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Interleukin-6 receptor blocking with intravenous tocilizumab in COVID-19 severe acute respiratory distress syndrome: A retrospective case-control survival analysis of 128 patients

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ABSTRACT

In cases of COVID-19 acute respiratory distress syndrome, an excessive host inflammatory response has been reported, with elevated serum interleukin-6 levels. In this multicenter retrospective cohort study we included adult patients with COVID-19, need of respiratory support, and elevated C-reactive protein who received intravenous tocilizumab in addition to standard of care. Control patients not receiving tocilizumab were matched for sex, age and respiratory support. We selected survival as the primary endpoint, along with need for invasive ventilation, thrombosis, hemorrhage, and infections as secondary endpoints at 30 days. We included 64 patients with COVID-19 in the tocilizumab group and 64 matched controls. At baseline the tocilizumab group had longer symptom duration (13 ± 5 vs. 9 ± 5 days) and received hydroxychloroquine more often than controls (100% vs. 81%). The mortality rate was similar between groups (27% with tocilizumab vs. 38%) and at multivariable analysis risk of death was not significantly influenced by tocilizumab (hazard ratio 0.61, 95% confidence interval 0.33–1.15), while being associated with the use at baseline of non invasive mechanical or invasive ventilation, and the presence of comorbidities. Among secondary outcomes, tocilizumab was associated with a lower probability of requiring invasive ventilation (hazard ratio 0.36, 95% confidence interval 0.16–0.83; P = 0.017) but not with the risk of thrombosis, bleeding, or infections. The use of intravenous tocilizumab was not associated with changes in 30-day mortality in patients with COVID-19 severe respiratory impairment. Among the secondary outcomes there was less use of invasive ventilation in the tocilizumab group.

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1. Introduction

As of May 10th, 2020 over 4 million cases of SARS-coronavirus-2 disease (COVID-19) have been reported worldwide with variable mortality rates and 218.000 cases in Italy, one of the most impacted Countries [1]. Current treatments proposed for COVID-19 are merely supportive as the main cause of death is the development of severe acute respiratory distress syndrome (ARDS) [2] with biochemical features resembling macrophage activation syndrome. These include the progressive increase in C reactive protein, ferritin, and D-dimer levels with reduced lymphocyte count [3]. Furthermore, increased levels of inflammatory cytokines, such as IL-1β, IL-6, GM-CSF, and TNF-α have been reported in the peripheral blood of hospitalized patients with COVID-19, with higher levels in those admitted to Intensive Care Units and an association between IL-6 levels and the probability of survival [4,5], as confirmed by a recent meta-analysis [6]. Previous reports suggested a beneficial effect of tocilizumab (TCZ), a humanized monoclonal antibody directed at the IL-6 receptor, in COVID-19 [7–10]. Similarly, proposed agents to treat COVID-19 include glucocorticoids [11], hydroxychloroquine [12], anakinra [13,14], baricitinib [15], and direct antivirals [16], with negative or inconclusive results reported to date largely from uncontrolled studies.

We report herein data from our retrospective study of death and other 30-day clinical outcomes in 128 patients treated for COVID-19-related ARDS and hyperinflammation with or without intravenous tocilizumab.

2. Methods

2.1. Setting

We conducted this study in two affiliated general hospitals in the Lombardy region of Northern Italy; these hospitals are located in two of the highest-impact cities (Milan and Bergamo) in Northern Italy, where COVID-19 was most incident. In both cases, hospitals were completely transformed over a two-week period, and wards fully dedicated to the care of patients with COVID-19 pneumonia [17]. Between February 23rd and May 9th, 2020, the two hospitals admitted over 2000 patients with such infection. In both hospitals, local recommendations were issued and updated regularly by a dedicated clinical and scientific committee. According to such recommendations, (i) all admitted patients were treated with subcutaneous enoxaparin at prophylactic dosage, (ii) direct antivirals including first lopinavir 400 mg + ritonavir 100 mg twice daily and subsequently, when this became unavailable, darunavir 800 mg + cobicistat 150 mg once a day were used in patients without contraindications, (iii) hydroxychloroquine 200 mg twice a day was used in all patients who could take oral treatments and had no contraindications (i.e. prolonged QT interval, retinopathies, advanced renal failure, known hypersensitivity). The same committee provided the criteria that candidates had to fulfill to be eligible for tocilizumab: (i) clinical worsening in the previous 24 h with increasing need for oxygen or ventilatory support, (ii) absence of clinical or biochemical signs of an active bacterial infection, (iii) elevated C reactive protein, (iv) a higher risk for mortality at blood tests, based on the odds ratios reported elsewhere [18] and including lymphocyte count, ferritin, creatine kinase, alanine aminotransferase, and D-dimer. Late intubation (over 24 h) was considered an exclusion criterion. The process of eligibility evaluation lasted a maximum of 2 days. Of major relevance for the design of this study, not all patients fulfilling these criteria could be treated based on the insufficient availability of the drug during the weeks of highest demand.

2.2. Data sources

Demographic, clinical, imaging, and laboratory data were obtained from the Humanitas clinical data warehouse which includes the information available on all inpatient admitted at Istituto Clinico Humanitas (Rozzano, Milan) or Cliniche Humanitas Gavazzeni (Bergamo). The extracted data included the patient demographic details, hourly vital signs, laboratory test results, medication administration dose and frequency, oxygen support needed, and outcome. The presence of comorbidities was manually abstracted from the electronic medical record or charts.

For each of the 64 patients treated with tocilizumab an extraction based on age, sex, and enrolling center led to 2–5 potential controls which had a proven COVID-19 pneumonia and were subsequently matched according to the respiratory support, as well as the presence or absence of exclusion criteria. To allow a uniform matching for all tocilizumab-treated patients, a 1:1 control:case ratio was ultimately chosen leading to the identification of 64 patients in the control group. As mentioned, these patients would have also been treated with tocilizumab if the drug had been widely available.

2.3. Variables assessed

Using the approach described above, we obtained for each patient at the time of tocilizumab administration or control matching: age, sex, daily recorded vital signs, the daily partial pressure of arterial oxygen to the fraction of inspired oxygen (PaO2:FiO2), estimated with the use of methods described elsewhere [19,20], required oxygen support which was grouped into low-medium flows (nasal canulae and facial masks with or without reservoir), high flows (non-invasive mechanical ventilation - NIMV) and invasive ventilation in the ICU, body mass index and smoking status, past and current diagnoses that were evaluated singularly and grouped into the Charlson comorbidity index [21], and laboratory tests.

2.4. Exposure to tocilizumab and other treatments

After providing written informed consent for the off-label use of tocilizumab, patients in the tocilizumab group received one intravenous infusion of 8 mg/kg tocilizumab, followed by a second dose 24 h later if no clinical worsening had occurred between infusions; 61/64 (95%) patients in the tocilizumab group received 2 infusions. The patients treated with tocilizumab were also included in the comparator arm of a National trial (ClinicalTrials.gov Identifier: NCT04317092), as for other previously reported series [8,9]. Other medications included hydroxychloroquine, direct antivirals (lopinavir and ritonavir, darunavir and cobicistat), antibiotics (ceftriaxone, azithromycin, piperacillin and tazobactam), glucocorticoids (IV methylprednisolone 1–2 mg/kg/day), prophylactic enoxaparin were used based on the internal guidelines.

2.5. Endpoints

The primary endpoint of the study was to compare mortality rates between the tocilizumab and control groups. Secondary endpoints included differences between groups in the incidence of invasive ventilation (only for patients who were not already being invasively ventilated at baseline), thromboembolic events (including pulmonary embolism and deep vein thrombosis), hemorrhagic events (defined as bleeding with the need for a therapeutic or diagnostic measure), and bacterial or fungal infection (defined as clinical evidence or serum procalcitonin >1 ng/ml).

2.6. Statistical analysis

Descriptive statistics included categorical variables reported as number (percentage) and continuous variables as mean (standard deviation). For missing data, we used listwise deletion for univariable and multivariable analysis. Chi-square test and Mann-Whitney test were used accordingly to the type of variable. We used time-to-event (survival) methods for censored observations to identify risk factors

associated with different outcomes. Time of exposure was defined as time from first day of tocilizumab infusion (for treatment group) or first day of clinical worsening (for control group) to the day of event. For the primary outcome, the event was death; for secondary outcome, the events were intubation, thrombosis, clinically relevant bleeding, bacterial or fungal infection. The only cause of right censoring was the transfer to a different hospital; in case of discharge from hospital due to clinical recovery, we implied no more events for the remaining time of exposure.

Kaplan–Meier estimates were used to draw the cumulative incidence curves, compared by log-rank tests. Furthermore, we utilized the univariable and multivariable Cox proportional hazard model of relevant prognostic factors for the primary outcome.

For multivariable survival analysis, variables were selected if the rate of missing values was very low (<5%) and proved significant in the univariable Cox analysis (P value < 0.1). After fitting the model, the PH assumption was examined on the basis of Schoenfeld residuals. Hazard ratios (HR) were presented with the 95% confidence intervals (CI) and respective P values. Adverse events were analyzed by treatment group and in survival analysis. P values lower than 0.05 were considered statistically significant; data were analyzed with STATA version 16.0 (StataCorp LLC, College Station, TX).

3. Results

3.1. Characteristics of the cohort

Between March 15 and April 22, 2020, 64 patients were treated with tocilizumab at the two participating hospitals (52 at Humanitas Gavazzeni Bergamo and 12 at Humanitas Milan). For each patient in the tocilizumab group, one patient was extracted from the data warehouse of the same hospital to compose the control group of 64 patients (52 at Humanitas Gavazzeni Bergamo and 12 at Humanitas Milan). Table 1 and Table 2 illustrate the clinical and laboratory features of the total study population and the tocilizumab and control groups. Of note, patients in the tocilizumab group reported a longer duration of symptoms at the time of infusion compared to controls at the time of matching (13 ± 5 vs. 9 ± 5 days, P < 0.001). Other clinically relevant differences, albeit not statistically significant, included the Charlson comorbidity index (2.2 ± 1.4 vs. 2.7 ± 1.7, P = 0.176) and the markedly reduced PaO2:FiO2 (104 ± 76 vs. 85 ± 54 in the control group, P = 0.057). The majority of patients in the study groups received hydroxychloroquine as indicated by the local recommendations and this was more frequent in the tocilizumab group (100% vs. 81% in the control group; P < 0.001). No differences were observed between groups in the use of glucocorticoids, direct antivirals or antibiotics.

Hemoglobin was significantly lower in the tocilizumab group, but mean values remained above 12 g/dl in both groups; platelet and lymphocyte counts did not differ between groups. Laboratory tests at baseline demonstrated higher serum aspartate aminotransferase (AST), creatine kinase (CK), and creatinine levels in the control group without reaching statistical significance.

3.2. Study endpoints

We performed univariable and multivariable analyses using death as the primary endpoint and HR with 95% CI observed for candidate variables are illustrated in Table 3.

In the univariable analysis, patient mortality was significantly associated with age (HR 1.06, 95% CI 1.01–1.10; P = 0.004 per additional year) but not male sex or a positive tobacco smoking history, either past or active, compared to non smokers. The Charlson comorbidity index significantly correlated with mortality (HR 1.42, 95% CI 1.20–1.67; P < 0.001 per additional point). Mortality was significantly influenced by a 1% increase in baseline oxygen saturation (HR 0.92, 95% CI 0.85–0.99; P = 0.22). The use of non invasive mechanical ventilation (HR 4.94, 95% CI 1.49–16.35; P = 0.009) and invasive ventilation (HR 8.14, 95% CI 2.31–28.7; P = 0.001) compared to a reservoir mask at baseline but not hydroxychloroquine, direct antivirals, glucocorticoids, or azithromycin were significantly associated with mortality. Among biochemical indices, higher aspartate aminotransferase (per 25 U/mL increase), d-dimer (per 1000 ng/mL increase), creatinine (per 1 mg/dl increase), and CRP levels directly correlated with mortality.

The use of tocilizumab and variables obtained from the univariable comparisons were included in the multivariable analysis which demonstrated that death was significantly associated with the Charlson comorbidity index (HR 1.46, 95% CI 1.21–1.76; P < 0.001), the need use of non invasive mechanical ventilation (HR 3.59, 95% CI 1.06–12.01; P = 0.039) and invasive ventilation (HR 9.50, 95% CI 2.67–33.85; P = 0.001) compared to a reservoir mask. The use of tocilizumab was not associated with a different risk of death over 30 days, as shown by the Kaplan-Meier curves in Fig. 1. As we observed that mortality at 5 days was equal in the two groups, and noticing that the effect of tocilizumab surged after some days (i.e. the nadir of CRP), we performed a *post hoc* analysis to compare death occurring between days 6 and 30 and observed a significant difference between groups, with a HR of 0.41 associated with tocilizumab (95% CI 0.17–0.96, P = 0.039).

Clinically relevant secondary outcomes, including the need for

Table 1
Demographic and clinical baseline characteristics of patients. Continuous variables are expressed as mean (standard deviation).

	Total (N = 128)	Tocilizumab (n = 64)	Controls (n = 64)	*P value*
Men (n)	94 (73%)	47 (73%)	47 (73%)	*1*
Age (years)	63 (10)	63 (12)	64 (8)	*0.993*
Time since symptom onset (days)	11 (6)	13 (5)	9 (5)	*<0.0001*
BMI (kg/m^2)	30 (6)	30 (6)	30 (7)	*0.682*
Smoking				*0.130*
- None	76 (74%)	33 (67%)	43 (80%)	
- Past	21 (20%)	14 (29%)	7 (13%)	
- Active	6 (6%)	2 (4%)	4 (7%)	
Charlson comorbidity index	2.4 (1.6)	2.2 (1.4)	2.7 (1.7)	*0.176*
Arterial hypertension	66 (52%)	33 (52%)	33 (52%)	*1*
Tympanic temperature (°C)	37.1 (0.6)	37.0 (0.56)	37.2 (0.6)	*0.144*
Respiratory function				
SpO2 (%)	93 (4)	93 (4)	93 (5)	*0.464*
PaO2:FiO2	94 (67)	104 (76)	85 (54)	*0.057*
Chest CT (n)				*0.924*
Normal	8/78 (10%)	3/35 (9%)	5/43 (12%)	
ground glass	35/78 (45%)	16/35 (46%)	19/43 (44%)	
consolidation	27/78 (5%)	13/35 (37%)	14/43 (33%)	
bilateral infiltrates	8/78 (10%)	3/35 (9%)	5/43 (12%)	
Respiratory support				*1*
Low-medium oxygen flow	10 (8%)	5 (8%)	5 (8%)	
High oxygen flow	92 (72%)	46 (72%)	46 (72%)	
Invasive intubation	26 (20%)	13 (20%)	13 (20%)	
Concomitant treatments				
Hydroxychloroquine	113 (90%)	63 (98%)	50 (81%)	*<0.001*
Direct antivirals[a]	103 (80%)	55 (86%)	48 (75%)	*0.119*
Glucocorticoids	57 (45%)	31 (48%)	26 (40%)	*0.374*
Ceftriaxone	85 (67%)	40 (63%)	45 (70%)	*0.414*
Azitromycin	81 (41%)	24 (38%)	28 (44%)	*0.517*
Piperacillin/tazobactam	56 (44%)	29 (46%)	27 (43%)	*0.720*

[a] Direct antivirals include lopinavir/ritonavir or darunavir/cobicistat.

Table 2
Biochemical baseline characteristics of patients Continuous variables are expressed as mean ± standard deviation. Upper limit of normal, ULN.

	Total (N = 128)	Tocilizumab (n = 64)	Controls (n = 64)	*P value*
Alanine aminotransferase (*IU/l*) (ULN 51)	64 (60)	60 (46)	68 (75)	*0.91*
Aspartate aminotransferase (*IU/l*) (ULN 51)	67 (70)	57 (38)	83 (99)	*0.092*
Creatine kinase (*U/ml*) (ULN 172)	178 (176)	154 (158)	217 (202)	*0.158*
Creatinine (*mg/dl*) (ULN 1.17)	0.92 (0.36)	0.85 (0.23)	1.01 (0.45)	*0.115*
D-dimer (*ng/ml*) (ULN 500)	4186 (7601)	3801 (6634)	5053 (9623)	*0.43*
Ferritin (*ng/ml*) (ULN 336)	1604 (1201)	1638 (1181)	1488 (1317)	*0.49*
Fibrinogen (*mg/dl*) (ULN 400)	624 (150)	613 (148)	662 (159)	*0.41*
Lactate dehydrogenase (*IU/l*) (ULN 248)	505 (177)	524 (188)	472 (153)	*0.36*
C reactive protein (*mg/dl*) (ULN 0.5)	19.1 (8.6)	19.8 (8.1)	18.1 (9.4)	*0.29*
Procalcitonin (*ng/ml*) (ULN 0.5)	0.34 (0.32)	0.34 (0.30)	0.31 (0.40)	*0.25*
Prothrombin time INR (ULN 1.18)	1.14 (0.14)	1.13 (0.11)	1.15 (0.17)	*0.82*
Interleukin-6 (*pg/ml*)	179 (193)	171 (175)	253 (358)	*0.94*
Hemoglobin (*g/dl*)	12.6 (1.5)	12.4 (1.2)	13.0 (1.7)	*0.022*
White blood cells (10^3/μl)	8.7 (3.1)	8.9 (3.0)	8.5 (3.1)	*0.69*
Lymphocytes (10^3/μl)	0.7 (0.4)	0.7 (0.3)	0.7 (0.4)	*0.93*
Platelets (10^3/μl)	285 (118)	301 (117)	265 (119)	*0.189*

Table 3
Univariable and multivariable survival analysis for 30-day mortality. Hazard ratios (HR) are reported with 95% confidence intervals (95% CI); in the case of continuous variables, absolute increments corresponding to the HR are specified.

	Univariable analysis		Mutivariable analysis	
	HR (95% CI)	*P value*	HR (95% CI)	*P value*
Tocilizumab (vs. controls)	0.61 (0.33–1.15)	0.129	0.82 (0.42–1.58)	0.55
Male sex (vs. female)	1.43 (0.68–2.99)	0.35		
Age (per year)	1.06 (1.01–1.10)	**0.004**		
Time since symptom onset (per day)	0.97 (0.92–1.03)	0.35		
Fever (per °C)	1.57 (0.93–2.67)	0.091		
BMI (per point)	0.97 (0.91–1.03)	0.29		
Smoke status (vs. no smoke)				
Past	0.87 (0.38–2.00)	0.74		
Active	0.40 (0.05–2.96)	0.37		
Charlson comorbidity index (per point)	1.42 (1.20–1.67)	**<0.001**	1.46 (1.21–1.76)	**<0.001**
Coronary heart disease	2.38 (0.93–6.10)	0.069		
Chronic obstructive lung disease	2.88 (0.89–9.33)	0.078		
Type II diabetes	1.77 (0.84–3.72)	0.130		
Respiratory status				
Baseline PaO_2/FiO_2 (per 25)	0.89 (0.78–1.03)	0.125		
Baseline Oxygen support *(vs. reservoir mask)*				
NIMV	4.94 (1.49–16.35)	**0.009**	3.59 (1.06–12.10)	**0.039**
Invasive ventilation	8.14 (2.31–28.7)	**0.001**	9.50 (2.67–33.85)	**0.001**
Baseline O_2 saturation (per 1%)	0.92 (0.85–0.99)	**0.022**		
Treatments				
Hydroxychloroquine	0.47 (0.20–1.13)	0.093		
Direct antiviral	0.77 (0.37–1.61)	0.49		
Systemic glucocrticoids	1.14 (0.62–2.12)	0.66		
Azithromycin	1.21 (0.66–2.25)	0.54		
Laboratory tests				
Alanine aminotransferase (per 25 IU/mL)	1.15 (0.97–1.37)	0.111		
Aspartate aminotransferase (per 25 IU/mL)	1.19 (1.05–1.35)	**0.005**		
C-reactive protein (per mg/dL)	1.05 (1.01–1.09)	**0.028**		
Creatine kinase (per 100 IU/mL)	1.12 (0.89–1.41)	0.33		
Creatinine (per mg/dl)	2.31 (1.17–4.58)	**0.016**		
D-dimer (per 1000 ng/mL)	1.05 (1.01–1.10)	**0.015**		
Fibrinogen (per 100 mg/dL)	0.74 (0.55–1.01)	0.061		
Lactate dehydrogenase (per 100 IU/L)	1.12 (0.87–1.45)	0.36		
INR (per 1.00)	3.24 (0.09–108.88)	0.51		
Lymphocytes (per 100/μl	0.32 (0.09–1.11)	0.074		
Platelets (per 10^5/μl)	0.73 (0.51–1.05)	0.091		

invasive ventilation, thrombotic events, major bleeding, and bacterial or fungal infections are illustrated in Table 4 in terms of both incidence over 30 days in the two groups and HR (95% CI) for tocilizumab vs. controls. The use of tocilizumab was associated with a lower risk to require invasive ventilation in patients who were not receiving this respiratory support at baseline (HR 0.36, 95% CI 0.16–0.83; P = 0.017) while not modifying the probability of thrombotic events, bleeding, or infections.

4. Discussion

Cases of pneumonia of unknown origin were first reported from the Hubei Province in China and later defined COVID-19 in association with the infection by SARS-coronavirus-2. Approximately 20% of cases develop severe respiratory symptoms and possibly need ventilatory support with variable mortality rates, in Italy currently estimated to be nearly 13% [1], depending on numerous individual factors [22]. Immunological alterations associated with different stages of COVID-19

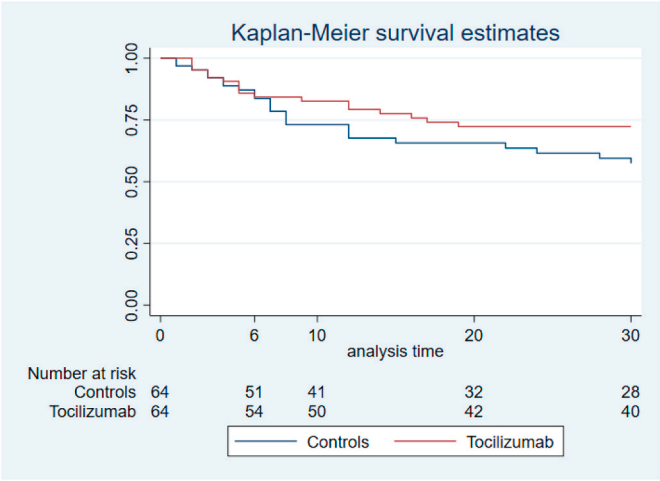

Fig. 1. Survival curves in patients with COVID-19 receiving tocilizumab (red) and those not receiving the drug (blue) are represented using Kaplan-Meier estimates. Data are censored at 30 days. Of note, after observing the CRP decrease by day 5 and the lack of differences between the two groups in the first 5 days of observation, we performed a post-hoc analysis which provided a hazard ratio for death occurring between days 6 and 30 of 0.41 (95% CI 0.17–0.96, P = 0.039) for TCZ vs controls. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

have been described since the earliest reports, which included the observed significant decrease in the lymphocyte count or the association of IL6 levels with disease outcome, suggesting the onset of a cytokine release syndrome [23,24]. SARS-coronavirus-2 in a subgroup of patients, mainly in aged subjects, trigger a vigorous inflammatory response, as represented by IL-6 levels, unbalanced by a rapid type I and III interferon, which would be crucial for virus clearance and is genetically determined [25–27]. These observations have prompted the use of available anti-rheumatic drugs, particularly tocilizumab, to treat COVID-19 [28]. Tocilizumab is a humanized monoclonal antibody directed at the interleukin-6 receptor and is widely used to treat rheumatoid arthritis and giant-cell arteritis; in the case of COVID-19, it was first used in 21 Chinese patients in critical conditions with remarkable improvements [29]. Since these first reports, IL-6 blockade strategy has been applied to treat other COVID-19 patients, including Italian patients in different areas of the Country, with conflicting results which led to a multicenter phase II clinical trial (ClinicalTrials.gov Identifier: NCT04317092) designed without a randomization and with broader inclusion criteria compared to the present study and a retrospective case-control study from Campochiaro and Colleagues failed to identify an advantage in survival [10].

Our data shows that the use of tocilizumab was not associated with a significant change the mortality rate at 30 days of patients with severe COVID-19 acute respiratory distress syndrome with hyperinflammation, after correction for pre-existing comorbidities and the need for respiratory support. These results are similar using different methods (i.e. survival analysis). Furthermore, a *post hoc* survival analysis between day 6 and day 30, following the identical mortality rates between groups until day 5 (when we also observed the nadir of CRP levels in the tocilizumab group), suggested that patients may significantly benefit from tocilizumab. We should note that these observations follow reports from single-center smaller studies with a shorter observation period and often with a limited use of controls [7–9].

We are aware of the strengths and weaknesses of the present study. Among the former, this is the first controlled study on a large number of patients with COVID-19 either receiving or potentially eligible for tocilizumab based on well-defined criteria for acute respiratory distress syndrome and signs of inflammation, with a significantly larger number of treated patients and matched controls compared to the published reports [8,9]. The matching criteria, furthermore, allowed to identify a control group which is comparable to the tocilizumab group and which could have been treated with tocilizumab if this had been widely available. Third, the study was performed in two Centers belonging to the same group, thus allowing a more uniform clinical management of patients in terms of indication for other medical treatments and ventilatory support. Fourth, the baseline differences between the two groups, which may have influenced the results, were either of negligible clinical relevance, as in the case of hemoglobin levels, or failed to prove significant in our univariable analysis, such as symptom duration. In this latter case, we should also note that patients receiving TCZ had a 2-day lag due to the delay in drug supply thus reducing the average differente in disease duration between groups.

Among weaknesses, the retrospective design of the study is predominant, but we should also be aware that the numerosity of the study population and the rigorous matching criteria allow to support our conclusions. Second, patients in the two groups differed for the use of hydroxychloroquine, but the univariable analysis demonstrated that no medical therapy correlated with mortality. Third, we may also hypothesize that the patients included in this study may have been too compromised to benefit from IL-6 modulation and we could not determine whether the limited occurrence of invasive ventilation may have been secondary to the allocation of the limited intensive care resources. It will be crucial to determine the immunological timeline of COVID-19 to ascertain the likely narrow window of opportunity for immunomodulatory treatments and achieve a more personalized treatment, as numerous authors have proposed that COVID-19 results from different immune mechanisms [30,31]. Fourth, our study did not account for potential genetic factors pointing at a different response to tocilizumab [27,32] despite the most recent genome-wide association data not yielding suggestive results [33], nor investigated a broader spectrum of immune activation markers or cytokines [34].

In conclusion, our data from a retrospective controlled study support that tocilizumab is not effective in modifying the 30-day mortality of patients with severe and critical COVID-19 with hyperinflammation while survival is significantly linked to comorbidities and the required respiratory support. Tocilizumab, on the other hand, led to a significantly better survival of patients who were alive after 5 days while reducing the risk of invasive ventilation over 30 days. No clear effect of tocilizumab was measured also on secondary outcome such as bleeding, thrombosis or infections. Prospective controlled randomized clinical

Table 4
Raw outcomes observed at 30 days. Chi-square and hazard ratios with 95% confidence intervals were reported for adverse events in tocilizumab vs. control groups. Intubation refers only to patients who were not intubated at baseline (n = 102); bleeding includes clinically relevant events that lead to diagnostic or therapeutic decision; thrombosis includes pulmonary embolism or deep vein thrombosis.

Outcome	Total (N = 128)	Tocilizumab (n = 64)	Controls (n = 64)	P value	HR (95% CI)	P value
Death	41 (32%)	17 (27%)	24 (38%)	0.185	0.61 (0.33–1.15)	0.129
Intubation	38 (34%)	9 (17%)	29 (48%)	0.001	0.36 (0.16–0.83)	0.017
Thrombosis	23 (18%)	12 (19%)	11 (17%)	0.82	0.89 (0.39–2.06)	0.79
Bleeding	19 (15%)	11 (17%)	8 (13%)	0.46	1.17 (0.47–2.92)	0.73
Infection	45 (35%)	20 (31%)	25 (39%)	0.36	0.71 (0.38–1.32)	0.28

trials are expected to determine whether IL-6 receptor blocking with tocilizumab may still be considered as an anti-inflammatory treatment for COVID-19 and further research should focus on the best timing for treatment.

Declaration of competing interest

None relevant to this manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jaut.2020.102511.

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