO_2 at 80% or more to maintain a~~n~~ SaO_2 level at 90%, or a PaO_2/FiO_2 < 100 mm Hg [31,32].

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Statistical analysis

Baseline characteristics of the participants treated with SoC with tocilizumab and SoC alone were compared. These included signs and symptoms, existing co-morbidities and blood count markers. Continuous variables were expressed as median (IQR) and compared by Kruskal Wallis test. Categorical variables were expressed as numbers (%) and compared by χ^2 test or Fisher's exact test across the SoC with tocilizumab and the SoC groups. In a secondary analysis, the group treated with SoC and tocilizumab was further split into those who received tocilizumab in its SC or IV formulation.

Standard survival analysis was performed. ~~with p~~ Participants' follow-up accrued from the date of entry into clinics until initiation of invasive mechanical ventilation or death. ~~In a sensitivity analysis, follow-up for the SoC with tocilizumab group accrued from the date of the first dose of tocilizumab while time zero for the SoC group was kept at the date of hospital admission.~~ Time to invasive mechanical ventilation or death by treatment ~~arms~~groups was compared using unweighted Kaplan-Meier curves and univariable and multivariable Cox regression analysis with baseline fixed covariates. The effect of treatment was shown by means of unadjusted and adjusted hazard ratio (HR) with 95% CI. ~~Three~~ Kkey confounders were identified as gender, age, ~~duration of symptoms,~~ recruiting centre, duration of symptoms, and baseline SOFA score as the most likely causes of both treatment assignment and outcome risk. A number of additional analyses have been performed to control for potential additional sources of time-fixed and time-varying confounding.

First of all, the analysis was adjusted for baseline level of inflammation and coagulation in a subset of participants with available C-reactive protein and D~~d~~-dimer values. Secondly, SOFA score was replaced by alternative measures of the extent of concomitant morbidities at baseline such as a binary indicator (≥1 of the comorbidities among: diabetes, hypertension, cardiovascular disease, chronic renal insufficiency or cancer) as well as the Charlson Comorbidity Index. Thirdly, with the aim of emulating a randomized trial with similar characteristics as well as appropriately controlling for the time varying confounder of glucocorticoids use, we also fitted a marginal structural Cox regression models with stabilized inverse probability weights constructed using gender, age, SOFA

score, recruiting ~~center~~centre, duration of symptoms and time varying use of glucocorticoids and inverse probability of censoring [33] ~~(ref)~~. A secondary analysis with endpoint death alone used both the cause-specific hazard approach, assuming non-informative censoring and a competing risk approach in which deaths, which occurred after initiation of invasive mechanical ventilation, have been included as events.

Finally, in order to test the hypothesis that the difference between treatment ~~arms~~groups might vary according to the baseline PaO_2/FiO_2 value, we formally included an interaction term in the Cox regression model. Results were shown after categorizing the population in two strata using a clinical threshold of PaO_2/FiO_2 = 150 mmHg. A similar stratification analysis has been also performed using age strata (18-64 vs. 65+ years) to further investigate the possible confounding/ effect modification due to age.

In the subset of participants from the Modena cohort alone, mean trajectories of IL-6 (in the log10 scale) and of AST (raw scale) over time were compared between tocilizumab and ~~control~~SoC using a linear mixed model with random intercept and slope.

A two-sided test of less than 0.05 was considered statistically significant. Statistical analyses were performed using the SAS software, version 9.4 (Carey USA), unless otherwise indicated.

Results

~~Study population~~

A subset of 544 patients with severe pneumonia were included in this analysis. Epidemiological and respiratory characteristics are shown in Table 1. The vast majority of patients were males (66%) with a median age of 67 years. All patients showed clinical deterioration with a median SOFA score of 2 (95% CI: 1-4), mainly driven by respiratory failure with a median PaO_2/FiO_2 <250 mmHg requiring oxygen support. SOFA score and PaO_2/FiO_2 at baseline differed substantially across centres, with patients in Modena being the most compromised (S~~s~~upplementary T~~t~~able 1).

Concerning treatment, 365 patients received SoC alone (67.2%), and 179 received additional treatment with tocilizumab (32.8%; in detail 16.1% by IV and 16.7% by SC) (Table 1). The ~~three~~

groups had different characteristics (Table 1). The ~~control~~comparator group included older patients with a less severe disease, and the group treated with IV tocilizumab included the most compromised patients as ~~tested~~ depicted by PaO_2/FiO_2 ratio and SOFA score. Post baseline, 53 out of 179 (29.6%) treated with tocilizumab started ~~-~~glucocorticoids vs. 61 out of 365 (16.7%) in the SoC group.

Comorbidities, signs and symptoms were available for the 354 patients from the Modena cohort (Table 2a). Tocilizumab treated patients had a higher burden of hypertension and of symptoms such as headache and cough. Among these patients, biochemical markers were available for a subset of 304 patients (Table 2b). Tocilizumab treated patients had a higher lactate dehydrogenase and worse baseline inflammatory profile with higher C-reactive protein and IL6 values.

~~Prospective analysis~~

Overall, invasive mechanical ventilation was started in 90 out of 544 patients (16.5%), but the percentage varied significantly across the centres (p=0.0283~~3~~). Eighty-six patients died (15.8%) and the risk of mortality did not differ significantly across centres (p=0.49) (Supplementary Table 1a). There were further 19 deaths which occurred after the date of initiation of mechanical ventilation for a total of 105 deaths which have been analysed using a competing risk approach. With regards to study outcomes, mortality was significantly higher in the ~~control~~ SoC group (20%) compared with both IV and SC tocilizumab groups (6.8% and 7.7%, respectively, p<0.001) (Table 1).

At 14 days from hospital admission, the overall cumulative Kaplan-Meier estimated probabilities amounted to 36.1% (95% CI:31.2-40.9%) for the primary composite endpoint of invasive mechanical ventilation or death, and 21.1% (95% CI: 16.3-25.8%) for death (Supplementary T~~t~~able 4, Figure 1).

~~Composite endpoint~~

Unweighted Kaplan-Meier estimates showed the beneficial effect of the use of tocilizumab compared to ~~control~~SoC (Figure 1). At day 14 from hospital admission the proportion of patients experiencing the composite outcome was 27.0% (95% CI: 19.6-34.4%)- for the tocilizumab group vs. 41.5% (95% CI: 35.1-47.9%) for ~~-~~SoC (log rank p=0.0023~~-~~, Figure 1a; Supplementary T~~t~~able 4). The difference was even larger and the association stronger when using the mortality endpoint both ~~when~~ with cause-specific hazard approach (log rank p<0.0001, Figure 1b) and competing risk

approach (p<0.0001). After splitting the tocilizumab group by administration route, both groups showed a benefit as compared to SoC with no marked difference between the IV and the SC group (log-rank p<0.0017, Figure 1c, 1d).

TheA 40% A significant reduction in risk of invasive mechanical ventilation or death was shown comparing patients receiving tocilizumab with those receiving SoC, as estimated by hazard ratio (HR) from the unadjusted Cox regression model (HR=0.40 0.60; 95% CI: 0.4343-0.8484, p<0.003=0.03, Table 3a).

After controlling for the key identified confounders of gender, age and SOFA score, the treatment effect was even larger (aHR=0.6152, 95% CI:0.4036-0.9273, p<0.0021, Table 3a). These results were confirmed in a number of analyses aiming to control for further sources of confounding, namely after adjusting for i) baseline CRP values (aHR=0.57, 95% CI:0.38-0.84, p=0.005); ii) baseline d-dimer levels (aHR=0.66, 95% CI:0.42-1.05, p=0.08), iii) after replacing the SOFA score with the Charlson Comorbidity Index (aHR=0.64, 95% CI:0.46-0.91, p=0.01) and iv) after controlling for time-varying confounding of using glucocorticoids and informative censoring (aHR=0.44, 95% CI:0.29-0,65, p<0.001) (Table 3).

These results were confirmed in a number of analyses aiming to control for further sources of confounding, namely after adjusting for i) baseline CRP values (aHR=0.57, 95% CI:0.38-0.84, p=0.005); ii) baseline d-dimer levels (aHR=0.66, 95% CI:0.42-1.05, p=0.08), iii) after replacing the SOFA score with the Charlson Comorbidity Index (aHR=0.64, 95% CI:0.46-0.91, p=0.01) and iv) after controlling for time-varying confounding of using glucocorticoids and informative censoring (aHR=0.44, 95% CI:0.29-0,65, p<0.001) (Table 3b). questo non va cancellato solo cambiato Tabella 3a/3b con 3!

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The largest difference was found when comparing IV tocilizumab with SoC.. with a, a 52% reduction in risk of invasive mechanical ventilation/death was found a After adjusting for the same set of identified confounders we estimated the following reduction in risk of invasive ventilation/death (aHR=0.55, 95% CI: 0.3110-0.9877, p=0.0402, Table 3). Still comparing the risk for the composite endpoint and regarding the potential difference by administration route, we found no evidence for

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a difference between ~~IV and~~ SC and IV (aHR=1.50 (95% CI:0.36; 6.24, p=0.58) ~~0.94, 95% CI:0.31-2.85), p=0.89~~, Table 3~~a~~).

Finally, the main results for this end point were similar ~~in a number of sensitivity analyses: i) when using for the intervention group the date of starting the first dose of tocilizumab as time zero instead of the date of hospital admission (aHR=0.57, 95% CI: 0.38-0,86, p=0.008). and ii)~~ after restricting the analysis to people enrolled in Modena, only (aHR=0.65, 95% CI: 0.43-0.99, p=0.04~~, data non shown~~).

The formal test for interaction and the stratified analyses showed evidence that this difference varied by baseline PaO_2/FiO_2 value (p=0.011). In particular, the effect of tocilizumab was two-fold higher in people with baseline PaO_2/FiO_2 value<150 (aHR=0.19~~21~~, 95% CI:0.08~~11~~;0.44~~38~~) (Table 3~~a~~). No difference in the results was found after controlling for age using stratification (18-65 vs. 65+ years, data not shown).

~~Mortality~~

Analysing the mortality endpoint, ~~the a 75%~~ a significant reduction in risk of death was found comparing tocilizumab with SoC after controlling for gender, age and SOFA score (aHR= 0.38~~25~~, 95% CI:0.17~~4~~;0.83~~47~~, p=0.015~~<0001~~, Table 4). Although there was little statistical evidence for a differential benefit of tocilizumab by baseline PaO_2/FiO_2 value (interaction p=0.12, Table 4), again the effect was much stronger in people with baseline PaO_2/FiO_2 <150.

We also repeated this analysis after controlling for the Charlson Comorbidity Index instead of SOFA and ~~again~~ results were similar (aHR=0.36, 95% CI:0.19-0.65, p=0.008; data not shown)~~.~~. Finally, after including the additional 19 deaths which occurred after the date of initiation of invasive ventilation, results from the competing risk analysis were again ~~almost identical~~ similar to those of the main analysis (aHR=0.27, 95% CI (0.16; 0.47, p<0.001).

~~Other analyses~~

The mixed linear models showed that IL-6 plasma levels were slightly higher at study entry in the intervention group vs. ~~control~~comparator (2.46 vs. 2.25 log10 mg/l, p=0.09) (Supplementary T~~t~~able 2). Over time, there was tendency for IL-6 to slowly increase in the intervention group and decrease

in the ~~control~~comparator group with a significant difference in slope (-0.02 log10 mg/l; 95% CI:-0.03;-0:00, p=0.004) (Supplementary F~~f~~igure 1).

~~***Safety endpoints***~~

Serious adverse events were carefully monitored during the study period. In the tocilizumab group a single episode of injection site reaction occurred with spontaneous resolution in a few hours. Moreover, one episode of severe neutropenia required Granulocyte-Colony Stimulating Factor administration. Finally, there was no evidence for a difference in the rate of increase of AST between treatment groups (Supplementary table 3 and Supplementary figure 2). Aside from, the case of acute liver failure (mentioned below), there was only one person in the SoC group whose AST increased from 19 U/l pre-treatment level to 139 six days post treatment, none in the tocilizumab group.

A great attention was given to new episodes of infections occurring both in tocilizumab and ~~control~~comparator group~~s~~. They included bloodstream infections (3 vs. 4), bacterial pneumonia (8 vs. 6), candidemia (2 vs. 2), urinary tract infection (1 vs. 1), ~~,~~*Pneumocistis jirovecii* pneumonia (1 vs. 1), invasive aspergillosis. (4 vs. 0), ~~,~~*Hepatitis B virus* reactivation (1 vs. 0), *Herpes simplex virus 1* (HSV1) reactivation (4 vs. 0). Of note, one episode of severe adverse event occurred in the tocilizumab group at 12 days after SC injection consisting of severe liver failure due to HSV1 reactivation, leading to death. This patient received high dose glucocorticoids after the administration of tocilizumab.

To summarize, 24 infections were diagnosed in 179 tocilizumab patients (13%) vs 14 out of 365 SoC patients (4%) (p<0.~~,~~001).

Discussion

In the real-life setting of the TESEO cohort, we reported a ~~48%~~significant reduction ~~of~~in in risk of invasive mechanical ventilation or death in patients with severe COVID-19 pneumonia treated with either IV or SC tocilizumab, compared to SoC. The ~~benefit of tocilizumab was strikingly higher (75%)~~ association with the use of tocilizumab was even stronger when overall mortality risk was analysed alone.

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Our results are consistent with those of a smaller retrospective case- controlled French study by Klopfesntein et al [20], in which death and/or ICU admissions were higher in patients without tocilizumab than in the tocilizumab group (72% vs 25%, p=0.002) vs 41.5 vs 27.0 % by 14 days in our study.

A press release of the ~~Corimuno~~CORIMUNO randomized clinical trial also anticipated a beneficial effect of tocilizumab when compared to SoC [34] ~~(ref press release)~~.

Natural history of severe COVID-19 pneumonia is thought to be driven by a "cytokine storm" [14]. Nevertheless, current recommendations do not comprise any immunologically active agent in routine clinical practice, while glucocorticoids use is still controversial [35,36]. Tocilizumab, administered both intravenously or subcutaneously, can be considered ~~together with as the first~~ together with anakinra as one of the immune-active agents which ~~has~~ have been tested in clinical care for the treatment of severe COVID-19 pneumonia~~,~~ [19–21,37] ~~(ref)~~. IL-6 levels increased after tocilizumab administration, compared to people receiving SoC. This is an expected finding since tocilizumab competitively blocks IL-6 receptors and leaves free IL-6 in plasma. Longer follow-up and larger sample are needed to better understand the prognostic role of IL-6 in patients with COVID-19 pneumonia treated with tocilizumab.

The real-life setting, including three different hospitals, accounted for the heterogenicity in clinical characteristics and disease severity across intervention ~~arms~~groups. In particular, as expected, the ~~control~~comparator group showed a higher baseline PaO_2/FiO_2 value than the intervention group. Thus, in the unadjusted analysis the magnitude of the beneficial effect associated with the use of tocilizumab could have been even underestimated. We attempted to control for this confounding bias by adjusting for SOFA score, which comprises baseline PaO_2/FiO_2 and indeed, the difference was larger after adjustment. In particular, the effect of tocilizumab was at least two-fold higher in people with baseline PaO_2/FiO_2 value<150, implying that the benefit of tocilizumab could be even higher 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.

~~The benefit of tocilizumab in reducing mortality and invasive mechanical ventilation was confirmed~~ Our results were similar after further adjusting for post-baseline use of glucocorticoid use. This

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analysis reinforces our findings and open the discussion for combination of immunomodulatory agents (i.e. monoclonal antibodies) with anti-inflammatory drugs (i.e. glucocorticoids and non-steroid anti-inflammatory drugs). Importantly, very similar results were obtained regardless of the route of administration. Nevertheless~~Of course~~, we cannot rule out the presence of other time-varying confounders affected by the chosen treatment strategy that have been not accounted for in the analysis. ~~O~~O ~~However, of~~ note, antiretroviral drugs (protease inhibitors, i.e. lopinavir/ritonavir and darunavir/cobicistat) were used both in SoC or tocilizumab group and were never started post baseline in the tocilizumab group.~~Of note, drugs from other classes (such as HIV drugs in the PI class were either used as part of SoC and never started post baseline in the tocilizumab group).~~

A major concern are~~is~~ adverse events. We observed a significant higher prevalence of infection in the tocilizumab vs SoC. The design and the short follow-up period of this study does not allow us to drive conclusions regarding early and long-term side effects of tocilizumab, eventually followed by glucocorticoids. This signal needs to be confirmed by the ongoing randomised clinical trials. ~~We observed a significant higher prevalence of infection in the tocilizumab vs SoC. The design and the~~ The short ~~short follow up period of this study does not allow us to drive conclusions regarding early and long-term side effects of tocilizumab, eventually followed by glucocorticoids. This signal needs to be confirmed by the ongoing randomized clinical trials.~~ Nevertheless, the case of a severe HSV1 hepatitis occurring in the tocilizumab group suggests screening for herpes virus reactivation especially if glucocorticoids are added.

We chose a composite outcome including both invasive mechanical ventilation and all-cause mortality. Crude fatality rate in our cohort was 16.8% (86 deaths over 544 diagnosed with severe pneumonia). A large multicentre cohort study from China, showed a fatality rate of 28% among hospitalized patients [3]. The reduction in mortality shown here in people receiving tocilizumab is particularly relevant because our patients were older by a median of 15 years than those included in the Chinese study. Moreover, in a study conducted in Wuhan, 84 out of 201 patients (41.8%) developed ARDS and 44 of them (52.4%) died [1]. In the European setting, a recent large study conducted in 1,591 patients admitted to ICUs in the Lombardy region, Italy, showed that 88% received mechanical ventilation and 11% non-invasive ventilation, while the fatality rate was 26%

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[38]. However, a latter analysis did not exclude patients still hospitalized and did not evaluate patients outside ICU, therefore it is not fully comparable to our findings.

Our composite end point allowed us to describe not only the most critical clinical event, but also the most burdensome issue for health care systems that need to rapidly increase their ICU resources availability. It is also important to note that many countries are facing the shortage of mechanical ventilators. This could lead to very difficult clinical choices about patients to be prioritized for treatment. As a consequence, a treatment that reduces ICU admission is highly relevant not only for ameliorating the prognosis of the hospitalized patients, but also to give more patients the opportunity to receive intensive care when needed. However, the largest effect was detected for mortality with little contribution to the difference provided by the rates of mechanical ventilation alone.

Due to a shortage of IV formulation, we were challenged to administer SC tocilizumab in a schedule trying to emulate the pharmacodynamic activity of the IV formulation. The SC route takes a few days to reach the peak plasma concentration after a single dose. This is the case because of the slow absorption through the lymphatic system into the systemic circulation [27]. To overcome all these limitations, higher doses for SC administration were provided through separate injections [27]. This choice was supported from the findings of a comparative pharmacokinetic/pharmacodynamic study of SC vs. IV tocilizumab. The study showed that, after a single 162 mg dose in healthy subjects, SC bioavailability amounted to 48.8%, while the pharmacodynamic activity of SC and IV tocilizumab against soluble IL-6 receptor was very similar over 1 week (AUC SC/IV ratio 1.09 at 162 mg) [28].

Our study suffers from a few limitations. To begin with, it is not a randomized comparison, ~~so that~~therefore unmeasured confounding cannot be ruled out. In addition, results rely on the usual assumptions about the model being correctly specified (i.e. we have adjusted for all sources of measured confounding). Participants in SoC were older, ~~therefore so~~ at higher baseline risk of invasive ventilation and death ~~but they were also more likely to be females~~. On the other hand they were also more likely to be females and female gender has been shown to be associated with better outcomes. ~~and female gender has been shown to be associated with better outcomes. On the other hand they were also more likely to be females and female gender has been shown to be associated with better outcomes.~~ Patients allocated to tocilizumab were mainly selected for availability of the

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drug (which was intermittent over the recruitment phase due to shortages) and they were more compromised patients with a lower PaO_2/FiO_2 ratio and higher SOFA score. The analysis was controlled for SOFA (which controls for both respiratory function, namely baseline PaO_2/FiO_2 ratio) and for the Charlson Comorbidity Index (which controls for the extent of comorbidities present at admission). Of course, because only one patient~~1 people~~ with cancer and two~~2~~ with renal insufficiency were allocated to tocilizumab, we cannot rule out residual confounding that cannot be controlled by regression interpolation.

Secondly, although the key measured confounders (gender, age, SOFA score and Charlson Comorbidity Index) were available for all participants in the cohort~~,t~~ this was not true for some of the biomarkers of inflammation and coagulation which were available only for a subset of the participants. However, results were similar when we repeated the analysis using the Modena centre dataset alone. Importantly, when using a marginal structural model instead of the standard estimate of the hazard ratio conditioned on covariates, which additionally controlled for glucocorticoids use post baseline the difference in risk between treatment strategies was even larger. This is a key result, given the wide use of these methods in situation of complicated time-varying structure of the data and while attempting to emulate the results of randomised comparisons [33]~~(ref)~~.

Another weakness of the study is the fact that it was open label so that staff involved knew which patients were receiving tocilizumab or not. Indeed, this knowledge might have led to variability in the decision of when to move a patient to invasive ventilation (faster for those who were receiving SoC). Moreover, it should be acknowledged that indication for mechanical ventilation, even if suggested by guidelines, still relies on clinical judgment that may vary according to centre experience and resources availability. Notwithstanding, ICU staffs involved in the study shared similar protocols and resources.

Last but not least, due to short period of follow-up, we were not able to assess long-term safety and adverse effects. Further studies are needed to define appropriate dosage of therapeutic effect and minimize the side effects.

Our study has also many strengths. First of all, this was a large study which included patients from a real-life hospital setting. Secondly, data were extremely rich with key confounding factors

collected in a standardized way daily for a minimum of 14 days and linked to the electronic charts of blood counts and clinical data.

Many questions still remain open. Generalizability of the results must be discussed in the light of different epidemiological settings with particular regards to tocilizumab dosage and use at the appropriate time point of the disease course. Other~~s monoclonal antibodies~~immune-active agents directly acting in the inflammatory response pathway triggered by SARS-CoV-2 are being tested. Tocilizumab use in severe COVID-19 pneumonia is still in its infancy and best strategies have yet to be developed. For instance, our experience also described SC tocilizumab, which paves the way to future studies of immune-active therapy in out-patient settings.

In conclusion, tocilizumab, regardless of IV or SC administration may be capable of reducing invasive mechanical ventilation or death in severe COVID-19 pneumonia. Although these results are encouraging, they should be confirmed in the large number of currently ongoing randomised studies.~~Our observation should be confirmed in the context of the large number of currently ongoing randomised studies.~~

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This study was not funded.

Authors' contribution

CM, GG, MaMes, ACL, JM, RT, CS, MaMass and PLV conceptualized and designed the study. CM, GG, MaMes, ACL, JM, RT wrote and revised the manuscript. CM, GG, MaMes, ACL and JM did the supervision of the final version of the manuscript. ACL did the statistical analysis. GG, GD and ST were in charge of the database of three centres. MarMen did the figures. All the authors contributed to data collection, clinical management of the patients and data interpretation.

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Conflict of interest

None to declare.

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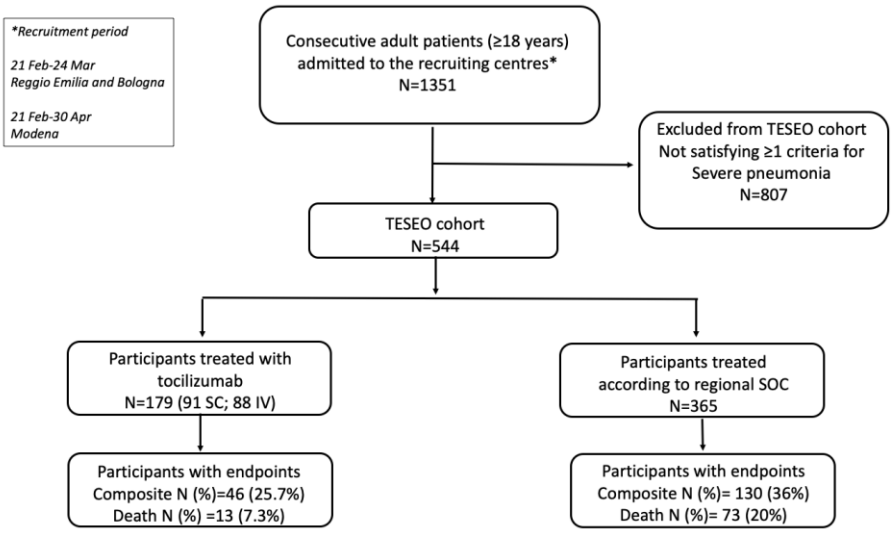
Flowchart


*Recruitment period
21 Feb-24 Mar
Reggio Emilia and Bologna
21 Feb-30 Apr
Modena
Consecutive adult patients (≥18 years) admitted to the recruiting centres*
N=1351
Excluded from TESEO cohort
Not satisfying ≥1 criteria for
Severe pneumonia
N=807
TESEO cohort
N=544
Participants treated with tocilizumab
N=179 (91 SC; 88 IV)
Participants treated according to regional SOC
N=365
Participants with endpoints
Composite N (%)=46 (25.7%)
Death N (%) =13 (7.3%)
Participants with endpoints
Composite N (%)= 130 (36%)
Death N (%)= 73 (20%)

TABLES AND FIGURES

Table 1. Key baseline factors – all centres combined. The reported p value refers to difference between tocilizumab (any) and SOC.

Characteristics	Intervention					
	Tocilizumab subcutaneous	Tocilizumab intravenous	Tocilizumab (any)	SOC	p-value‡	Total
	N= 91	N= 88	N= 179	N= 365		N= 544
Age, years					0.006	
Median (IQR)	67 (55, 73)	63 (54, 72)	64 (54, 72)	69 (57, 78)		67 (56, 77)
Gender, n(%)					0.088	
Female	28 (30.8%)	24 (27.3%)	52 (29.1%)	133 (36.4%)		185 (34.0%)
Baseline Po2/Fo2	199 (123, 262)	145 (102, 229)	169 (106, 246)	277 (191, 345)	<.001	239 (139, 306)
SOFA Score	2 (1, 3)	3 (2, 4)	3 (2, 4)	2 (0, 3)	<.001	2 (1, 4)
Follow-up, days	12 (6, 17)	13 (7, 18)	12 (6, 17)	8 (4, 14)	<.001	9 (4, 15)
Events, n(%)						
Mechanical ventilation	17 (18.7%)	16 (18.2%)	33 (18.4%)	57 (15.6%)	0.406	90 (16.5%)
Death	7 (7.7%)	6 (6.8%)	13 (7.3%)	73 (20.0%)	<.001	86 (15.8%)

‡Chi-square or Kruskal-Wallis test as appropriate

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TABLE 2A Signs and symptoms and baseline comorbidities - Modena center only

	Treatment started				
Characteristics	**Tocilizumab subcutaneous**	**Tocilizumab intravenous**	**SOC**	**p-value***	**Total**
	N= 84	N= 48	N= 222		N= 354
Age, years				0.357	
Median (IQR)	67 (56, 73)	61 (52, 74)	67 (55, 78)		66 (55, 76)
Gender, n(%)				0.996	
Female	26 (31.0%)	15 (31.3%)	68 (30.6%)		109 (30.8%)
Any comorbidity, n(%)				<.001	
Yes	39 (46.4%)	24 (50.0%)	36 (16.2%)		99 (28.0%)
Comorbidities, n(%)					
Diabetes	11 (13.1%)	6 (12.5%)	7 (3.2%)	0.002	24 (6.8%)
Hypertension	37 (44.0%)	22 (45.8%)	30 (13.5%)	<.001	89 (25.1%)
Cardiovascular Disease	9 (10.7%)	6 (12.5%)	12 (5.4%)	0.117	27 (7.6%)
Chronic Renal Insufficiency	2 (2.4%)	5 (10.4%)	7 (3.2%)	0.045	14 (4.0%)
Cancer	2 (2.4%)	0 (0.0%)	8 (3.6%)	0.379	10 (2.8%)
Hepatitis B/C	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)
Disease Duration					
Days from symptoms onset to hospitalisation, median(IQR)	3 (0, 5)	2 (1, 4)	2 (0, 3)	0.289	2 (0, 4)
Days from hospitalisation to intubation, median(IQR)	7 (4, 10)	6 (1, 7)	6 (3, 7)	0.493	6 (3, 8)
Sign and symptoms, n(%)					
Fever, median(IQR)	37 (36, 38)	37 (36, 38)	37 (36, 37)	0.541	37 (36, 37)
Cough	42 (50.0%)	20 (41.7%)	55 (24.8%)	<.001	117 (33.1%)
Myalgia	5 (6.0%)	5 (10.4%)	7 (3.2%)	0.088	17 (4.8%)
Sputum	5 (6.0%)	0 (0.0%)	4 (1.8%)	0.059	9 (2.5%)
Headache	5 (6.0%)	7 (14.6%)	10 (4.5%)	0.032	22 (6.2%)
Haemptysis	0 (0.0%)	1 (2.1%)	2 (0.9%)	0.451	3 (0.8%)
Diaharrea	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)
Systolic pressure, mmHg median(IQR)	130 (118, 138)	120 (110, 135)	124 (110, 140)	0.361	125 (110, 138)

*Chi-square or Kruskal-Wallis test as appropriate

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Table 2b. Baseline blood count and biochemical parameters (Modena only).

	Treatment started				
Blood tests	Tocilizumab subcutaneous	Tocilizumab intravenous	SOC	p-value*	Total
-	N= 78	N= 47	N= 179	-	N= 304
Markers, Median (IQR)	-	-	-	-	-
Haemoglobin, g/l	12.8 (11.5, 13.7)	13.0 (11.6, 13.7)	12.7 (11.2, 14.2)	0.966	12.7 (11.4, 14.0)
White cells, mm3	7195 (5470, 10380)	6840 (5140, 9380)	6200 (4570, 9360)	0.224	6700 (4890, 9560)
Lymphocytes, %	22.1 (9.7, 36.8)	18.1 (12.5, 25.8)	23.1 (9.9, 39.7)	0.598	20.6 (9.9, 36.6)
Total lymphocytes, mm3	1580 (1390, 2142)	2459 (1852, 3348)	1390 (1000, 2815)	0.297	1852 (1120, 2726)
Platelets, 10^9/l	257.5 (183.0, 374.0)	211.0 (156.0, 294.0)	209.0 (155.0, 298.0)	0.003	221.5 (163.0, 317.0)
Alanine amino-transferase, U/l	37.0 (27.0, 71.0)	35.0 (21.5, 62.5)	31.0 (19.0, 48.0)	0.007	33.0 (22.0, 56.0)
Bilirubin, mg/l	0.6 (0.4, 0.7)	0.6 (0.4, 0.8)	0.6 (0.4, 0.8)	0.735	0.6 (0.4, 0.8)
Calcium, mg/l	8.6 (8.4, 9.1)	8.5 (8.1, 8.9)	8.6 (8.3, 9.1)	0.264	8.6 (8.3, 9.1)
Creatine Kinase, U/l	63.0 (33.0, 159.0)	130.0 (41.5, 312.0)	71.0 (39.0, 202.0)	0.053	76.0 (38.0, 197.5)
Chloride, mmol/l	101.0 (98.0, 105.0)	100.0 (99.0, 103.0)	101.0 (97.0, 103.0)	0.906	101.0 (98.0, 103.0)
Creatinine, mg/l	0.8 (0.6, 0.9)	0.9 (0.7, 1.2)	0.9 (0.7, 1.1)	0.005	0.8 (0.7, 1.1)
D-dimer, mg/l	1210 (820.0, 2290)	1000 (780.0, 2730)	1240 (610.0, 2480)	0.636	1200 (690.0, 2480)
Lactate dehydrogenase, U/l	600.0 (505.0, 761.0)	676.0 (536.0, 765.0)	507.5 (419.5, 705.5)	<.001	564.0 (454.0, 745.0)
Potassium, mmol/l	3.9 (3.5, 4.3)	3.8 (3.6, 4.0)	3.9 (3.5, 4.3)	0.338	3.9 (3.5, 4.2)
Sodium, mmol/l	137.5 (136.0, 139.0)	137.0 (135.0, 138.0)	138.0 (135.0, 141.0)	0.019	138.0 (135.0, 140.0)
Ferritine, mg/l	1168 (543, 1214)	-	423 (355, 993)	0.135	447 (355, 1141)
C-reactive protein, mg/l	3.4 (0.6, 7.8)	6.1 (1.8, 15.3)	5.4 (1.8, 14.6)	0.022	5.3 (1.4, 13.6)
IL-6, mg/l	190.2 (86.6, 401.0)	238.3 (140.2, 731.9)	144.1 (41.1, 385.8)	0.045	178.6 (67.6, 402.0)

*Kruskal-Wallis test

TABLE 3a Hazard ratio from fitting a Cox regression model

	Unadjusted and adjusted relative hazards of mechanical ventilation/death[&]					
	Unadjusted HR (95% CI)	p-value	Adjusted[‡] HR (95% CI)	p-value	Adjusted[‡‡] HR (95% CI)	p-value
Overall - 2 arms contrasts						
SOC	1	-	1	-	1	-
Tocilizumab (any)	0.60 (0.43, 0.84)	0.003	0.64 (0.45, 0.91)	0.012	0.52 (0.36, 0.73)	<.001
Stratum baseline Po2/Fo2 below 150 - 2 arms contrasts						
SOC	1	-	1	-	1	-
Tocilizumab (any)	0.30 (0.17, 0.52)	-	0.20 (0.11, 0.36)	-	0.21 (0.11, 0.38)	-
Interaction p-value	-	-	-	-	-	0.011
Stratum baseline Po2/Fo2 above 150 - 2 arms contrasts						
SOC	1	-	1	-	1	-
Tocilizumab (any)	0.31 (0.16, 0.59)	-	0.39 (0.20, 0.77)	-	0.40 (0.20, 0.79)	-
Overall 3 arms contrasts						
SOC	1	-	1	-	1	-
Tocilizumab subcutaneous	0.63 (0.41, 0.97)	0.036	0.69 (0.44, 1.08)	0.102	0.55 (0.35, 0.87)	0.011
Tocilizumab intravenous	0.57 (0.36, 0.90)	0.016	0.60 (0.38, 0.95)	0.030	0.48 (0.30, 0.77)	0.002
Overall 3 arms contrasts						
Tocilizumab intravenous	1	-	1	-	1	-
Tocilizumab subcutaneous	1.19 (0.40, 3.55)	0.751	1.08 (0.36, 3.25)	0.886	0.94 (0.31, 2.85)	0.919
SOC	3.95 (1.72, 9.09)	0.001	2.89 (1.25, 6.70)	0.013	3.84 (1.64, 8.99)	0.002

[‡]Adjusted for age, gender and recruiting center

[‡‡]Adjusted for age, gender, recruiting center and SOFA Score

[&]Initiation of invasive mechanical ventilation or death

TABLE 3b Hazard ratios from fitting a marginal Cox regression model

	Unadjusted and adjusted marginal relative hazards of mechanical ventilation/death[&]			
	Unadjusted HR (95% CI)	p-value	Adjusted[‡] HR (95% CI)	p-value
SOC	1.00	-	1.00	-
Tocilizumab	0.54 (0.37, 0.78)	<.001	0.44 (0.29, 0.65)	<.001

[‡]adjusted for age, gender, recruiting center, SOFA score, use of steroids post-baseline and censoring using IPW

[&]Initiation of invasive mechanical ventilation or death

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TABLE 4 RH from fitting a Cox regression model

	Unadjusted and adjusted relative hazards of death[&]					
	Unadjusted HR (95% CI)	p-value	Adjusted[‡] HR (95% CI)	p-value	Adjusted[‡‡] HR (95% CI)	p-value
Overall - 2 arms contrasts						
SOC	1	-	1	-	1	-
Tocilizumab (any)	0.28 (0.15, 0.50)	<.001	0.36 (0.20, 0.66)	<.001	0.25 (0.14, 0.47)	<.001
Stratum baseline Po2/Fo2 below 150 - 2 arms contrasts						
SOC	1	-	1	-	1	-
Tocilizumab (any)	0.11 (0.04, 0.27)	-	0.09 (0.03, 0.24)	-	0.08 (0.03, 0.22)	-
Interaction p-value	-	-	-	-	-	0.116
Stratum baseline Po2/Fo2 above 150 - 2 arms contrasts						
SOC	1	-	1	-	1	-
Tocilizumab (any)	0.22 (0.08, 0.63)	-	0.39 (0.12, 1.20)	-	0.38 (0.12, 1.19)	-
Overall 3 arms contrasts						
SOC	1	-	1	-	1	-
Tocilizumab subcutaneous	0.30 (0.14, 0.66)	0.003	0.37 (0.17, 0.83)	0.016	0.25 (0.11, 0.55)	<.001
Tocilizumab intravenous	0.25 (0.11, 0.58)	0.001	0.35 (0.15, 0.80)	0.013	0.26 (0.11, 0.61)	0.002

[‡]Adjusted for age, gender and recruiting center

[‡‡]Adjusted for age, gender, recruiting center and SOFA Score

[&]All-cause mortality

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~~**Figure 1** depicts Kaplan-Mayer curves and impact of tocilizumab overall compared to control on initiation of invasive mechanical ventilation/death (panel A) and all-cause mortality (panel B), while the effect of IV and SC tocilizumab on initiation of invasive mechanical ventilation/death and all-cause mortality is shown in panels C and D respectively.~~

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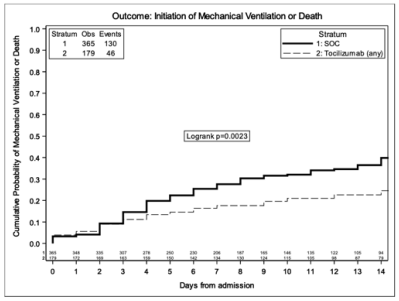

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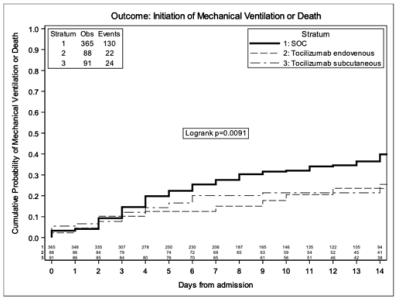

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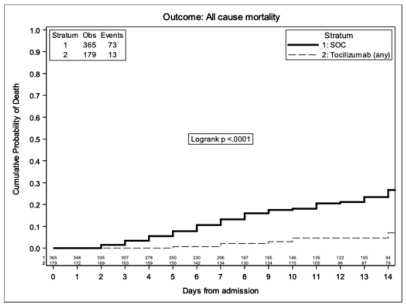

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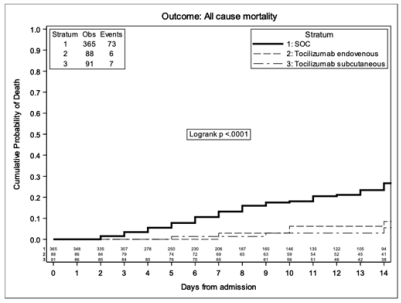

Supplementary TABLE 1A Key baseline factors by cohort

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	recruiting center				
Characteristics	**Modena**	**Bologna**	**Reggio Emilia**	**p-value***	**Total**
	N= 354	N= 80	N= 110		N= 544
Age, years				0.653	
Median (IQR)	66 (55, 76)	68 (56, 78)	68 (59, 77)		67 (56, 77)
Gender, n(%)				<.001	
Female	109 (30.8%)	44 (55.0%)	32 (29.1%)		185 (34.0%)
Baseline Po2/Fo2	227 (126, 289)	255 (213, 310)	255 (155, 340)	0.006	239 (139, 306)
SOFA Score	2 (0, 3)	2 (2, 3)	3 (2, 4)	<.001	2 (1, 4)
Follow-up, days	9 (4, 15)	12 (6, 18)	8 (4, 14)	0.039	9 (4, 15)
Intervention, n(%)				<.001	
Tocilizumab subcutaneous	84 (23.7%)	4 (5.0%)	3 (2.7%)		91 (16.7%)
Tocilizumab intravenous	48 (13.6%)	24 (30.0%)	16 (14.5%)		88 (16.2%)
SOC	222 (62.7%)	52 (65.0%)	91 (82.7%)		365 (67.1%)
Events, n(%)					
Mechanical ventilation	54 (15.3%)	9 (11.3%)	27 (24.5%)	0.028	90 (16.5%)
Death	52 (14.7%)	16 (20.0%)	18 (16.4%)	0.494	86 (15.8%)

*Chi-square or Kruskal-Wallis test as appropriate

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~~Supplementary TABLE 1B Key baseline factors by intervention and cohort~~

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	~~Intervention~~				
~~Characteristics~~	~~Tocilizumab subcutaneous~~	~~Tocilizumab intravenous~~	~~SOC~~	~~p-value~~	~~Total~~*
	~~N= 91~~	~~N= 88~~	~~N= 365~~		~~N= 544~~
~~Age, years~~				~~0.017~~	
~~Median (IQR)~~	~~67 (55, 73)~~	~~63 (54, 72)~~	~~69 (57, 78)~~		~~67 (56, 77)~~
~~Gender, n(%)~~				~~0.206~~	
~~Female~~	~~28 (30.8%)~~	~~24 (27.3%)~~	~~133 (36.4%)~~		~~185 (34.0%)~~
~~Baseline Po2/Fo2~~	~~199 (123, 262)~~	~~145 (102, 229)~~	~~277 (191, 345)~~	~~<.001~~	~~239 (139, 306)~~
~~SOFA Score~~	~~2 (1, 3)~~	~~3 (2, 4)~~	~~2 (0, 3)~~	~~<.001~~	~~2 (1, 4)~~
~~Follow-up, days~~	~~12 (6, 17)~~	~~13 (7, 18)~~	~~8 (4, 14)~~	~~<.001~~	~~9 (4, 15)~~
~~Events, n(%)~~					
~~Mechanical ventilation~~	~~17 (18.7%)~~	~~16 (18.2%)~~	~~57 (15.6%)~~	~~0.705~~	~~90 (16.5%)~~
~~Death~~	~~7 (7.7%)~~	~~6 (6.8%)~~	~~73 (20.0%)~~	~~<.001~~	~~86 (15.8%)~~

~~*Chi-square or Kruskal-Wallis test as appropriate~~

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~~Supplementary TABLE 2 Means IL-6 values (log10 scale) from fitting a mixed linear model~~

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	~~Estimates from the mixed model (Mean values 95% CI) log10 scale~~					
	~~Baseline IL6~~	~~Difference in baseline~~	~~IL6 change/year~~	~~Difference in change/year~~	~~Adjusted[±] IL6 change/year~~	~~Adjusted[±] differencein change/year~~
~~*Treatment*~~						
~~Tocilizumab (any)~~	~~2.46 (2.35, 2.57)~~		~~0.00 (0.00, 0.01)~~		~~0.00 (0.00, 0.01)~~	
~~SOC~~	~~2.25 (2.11, 2.39)~~	~~-0.21 (-0.39, -0.03)~~	~~-0.01 (-0.02, 0.00)~~	~~-0.01 (-0.02, 0.00)~~	~~-0.01 (-0.02, 0.00)~~	~~-0.02 (-0.03, 0.00)~~
		~~0.020~~		~~0.006~~		~~0.004~~

~~[±]Adjusted for age, gender and total SOFA Score~~

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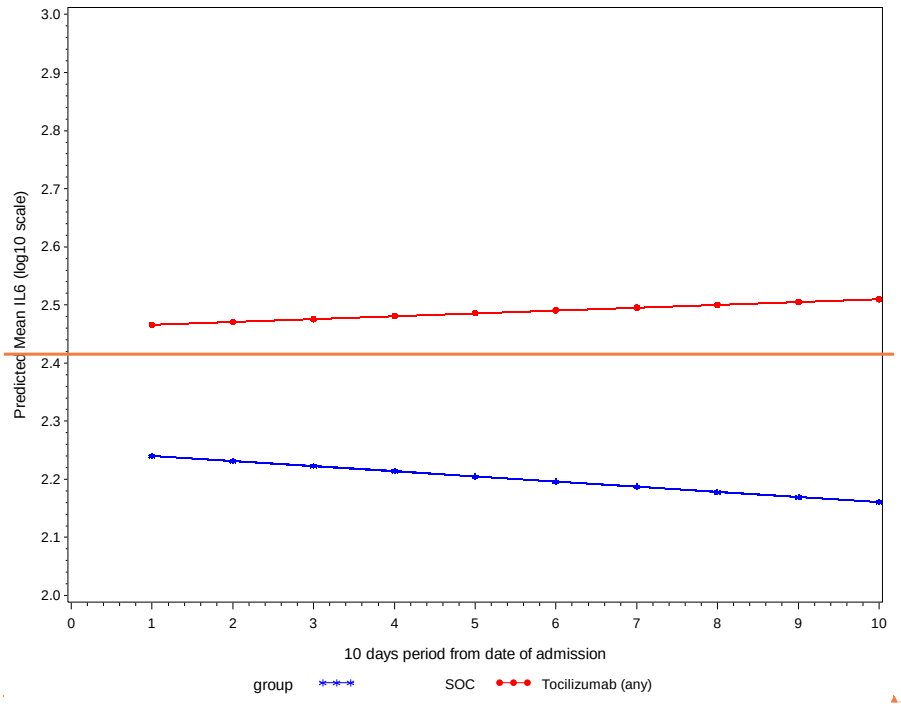
~~Supplementary Figure 1 Means IL-6 values (log10 scale) from fitting a mixed linear model~~



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Supplementary TABLE 3 Means AST values from fitting a mixed linear model

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	Estimates from the mixed model (Mean values 95% CI)					
	Baseline AST	Difference in baseline	AST change/year	Difference in change/year	Adjusted[a] AST change/year	Adjusted[a] differencein change/year
Treatment						
Tocilizumab (any)	44.05 (26.60, 61.51)		4.12 (0.97, 7.27)		4.12 (0.88, 7.36)	
SOC	1.48 (1.43, 1.53)	7.88 (30.8, 15.07)	0.00 (0.00, 0.01)	2.47 (6.57, 1.63)	0.01 (0.00, 0.01)	2.26 (6.57, 2.04)
		0.501		0.237		0.302

[a]Adjusted for age, gender and total SOFA Score

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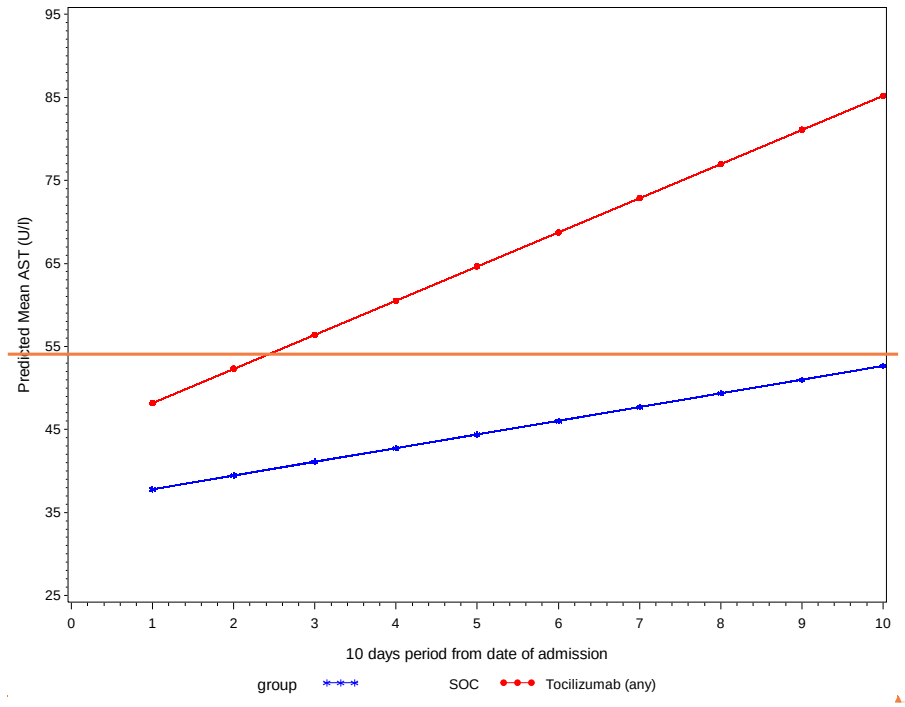
~~Supplementary Figure 2. Means AST values from fitting a mixed linear model~~



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Supplementary table 4. Kaplan-Meier estimates reported in text of Results section

	Kaplan-Meier estimates of probability of outcomes			
	No. events by 7 days	Day 7 percent (95% CI)	No. events by 14 days	Day 14 percent (95% CI)
Endpoints - Overall				
Mechanical ventilation	84	16.4 (13.2, 19.6)	90	18.8 (15.1, 22.5)
Composite	129	25.7 (21.9, 29.6)	159	36.1 (31.2, 40.9)
Death	45	11.0 (7.9, 14.0)	69	21.1 (16.3, 25.8)
Endpoints - Stratified				
Mechanical ventilation - SOC	98	30.3 (25.2, 35.4)	117	41.5 (35.1, 47.9)
Mechanical ventilation - TCZ	34	19.6 (13.7, 25.5)	42	27.0 (19.6, 34.4)
Death - SOC	42	16.0 (11.5, 20.5)	60	28.7 (21.9, 35.4)
Death - TCZ	4	2.9 (0.1, 5.8)	9	8.5 (3.0, 14.1)

Modena, 21 May 2020

Dear Dr Van Epps,

We are very grateful for another round of constructive comments and suggestions to our paper.

We here provide a point-by-point reply to the comments and we have incorporated the related changes in the manuscript.

We thank the reviewers for their thoughtful insights which helped to, once again, significantly improve the manuscript and prepare it for publication.

Reviewers' comments:

Reviewer #1:

Thank you for the comprehensive revision. I have the following additional comments:

1. p.1, under 'Added value of this study', you wrote, "The all-cause mortality was reduced by 75%." I don't believe this is an appropriate conclusion for a retrospective study. The language implies causation rather than association. Similarly, in the opening paragraph of the Discussion, "The benefit of tocilizumab was strikingly higher...". I suggest rephrasing this. Later in the Discussion, you assert "The benefit of tocilizumab in reducing mortality and invasive mechanical ventilation was confirmed after adjusting for glucocorticoid use". I don't believe you have confirmed any putative benefit at all. You can't use statistical adjustment to confirm the benefit of an intervention using your study design. I think a more appropriate conclusion would be that your findings provide support to proceed with a randomised controlled trial of tocilizumab in COVID-19 pneumonia. [Editors' note: we agree strongly with this comment; see Editors' comments below]

Authors' answer:
According to the reviewer's suggestion, all the sentences have been rephrased.
In the "Added value of this study", the following sentence was modified to read as below:
We also found a strong association between use of tocilizumab and the risk of death (aHR=0.38 95% CI:0.17-0.83, p=0.02).
In the Discussion, the following sentences were modified accordingly:
"The association with the use of tocilizumab was even stronger when overall mortality risk was analysed alone."
"Our results were similar after further adjusting for post-baseline use of glucocorticoid use."
We agree with the reviewer that the tone of conclusions of the manuscript should be lowered, therefore we changed both abstract and conclusions according to this suggestion:
"Although these results are encouraging, they should be confirmed in the large number of currently ongoing randomised studies."

2. At times, I find the language used hard to understand, eg. p.3 "The second dose of Tocilizumab was chosen joining the real-life experience which indicated difficult to assess benefit in 12 hours and according to PK data which suggest plasma level is obtained in two subsequent doses"

Authors' answer:
We rewrote the sentence in order to make it clearer:
"The second dose was given because PK data were suggesting that adequate plasma levels of the drug could be obtained only after two doses."

3. I still find the Discussion of limitations missing some key material. My earlier concern that some patients with chronic illnesses were excluded from receiving tocilizumab and the resultant treatment selection bias has not been addressed, despite adopting the Charlson Comorbidity Index in your model.

Authors' answer:
Table 1 shows that the burden of co-morbidities and chronic illnesses was higher in groups receiving tocilizumab. However, we added a sentence in the Discussion to acknowledge that people with cancer and renal insufficiency were not given tocilizumab and this could have biased the results:
"Of course, because only one patient with cancer and two with renal insufficiency were allocated to tocilizumab, we cannot rule out residual confounding that cannot be controlled by regression interpolation."

Reviewer #3 (statistics):

Below are my further comments for the authors:

Minor comments:

1. The authors should consider defining SoC at first use in the abstract and introduction sections. Consider defining SOFA in the abstract.

Authors' answer:
According to the reviewer's suggestion, these were corrected in the abstract and introduction.

2. Authors should revise the following to include the treatment: 'A 48% reduction in risk of invasive mechanical ventilation/death was shown from fitting a Cox regression analysis adjusted for gender, age and total SOFA score.'

Authors' answer:

According to the reviewer 1 and 3' suggestion, the following sentence was rephrased in the abstract:
"A reduced risk of invasive mechanical ventilation/death was shown for participants treated with tocilizumab from fitting a Cox regression analysis adjusted for gender, age and SOFA score (aHR=0.61, 95% CI:0.40-0.92; p=0.02)."

3. Remove ref from: 'The time course of the disease is characterized by an initial phase of viral replication that may be followed by a second phase driven by the host inflammatory response [6] (ref).'

Authors' answer:
According to the reviewer's suggestion, "(ref)" was removed.

4. Indicate in table 1 that the values against: 'Baseline Po2/Fo2', 'SOFA score' and 'Follow-up, days' are median and IQRs.

Authors' answer:
According to the reviewer's suggestion, median and IQRs were added in the Table 1.

5. Under study settings, authors should include a brief description and the contributions of the participating centres (or recruiting sites): Modena, Bologna and Reggio Emilia. Authors seem to have only mentioned Modena. [Editors' note: please add to the appendix a full list of the patients who were included at each centre, including the numbers, and a breakdown of the clinical information for each group.]

Authors' answer:
The following sentence was added:
The contribution of each recruiting centre is specified in Supplementary Table 1.

6. I think the first paragraph under the 'statistical analysis' should be broken down (spaced) into multiple paragraphs, as it seems a bit long for a single paragraph.

Authors' answer:
According to the reviewer's suggestion, the paragraph regarding statistical analysis was broken down into multiple paragraphs.

Reviewer #4:

The authors have updated their dataset with including 544 severe COVID-19 patients instead of 411 patients in the previous version. In general, they revised the manuscript according to the comments of the reviewers and improved this paper. I have some further points left for them to consider.

1. The authors mentioned patients were consecutively enrolled. However, it seems the number of COVID-19 patients documented was changed from 5744 (reported in previous version) to 1351. This should be clarified.

Authors' answer:

5,744 referred to a total number of positive nasopharyngeal swabs registered in three provinces (Modena, Reggio Emilia and Bologna) of Emilia-Romagna region up to the first submission date of the manuscript (30 March 2020). This was not the correct universe from where participants were extracted for the analysis as it included people with mild symptoms who were never referred to hospital. Moreover, aside from the asymptomatic patients included in this number, there were also some other small hospitals in three provinces that were admitting patients with COVID-19 infection which should not be included in the universe in the first place. The corrected number of 1,351 now refers to the number patients admitted only to the three recruiting sites and can be traced in the flowchart of the study.

2. The time from symptom onset to clinic entry was very important, which indicates the stage of disease process for patients receiving or not receiving tocilizumab. Although not being able to perform the analysis accruing from the date of symptom onset, the information should be listed (if possible; now only listed for patients from Modena centre). This indicates whether stage of disease process of patients at clinic entry is balanced between two groups.

Authors' answer:

We have now made a further extra effort and extracted the duration of symptoms for all recruiting sites. According to the reviewer's suggestion, this has been added in the Table 1. Moreover, all our main analyses in Table 3 and Table 4 were adjusted for the duration of symptoms.

3. I suggest to remove the sensitivity analysis with follow-up for the SoC with tocilizumab group accruing from the date of the first dose of tocilizumab while time zero for the SoC group being kept at the date of hospital admission. This does not make sense to me, as the time of receiving tocilizumab may be few days after admission, which could make the stage of disease process between groups not comparable.

Authors' answer:

According to reviewer's suggestion, this analysis and the corresponding sentence of the results below were removed from the Methods and Results section:

"In a number of sensitivity analyses: i) when using for the intervention group the date of starting the first dose of tocilizumab as time zero instead of the date of hospital admission (aHR=0.57, 95% CI: 0.38-0,86, p=0.008)."

4. Speculated from the duration of follow-up, which was relatively short (12 vs 8 days) and duration from symptom onset to hospital admission (only listed for patients from Modena center), a majority of patients were still in hospital when the outcome was recorded. This makes it challenging to interpret the results.

Authors' answer:
Unfortunately, there was a mistake so that the lines for the duration of symptoms and the time from hospital admission to the date of mechanical ventilation have been swapped in Table 2. These have been now corrected. The median time from admission to mechanical ventilation in those who experienced ventilation cannot be taken as a measure of the median time of follow-up. This is instead reported for the whole TESEO cohort in Table 1. We have now also added in the same table the information of the duration of symptoms for the whole cohort.

5. The authors took use of steroids post-baseline into consideration, which was good. Some other treatments post-baseline may also be important confounders. If these factors are not evaluated, the effect of tocilizumab shown may be due to the combined contribution of different treatments. For example, although lopinavir-ritonavir did not show statistically significant association with reduced mortality in previous RCT, the numerically lower mortality in lopinavir-ritonavir group indicated lopinavir-ritonavir can be a potential confounder for this study.

Authors' answer:
We agree that there could be other time-varying confounders affected by prior treatment have not been accounted for in the analysis and we have added a sentence to acknowledge this potential shortfall. Nevertheless, both study groups received lopinavir/ritonavir or darunavir/cobicistat during the first month of the observational period, therefore, we can exclude it as a confounder. Moreover, none of the patients received lopinavir/ritonavir after the administration of tocilizumab. The sentence below was added in the Discussion:
"Nevertheless, we cannot rule out the presence of other time-varying confounders affected by the chosen treatment strategy that have been not accounted for in the analysis. Of note, antiretroviral drugs (protease inhibitors, i.e. lopinavir/ritonavir and darunavir/cobicistat) were used both in SoC or tocilizumab group and were never started post baseline in the tocilizumab group".

6. Considering the limitations regarding the limited information for stage of disease process, short duration of follow-up, and confounders including but not limited to treatments post-baseline, the "large" effect of tocilizumab evaluated by this article should be paid attention. Strong evidence needs to be fulfilled by further studies.

Authors' answer:
We agree with the reviewer that the tone of conclusions of the manuscript should be lowered, therefore we changed both abstract and conclusions according to this suggestion:
"Although these results are encouraging, they should be confirmed in the large number of currently ongoing randomised studies."

Reviewer #5:

The authors have nicely addressed the reviewers' concerns. A few suggestions remain:

1. It is unclear in their response what was meant by CRP and LDH do not predict outcome. I think there are several examples of this. Here is one: Infect Dis (Lond) 2020 May 6;1-8. doi: 10.1080/23744235.2020.1759817. Online ahead of print. Risk Factors for Disease Progression in Hospitalized Patients With COVID-19: A Retrospective Cohort Study. Wei Hou et al.

Authors' answer:
We apologize for the typo in the previous round of answers to the reviewers, as we wrote that CRP and LDH do NOT predict outcome. Actually, our findings on the prognostic values of CRP and LDH confirmed some of the previously published papers. Nevertheless, we agree with the reviewer that conflicting results still exist regarding the prognostic value of biomarkers in patients with COVID-19.
In the previous round of our answer, we wrote:
"Previous reports did ~~not~~ find an association between CRP, LDH and clinical outcome. However, we did fit a model in a subset of the study population after controlling for baseline CRP and results were similar (aHR for the composite endpoint =0.57, 95% CI:0.38-0.84, p=0.005)."

2. The following sentence in the 2nd to last paragraph of the manuscript should be modified. It currently reads, "Others monoclonal antibodies directly acting in the inflammatory response pathway triggered by SARS-CoV-2 are being tested." "Others" is a typo; should be "Other". More importantly, several of the therapies in clinical trials are NOT monoclonal antibodies (e.g., anakinra = rhIL-1 receptor antagonist; also, small molecule inhibitors of the JAK-STAT pathway).

Authors' answer:
We thank the reviewer for pointing out this error. The typo was corrected and the sentence was changed as following:
"Other immune-active agents directly acting in the inflammatory response pathway triggered by SARS-CoV-2 are being tested."

3. There are now several published manuscripts reporting benefit of tocilizumab in treating Covid-19. These should at least be mentioned/addressed: (1) Med Mal Infect. 2020 May 6:S0399-077X(20)30129-3. doi: 10.1016/j.medmal.2020.05.001; online ahead of print; (2) Klopfenstein T et al.b. Autoimmun Rev. 2020 May 3:102568. doi: 10.1016/j.autrev.2020.102568; online ahead of print; (3) Toniati P et al. Clinical and Experimental Rheumatology 2020; 38: in press; (4) Sciascia S et al. Proc Natl Acad Sci U S A. 2020 Apr 29:202005615. doi: 10.1073/pnas.2005615117. Online ahead of print. Xu X et al.

Authors' answer:
We thank the reviewer. The suggested references were added. The following sentence was added citing the suggested references:
"More recently, an increasing number of studies are reporting use of tocilizumab in COVID-19 [20,21]."
Also, these studies were commented in the discussion in the light of our results:

“Our results are consistent with those of a smaller retrospective case-controlled French study by Klopfesntein et al [20], in which death and/or ICU admissions were higher in patients without tocilizumab than in the tocilizumab group (72% vs 25%, p=0.002) vs 41.5 vs 27.0 % by 14 days in our study. A press release of the CORIMUNO randomized clinical trial also anticipated a beneficial effect of tocilizumab when compared to SoC [34].”
“Tocilizumab, administered both intravenously or subcutaneously, can be considered together with anakinra as one of the immune-active agents which have been tested in clinical care for the treatment of severe COVID-19 pneumonia [19–21,37].”

Reviewer #7:

I thank the authors for their rapid response to an exhaustive list of comments. I appreciate the authors responses to my comments and concerns. I have two notes to consider adding to the text:

The authors addressed my comment about the borderline significance of less females treated with TCZ (p=0.053), noting there is reason to think females may have better survival and therefore may have been systematically had less access to the study drug (though authors believe this was a chance finding). I would add this note to the discussion of patients selected for treatment as this is a potentially important and worrisome features.

Authors’ answer:
The p value of 0.053 referred to the Table 1 of the first version of our paper. After updating the database, we included 544 patients in which females were not treated less with tocilizumab (p=0.088). Nevertheless, the percentage of females by treatment strategy was rather different and we have kept in the Discussion the possibility of residual confounding due to gender.

The authors now have infection data which I appreciate. However, they separate by infection type and do not provide a summary. By my count there were 24 infections in 179 TCZ patients (13%) vs 14 in 365 SoC patients (4%). Presenting it as such is more accurate and points to a potential concern - should also see if this is a significant difference but I suspect it is.

Authors’ answer:
The following sentence was added in the results:
To summarize, 24 infections were diagnosed in 179 tocilizumab patients (13%) and 14 out of 365 SoC patients (4%) (p<0.001).
The following sentence was changed in the discussion:
“A major concern are adverse events. We observed a significant higher prevalence of infection in the tocilizumab vs SoC. The design and the short follow-up period of this study does not allow us to drive conclusions regarding early and long-term side effects of tocilizumab, eventually followed by glucocorticoids. This signal needs to be confirmed by the ongoing randomised clinical trials.”

//////////////////////////////////

Editors' specific points:

1. Please present the conclusions of the study with more caution, as we feel that the positive outcomes with tocilizumab treatment are still overstated given the limitations of the study. For instance, throughout the paper, we ask that authors not translate HRs/RRs/ORs into percentages, since this can be misleading. Simply indicate the HRs/ORs/RRs and let the reader interpret the data.

Authors' answer:
According to the concerns raised by reviewer 1 regarding lowering the tone of the conclusions, the wording was mitigated. Also, we have removed from the text all the translations from the HR estimates to percentages of risk reduction.

2. Please avoid language that evokes the notion of a randomised trial. For example, please refer to the patients treated with standard of care as the 'comparator' group rather than the 'control' group. Also, please avoid using the terms 'treatment arms'.

Authors' answer:
According to the Editor's suggestion, the "control group" and "treatment arms" were replaced with the "comparator group" and "treatment groups", respectively.

3. Results: Please add number at risk and the number of patients censored in each group for each time point on your K-M curves and cumulative incidence plots. Please ensure both are cumulative and please use the format "number at risk (number censored)".

Authors' answer:
According to the Editor's suggestion, K-M curves have been modified by moving the cumulative number of people at risk at each day as a footnote below the figures. We have also added in brackets the cumulative number of people censored at each day.

4. When you submit your revision, please supply the figure as an individual, editable figure file.

Authors' answer:
According to the Editor's suggestion, Figure 1 is supplied in an individual, editable file.

5. Please supply the tables as a separate Word file, and please present table 3 as a single table (ie, do not divide it into panels A and B).

Authors' answer:
According to the Editor's suggestion, all tables are provided as a separate file. Division into the parts A and B are removed and Table 3 is a single table.

6. We require each author to submit an ICMJE conflict of interest form. These forms should be uploaded as a continuous PDF file with all authors' forms included when you submit the final revision. The file is attached here.

Authors' answer:
According to the Editor's suggestion, ICMJE files are uploaded as a continuous PDF file.

7. We also require that all authors complete and sign the author signature/contributions form (also attached here). Both hand-written and electronic signatures are acceptable.

Authors' answer:
According to the Editor's suggestion, signature/contributions files are uploaded as a continuous PDF file.

8. In the authorship list, please indicate ONE higher degree for each author; please also indicate if any authors are full professors.

Authors' answer:
According to the Editor's suggestion, degrees and professors are indicated in the main document. Full professors are: Cristina Mussini, Pier Luigi Viale, Andrea Cossarizza, Enrico Clini, Carlo Salvarani and Antonello Pietrangelo. Associated professors are: Giovanni Guaraldi, Massimo Girardis, Federico Pea, Alessandro Cozzi-Lepri and Maddalena Giannella.

9. You list various groups at the end of the manuscript. Please clarify whether you intend for any of these groups to be credited as authors (ie, 'on behalf of the XXX study group' at the end of the authorship list). If yes, you then need to clarify whether each of the individuals in this group should appear on Pubmed. If so, you will need to upload with your revision a separate Word document with a list of names of the study group members presented as a two-column table. First and middle names or initials should be placed in the first column, and surnames in the second column. Names should be ordered as you wish them to appear on PubMed. The table will not be included in the paper itself - it's simply used to make sure that PubMed adds the names correctly. We reserve the right to not make further changes to the collaborator list after the paper has passed for publication.

Authors' answer:
The final list is already provided in the main document and in the Editorial Manager as well.

10. As above, please clarify the multiple lists of individuals. For those not involved in authorship, these names should simply be included in the acknowledgements section.

Authors' answer:
The final list is already provided in the main document and in the Editorial Manager as well.

11. Please note that for individuals named in acknowledgements, we require permission from that person. They can provide permission via email. If multiple individuals are listed, please provide permissions statements as a single, concatenated PDF when you resubmit. The permission statement should use the language "I permit Giovanni Guaraldi et al to list my name in the acknowledgments section of their manuscript and I have seen a copy of the paper, “Tocilizumab in patients with severe COVID-19: a retrospective cohort study”

Authors’ answer:
COVID-19 epidemic has involved many clinicians, pharmacists and data managers in our provinces. They work extremely hard and we would like to thank them even if they did not directly contribute to the manuscript. Unfortunately, it is not feasible for us to obtain permissions from all of them. Nevertheless, we are sure that they would appreciate our gesture.

12. Please remove the subheadings from the Results section, per Lancet style.

Authors’ answer:
This has been done.

13. In the discussion, please remove the claim about tocilizumab being ‘the first immune-active agent that has been tested in clinical care for the treatment of severe COVID-19 pneumonia,’ as this is not strictly true (eg, The Lancet Rheumatology has published data from a cohort of patients treated with anakinra).

Authors’ answer:
According to the Editor’s suggestion, this was modified to the following sentence:
“Tocilizumab, administered both intravenously or subcutaneously, can be considered together with anakinra as one of the immune-active agents which have been tested in clinical care for the treatment of severe COVID-19 pneumonia [19–21,37].”

14. Please add an Author contributions section to the end of your paper before the references, as per Lancet style. These statements should exactly match those given on your signed author contribution forms. Authors should be referred to by their initials in this section.

According to the Editor’s suggestion, author contributions are provided to the end of paper and in signed contribution forms.

Best regards,
Giovanni Guaraldi, Marianna Meschiari, Alessandro Cozzi-Lepri, Jovana Milic and Cristina Mussini

Table 1. Key baseline factors - all centres combined. The reported p value refers to difference between tocilizumab (any) and standard of care (SoC).

Characteristics	Intervention					
	Tocilizumab subcutaneous	**Tocilizumab intravenous**	**Tocilizumab (any)**	**SoC**	**p-value***	**Total**
	N= 91	N= 88	N= 179	N= 365		N= 544
Age, years					0.006	
Median (IQR)	67 (55, 73)	63 (54, 72)	64 (54, 72)	69 (57, 78)		67 (56, 77)
Gender, n(%)					0.088	
Female	28 (30.8%)	24 (27.3%)	52 (29.1%)	133 (36.4%)		185 (34.0%)
Baseline PaO_2/FiO_2					<.001	
Median (IQR)	199 (123, 262)	145 (102, 229)	169 (106, 246)	277 (191, 345)		239 (139, 306)
SOFA Score					<.001	
Median (IQR)	2 (1, 3)	3 (2, 4)	3 (2, 4)	2 (0, 3)		2 (1, 4)
Duration of Symptoms					0.003	
Median (IQR)	8 (5, 10)	4 (3, 8)	7 (4, 10)	5 (2, 9)		6 (3, 9)
Follow-up, days					<.001	
Median (IQR)	12 (6, 17)	13 (7, 18)	12 (6, 17)	8 (4, 14)		9 (4, 15)
Events, n(%)						
Mechanical ventilation	17 (18.7%)	16 (18.2%)	33 (18.4%)	57 (15.6%)	0.406	90 (16.5%)
Death	7 (7.7%)	6 (6.8%)	13 (7.3%)	73 (20.0%)	<.001	86 (15.8%)

*Chi-square or Kruskal-Wallis test as appropriate

Table 2a. Signs and symptoms and baseline comorbidities (Modena centre only)

Characteristics	Treatment started				
	Tocilizumab subcutaneous	**Tocilizumab intravenous**	**SoC**	**p-value***	**Total**
	N= 84	N= 48	N= 222		N= 354
Age, years				0.357	
Median (IQR)	67 (56, 73)	61 (52, 74)	67 (55, 78)		66 (55, 76)
Gender, n(%)				0.996	
Female	26 (31.0%)	15 (31.3%)	68 (30.6%)		109 (30.8%)
Any comorbidity, n(%)				<.001	
Yes	39 (46.4%)	24 (50.0%)	36 (16.2%)		99 (28.0%)
Comorbidities, n(%)					
Diabetes	11 (13.1%)	6 (12.5%)	7 (3.2%)	0.002	24 (6.8%)
Hypertension	37 (44.0%)	22 (45.8%)	30 (13.5%)	<.001	89 (25.1%)
Cardiovascular Disease	9 (10.7%)	6 (12.5%)	12 (5.4%)	0.117	27 (7.6%)
Chronic Renal Insufficiency	2 (2.4%)	5 (10.4%)	7 (3.2%)	0.045	14 (4.0%)
Cancer	2 (2.4%)	0 (0.0%)	8 (3.6%)	0.379	10 (2.8%)
Disease Duration					
Days from symptoms onset to hospitalisation, median(IQR)	8 (6, 11)	5 (3, 9)	5 (2, 9)	0.002	7 (3, 10)
Days from hospitalisation to intubation, median(IQR)	3 (0, 5)	2 (1, 4)	2 (0, 3)	0.493	2 (0, 4)
Sign and symptoms, n(%)					
Fever, median(IQR)	37 (36, 38)	37 (36, 38)	37 (36, 37)	0.541	37 (36, 37)
Cough	42 (50.0%)	20 (41.7%)	55 (24.8%)	<.001	117 (33.1%)
Myalgia	5 (6.0%)	5 (10.4%)	7 (3.2%)	0.088	17 (4.8%)
Sputum	5 (6.0%)	0 (0.0%)	4 (1.8%)	0.059	9 (2.5%)
Headache	5 (6.0%)	7 (14.6%)	10 (4.5%)	0.032	22 (6.2%)
Haemptysis	0 (0.0%)	1 (2.1%)	2 (0.9%)	0.451	3 (0.8%)
Systolic pressure, mmHg median(IQR)	130 (118, 138)	120 (110, 135)	124 (110, 140)	0.361	125 (110, 138)

*Chi-square or Kruskal-Wallis test as appropriate

Table 2b. Baseline blood count and biochemical parameters (Modena centre only).

Blood tests	Treatment started				
	Tocilizumab subcutaneous	**Tocilizumab intravenous**	**SoC**	**p-value***	**Total**
	N= 78	N= 47	N= 179		N= 304
Markers, Median (IQR)					
Haemoglobin, g/l	12.8 (11.5, 13.7)	13.0 (11.6, 13.7)	12.7 (11.2, 14.2)	0.966	12.7 (11.4, 14.0)
White cells, mm3	7195 (5470, 10380)	6840 (5140, 9380)	6200 (4570, 9360)	0.224	6700 (4890, 9560)
Lymphocytes, %	22.1 (9.7, 36.8)	18.1 (12.5, 25.8)	23.1 (9.9, 39.7)	0.598	20.6 (9.9, 36.6)
Total lymphocytes, mm3	1580 (1390, 2142)	2459 (1852, 3348)	1390 (1000, 2815)	0.297	1852 (1120, 2726)
Platelets, 10^9/l	257.5 (183.0, 374.0)	211.0 (156.0, 294.0)	209.0 (155.0, 298.0)	0.003	221.5 (163.0, 317.0)
Alanine amino-transferase, U/l	37.0 (27.0, 71.0)	35.0 (21.5, 62.5)	31.0 (19.0, 48.0)	0.007	33.0 (22.0, 56.0)
Bilirubin, mg/l	0.6 (0.4, 0.7)	0.6 (0.4, 0.8)	0.6 (0.4, 0.8)	0.735	0.6 (0.4, 0.8)
Calcium, mg/l	8.6 (8.4, 9.1)	8.5 (8.1, 8.9)	8.6 (8.3, 9.1)	0.264	8.6 (8.3, 9.1)
Creatine Kinase, U/l	63.0 (33.0, 159.0)	130.0 (41.5, 312.0)	71.0 (39.0, 202.0)	0.053	76.0 (38.0, 197.5)
Chloride, mmol/l	101.0 (98.0, 105.0)	100.0 (99.0, 103.0)	101.0 (97.0, 103.0)	0.906	101.0 (98.0, 103.0)
Creatinine, mg/l	0.8 (0.6, 0.9)	0.9 (0.7, 1.2)	0.9 (0.7, 1.1)	0.005	0.8 (0.7, 1.1)
D-dimer, mg/l	1210 (820.0, 2290)	1000 (780.0, 2730)	1240 (610.0, 2480)	0.636	1200 (690.0, 2480)
Lactate dehydrogenase, U/l	600.0 (505.0, 761.0)	676.0 (536.0, 765.0)	507.5 (419.5, 705.5)	<.001	564.0 (454.0, 745.0)
Potassium, mmol/l	3.9 (3.5, 4.3)	3.8 (3.6, 4.0)	3.9 (3.5, 4.3)	0.338	3.9 (3.5, 4.2)
Sodium, mmol/l	137.5 (136.0, 139.0)	137.0 (135.0, 138.0)	138.0 (135.0, 141.0)	0.019	138.0 (135.0, 140.0)
Ferritine, mg/l	1168 (543, 1214)	-	423 (355, 993)	0.135	447 (355, 1141)
C-reactive protein, mg/l	3.4 (0.6, 7.8)	6.1 (1.8, 15.3)	5.4 (1.8, 14.6)	0.022	5.3 (1.4, 13.6)
IL-6, mg/l	190.2 (86.6, 401.0)	238.3 (140.2, 731.9)	144.1 (41.1, 385.8)	0.045	178.6 (67.6, 402.0)

*Kruskal-Wallis test

Table 3 Hazard ratio from fitting a Cox regression model

	Unadjusted and adjusted relative hazards of mechanical ventilation/death[&]					
	Unadjusted HR (95% CI)	**p-value**	**Adjusted[*] HR (95% CI)**	**p-value**	**Adjusted[**] HR (95% CI)**	**p-value**
	Overall - 2 arms contrasts					
SoC	1		1		1	
Tocilizumab (any)	0.60 (0.43, 0.84)	0.003	0.64 (0.45, 0.91)	0.012	0.61 (0.40, 0.92)	0.020
SoC	1				1	
Tocilizumab	0.54 (0.37, 0.78)	<.001			0.53[$] (0.31, 0.89)	0.016
	Stratum baseline PaO_2/FiO_2 below 150 - 2 arms contrasts					
SoC	1		1		1	
Tocilizumab (any)	0.30 (0.17, 0.52)		0.20 (0.11, 0.36)		0.19 (0.08, 0.44)	
Interaction p-value						0.011
	Stratum baseline PaO_2/FiO_2 above 150 - 2 arms contrasts					
SoC	1		1		1	
Tocilizumab (any)	0.31 (0.16, 0.59)		0.39 (0.20, 0.77)		0.46 (0.21, 0.99)	
	Overall 3 arms contrasts					
SoC	1		1		1	
Tocilizumab subcutaneous	0.63 (0.41, 0.97)	0.036	0.69 (0.44, 1.08)	0.102	0.65 (0.39, 1.11)	0.114
Tocilizumab intravenous	0.57 (0.36, 0.90)	0.016	0.60 (0.38, 0.95)	0.030	0.55 (0.31, 0.98)	0.042
Tocilizumab endovenous	1		1		1	
Tocilizumab subcutaneous	1.19 (0.40, 3.55)	0.751	1.08 (0.36, 3.25)	0.886	1.50 (0.36, 6.24)	0.578
SOC	3.95 (1.72, 9.09)	0.001	2.89 (1.25, 6.70)	0.013	3.40 (1.01, 11.44)	0.048

[*]Adjusted for age, gender and recruiting centre
[**]Adjusted for age, gender, recruiting centre, duration of symptoms and SOFA score
[$]Adjusted for age, gender, recruiting centre, duration of symptoms, SOFA score, use of steroids post-baseline and censoring using IPW
[&]Initiation of invasive mechanical ventilation or death

Table 4. Hazard Ratio from fitting a Cox regression model

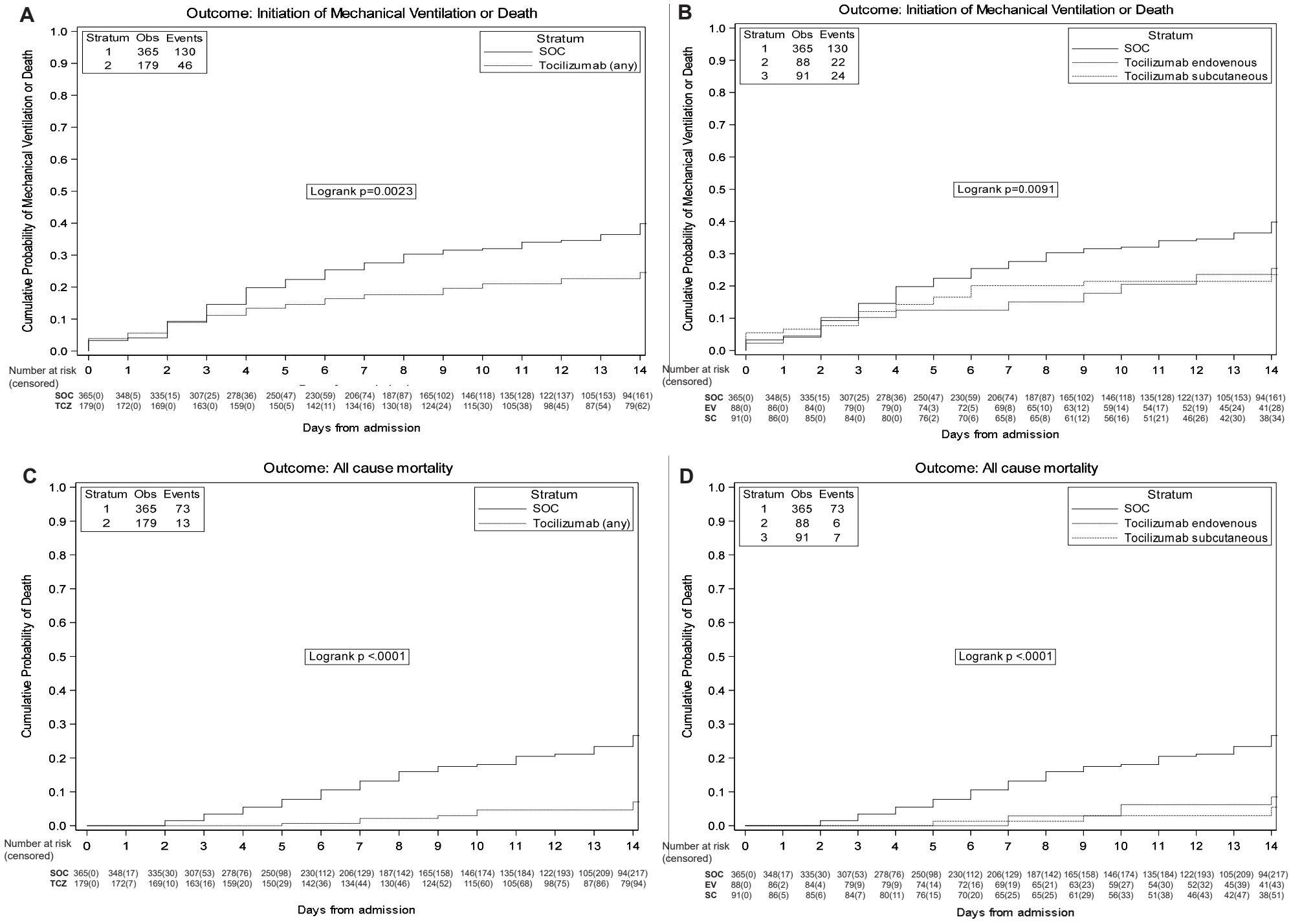
	Unadjusted and adjusted relative hazards of death[&]					
	Unadjusted HR (95% CI)	**p-value**	**Adjusted[*] HR (95% CI)**	**p-value**	**Adjusted[**] HR (95% CI)**	**p-value**
	Overall - 2 arms contrasts					
SoC	1		1		1	
Tocilizumab (any)	0.28 (0.15, 0.50)	<.001	0.36 (0.20, 0.66)	<.001	0.38 (0.17, 0.83)	0.015
	Stratum baseline PaO_2/FiO_2 below 150 - 2 arms contrasts					
SoC	1		1		1	
Tocilizumab (any)	0.11 (0.04, 0.27)		0.09 (0.03, 0.24)		0.03 (0.00, 0.24)	
Interaction p-value						0.116
	Stratum baseline PaO_2/FiO_2 above 150 - 2 arms contrasts					
SoC	1		1		1	
Tocilizumab (any)	0.22 (0.08, 0.63)		0.39 (0.12, 1.20)		0.44 (0.11, 1.73)	
	Overall 3 arms contrasts					
SoC	1		1		1	
Tocilizumab subcutaneous	0.30 (0.14, 0.66)	0.003	0.37 (0.17, 0.83)	0.016	0.44 (0.17, 1.14)	0.091
Tocilizumab intravenous	0.25 (0.11, 0.58)	0.001	0.35 (0.15, 0.80)	0.013	0.29 (0.09, 0.99)	0.048

[*]Adjusted for age, gender and recruiting centre

[**]Adjusted for age, gender, recruiting centre, duration of symptoms and total SOFA Score

[&]All-cause mortality

Figure 1


A
Outcome: Initiation of Mechanical Ventilation or Death
Stratum Obs Events
1 365 130
2 179 46
Stratum
SOC
Tocilizumab (any)
Logrank p=0.0023
Cumulative Probability of Mechanical Ventilation or Death
Number at risk (censored)
SOC 365(0) 348(5) 335(15) 307(25) 278(36) 250(47) 230(59) 206(74) 187(87) 165(102) 146(118) 135(128) 122(137) 105(153) 94(161)
TCZ 179(0) 172(0) 169(0) 163(0) 159(0) 150(5) 142(11) 134(16) 130(18) 124(24) 115(30) 105(38) 98(45) 87(54) 79(62)
Days from admission
B
Outcome: Initiation of Mechanical Ventilation or Death
Stratum Obs Events
1 365 130
2 88 22
3 91 24
Stratum
SOC
Tocilizumab endovenous
Tocilizumab subcutaneous
Logrank p=0.0091
Cumulative Probability of Mechanical Ventilation or Death
Number at risk (censored)
SOC 365(0) 348(5) 335(15) 307(25) 278(36) 250(47) 230(59) 206(74) 187(87) 165(102) 146(118) 135(128) 122(137) 105(153) 94(161)
EV 88(0) 86(0) 84(0) 79(0) 79(0) 74(3) 72(5) 69(8) 65(10) 63(12) 59(14) 54(17) 52(19) 45(24) 41(28)
SC 91(0) 86(0) 85(0) 84(0) 80(0) 76(2) 70(6) 65(8) 65(8) 61(12) 56(16) 51(21) 46(26) 42(30) 38(34)
Days from admission
C
Outcome: All cause mortality
Stratum Obs Events
1 365 73
2 179 13
Stratum
SOC
Tocilizumab (any)
Logrank p <.0001
Cumulative Probability of Death
Number at risk (censored)
SOC 365(0) 348(17) 335(30) 307(53) 278(76) 250(98) 230(112) 206(129) 187(142) 165(158) 146(174) 135(184) 122(193) 105(209) 94(217)
TCZ 179(0) 172(7) 169(10) 163(16) 159(20) 150(29) 142(36) 134(44) 130(46) 124(52) 115(60) 105(68) 98(75) 87(86) 79(94)
Days from admission
D
Outcome: All cause mortality
Stratum Obs Events
1 365 73
2 88 6
3 91 7
Stratum
SOC
Tocilizumab endovenous
Tocilizumab subcutaneous
Logrank p <.0001
Cumulative Probability of Death
Number at risk (censored)
SOC 365(0) 348(17) 335(30) 307(53) 278(76) 250(98) 230(112) 206(129) 187(142) 165(158) 146(174) 135(184) 122(193) 105(209) 94(217)
EV 88(0) 86(2) 84(4) 79(9) 79(9) 74(14) 72(16) 69(19) 65(21) 63(23) 59(27) 54(30) 52(32) 45(39) 41(43)
SC 91(0) 86(5) 85(6) 84(7) 80(11) 76(15) 70(20) 65(25) 65(25) 61(29) 56(33) 51(38) 46(43) 42(47) 38(51)
Days from admission

Supplementary Table 1a. Key baseline factors by cohort

Characteristics	Recruiting center				
	Modena	**Bologna**	**Reggio Emilia**	**p-value***	**Total**
	N= 354	N= 80	N= 110		N= 544
Age, years				0.653	
Median (IQR)	66 (55, 76)	68 (56, 78)	68 (59, 77)		67 (56, 77)
Gender, n(%)				<.001	
Female	109 (30.8%)	44 (55.0%)	32 (29.1%)		185 (34.0%)
Baseline PaO_2/FiO_2 Median (IQR)	227 (126, 289)	255 (213, 310)	255 (155, 340)	0.006	239 (139, 306)
SOFA Score Median (IQR)	2 (0, 3)	2 (2, 3)	3 (2, 4)	<.001	2 (1, 4)
Follow-up, days Median (IQR)	9 (4, 15)	12 (6, 18)	8 (4, 14)	0.039	9 (4, 15)
Intervention, n(%)				<.001	
Tocilizumab subcutaneous	84 (23.7%)	4 (5.0%)	3 (2.7%)		91 (16.7%)
Tocilizumab intravenous	48 (13.6%)	24 (30.0%)	16 (14.5%)		88 (16.2%)
SoC	222 (62.7%)	52 (65.0%)	91 (82.7%)		365 (67.1%)
Events, n(%)					
Mechanical ventilation	54 (15.3%)	9 (11.3%)	27 (24.5%)	0.028	90 (16.5%)
Death	52 (14.7%)	16 (20.0%)	18 (16.4%)	0.494	86 (15.8%)

*Chi-square or Kruskal-Wallis test as appropriate

Supplementary Table 1b. Key baseline factors by intervention and cohort

Characteristics	Intervention				
	Tocilizumab subcutaneous	**Tocilizumab intravenous**	**SOC**	**p-value***	**Total**
	N= 91	N= 88	N= 365		N= 544
Age, years				0.017	
Median (IQR)	67 (55, 73)	63 (54, 72)	69 (57, 78)		67 (56, 77)
Gender, n(%)				0.206	
Female	28 (30.8%)	24 (27.3%)	133 (36.4%)		185 (34.0%)
Baseline PaO_2/FiO_2 Median (IQR)	199 (123, 262)	145 (102, 229)	277 (191, 345)	<.001	239 (139, 306)
SOFA Score Median (IQR)	2 (1, 3)	3 (2, 4)	2 (0, 3)	<.001	2 (1, 4)
Follow-up, days Median (IQR)	12 (6, 17)	13 (7, 18)	8 (4, 14)	<.001	9 (4, 15)
Events, n (%)					
Mechanical ventilation	17 (18.7%)	16 (18.2%)	57 (15.6%)	0.705	90 (16.5%)
Death	7 (7.7%)	6 (6.8%)	73 (20.0%)	<.001	86 (15.8%)

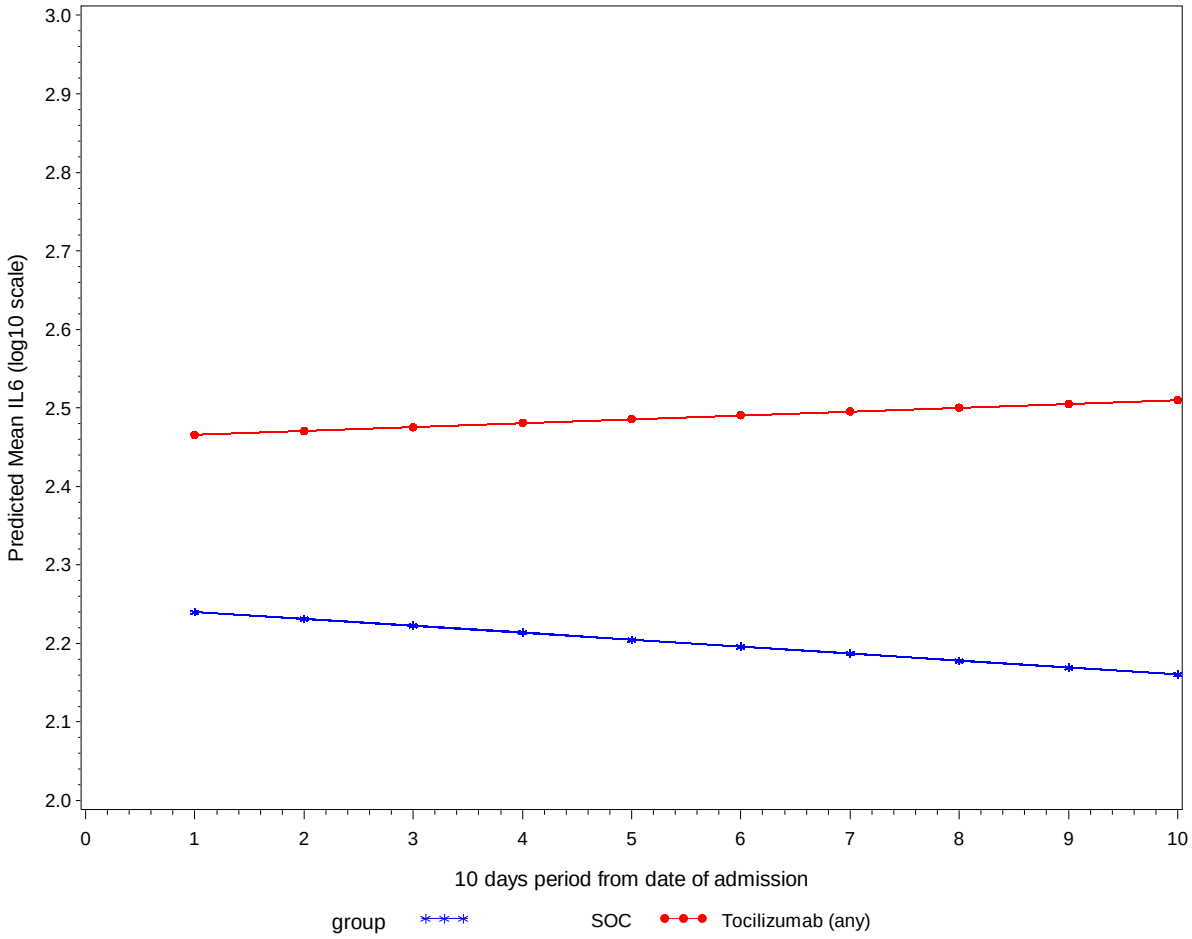
*Chi-square or Kruskal-Wallis test as appropriate

Supplementary Table 2. Means IL-6 values (log10 scale) from fitting a mixed linear model

	Estimates from the mixed model (Mean values 95% CI) - log10 scale					
	Baseline IL6	**Difference in baseline**	**IL6 change/year**	**Difference in change/year**	**Adjusted* IL6 change/year**	**Adjusted* difference in change/year**
Treatment						
Tocilizumab (any)	2.46 (2.35, 2.57)		0.00 (-0.00, 0.01)		0.00 (-0.00, 0.01)	
SoC	2.25 (2.11, 2.39)	-0.21 (-0.39, -0.03) 0.020	-0.01 (-0.02, -0.00)	-0.01 (-0.02, -0.00) 0.006	-0.01 (-0.02, -0.00)	-0.02 (-0.03, -0.00) 0.004

*Adjusted for age, gender and SOFA Score

Supplementary Figure 1. Means IL-6 values (log10 scale) from fitting a mixed linear model

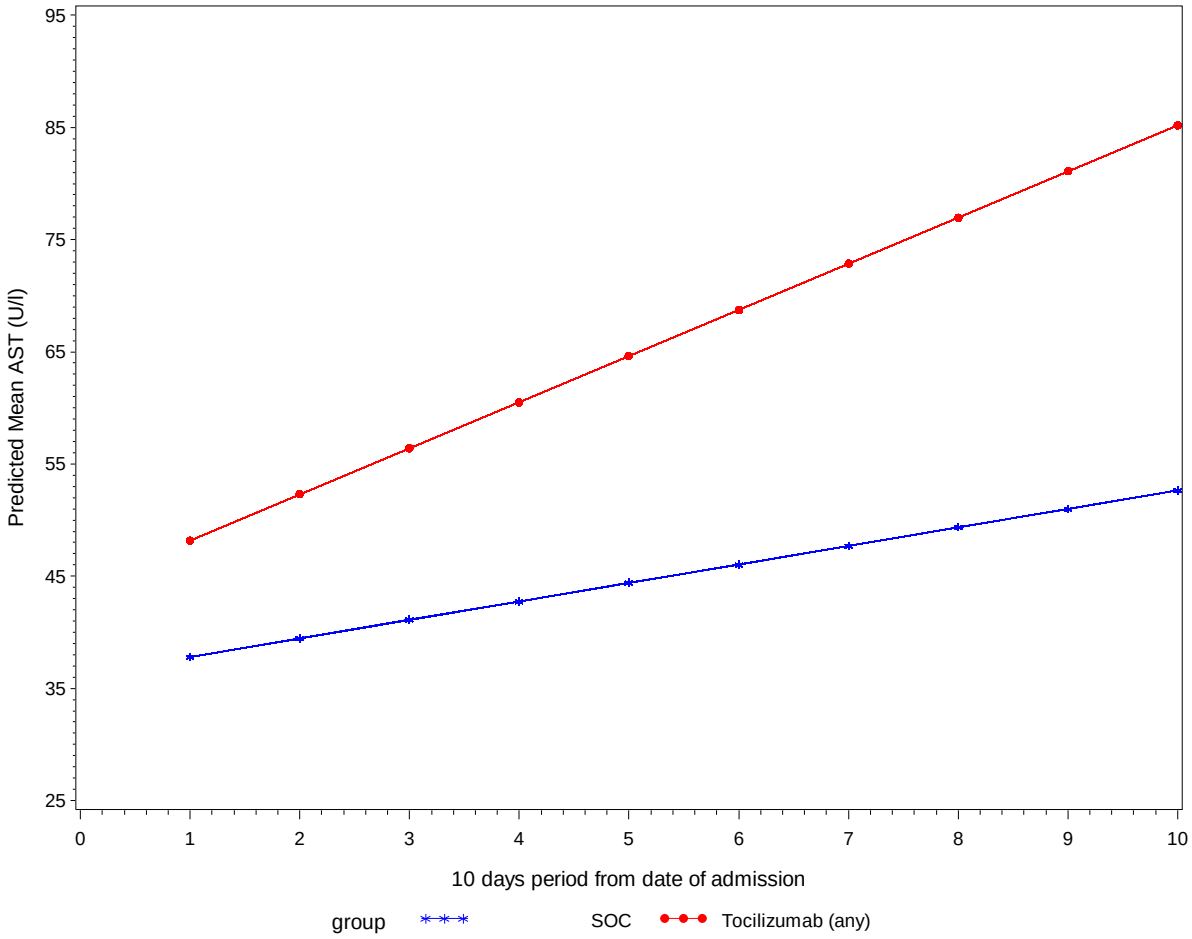


Supplementary Table 3. Means of aspartate transaminase (AST) values from fitting a mixed linear model

	Estimates from the mixed model (Mean values 95% CI)					
	Baseline AST	**Difference in baseline**	**AST change/year**	**Difference in change/year**	**Adjusted* AST change/year**	**Adjusted* difference in change/year**
Treatment						
Tocilizumab (any)	44.05 (26.60, 61.51)		4.12 (0.97, 7.27)		4.12 (0.88, 7.36)	
SoC	1.48 (1.43, 1.53)	-7.88 (-30.8, 15.07) 0.501	0.00 (0.00, 0.01)	-2.47 (-6.57, 1.63) 0.237	0.01 (0.00, 0.01)	-2.26 (-6.57, 2.04) 0.302

*Adjusted for age, gender and SOFA Score

Supplementary Figure 2. Means AST values from fitting a mixed linear model



Supplementary table 4. Kaplan-Meier estimates reported in text of Results section

	Kaplan-Meier estimates of probability of outcomes			
	No. events by 7 days	**Day 7 percent (95% CI)**	**No. events by 14 days**	**Day 14 percent (95% CI)**
Endpoints - Overall				
Mechanical ventilation	84	16.4 (13.2, 19.6)	90	18.8 (15.1, 22.5)
Composite	129	25.7 (21.9, 29.6)	159	36.1 (31.2, 40.9)
Death	45	11.0 (7.9, 14.0)	69	21.1 (16.3, 25.8)
Endpoints - Stratified				
Mechanical ventilation -SOC	98	30.3 (25.2, 35.4)	117	41.5 (35.1, 47.9)
Mechanical ventilation -TCZ	34	19.6 (13.7, 25.5)	42	27.0 (19.6, 34.4)
Death -SOC	42	16.0 (11.5, 20.5)	60	28.7 (21.9, 35.4)
Death -TCZ	4	2.9 (0.1, 5.8)	9	8.5 (3.0, 14.1)