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Short communication

Predictive factors of mortality in patients treated with tocilizumab for acute respiratory distress syndrome related to coronavirus disease 2019 (COVID-19)

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ABSTRACT

COVID-19 patients (n = 34) suffering from ARDS were treated with tocilizumab (TCZ). Outcome was classified in two groups: "Death" and "Recovery". Predictive factors of mortality were studied. Mean age was 75.3, mean oxygen (O_2) requirements 10.4 l/min. At baseline, all patients had multiple biological abnormalities (lymphopenia, increased CRP, ferritin, fibrinogen, D-dimer and liver enzymes). 24 patients (70.5%) recovered after TCZ therapy and 10 died (29.5%). Deceased subjects differed from patients in whom treatment was effective with regard to more pronounced lymphopenia (0.6 vs 1.0 G/l; $p = 0.037$), lower platelet number (156 vs 314 G/l; $p = 0.0001$), lower fibrinogen serum level (0.6 vs 1.0 G/l; $p = 0.03$), higher aspartate-amino-transferase (108 vs 57 UI/l; $p = 0.05$) and greater O_2 requirements (11 vs 8 l/min; $p = 0.003$).

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The outbreak of coronavirus disease 2019 (COVID-19) [1] due to SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) has left behind tens of thousands deaths worldwide, in only a few months. On March 11th, 2020, COVID-19 outbreak was declared a pandemic by the World Health Organization [1]. Data from China, the cradle of the pandemic, pointed out that older and frail patients may develop severe disease with respiratory distress and multi-organ failure, resulting in death in about 4% of the cases [2]. Acute respiratory distress syndrome (ARDS) related to SARS-CoV-2 is likely due to a cytokine dysregulation, occurring during the second week of the disease. This cytokine "storm" is characterized by a major release of pro-inflammatory cytokines, including interleukine-6 (IL-6), IL-2, IL-7, IL-10, granulocyte-colony-stimulating factor (G-CSF), interferon γ inducible protein (IP10) and tumor necrosis factorα [2–4]. Blocking this excessive cytokine production might be the key to the COVID-19-ARDS treatment. Elevated IL-6 levels, correlated with clinical severity, were found in patients with other cytokine release syndrome (CRS) [5]. Remarkable beneficial effects of IL-6 blockade therapy using a humanized anti-IL-6 receptor antibody, tocilizumab (TCZ) were observed in patients with CRS due to chimeric antigen receptor T-cell therapy [6]. Many reports underlined the major interest of TCZ, and other cytokine blockade, in the treatment of the COVID-19 related CRS [2,4,7–15]. Due to increasing evidence that TCZ might reduce mortality in patients with COVID-19 related CRS, a scientific committee was constituted within the *Nord Franche-Comté* (NFC) Hospital on the very last days of March 2020. The committee, including infectious diseases specialists, rheumatologists, pharmacists and intensivists, proposed to authorize the off-label use of TCZ in

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patients who were developing ARDS with rapid increase of O_2 requirements (i.e. exceeding 5 L/min with high concentration mask), after failure of a treatment including hydroxychloroquine and/or IV corticosteroids, still within the window of opportunity for TCZ treatment (day 7 to day 17 after onset of symptoms). To be eligible for TCZ therapy each case had to be discussed during a daily multidisciplinary consultation meeting (MCM) including infectious diseases specialists rheumatologist, pharmacists, biologists and intensivists. Between April 1st and May 11th, 2020, 40 severe COVID-19 patients were treated with TCZ in our hospital. Contra-indications for TCZ were bacterial superinfection, latent tuberculosis infection, macrophage activation syndrome and hypersensitivity to tocilizumab.

We have recently published a retrospective case–control study on our first sample of patients treated with TCZ [16]. Despite a Charlson comorbidity index higher than that of patients from the control group, more severe disease, higher oxygen requirement and poorer biological findings, patients treated with TCZ had a better prognosis than control with a death rate of 25% and 48% respectively. However the percentage of deceased patients remains rather high and justifies looking for the reasons of the treatment failure in about 1 patient out of 4.

The aim of the present work was to identify prognostic factors of mortality in subjects treated with TCZ for COVID-19 severe pneumonia, in order to better select further patients, and consequently to optimize the rate of success of TCZ therapy.

1. Methods

The present study is a retrospective analysis of the demographic, clinical, biological and Computed Tomography (CT)-scan data of all the consecutive patients, treated with TCZ for COVID-19 severe pneumonia in the conventional medicine units of the *NFC* Hospital (Belfort, France). The first patient was treated on April 1st 2020, the last one on May 11th, 2020 and the follow-up was stopped on May 28th, 2020.

1.1. Treatment

Tocilizumab treatment consisted in two intra-venous (IV) infusions of TCZ, at 24 h interval, at a dosing regimen of 8 mg per kilogram with a maximum dose of 800 mg per infusion. Before treatment administration, patients had to give their informed consent for the off-label use of TCZ. Before TCZ treatment, most of patients received standard therapy including IV antibiotics (i.e. amoxicillin/clavulanic acid or cefotaxime or levofloxacin), hydroxychloroquine (800 mg/day at day 1, then 400 mg/day from day 2 to day 10), enoxaparin at anticoagulant dose and paracetamol (1–3 g/d). Some of them received IV methylprednisolone at a dosing regimen of 0.5–2 mg/kg/day, 1 to 5 consecutive days.

All patients received oxygen therapy, using high concentration mask. The oxygen flow was calculated to obtain oxygen saturation between 92 and 96%. Oxygen saturation was measured every 4 h using a digital saturometer.

1.2. Data collection

The following data were collected from the patients' medical file: demographic characteristics (age, gender, height, weight, body mass index), comorbidities, concomitant treatments for comorbidities, previous and current treatments for COVID-19, clinical characteristics, oxygen requirements, oxygen saturation, lung CT-scan lesions and biological data, before TCZ treatment. In all patients admitted in the *NFC* Hospital for COVID-19, regardless the severity of the disease, the laboratory tests at entry included blood count, blood electrolytes, glycaemia, creatinine and clearance, C reactive protein (CRP), bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatases, gamma glutamyltransferase, ferritin, fibrinogen, D-dimer, prothrombin, triglycerides, lactate dehydrogenase (LDH), creatin kinase (CK) and arterial blood PO_2 and PCO_2. The same blood tests were repeated every 2 or 3 days until patients got out of the hospital.

Lung CT-scan was performed to confirm diagnosis and to assess pneumonia severity by measuring the extent of the lung lesions (especially plaque-like and ground-glass opacities. Condensation. Crazy paving). Extent was classified <10%, 10–25%, 25–50%, 50–75% or >75%.

Diagnosis of COVID-19 had to be proven by real-time RT-PCR on respiratory samples, mainly nasopharyngeal swabs, sputum and bronchial aspirates. Briefly, viral RNA was extracted using the NucleoSpin® RNA Virus kit (Macherey–Nagel) according to the manufacturers' instructions, and amplified by RT-PCR protocols developed by the Charité (E gene) [17] and the Institut Pasteur (RdRp gene) [18] on LightCycler 480 (Roche). In case of a negative RT-PCR result the CT-scan had to be suggestive enough to be certain of the diagnosis.

1.3. Outcome measurement

All patients treated with TCZ for COVID-19-ARDS in the medicine departments of *NFC* Hospital (patients treated in ICU were excluded from the analysis) were classified into 2 groups: Death and Recovery. We compared baseline values (demographics, biomarkers, lung CT-scan) in patients from the 2 groups.

1.4. Statistics

A descriptive analysis was performed on the collected data. Qualitative variables were described using frequencies and percentages. Quantitative variables were described using mean standard deviation and characteristics of their distribution (minimum, maximum and median). For between group comparisons, Chi-square test or Fisher's exact test, and Student's t-test or Wilcoxon–Mann–Whitney test were used. A multivariate analysis was achieved including all variables with $p < 0.10$ in the univariate analysis. All statistical tests were carried out two tailed at the 5% level of significance. The statistical analysis was carried out using XLstats© software version 2019.3.2 (Addinsoft, Paris, France).

1.5. Ethical approval

Due to the retrospective nature of the study, the Ethics & Scientific Committee of *NFC* Hospital determined that patient consent was required for the off-label use TCZ.

1.6. Informed consent

Due to the retrospective nature of the study, the Ethics Committee determined that no patient consent was required. We make sure to keep patient data confidential and compliance with the Declaration of Helsinki.

2. Results

Between April 1st and May 11th 2020, 40 patients fulfilled the criteria for being treated with TCZ. Six patients were treated in ICU and were therefore excluded from the present analysis, because in such patients it was not possible to attribute with certainty recovery to TCZ treatment. In the remaining 34 patients, unsurprisingly, there was an overwhelming majority of males (24 males vs 10

females). The average (SD) age was 75.3 (11.4) ranging from 52 to 93. At time of the first TCZ injection, the mean oxygen requirement was 10.4 l/min ranging from 4 to 15 l/min. On CT-scan, extend of lung lesions was <50% and >50% in 18 and 16 patients respectively. It was unrelated to oxygen requirement ($p = 0.89$), lymphocytes number ($p = 0.38$), hemoglobin ($p = 0.62$), ferritin ($p = 0.46$), CRP ($p = 0.29$), ALT ($p = 0.29$), AST ($p = 0.33$), D-dimer ($p = 0.90$), prothrombin ($p = 0.42$), fibrinogen ($p = 0.22$) and LDH ($p = 0.67$). All but one patient suffered from one or more comorbidity. The most frequently observed comorbidities were arterial hypertension (18 patients), other cardio-vascular diseases (10 patients), diabetes (6 patients), cancers (7 cases), obesity (5 cases), chronic obstructive pulmonary disease (4 cases), hematological malignancy (2 cases), Parkinson disease (2 cases) and rheumatoid arthritis (1 case).

As previously reported in a number of other series [18–21], all patients had multiple biological abnormalities, with a high frequency of lymphopenia (mean 0.87 Giga/l; normal range-NR = 1.0 to 4.8 Giga/l), high serum levels of CRP (mean 146.0 mg/l; NR < 10), ferritin (mean 1474.7 ng/ml; NR = 10 to 291), fibrinogen (5.8 g/l; NR = 1.7 to 4.2), LDH (mean 527 UI/l; NR = 120 to 246) and D-dimer (mean 7095.1 ng/l; NR < 500). Liver enzymes, AST and ALT, were moderately increased (mean 71.0 and 54.8 UI/l; NR = 13 to 40 and 7 to 40 respectively). Details are given in Table 1.

Deceased patients (n = 10) differed from patients who recovered (n = 24) with respect to a number of factors (Table 2). There was a trend for older age (80 vs 73.1 years; $p = 0.11$) that did not reach the statistical significance. Deceased patients had statistically significant higher O_2 requirements (11.0 vs 8.3 l/min; $p = 0.003$), much more severe lymphopenia (0.57 vs 1.0 G/l; $p = 0.037$), lower platelet count (155.9 vs 314.2 G/l; $p = 0.0001$), lower fibrinogen (0.57 vs 1.0 G/l; $p = 0.03$) and higher AST levels (108.2 vs 56.8 UI/l; $p = 0.05$). The between group difference was not significant for gender, ferritin, CRP, D-dimer and the other studied biomarkers (all $p > 0.05$). Pretreatment with hydroxychloroquine (9/10 patients in the death group and 19/24 in the recovery group) was unrelated to TCZ result. Patients who recovered were as frequently treated with IV corticosteroids than those in whom treatment failed (10/24 vs 6/10). Moreover, there was no between group difference in methylprednisolone dose (mean dose 259 mg; range 120–480 mg in the Death group versus 333 mg; range 120–840 mg in the Recovery group). Response to treatment was unrelated to the extent of lung lesions on CT-scan (>or <50%) ($p = 0.88$) and to the time since onset of symptoms ($p = 0.54$). In multivariate analysis we identified 3 independent factors of mortality: lymphopenia, platelets number and AST ($p = 0.034$).

Table 1
Patients characteristics at baseline (COVID-19 patients treated with tocilizumab (n = 34), *Nord Franche-Comte* Hospital, France, 2020).

Variables	Mean	Range
Age (years)	75.3	52–93
Symptoms duration (days)	13	4–21
Oxygen requirement (l/min)	10.4	5–15
Leucocytes (Giga/l)	10.1	2.4–17.2
Hemoglobin (g/dl)	12.2	9–15.2
Platelets (Giga/l)	292.7	63–521
Lymphocytes (Giga/l)	0.87	0.02–1.51
Prothrombin (%)	84.3	5–100
Fibrinogen (g/l)	5.8	2.3–10
D-dimer (ng/ml)	7095.1	409–35,200
Creatinin (μmol/l)	88.1	38–119
Bilirubin (μmol/l)	10.9	2–35.2
CRP (mg/l)	146.0	23.8–283.1
Ferritin (ng/ml)	1474.7	156–6115
AST (U/l)	71.0	17–154
ALT (U/l)	54.8	14–193
LDH (UI/l	527	292–695

Abbreviations: CRP: C reactive protein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; LDH: lactate dehydrogenase.

Table 2
Comparison between the two groups in COVID-19 patients treated with tocilizumab (n = 34), *Nord Franche-Comte* Hospital, France, 2020.

Variables	Recovery (n = 24) Mean (SD)	Death (n = 10) Mean (SD)	P-values
Age [years]	73.1 (11.1)	80.0 (10.1)	0.11
Symptoms duration [days]	11.7 (5.6)	12.4 (6.7)	0.79
Oxygen [l/min]	**8.3 (4.8)**	**11.0 (4.4)**	**0.003**
Leucocytes [G/l]	10.2 (3.6)	10.6 (4.8)	0.86
Hemoglobin [g/dl]	11.9 (1.7)	13.0 (1.5)	0.17
Platelets [G/l]	**314.2 (112.0)**	**155.9 (78.8)**	**0.0001**
Lymphocytes [G/l]	**01.0 (0.58)**	**0.57(0.38)**	**0.037**
Prothrombin [%]	79 (30.4)	82.2 (15.5)	0.83
Fibrinogen [g/l]	**6.4 (1.9)**	**4.8 (1.4)**	**0.032**
D-dimer [ng/ml]	4768.7 (6424.5)	8367.8 (15,052.5)	0.49
Creatinine [μmol/l]	90.6 (57.2)	93.4 (33.9)	0.89
Bilirubin [μmol/l]	10.6 (7.3)	13.1 (4.6)	0.50
CRP [mg/l]	154.3 (66.9)	138.3 (52.3)	0.53
Ferritin [ng/ml]	1722.7 (1398.8)	1639.2 (828.4)	0.87
AST [U/l]	**56.8 (29.4)**	**108.2 (74.6)**	**0.05**
ALT [U/l]	55.1 (40.4)	53.0 (41.4)	0.65
LDH [U/l]	459.6 (113.7)	531.3 (151.4)	0.27

Abbreviations: SD: standard deviation; CRP: C reactive protein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; LDH: lactate dehydrogenase.

3. Discussion

This case series of patients treated with TCZ for SARS-CoV-2 severe pneumonia is, to our knowledge, the first one specifically focusing on the predictive factors of mortality. Despite the small sample size, it gives some interesting information especially on the profile of patients who may or may not benefit from IL-6 blockade therapy. Patients who recovered were slightly younger -about 7 years-than those in whom treatment failed, although good results have been obtained in several patients older than 85. The biological inflammatory syndrome was slightly more marked in the “Recovery” group, as demonstrated by higher fibrinogen serum levels and higher platelet count. This confirms the rationale of using an IL-6 targeted therapy, well known for its powerful anti-inflammatory effect. Surprisingly baseline CRP was not predictive of response to therapy, conversely to what has been shown in RA [23]. Patients with higher AST levels and those severe lymphopenia, both predictors of mortality [20], were less likely to benefit from TCZ. High ferritin levels, that have been shown to be a prognostic factor for mortality in patients with COVID-19 [20], were not identified as a biomarker of poor prognostic in our study. Surprisingly, we did not found any relationship between response to treatment and lung lesions extent. The lack of correlation between response to TCZ and time since onset of symptoms can be explained by the criteria of eligibility to treatment that stipulates patients must be within the “window of opportunity” [24]. Our case series suffers from some limitations. The sample size was probably too small to determine “cut-off values”, which would allow precise identification of patients to be treated with high chances of success. All patients were treated within a short period of time, considered as the best “window of opportunity” for TCZ treatment (i.e. day 7–17), and we do not have information on the benefit to treat earlier or later. Lastly, our patients were all suffering from very severe disease with multiple risk factors for mortality [19–22], and most of them were not eligible for resuscitation because of age and/or too severe comorbidities. So we cannot draw a conclusion for the interest of TCZ in mild and moderate COVID-19 cases, as well as in patients not suffering from other chronic and debilitating diseases.

In summary, our short single-center experience encourages us to continue treating with TCZ patients suffering from SARS-CoV-2 severe pneumonia. In patients with COVID-19-ARDS, whose life was threatened, treatment with TCZ resulted in favorable evolution in 70% of the cases. However, older patients with higher O_2 needs, more severe lymphopenia, lower fibrinogen and higher AST levels were less likely to benefit from TCZ. Further larger scale prospective trials are mandatory to conclude with certainty on the best patient profile and the right time to initiate the treatment.

Declaration of Competing Interest

The authors declare that they have no conflict interests.

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