EFFICACY OF TOCILIZUMAB IN THE TREATMENT OF PATIENTS WITH SEVERE INFECTION

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Abstract
Since the revealing of the SARS-CoV-2 virus, the broad clinical spectrum of COVID-19 and, especially, the acute severe respiratory syndrome, has alarmed the whole medical world. The pathogenesis of COVID-19 is complex and unknown since the beginning of the pandemic. Many proinflammatory cytokines, including interleukine-6 (IL-6), are involved in the occurrence of cytokine release syndrome, having an important role in the severe evolution and death of COVID-19 patients. For evaluating the role of tocilizumab (TCZ), an anti-IL-6 agent, we analysed the survival in 149 COVID-19 patients from the first day of therapy until the next 90 days of follow-up. Less than half of patients (18.79%) died. The mean survival period was 75.05 days. The evolution of the inflammatory syndrome was favourable at 3 and 7 days, after TCZ administration, with a statistically significant decreased amount of fibrinogen, erythrocyte sedimentation rate, and C-reactive protein. The risk factors of death rate were age over 60 and pre-existing co-morbidities, as heart disease and obesity. The administration of TCZ in severe COVID-19 patients as in other studies was beneficial.

Rezumat
După apariția virusului SARS-CoV-2, spectrul clinic larg al COVID-19 și, în special, sindromul respirator acut sever au ridicat un mare semn de îngrijorare în întreaga lume medicală. Patogenia COVID-19 a fost complexă și necunoscută la începutul pandemiei. Multe citokine proinflamatorii, inclusiv interleukinele IL-6, sunt implicate în sindromul de eliberare a citokinelor, cu rol important în evoluția severă și decesul bolnavilor COVID. Pentru evaluarea rolului tocilizumab-ului (TCZ) ca medicație anti-IL-6, am analizat supraviețuirea în rândul a 149 pacienții COVID-19 monitorizați 90 de zile de la inițierea tratamentului. Mai puțin de jumătate dintre pacienții (18,79%) au decedat. Durata medie de supraviețuire a fost de 75,05 zile. Evoluția sindromului inflamațor a fost favorabilă, cu reducerea semnificativă statistică a valorilor fibrinogenui, vitezii de sedimentare a hematilor și proteinei C reaktive. Factorii de risc ai ratei de mortalitate au fost vârsta peste 60 de ani, afecțiunile cardiace preexistente și obezitatea. Administrarea de TCZ la pacienții cu forme severe COVID-19, ca și în alte studii publicate, s-a dovedit a fi benefică.

Keywords: SARS-CoV-2, COVID-19, tocilizumab, survival rate, cytokine release syndrome

Introduction
The severe acute respiratory syndrome (SARS), caused by the new coronavirus (SARS-CoV-2), first discovered in December 2019 in the Wuhan region in China, contributed to a pandemic with potentially lethal individual implications [4], and had a strong impact on both health and socio-economic systems, emphasizing the limits of the majority of developed countries. The mortality rate of COVID-19 was estimated at 3.7%, by the World Health Organization (WHO), more than 10 times higher than the flu mortality rate [19]. COVID-19 patients presenting certain risk factors of severe evolution have died because of a virus-induced intensified immune system reaction, releasing numerous cytokines, including IL-6, causing a true cytokine storm, even a macrophage activation syndrome, inducing a progressive lungs involvement to acute respiratory distress syndrome (ARDS) [2, 5, 13]. Several pro-inflammatory cytokines have elevated serum levels in patients with severe forms of COVID-19, as well as hyperinflammation, high levels of ferritin and D-dimers, low levels of lymphocytes and platelets. There are more and more data that biological therapies are capable to reduce the cytokine storm, macrophage activation or sepsis in COVID-19 patients. Tocilizumab (TCZ), a biological anti-IL-6 therapy, has been approved for treating patients with cytokine storms, or macrophage activation, and other rheumatic conditions, such

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as rheumatoid arthritis (RA) or giant cell arthritis (GCA), with a good safety profile in the elderly and it is given, off-label, in some categories of COVID-19 patients. Based on data from various communications, it is assumed that TCZ may reduce mortality caused by severe forms of COVID-19 with cytokine release syndrome (CRS) and ARDS. Although early studies on few cases have suggested little benefits, there are no conclusive data to prove TCZ usefulness [1, 2, 8, 23]. The aim of the study was to assess the survival time and the risk factors of mortality among severe COVID-19 patients treated with tocilizumab.

Materials and Methods

Study design

A retrospective survival study, by Kaplan Meier method, was conducted on 1,436 COVID-19 patients, hospitalized in the Infectious Diseases Clinical Hospital of Constanța, Romania, between April and September 2020. The inclusion criteria consisted in severe forms of COVID-19 pneumonia, with varying degrees of lung infiltrates, PaO₂/FiO₂ ratio of less than 200 and elevated inflammatory biomarkers, requiring biological therapy. TCZ administered dose was 8 mg/kg body weight (b.w.), one or two doses (at 12 h interval), intravenous. The survival time was analysed among 149 COVID-19 patients (deceased and survivors) treated with TCZ alone or combined with remdesivir. Remdesivir (REM) is a nucleotide prodrg derived from an adenosine analogue, which is capable to bind to SARS CoV2 RNA polymerase, causing inhibition of viral replication. Follow-up to 90 days was started from the first administration of TCZ ± REM. The evolution of inflammatory syndrome was monitored by the values of erythrocyte sedimentation rate (ESR), fibrinogen (FIB), C-reactive protein (CRP), lactate dehydrogenase (LDH) in the 3rd and 7th day after tocilizumab ± remdesivir intravenous administration.

Statistical analysis

Experimental data were processed using the IBM SPSS Statistics 23 statistical processing program. The procedures used were descriptive statistics (for characterizing discrete and continuous variables defined at the database level), graphs, parametric statistical tests (Independent Samples t-Test), non-parametric statistical tests - addressed to categorical variables (Chi-Square Test of Independence/ association between categorical variables, Chi-Squared Test for the comparison of two proportions), non-parametric statistical tests addressed numerical variables, when the condition of normality is not satisfied, Mann-Whitney U Test), Kaplan-Meier analysis. The chosen significance level was α = 0.05.

Results and Discussion

During the year 2020, between April 1\textsuperscript{st} and September 30\textsuperscript{th}, 1,436 patients with positive RT-PCR confirmation of SARS-CoV2 infection were hospitalized in the Clinical Hospital of Infectious Diseases of Constanța, Romania. 149 patients (10.39%), diagnosed with severe forms of the disease, received treatment with tocilizumab and less than a half (n = 28/149; 18.79%) died. So, the median survival time could not be estimated, but the overall mean survival time was 75.05 days ± 2.566 std. error (limits of IC95% for median were between 69.98 - 80.04 days). Of the 149 patients, 122 received TCZ alone and 27 received both TCZ and REM. The rate of mortality was greater in the group treated by TCZ and REM (n = 7/27; 25.92%) compared to 9.83% in the group of patients treated only with TCZ, without significant statistical differences (χ\textsuperscript{2} = 1.1349; DF = 1; p = 0.2867) (Figure 1).

![Figure 1](image)

Survival analysis in COVID-19 patients treated by tocilizumab ± remdesivir

The association of remdesivir and tocilizumab compared to tocilizumab alone did not significantly influence the survival time. Therefore, in the study group, the supplemental administration of remdesivir (antiviral medication) did not show an advantage compared to tocilizumab (biological therapy) in the survival of COVID-19 patients. This aspect can be influenced by the delay in administering remdesivir in the late phase of disease, when its effectiveness decreases.

Based on the known negative prognostic factors of lung diseases [6, 15, 16] and recorded data about COVID-19 evolution, several risk factors were analysed. In our study sample, the identified risk factors of mortality in patients treated with tocilizumab, were age over 60 (p = 0.009), previous heart disease (p = 0.020) and obesity (p = 0.018). Other factors like the gender of patients (p = 0.112), environmental area of residence (p = 0.882), previous renal injury (p = 0.691), pre-existing lung disease (p = 0.125), diabetes mellitus (p = 0.270) or other medical conditions (p = 0.425), did not influence the survival of patients treated by tocilizumab.

The mean age of the cases was 65.36 (limits between 36 and 83 years), with a median value of 66.50 years, and an interquartile range (IQR) of 47 years. Mann-
Whitney U test revealed significant differences between the median age of the survivors (60-year-old with limits between 29 to 88 years and IQR of 59-year-old), compared to deceased median age of 66.5 years old (limits between 36 to 83 years), with IQR of 47 years old (p < 0.001) (Figure 2).

So, the cut off of 60 years was selected for splitting the cases of survivors and deceased, and significant differences between groups ($\chi^2 = 6.907$; DF = 1; p = 0.009) were found, and a risk of mortality 3.489 times higher in the group of patients over 60 years old compared to younger patients [OR = 3.489; 95% IC = (1.322, 9.209)] (Figure 3).

The mortality rate in our case, treated with TCZ, was low (12.75%) compared to a recent Turkish study [9], which presented a 28 days-mortality rate in patients treated with TCZ (16.1%), for a similar median age (59 years old). Age over 65 years old was a significant risk factor for mortality, in our study. We found that older patients, over 60 years old, had a 3.5 times higher risk of death than patients younger than 60.

According to patients' body mass index (higher or lower than 30 kg/m$^2$), the risk of death was 2.705 times significantly statistic higher in the group of obese patients than the risk of death in the group of non-obese patients [OR =2.705; 95% IC = (1.163, 6.290); ($\chi^2_{\text{calc}} = 5.576$, df = 1, p = 0.018)] (Figure 4).

The immune and metabolic derangement in obesity can produce a more severe clinical COVID-19 evolution [12]. Obesity, especially higher BMI > 40 kg/m$^2$, was a strong predictor factor for hospitalization in an American study [18], with an increased odds ratio (O.R.) of 6.2. A meta-analysis [20] showed that the risk ratio (RR) of mortality among COVID-19 obese patients was 1.42 compared to 2.42 in our study, and a similar mortality risk in their study as ours (RR = 2.54) among patients with obesity and age over 65. The RR differences are explained by the specific profile of our hospital designated to treat severe forms of infection.
the two variables under study, the death and age of the patients ($\chi^2_{calc} = 5.405, df = 1, p = 0.020$). Patients with pre-existing cardiovascular diseases are at greater risk of developing more severe forms of COVID-19, which may adversely affect the survival. Cardiomyocytes are directly damaged by SARS-CoV-2 virus. Also, ACE-2 receptors are expressed in cardiovascular, gastro-intestinal and renal systems, representing an important target for SARS-CoV-2 virus. Severe pneumonia causes gas exchange obstruction, hypoxemia, anaerobic fermentation, intracellular acidosis [22] and all of these cause injury and apoptosis of cardiomyocytes [9]. A Chinese meta-analysis [14] showed the incidence of cardio-metabolic diseases in patients with severe forms of COVID-19, hospitalized in ICU (intensive-care units) for arterial hypertension, was twofold higher, cardio-cerebrovascular diseases threefold higher and for diabetes twofold higher, than non-ICU hospitalized cases. Our study confirmed those results; the mortality of our COVID-19 patients with cardiovascular comorbidities was 3.5 times higher than the others.

To document the efficiency of TCZ, the mean values of lactate dehydrogenase (LDH), fibrinogen (FIB), C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were calculated at 3 different moments: initially, at the time of the first administration = M0, 3 days after administration = M3 and one week (7 days) after administration = M7 (Figure 6).

The LDH value at M0 for TCZ administration was between 291 and 1,491, with a median value of 618 and an IQR of 1,200. The distribution of LDH values was not statistically different in the first moment (M0) versus M3, and M7 moments of the follow-up ($p = 0.06$), as well as the test comparing the median values of LDH at the 3 different times ($p = 0.31$). Although most patients experienced a better evolution after TCZ, LDH changes after administration are not essential in the first week of follow-up after administration. The evolution of inflammatory tests after TCZ administration was favourable, presenting a statistical significance in terms of overall fibrinogen level, and ESR and CRP values ($p < 0.001$). The FIB value at the time of TCZ administration (M0) was between 191 and 1,092, with a median value of 612 and an IQR of 901. The distribution of CRP was statistically different at the 3 times (M0, M3, and M7) ($p < 0.001$). The test comparing the median values of FIB in the 3 different moments revealed significant differences between their values ($p < 0.001$). The CRP value at the time of TCZ first administration was between 2 and 343, with a median value of 92 and IQR of 341. The distribution of CRP values at the time of TCZ administration was statistically different at the 3 times (M0, M3 and M7), with $p < 0.001$. The test comparing the median values of CRP at the 3 different times showed significant differences between their values.

Figure 6.

Distribution of mean values of lactate-dehydrogenase and inflammatory tests (fibrinogen, erythrocytes sedimentation rate, reactive C protein) in patients treated with tocilizumab, according to the moment of evaluation M0 to M3 and M7

M0 = first administration of tocilizumab; M3= follow up after 3 days; M7 = follow-up after 7 days
(p < 0.001). The ESR value, at the time of M0 TCZ administration, was between 5 and 120, with a median value of 60 and an IQR of 115. The distribution of ESR values, at the time of TCZ administration, was statistically different at 3 the times (M0, M3 and M7), with p < 0.001. The test comparing the median values of ESR, in the 3 different moments, showed significant differences between values (p < 0.001).

Our study found similar initial value of CRP in COVID-19 patients treated with TCZ, with a mean value of 107.83 mg/dL in M0, compared to 120 mg/dL in other study, as well as similar mortality rates in severe forms reported (in our data 74.08% versus 78%) [21]. LDH, a marker of lung injury, was not significantly reduced in our study.

COVID-19 has become a global threat since its first emerging in late 2019, SARS-CoV-2 virus has infected millions of persons in the whole world, and the human lives losses, associated with COVID-19 pandemic, have frightened medical practitioners and researchers [7, 10, 17]. Severe COVID-19 pneumonia appeared in the second disease phase, after the initial phase of viral replication, when the immune system dysregulation can induce a cytokine storm, by hypersecretion of proinflammatory cytokines and chemokines [3, 13, 24], contributing to tissue damage and severe lung injury. The benefic role of tocilizumab (a monoclonal anti-IL-6 receptor alpha) in COVID-19 treatment was sustained by the Romanian National Treatment Protocol [11], which recommended TCZ from the beginning of pandemic in Romania (March 2020). The availability of the drug was, unfortunately, inconstant and IL-6 level assessment was irregularly determined in our hospital, but increased acute-phase proteins (CRP, ferritin, FIB, ESR) served as surrogate biomarkers for elevated IL-6. Our study indicates that severe COVID-19 patients equally responded to treatment with TZM or TZM + REM because all of the cases were in the second phase of the disease (10 - 14 days from the beginning of symptoms), when most of antivirals are less efficient. TCZ led to a sharp resolution of cytokines storm, demonstrating a significant reduction of inflammatory tests at treatment days 3 and 7. TCZ administration was associated with a dramatic decline in inflammatory markers, radiological improvement and reduced ventilatory support requirements in COVID-19 patients [25]. Given the study's limitations, the results require future assessment in adequately powered randomized controlled trials.

Conclusions

Previous cardiovascular diseases, obesity and older age are risk factors of severity and increased risk of mortality among patients infected with SARS-CoV-2. The treatment with the IL-6 cytokine inhibitor showed statistically significant beneficial impact in severe COVID-19 forms, reducing inflammatory markers and cytokine storm worsen predictive outcome of death in 81.8% of cases with severe COVID-19.

Conflict of interest

The authors declare no conflict of interest.

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