Acute-phase reactants during tocilizumab therapy for severe COVID-19 pneumonia

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Abstract

Objective

To identify predictors of clinical improvement and intubation/death in tocilizumab-treated severe COVID19, focusing on IL6 and CRP longitudinal monitoring.

Method

173 consecutive patients with severe COVID-19 pneumonia receiving tocilizumab in Reggio Emilia province Hospitals between 11 March and 3 June 2020 were enrolled in a prospective cohort study. Clinical improvement was defined as status improvement on a six-category ordinal scale or discharge from the hospital, whichever came first. A composite outcome of intubation/death was also evaluated. CRP and IL-6 levels were determined before TCZ administration (T0) and after 3 (T3), and 7 (T7) days.

Results

At multivariate analysis T0 and T3 CRP levels were negatively associated with clinical improvement (OR 0.13, CI 0.03–0.55 and OR 0.11, CI 0.0–0.46) (p=0.006 and p=0.003) and positively associated with intubation/death (OR 17.66, CI 2.47–126.14 and OR 5.34, CI: 1.49–19.12) (p=0.01 and p=0.004). No significant associations with IL-6 values were observed. General linear model analyses for repeated measures showed significantly different trends for CRP from day 3 to day 7 between patients who improved and those who did not, and between patients who were intubated or died and those who were not (p<0.0001 for both). ROC analysis identified a baseline CRP level of 15.8 mg/dl as the best cut-off to predict intubation/death (AUC = 0.711, sensitivity = 0.67, specificity = 0.71).

Conclusion

CRP serial measurements in the first week of TCZ therapy are useful in identifying patients developing poor outcomes.

Key words

clinical improvement, death, COVID-19, tocilizumab, CRP, IL-6

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Data availability: the data that support the findings of this study are available from the corresponding author [C.S.]. Access to these data will be provided in a secured analysis environment to qualified external researchers depending on the nature of the request, the merit of the research proposed, availability of the data and the intended use of the data. To gain access, approved requestors will need to sign a data sharing agreement.

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Introduction

The clinical presentation of coronavirus disease 2019 (COVID-19) ranges from asymptomatic cases to severe pneumonia with respiratory failure (1-3). In patients with severe lung disease, an exaggerated inflammatory response induced by the virus represents one of the most important negative prognostic factors (4-8).

At the present, dexamethasone is the only treatment that seems to reduce the mortality in hospitalised patients with severe COVID-19 (9). However, given the association of elevated IL-6 levels with the severity and mortality of COVID-19 (8, 10), therapy with tocilizumab (TCZ), a recombinant humanised monoclonal antibody against both the soluble (sIL-6R) and the membrane bound IL-6 receptor (mIL-6R), seems to be a promising therapy able to reduce the risk of invasive mechanical ventilation or death in severe COVID-19 pneumonia (11-15).

IL-6 is an important pro-inflammatory cytokine that has a key role in sustaining inflammation, and is a potent inducer of hepatic synthesis of acute phase proteins, particularly C-reactive protein (CRP) (16, 17). In rheumatic conditions, some studies demonstrated an association between pre-treatment elevated levels of CRP and/or IL-6 and treatment response to TCZ (18-20).

Early identification of personalised and efficacious treatment options for COVID19 pneumonia, according to specific parameters, could lead to a beneficial effect on prognosis and long-term outcome (21-22). The aim of this study was to identify predictors of clinical improvement and mortality/intubation in patients with COVID19 treated with TCZ, focusing on baseline IL6 and CRP plasma levels and their longitudinal monitoring.

Patients and methods

Patients and treatment

This prospective, observational study on 173 consecutive patients treated with TCZ was performed at Reggio Emilia and Guastalla Hospitals (Reggio Emilia province, Italy), which were designated to treat COVID19 patients. All patients had a chest computed tomography (CT) scan at diagnosis showing the typical findings of COVID 19 pneumonia (23). SARS-CoV-2 infection was diagnosed at Hospital admission by a positive RT-PCR assay for SARS-CoV-2 in a respiratory tract specimen. Tocilizumab was administered in case of presence of severe pneumonia [oxygen saturation at rest on room air ≤93% and/or arterial oxygen partial pressure (PaO2)/oxygen concentration (FiO2) ≤300 mmHg], and absence of contraindications to TCZ. There were no age limits. Previously intubated patients were included only if intubated for less than 24 hours. 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. 84 patients received iv TCZ that 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. When iv TCZ was not available, the sc formulation was used, usually at the dose of 162 mg administered twice simultaneously, one per each thigh (81 patients). Differently, 4 patients received 4 sc doses simultaneously and other 4 patients 3 doses. This approach was used to mimic, as much as possible, the pharmacokinetic activity of iv formulation to achieve similar levels of drug exposure. Pharmakocinetically, 2 doses of 162 mg of sc TCZ administered simultaneously are similar to 4 mg/kg iv (24). The first patient was treated with TCZ on March 11, and the last on April 14, 2020. Patients were followed up until June 18, 2020. Glucocorticoids (GCs) use before or after TCZ administration during the follow-up time was recorded.

In Reggio Emilia province, a standardised protocol with predefined laboratory tests at admission and during the follow-up was followed for all hospitalised COVID-19 patients and the data recorded in an electronic medical record. We also collected information about the type of respiratory support.

The study was approved by local Ethics Committee on 07/04/2020 n° 2020/0045199. Informed consent was collected from all patients whenever possible.

Table I. Characteristics of the 173 patients treated with tocilizumab and comparisons between patients who improved and did not improve, and between patients who were intubated or died and those who were not intubated and survived[&].

Variable	All n=173	Responders n=124	Non-responders n=49	*p*	Non-intubated/ surviving n=141	Intubated/death n=32	*p*
Age, mean ± SD, years	64 ± 11	63 ± 11	64 ± 11	0.573	63 ± 11	67 ± 8	0.034
Sex, males/females	135 (78)/38 (22)	100 (81)/24 (19)	35 (71)/14 (29)	0.187	112 (79)/29 (21)	23 (72)/9 (28)	0.351
Comorbidities							
Obesity	59 (34)	41 (33)	18 (37)	0.722	39 (28)	20 (62.5)	0.0003
Hypertension	112 (65)	75 (60)	37 (75.5)	0.077	76 (53.9)	29 (90.6)	0.0001
Ischaemic cardiopathy	14 (8)	10 (8)	4 (8)	1.000	10 (7)	4 (12.5)	0.295
Diabetes	42 (24)	28 (22.5)	14 (28.5)	0.434	30 (21)	12 (37.5)	0.067
Cancer	3 (2)	3 (2)	0	0.559	3 (2)	0	1.000
TCZ formulation							
iv	84 (49)	60 (48)	24 (49)	0.944	69 (49)	15 (47)	0.833
sc	89 (51)	64 (52)	25 (51)		72 (51)	17 (53)	
Time from symptom onset to TCZ administration, days (median, interquartile range)	10 (7.5, 14)	10 (7.7, 14)	10 (7.5, 14)	0.980	10 (7, 14)	9.5 (8, 14)	0.930
Glucocorticoids, before TCZ	40 (23)	29 (23)	11 (22)	0.895	31 (22)	9 (28)	0.457
Glucocorticoids, after TCZ	74 (43)	53 (43)	21 (44)	0.905	59 (42)	15 (48)	0.505
IL-6 values before TCZ*, pg/ml, mean ± SD	198.6 ± 459.0	207.2 ± 513.2	173.7 ± 249.9	0.260	204.5 ± 484.0	164.4 ± 284.6	0.763
IL-6 values 3 days after TCZ**, pg/ml, mean ± SD	473.8 ± 653.1	406.2 ± 563.8	697.7 ± 873.2	0.029	416.2 ± 540.3	813.6 ± 1091.7	0.201
IL-6 values 7 days after TCZ***, pg/ml, mean ± SD	321.7 ± 827.1	159.1 ± 244.7	791.6 ± 1510.8	0.006	232.7 ± 628.9	1011.7 ± 1638.0	0.006
CRP values before TCZ^, mg/dl, mean ± SD	14.5 ± 8.3	13.2 ± 7.6	17.8 ± 9.2	0.003	13.5 ± 7.9	19.0 ± 8.7	0.001
CRP values 3 days after TCZ^^, mg/dl, mean ± SD	4.2 ± 4.3	3.3 ± 3.3	6.0 ± 5.3	<0.0001	3.7 ± 3.5	5.9 ± 6.3	0.043
CRP values 7 days after TCZ^^^, mg/dl, mean ± SD	1.3 ± 3.6	0.5 ± 0.6	2.7 ± 5.9	<0.0001	0.6 ± 0.6	4.2 ± 7.6	<0.0001

[&]Except where indicated otherwise, values are the number of patients who were positive (%).
Data were available for *82, **70, and ***70 patients; data were available for ^167, ^^148, and ^^^148 patients.
TCZ: tocilizumab; iv: intravenous; sc: subcutaneous; IL-6: interleukin-6; CRP: C-reactive protein.

Outcome measures

Patients were assessed daily during the hospitalisation. The patient's clinical status was recorded every day using a six-category ordinal scale defined as follows: 1) not hospitalised; 2) hospitalised, not requiring supplemental oxygen; 3) hospitalised, requiring any supplemental oxygen; 4) hospitalised, requiring non-invasive ventilation or use of high-flow oxygen devices; 5) hospitalised, receiving invasive mechanical ventilation or extracorporeal membrane oxygenation (ECMO); 6) death. Clinical improvement was defined as an improvement of one point (from the status at the beginning of TCZ treatment) during the follow-up on a six-category ordinal scale or live discharge from the hospital, whichever came first. This six-category ordinal scale was similar to that used and validated by Cao *et al*. in the lopinavir–ritonavir trial (25).

A composite outcome of worsening during TCZ therapy included the patients who died or were intubated during the follow-up; patients already intubated at the moment of TCZ administration were not included for analyses regarding this outcome.

Laboratory methods and predictor monitoring

C-reactive protein (CRP) levels were measured by an immunoturbidimetric assay (Siemens Healthineers, Erlangen, Germany). The upper limit of the normal reference range is 0.5 mg/dl.

Serum IL-6 concentrations were evaluated by chemiluminescent microparticle immunoassay (Roche Diagnostics, Basel, Switzerland). The sensitivity of the test is 1.5 pg/mL. Normal IL-6 levels are considered <7 pg/mL (95° percentile).

CRP and IL-6 levels were determined before TCZ administration (T0) and after 3 (T3), and 7 (T7) days. IL-6 determination was introduced later during the study period, therefore only almost half of the patients had serial IL-6 determinations.

Statistical analysis

CRP and IL-6 data were log-transformed (base 10) to approximately conform to normality the distributions before analyses (14). All subsequent references to IL-6 and CRP refer to the

log-transformed variable. Continous variables were compared using a 2-sided 2-sample t-test or a Mann-Whitney test when the distributions were skewed. Categorical variables were compared by chi-square test or Fisher's exact test when cell counts were small. Spearman's correlations were used to evaluate relationship between clinical improvement or intubation/death during TCZ treatment and the different parameters.

Logistic regression models were used to identify characteristics that increased the odds of clinical improvement and intubation/death. The potential predictors were represented by CRP and IL-6 levels before starting TCZ and after 3 days, sex, age, glucocorticoid treatment (before starting TCZ), and sc TCZ *versus* iv TCZ. Because the number of patients with IL-6 levels at T0 and T3 were relatively small (82 and 70 patients), missing IL-6 data were imputed using multiple imputation (MI) (n=5 imputations), under the assumption that were missing at random. Complete case analyses (CCA) were additionally performed. We also performed logistic regression analyses without considering IL-6. Both multivariate models were corrected by age, sex, TCZ formulation and glucocorticoid treatment. Odds ratios (ORs) and 95% CIs were reported.

Fatality rate with follow up at 18/06/2020 of the cohort has been compared with that of patients non-treated with TCZ hospitalised in Reggio Emilia area for COVID-19 in the same period: we predicted the probability of death of patients treated with TCZ, with relative 95%CI, if they had the same distribution of covariate (age, sex and calendar time of hospitalisation) as the whole hospitalised population with a logistic mulitvariate model.

Time-dependent analysis of CRP and IL-6 levels during TCZ therapy was performed by means of general linear model (GLM) for repeated-measures. Two time points were included in the analysis (3 and 7 days after treatment) with baseline value as covariate.

Receiver operating characteristic (ROC) analysis was used to identify the cut-off CRP values associated with the highest sensitivity and specificity for intubation/death.

A p-value ≤0.05 was evaluated to indicate statistical significance. Statistical analysis was performed using SPSS v. 26 (IBM Statistics) for Windows.

Table II. Characteristics associated with clinical improvement in patients with baseline CRP and IL-6 values.

Characteristic	Univariate		Multivariate CCA*		Multivariate MI**	
	OR (95%CI)	*p*	OR (95%CI)	*p*	OR (95%CI)	*p*
Log CRP T0	0.13 (0.03-0.53)	0.004	0.21 (0.03-1.48)	0.11	0.08 (0.01-0.53)	0.03
Log IL-6 T0***	0.67 (0.30-1.49)	0.33	0.67 (0.13-1.71)	0.40	0.64 (0.28-1.45)	0.28
Age	0.99 (0.96-1.02)	0.53	1.00 (0.95-1.05)	0.89	1.00 (0.96-1.04)	0.94
Female *vs*. male	1.67 (0.78-3.58)	0.19	0.40 (0.12-1.26)	0.12	0.28 (0.09-0.83)	0.02
TCZ sc *vs*. TCZ iv	1.02 (0.53-1.98)	0.94	1.49 (0.47-4.74)	0.51	1.18 (0.44-3.19)	0.74
GC therapy before TCZ	1.05 (0.48-2.32)	0.07	0.38 (0.10-1.51)	0.17	0.30 (0.09-1.02)	0.05

Univariate and multivariate logistic regression models (age-, sex-, TCZ formulation- and glucocorticoid-corrected analyses); *complete case analysis on 82 patients.
**missing IL-6 data were imputed using multiple imputation, the analysis was performed on 107 patients; ***82 patients had available IL-6 values at the beginning of TCZ therapy.
CRP: C-reactive protein; IL-6: interleukin-6; TCZ: tocilizumab; sc: subcutaneous; iv: intravenous.

Results

Table I shows the characteristics of the 173 patients treated with TCZ. The mean age was 64±11 years, most patients were males (78%), iv and sc TCZ were equally administered (49% *vs*. 51%), and 23% were treated with GCs before TCZ, while 43% were started on GCs following TCZ treatment. Most patients clinically improved during TCZ therapy (72%). 25 (14%) patients died, 9 were intubated at admission, while 12 (7%) were intubated during hospitalisation, and 5 (42%) of them latter died. Totally, 32 (19%) patients died or were intubated during the follow-up, and 144 (83%) were discharged at the end of follow-up.

Table I also compares the characteristics between patients who improved and not improved and between patients who were intubated or died and those who were not intubated and survived. Patients who were intubated or died were significantly older (p=0.03) and had more frequently obesity (p=0.0003) and hypertension (p=0.0001). T3 and T7 IL-6 values (p=0.03 and p=0.006, respectively) and T0, T3 and T7 CRP values (p=0.003, p<0.0001, and p<0.0001, respectively) were significantly higher in patients not improving, while T0, T3 and T7 CRP and T7 IL-6 values (p=0.001, p=0.04, p<0.0001, and p=0.006, respectively) were significantly higher in patients who were intubated or died. No differences in GC treatment before and after TCZ were observed.

In the same time period (March/April 2020), excluding the 173 patients treated with TCZ, 1159 patients with COVID-19 were hospitalised in Reggio Emilia area. 356 (31%) of these 1159 patients died compared to 25 (14%) of the 173 patients treated with TCZ; when taking into account the different distribution by age, sex and calendar time of the hospitalisation, the estimated probability of death in the patients treated with TCZ was 27% (95% CI: 19.4–34.0) and in those not treated was 29% (95% CI: 26.6–31.2).

Correlations were sought between the parameters examined and treatment response. Age positively correlated with IL-6 and CRP levels before treatment (r=0.23, p=0.04 and r=0.14, p=0.05). Lower CRP levels before TCZ were associated with clinical improvement (r= -0.24, p=0.002), and GC treatment before TCZ with lower IL-6 and CRP baseline levels (r= -0.34, p=0.002 and r= -0.24, p=0.002). Risk of death or intubation positively correlated with age (r=0.20, p=0.01) and baseline CRP (r=0.24, p=0.002).

Logistic regression analysis adjusted for age, sex, TCZ formulation, and GC

therapy at admission was used to assess the association of IL-6 and CRP values at the beginning of TCZ therapy with clinical improvement and intubation/death. For the intubation/death analyses, we excluded the 9 patients already intubated when TCZ was administered. Higher baseline CRP values were significantly negatively associated with clinical improvement at univariate analysis (OR=0.13, p=0.004), the association was maintained at multivariate MI analysis (OR=0.08, p=0.03) (Table II). No association was observed between baseline IL-6 levels and clinical improvement. At multivariate MI analysis female gender was negatively associated with clinical improvement (OR 0.28, p=0.02). Considering the 167 patients with available CRP determination before starting TCZ, multivariate analysis confirmed the significantly negative association between CRP levels and clinical improvement (OR=0.13, p=0.006) (Table III). Table IV shows the findings associated with intubation/death. Higher CRP values before starting TCZ (OR=17.66, p=0.004) and older age (OR for one year increase =1.05, p=0.02), and GC therapy started before TCZ (OR=2.90, p=0.04) were associated with an increased risk to die or to be intubated, after adjusting for gender and TCZ formulation.

In the multivariate MI models including CRP and IL-6 values after 3 days of TCZ treatment as covariates, higher CRP values and female gender resulted significant and independent predictors of failure to achieve clinical improvement [OR=0.11 (95% CI: 0.03-0.46), p=0.003; and OR=0.25 (95% CI: 0.07-0.88), p=0.03, respectively]. Intubation/death was positively associated with CRP levels after 3 days of TCZ and increasing age [OR=5.34 (95% CI: 1.49–19.12), p=0.01; and OR=1.05 (95% CI: 1.00–1.10), p=0.04, respectively]. No association was observed between T3 IL-6 levels clinical improvement and intubation/death

GLM for repeated measures of CRP levels from day 3 to day 7, after adjusting for baseline CRP values, showed a steady and significant decrease of CRP levels during time (p<0.0001), significantly more pronounced in patients who improved compared to those who did not improve (p<0.0001). Similarly, GLM confirmed a different pattern in CRP levels among patients who were intubated or died and those who were not (p<0.0001) (Fig. 1A-B).

ROC analysis identified a baseline CRP level of 15.8 mg/dl as the best cut-off to predict intubation/death (area under the curve = 0.711, sensitivity = 0.67, specificity = 0.71, negative predictive value = 0.92) (Fig. 2).

Analyses stratifying patients according to iv and sc tocilizumab

No differences were observed between patients treated with iv and sc TCZ regarding the frequencies of responders (71.4% *vs*. 71.9%) and those of patients who were intubated or died (17.8% *vs*. 19.1%). Supplementary Tables S1 and S2 also compare IL-6 and CRP levels at different times before and after the beginning of the two TCZ formulations between responders and non-responders and between patients who were intubated or died and those who were not intubated and survived. For iv formulation T0, T3 and T7 CRP values (p=0.009, p=0.017, and p=0.001, respectively) and for sc formulation T3 and T7 CRP values (p=0.003 and p=0.004) were significantly higher in non-responders, while T0 and T7 CRP values for both iv TCZ (p=0.014 and p<0.0001) and sc TCZ (p=0.022 and p=0.034) were significantly higher in patients who were intubated or died. For sc TCZ T7 IL-6 values were significantly higher in non-responders (p=0.015) and in patients who were intubated or died (p=0.005).

GLM for repeated measures of CRP levels from day 3 to day 7, after adjusting for baseline CRP values, showed for both TCZ formulations a significant decrease of CRP levels during time (p=0.03), significantly more pronounced in patients who improved compared to those who did not improve (p=0.01 and p=0.003, respectively) (Suppl. Fig. S1A-B). GLM confirmed a different pattern in CRP levels, similar

Table III. Characteristics associated with clinical improvement in 167 patients.

Findings	Univariate		Multivariate*	
	OR (95%CI)	p	OR (95%CI)	p
Log CRP T0	0.13 (0.03-0.53)	0.004	0.13 (0.03-0.55)	0.006
Age	0.99 (0.96-1.02)	0.53	0.99 (0.96-1.03)	0.60
Female *vs*. male	1.67 (0.78-3.58)	0.19	0.59 (0.25-1.36)	0.21
TCZ sc *vs*. TCZ iv	1.02 (0.53-1.98)	0.94	0.96 (0.47-1.97)	0.91
GC therapy before TCZ	1.05 (0.48-2.32)	0.07	0.66 (0.28-1.56)	0.34

Univariate and multivariate logistic regression models (age-, sex-, TCZ formulation- and glucocorticoid-corrected analyses).
*complete case analysis on the 167 patients with complete data at the beginning of TCZ.
CRP: C-reactive protein; TCZ: tocilizumab; sc: subcutaneous; iv: intravenous.

Table IV. Characteristics associated with intubation/death[&].

Characteristic	Univariate		Multivariate*	
	OR (95%CI)	p	OR (95%CI)	p
Log CRP T0	16.87 (2.62-108.68)	0.003	17.66 (2.47-126.14)	0.004
Log IL-6 T0**	1.11 (0.42-2.89)	0.83	-	-
Age	1.05 (1.01-1.09)	0.03	1.05 (1.00-1.10)	0.02
Female *vs*. male	1.51 (0.63-3.62)	0.35	1.58 (0.57-4.36)	0.38
TCZ sc *vs*. TCZ iv	1.00 (0.46-2.15)	0.32	0.87 (0.36-2.10)	0.75
GC therapy before TCZ	1.33 (0.56-3.16)	0.52	2.90 (1.05-8.04)	0.04

Univariate and multivariate logistic regression models were used (age-, sex-, TCZ formulation- and glucocorticoid-corrected analyses.
[&]9 patients already intubated when TCZ was administered were excluded; *158 patients with complete data at the beginning of TCZ therapy were included in the multivariate analysis; **73 patients had available IL-6 values at the beginning of tocilizumab therapy.
CRP: C-reactive protein; IL-6: interleukin-6; TCZ: tocilizumab; sc: subcutaneous; iv: intravenous.

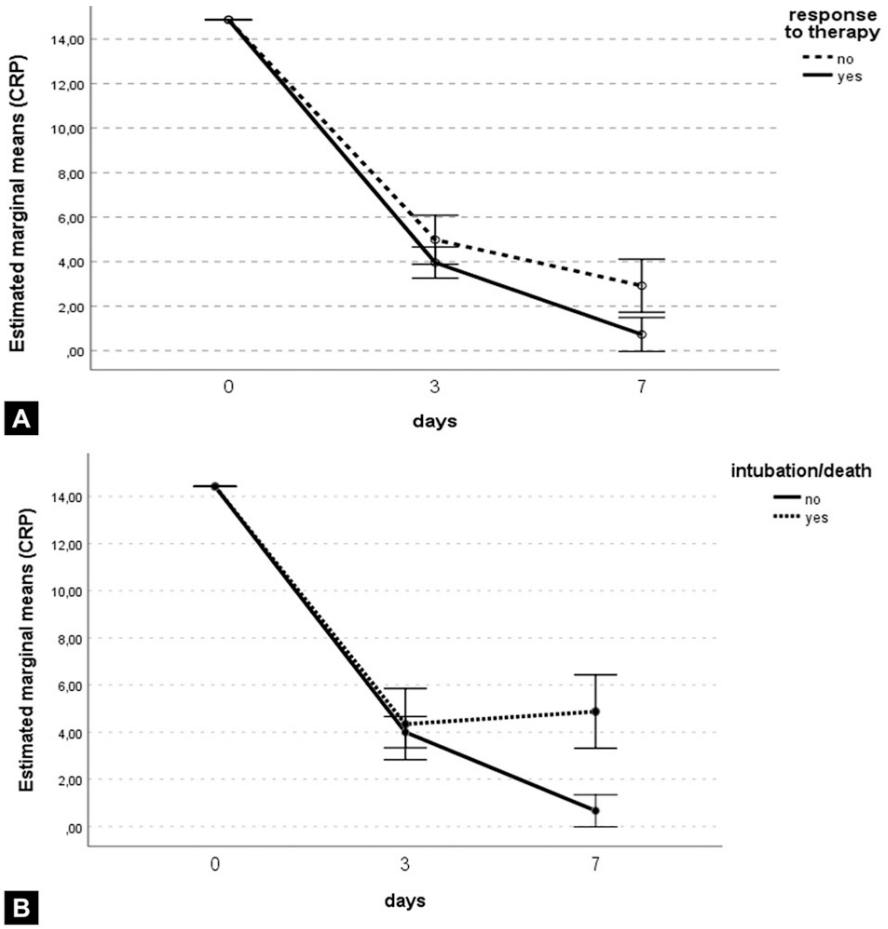

Fig. 1. GLM for repeated measures of CRP levels from day 3 to day 7:
A: after adjusting for baseline CRP values, a steady and significant decrease of CRP levels during time was observed (p<0.0001), significantly more pronounced in responders compared to non-responders (p<0.0001).
B: Similarly, GLM confirmed a different pattern in CRP levels among patients who were intubated or died and those who were not (p<0.0001). Interestingly at day 7, a steady decrease continued to be observed in patients with a more favourable outcome, whereas in patients who died or were intubated CRP levels increased again and remained elevated.

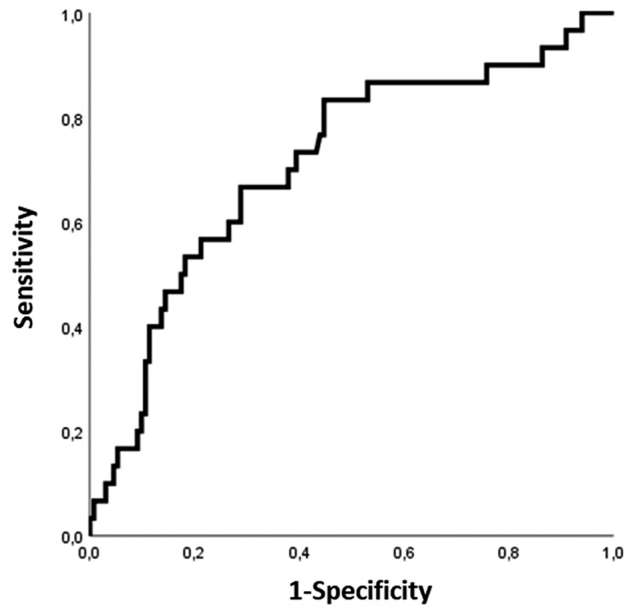

Fig. 2. Receiver operating characteristic (ROC) curve of baseline CRP level for intubation/death. A CRP value of 15.8 mg/dl was the best cut-off to predict intubation/death (area under the curve=0.711, sensitivity=0.67, specificity=0.71, negative predictive value = 0.92).

for both formulations, among patients who were intubated or died and those who were not (p<0.0001 and p=0.04, respectively) (Suppl. Fig. S1C-D).

Discussion

Our study showed that most hospitalised patients treated with TCZ during the period March-April 2020 improved after TCZ administration (79%), while 25 died. No differences were found between iv and sc TCZ. The percentage of patients who died after TCZ therapy was similar, taking into account different age and sex distribution, to COVID-19 patients hospitalised in the same time period and not treated with TCZ after a follow-up of more than one month. In Reggio Emilia hospitalised COVID-19 patients the fatality rate reached a plateau one month after hospitalisation (26), therefore our study had an adequate follow-up period to properly observe COVID-19 related deaths. However, this study was not designed to evaluate the efficacy of TCZ, but to evaluate if acute inflammatory markers, specifically IL-6 and CRP, may be used in clinical practice to predict clinical improvement and intubation/mortality in hospitalised patients with severe COVID-19 pneumonia treated with TCZ.

Some interesting findings emerged from the correlations of IL-6 and CRP. We observed that age positively correlates with baseline CRP and IL-6 levels. IL-6 and CRP are inflammatory mediators commonly used as indicators of inflammaging because their levels systematically increase in an age-dependent manner (27). This chronic, sterile, low-grade inflammation contributes to the pathogenesis of age-related diseases and is a significant risk factor for both morbidity and mortality in the elderly people (27-31). Therefore, despite the cytokine storm, the persistence in COVID-19 of the age-related upregulation of inflammatory markers may contribute to the observed relationship between increased mortality, older age on the one hand, and higher levels of IL-6 and/or CRP, on the other hand (32).

Multivariate analyses clearly demonstrated that higher CRP levels before starting TCZ and 3 days after the onset were associated with a reduced clinical

improvement and an increased risk of being intubated or die. We did not observe any association with baseline and T3 IL-6 levels also using multiple imputation analyses for both outcomes. In our study, as observed in autoimmune diseases, serum IL-6 levels markedly increased after TCZ administration and this increase in free IL-6 probably more closely reflects the endogenous IL-6 production and then the true inflammatory response (33). Interestingly, T7 IL-6 and CRP levels were significantly higher in patients who did not improve and in those who were intubated or died, and their levels in these two groups remained persistently elevated one week after starting TCZ. These observations point out that TCZ is probably less effective when the cytokine storm reaches its peak and the drug is no longer able to abrogate the hyperinflammatory response triggered by the virus. An association between elevated levels of CRP and/or IL-6 at diagnosis and disease severity and mortality, regardless of treatment, was also observed in hospitalised Chinese, Korean and American patients with COVID-19 (1, 8, 34-38).

We considered as covariate only GC therapy before TCZ administration because GCs started after TCZ were usually used in patients who did not improve with TCZ therapy, introducing a bias in the analyses. Despite GC treatment before TCZ was correlated with lower IL-6 and CRP baseline levels, we observed at multivariate analysis a positive association between GC use and intubation/death. This is probably related to GC effects that reduce CRP and IL-6 levels before starting TCZ therapy. These findings suggest that early GCs administration targeted patients with poor outcomes despite lower levels of inflammatory biomarkers.

The role of monitoring acute phase reactants levels over time in gauging the clinical improvement and disease outcomes has not yet been investigated in COVID-19 patients. Interestingly, from T0 to T7 the decrease in CRP levels was significantly more prominent in patients who improved compared to those who did not. In particular, in patients who improved from day 3 to day 7, CRP levels continued to decrease and normalised, while in those who did not improve the decrease was less marked and at day 7 they remained elevated. Differences in the pattern of serial CRP measurements during the first week following TCZ therapy also predicted intubation/death. Figure 1B clearly shows a similar marked reduction in CRP levels at day 3 between patients intubated or who died and those with a better outcome at the end of follow-up. However, at day 7, a significant and steady decrease continued to be observed only in patients with a more favourable outcome, whereas in patients who died or were intubated CRP levels increased again and remained elevated. Supplementary Figure 1 shows similar CRP patterns related to the outcomes for both iv and sc TCZ, showing a similar effect on inflammatory markers and confirming their interchangeability in COVID-19 (15).

Therefore, in TCZ-treated patients the pattern of CRP response to therapy provides useful information about individual clinical course and outcome. Patients showing a rapid and persistent CRP decline with CRP normalisation at day 7 have clinical response and better prognosis, while patients with less rapid decline and particularly those with increasing levels after day 3 had a poor outcome. Therefore, such patients need an aggressive and prompt therapeutic intervention with GCs and/or other cytokine targeting therapy to curb the COVID-19-induced cytokine storm in order to prevent further clinical deterioration.

ROC analysis identified a baseline CRP level of 15.8 mg/dl as the best cut-off to predict intubation/death with a sensitivity of 67% and a specificity of 71%. Other studies have defined CRP cut-off values separating patients with better and worse outcomes (36, 39-43). Similarly to our results, in a recent Italian study ROC curve showed a sensitivity of 72% and specificity of 71% for the CRP cut-off value of 11 mg/dl in identifying CoVID-19 patients with moderate/severe ARDS who require closer respiratory monitoring and more aggressive supportive therapies to avoid poor prognosis (41). Also, in Chinese and American studies CRP values higher than 10 mg/dL identified the patients with more severe inflammation and worst outcomes (36, 42, 43).

The current study has many limitations, but also some strengths. First, this prospective study was limited by the sample size, however, it represents one of the largest series of TCZ-treated patients and the patients were homogeneously followed-up using a common standardised protocol. Secondly, IL-6 determination was performed in only half of the patients, limiting the analyses on this cytokine. Furthermore, not having an untreated control group, we cannot distinguish if the biomarkers we identified are predictors of COVID-19 prognosis even independently of TCZ treatment. Nevertheless, our time dependent analysis clearly suggests a way to identify patients needing further interventions after about one week of TZ treatment.

In conclusion, CRP serial measurements in the first week of TCZ therapy are useful in identifying patients who did not have clinical improvement, particularly those patients developing poor outcomes. In these patients an aggressive therapeutic intervention should be implemented in order to prevent further clinical deterioration.

Key messages

- A baseline CRP cut-off of 15.8 mg/dl identifies COVID-19 with the worst outcome;
- A rapid and persistent CRP decline predicts better prognosis;
- Monitoring CRP during TCZ provides useful information about course and outcomes.

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