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Article

Efficacy of tocilizumab therapy in different subtypes of COVID-19 cytokine storm syndrome.

Oleksandr Oliynyk [1], Wojciech Barg[2], Anna Slifirczyk[3], Yanina Oliynyk[4], Vitaliy Gurianov[5], Marta Rorat[6,*]

[1] Department of Anaesthesiology and Intensive Care, Bogomolets National Medical University, Kyiv, Ukraine; Department of Emergency Medicine, Pope John II State School of Higher Education in Biala Podlaska, Biala Podlaska, Poland; alexanderoliynyk8@gmail.com
[2] Department of Internal Medicine, Pneumonology and Allergology, Wroclaw Medical University, Wroclaw, Poland; wojciech.barg@umed.wroc.pl
[3] Department of Emergency Medicine, Pope John II State School of Higher Education in Biala Podlaska, Biala Podlaska, Poland; aslifirczyk1@gmail.com
[4] Department of Immunology and Allergology, Bogomolets National Medical University, Kyiv, Ukraine; janinaoliynyk@gmail.com
[5] Department of Medical Statistics, Bogomolets National Medical University, Kyiv, Ukraine; i_@ukr.net
[6] Department of Forensic Medicine, Wroclaw Medical University, Wroclaw, Poland; marta.rorat@gmail.com
* Correspondence: marta.rorat@gmail.com

Abstract: Background: Cytokine storm in COVID-19 is heterogenous. There are at least three subtypes: cytokine release syndrome (CRS), macrophage activation syndrome (MAS), and sepsis. Methods: A retrospective study comprising 276 patients with SARS-CoV-2 pneumonia. All patients were tested for ferritin, interleukin-6, D-Dimer, fibrinogen, calcitonin, and C-reactive protein. According to the diagnostic criteria, three groups of patients with different subtypes of cytokine storm syndrome were identified: MAS, CRS or sepsis. In each group, treatment results were assessed depending on whether or not tocilizumab was used. Results: MAS was diagnosed in 9.1% of the patients examined, CRS in 81.8%, and sepsis in 9.1%. Median serum ferritin in patients with MAS was significantly higher (5894 vs. 984 vs. 957 ng/ml, $p < 0.001$) than in those with CRS or sepsis. Hypofibrinogenemia and pancytopenia were also observed in MAS patients. In CRS patients, a higher mortality rate was observed among those who received tocilizumab, 21 vs. 10 patients ($p=0.043$), RR = 2.1 (95% CI 1.0-4.3). In MAS patients, tocilizumab decreased the mortality, 13 vs. 6 patients ($p=0.013$), RR = 0.50 (95% CI 0.25-0.99). Conclusions: Tocilizumab therapy in patients with COVID-19 and CRS was associated with increased mortality, while in MAS patients it contributed to reduced mortality.

Keywords: monoclonal antibodies; ARDS; cytokine storm syndrome; inflammation

1. Introduction

The COVID-19 pandemic means there is a lot of attention on the immunopathology problems caused by this disease. Many studies discussing an altered immune status in COVID-19 mention a cytokine storm syndrome (CSS) [1]. Activation of macrophages by SARS-CoV-2 infection, which starts in the lungs, is a primary source of pro-inflammatory cytokines. Development and exacerbation of the inflammatory process, which consequently leads to lung failure and other organ dysfunction is the result of a dysregulated macrophage response [2]. Diagnosis of CSS is based on three criteria:

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elevated circulating cytokine levels, acute systemic inflammatory symptoms, and secondary organ failure (mostly pulmonary, renal, and hepatic) due to hyperinflammation beyond a normal response to the pathogen [1]. Only a handful of researchers indicate that CSS is heterogenic and comprises subtypes. This includes macrophage activation syndrome (MAS), cytokine release syndrome (CRS), and sepsis [3]. Despite similar clinical symptoms, these subtypes differ in their pathomechanism and may consequently require different therapeutic options [4,5]. Some researchers suggest that improper qualification in the treatment of these subtypes may result in an increased mortality rate [4].

One of the treatment options studied in patients with CSS in the course of COVID-19 is based on the use of anti-interleukin-6 receptor monoclonal antibodies from immunoglobulin subclass IgG1, among which tocilizumab is the most commonly used [6,7]. The effectiveness of tocilizumab has been demonstrated in cytokine storm disorders, including HLH, idiopathic multicentric Castleman disease, and CAR T-cell–induced cytokine storm [8]. The role of interleukin 6 (Il-6) in controlling viral infections has been proven in influenza A, herpesvirus, and SARS-CoV-1 infections [9]. As the increased level of Il-6 also correlates with COVID-19 severity and mortality, researchers hypothesise that blocking cytokine signalling may impair clearance of the SARS-CoV-2 virus [1,10].

Tocilizumab is mainly prescribed to patients with severe COVID-19 and is reported to have several significant side effects. Opinions about its usefulness in COVID-19 are ambiguous and the majority of researchers consider more studies on the feasibility and effectiveness of this drug to be required [7,11,12]. The purpose of this research was to study the outcome of treatment with tocilizumab in different CSS subtypes.

2. Materials and Methods

In a retrospective study, we analysed the medical records of 276 consecutive patients with severe COVID-19, hospitalised from 01.02.2020 to 01.11.2020 in an infectious diseases intensive care unit at Kyiv City Clinical Hospital № 4.

Inclusion criteria comprised:

1. confirmed SARS-CoV-2 infection (positive RT PCR test),
2. presence of bilateral interstitial pneumonia on a CT scan, and
3. respiratory failure with arterial partial pressure of oxygen < 60 mm Hg on room air.

Exclusion criteria comprised: serious comorbidities that could potentially affect the course of the disease (cardiogenic pulmonary oedema, pulmonary embolism, recent brain stroke, advanced chronic pulmonary diseases, malignancies, diabetic ketoacidosis, decompensated chronic kidney or liver diseases, and autoimmune diseases), pregnancy, and participation in other clinical studies.

Patients were split into three groups: patients with MAS (n=28, 10.1%), with sepsis (n=24, 8.7%) and with CRS (n=224, 81.2%).

The diagnostic criteria of the Histiocyte Society (HLH-2004 criteria) [13] were used to diagnose MAS. According to this definition, the patient must meet at least 5 out of 8 of the following criteria:

1. Fever over 38.5°C for more than 7 days

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2. Splenomegaly
3. Cytopenia (affecting ≥ 2 of 3 peripheral blood lineages):
   - Haemoglobin (< 9 g/dl)
   - Platelets (<100 x 10^9/l)
   - Neutrophils (<1.0 x 10^9/l)
4. Hypertriglyceridemia (>3 mmol/l)
5. Hypofibrinogenemia (<1.5 g/l)
6. Haemophagocytosis in bone marrow or spleen or lymph nodes
7. Ferritin >500 μg/l
8. Elevated IL-6

In our group, criterion 6 was not taken into account - patients did not have a bone marrow or lymph node biopsy.

Sepsis was diagnosed according to the commonly used definition of sepsis [14]: Sepsis is life-threatening organ dysfunction caused by a dysregulated host response to infection. Organ dysfunction is identified as an acute change in total SOFA score ≥2 points consequent to the infection. Additionally, patients with procalcitonin ≥2.0 ng/ml were included in that group. CRS was diagnosed in patients who also had clinical (hyperthermia, general weakness, myalgia) and laboratory (C-reactive protein, ferritin, and IL-6) markers of severe inflammation but had procalcitonin <0.2 ng/ml [15,16].

The medical report comprised the patient's medical history, physical examination, and laboratory tests: complete blood count, arterial blood gases, C-reactive protein (CRP), procalcitonin (PCT), fibrinogen, D-dimer, Il-6, and ferritin.

Treatment with tocilizumab is not recommended in COVID-19 patients with an increased level of procalcitonin [17]. Consequently, this approach was offered to all patients with CSS and MAS, but not to patients suspected of having sepsis. Only 55/252 (21.8%) patients gave written, informed consent to the treatment. Treatment with tocilizumab was started between day 8 and day 14 from the onset of symptoms. Tocilizumab was administered intravenously at a dose of 400 mg for two consecutive days. All patients were treated with dexamethasone 6 mg i.v. daily, low molecular weight heparin administered subcutaneously in prophylactic doses, antibiotics (in case of suspected or confirmed bacterial infection), and balanced fluid therapy.

Statistical analysis

MedCalc® Statistical Software version 19.5.6 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2020) was used for the statistical analysis. To present quantitative data, the median of the indicator (Me) and the interquartile range (QI-QIII) were calculated; the distribution differed from the normal one by the Shapiro-Wilk criterion. Frequency (%) was calculated for qualitative indices. For comparison of quantitative features between groups, the Kruskal-Wallis criterion was used, posterior comparisons were made according to the Dann criterion. For comparison of qualitative features in more than two groups, the chi-square criterion was used posterior comparisons were made by the Fisher exact test with the Bonferroni correction taken into account. Fisher's exact test was used to compare frequencies in two groups. To quantify the clinical effect, the Risk Ratio (RR) and its 95% confidence interval (95% CI) were

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calculated. To quantitatively assess the degree of influence of factor signs on the risk of a fatal outcome, the method of building and analysing logistic regression models was used. The impact of the factors was measured by the value of the odds ratio (OR) for which 95% CI was calculated. For all statistical tests, the P-value <0.05 was considered significant.

3. Results

Table 1 presents the medical data of the groups studied. There were no statistically significant differences in age and sex between the groups. In our patients, CRS was the most common (224/276, 81.2%) CSS subtype and seems to be the mildest of the three subtypes considered. Compared to the MAS and sepsis subtypes, clinical symptoms in CRS were less pronounced, with lower body temperature and without hepato- or splenomegaly. All 38/276 (13.8%) patients who were not diagnosed with ARDS had CRS. Laboratory parameters were also less pronounced, with no leukocytopenia and relatively mild thrombocytopenia, hyperferritinemia, and interleukinemia (Table 1). Most importantly, mortality in CRS patients was 14.4% compared to 67.9% and 50% in MAS and sepsis, respectively.

In contrast, patients with MAS and sepsis demonstrated a more severe course of COVID-19. The median body temperature was 38.1ºC and 39.2ºC in sepsis and MAS, respectively. Hepato- and splenomegaly were observed in MAS patients. All patients with sepsis or MAS had ARDS. We also observed significant differences in laboratory tests between the groups studied (Table 1). Patients with MAS demonstrated severe hyperferritinemia, hypofibrinogenemia, and pancytopenia i.e. anaemia, leukopenia, and lymphocytopenia. D-dimers were also higher in those patients.

Table 1. Laboratory features of cytokine storm syndrome subtypes, median (QI–QIII)

	CRS (n=224)	**MAS** (n=28)	**Sepsis** (n=24)
Age, years	68 (66-71)	69.5 (66-71)	68 (66-71)
Female, (%)	108 (48.2)	13 (46,4)	13 (54.2)
Temperature (on admission), ^{o}C	37.5 (37.3-37.8)	39.2* (39.2-39.2)	38.1* (38-38.3)
Ferritin, ng/ml	984 (626.5-1314)	5894*,** (5537-6595)	957 (868-1221)
Interleukin-6, pg/ml	60 (47-72)	64** (60.5-66)	95.45* (88.5-103.45)
Procalcitonin, ng/ml	1.4 (1-1.7)	0.6** (0.6-0.7)	5.6* (3.95-7.1)
Fibrinogen, g/l	2.5 (2.1-3.3)	1.55*,** (1.5-1.6)	2.7 (2.25-3.15)
CRP, mg/l	52 (48-72)	32*,** (28-44)	79* (76-97)
D-Dimer, ng FEU/ml	1246 (435-1423)	2485.5* (1978-3115.5)	2005* (1567.5-2448.5)
Leukocytes, x10^{9}/l	4.2 (3.95-4.3)	1.8*,** (1.7-1.8)	14.6* (12.3-15.65)
Thrombocytes, x10^{9}/l	126 (102-138)	60*,** (58-66)	87.75* (83-95)
Lymphocytes, %	24 (22-26)	17*,** (16-18)	23.5 (22-3)
Erythrocytes, x10^{12}/l	3.2 (2.6-3.7)	2.2*,** (2.2-2.3)	3 (2.85-3.15)
PaO_2/FiO_2, mm Hg	276 (249-287)	120* (112-124)	91* (86-116.5)

* - statistically significant difference from the group of patients with cytokine release syndrome, p < 0.001

** - statistically significant difference from the group of patients with sepsis, p <0.001

Table 2 presents the differences in clinical effects of tocilizumab on the course of MAS and CRS. In MAS patients, tocilizumab improved the course of COVID-19 with respect to

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mortality and the need for intubation, while in patients with CRS it increased the risk of both death and intubation.

Table 2. Clinical course of MAS and CRS depending on the administration of tocilizumab. To compare the risk of an event depending on tocilizumab administration, Fisher's exact test has been used for each group.

	MAS (n=28)		**CRS** (n=224)	
	Tocilizumab administration			
	No (n=14)	Yes (n=14)	No (n=183)	Yes (n=41)
Deceased, (%)	13 (92.9)	6 (42.9)	21 (11.5)	10 (24.4)
P value	**0.013**		**0.043**	
Intubated, (%)	14 (100)	8 (57.1)	27 (14.8)	12 (29.3)
P value	**0.016**		**0.039**	

An in-depth analysis of features that may be associated with the risk of intubation or death was conducted. Single-factor and multi-factor logistic regression models were used for analysis. Table 3 shows the results of a single-factor analysis and table 4 the ones of a multifactor logistic regression.

Table 3. Results of a single-factor logistic regression analysis of the risk factors for intubation and death.

	Intubation			**Death**		
N=252	**OR**	**95% CI**	**P value**	**OR**	**95% CI**	**P value**
Age, years	6.92	1.20-39.6	**0.029**	3.41	0.54-21.4	0.188
Temperature (on admission), ^{o}C	115.1	20.8-637.8	**<0.001**	73.6	14.9-363.2	**<0.001**
Ferritin, ng/ml				6.01	2.44-14.8	**<0.001**
Interleukin-6, pg/ml	2.93	1.05-8.2	**0.04**	2.26	0.76-6.71	0.140
Procalcitonin, ng/ml	0.797	0.333-1.91	0.640	0.721	0.28-1.84	0.491
Fibrinogen, g/l	0.0334	0.00945-0.118	**<0.001**	0.0567	0.0159-0.202	**<0.001**
CRP, mg/l	1.04	0.301-3.57	0.953	0.857	0.225-3.26	0.821
D-Dimer, ng FEU/ml	2.68	1.43-4.99	**0.002**	2.21	1.14-4.27	**0.018**
Leukocytes, x10^{9}/l	0.0811	0.0114-0.575	**0.012**	0.0222	0.00218-0.226	**0.001**
Thrombocytes, x10^{9}/l	0.00749	0.00148-0.0377	**<0.001**	0.0082	0.00149-0.0452	**<0.001**
Lymphocytes, %	0.0034	0.00042-0.0275	**<0.001**	0.00383	0.00042-0.0347	**<0.001**
Erythrocytes, x10^{12}/l	0.129	0.0474-0.35	**<0.001**	0.181	0.0637-0.514	**0.001**
PaO_2/FiO_2, mm Hg	0.00483	0.00091-0.0256	**<0.001**	0.00743	0.00159-0.0348	**<0.001**

Among all patients treated with tocilizumab, no statistically significant differences were found between ferritin and Il-6 levels in the group of intubated vs. non-intubated patients (p=0.095, p=0.309, respectively) as well as in the deceased vs. the survivors (p=0.074, p=0.114, respectively). To identify a pool of independent risk factors of intubation and death, a set of features was selected in multi-factor logistic regression models (stepwise method, parameters with p<0.1 were included in the analysis). Due to the small groups, no separate analysis was performed for the CRS and MAS groups.

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From all the biomarkers studied (Table 3) multivariate logistic regression analysis revealed few that are independent risk factors of intubation: leucocyte count, fibrinogen and D-dimer concentration; and death: ferritin concentration, leucocytes and lymphocytes count (Table 4).

Table 4. Results of a multivariate logistic regression analysis of the risk factors for intubation and death.

	Intubation, χ^2_3=55.8 p=0.00000				**Death,** χ^2_3=47.4 p=0.00000			
N=252	**Estimate**	**OR**	**95% CI**	**P value**	**Estimate**	**OR**	**95% CI**	**P value**
Ferritin, ng/ml					1.07	2.91	1,00-8,42	**0.048**
Fibrinogen, g/l	-2.58	0.00446	0.00053-0.0375	**<0.001**				
D-Dimer, ng FEU/ml	-1.30	0.273	0.0899-0.0153	**0.02**				
Leukocytes, x10^9/l	-0.332	0.0805	0.0153-0.424	**0.003**	-0.426	0.0392	0,00594-0,259	**<0.001**
Lymphocytes, %					-0.166	0.0134	0,00085-0,210	**0.002**

4. Discussion

A severe course of COVID-19 is generally combined with one of the CSS subtypes, usually CRS, but the most severe cases are usually complicated by sepsis or MAS. All CSS subtypes are characterised by life-threatening hyperinflammation which supports a cytokine storm and ultimately leads to multiple organ failure [18]. In the spectrum of cytokines involved in the pathogenesis of CSS, IL-6 and ferritin are of great importance [19].

The role of IL-6 in the immunopathogenesis of COVID-19 is supported by extensive research data showing an increase in the concentration of this cytokine in patients' serum. It is proposed for monitoring the severity of COVID-19 [10,20]. According to Otsuka and Seino [21] in patients (n=1302) with severe COVID-19, IL-6 was 3 times higher than in patients with a mild or moderate course (p <0.001). Its concentration was associated with bilateral lung damage (p=0.001) and fever (p=0.001). Other studies show that elevated IL-6 correlates with a progression of ARDS (p=0.03), the requirement for mechanical ventilation, and mortality risk [10,15,22]. According to Ruan et al. [23], the average concentration of IL-6 (11.4 ± 8.5 mg/ml) in deceased patients is significantly higher (p<0.001) than that of survivors (6.8 ± 3.6 mg/ml). In our study, IL-6 concentrations differ statistically significantly within the investigated groups although the difference in absolute values between MAS and CRS is small (64 v 60 pg/ml, p<0.001) (Table 1).

Ferritin concentration is increased in all subtypes of CSS but in MAS it rises sharply and may exceed the normal value even by a factor of several hundred [24]. The highest ferritin level in our patients with MAS was as high as 129,000 ng/ml, and the average value was 5894 ng/ml. According to the HLH-2004 recommendations [13], the characteristic feature of MAS is an increase in ferritin levels above 500 ng/ml, but some authors [21] suggest that the average ferritin level in MAS exceeds 3000 ng/ml. In contrast, ferritin levels in sepsis patients are much lower and, in our study, the median was 957 ng/ml. This data is consistent with the results of Giamarellos-Bourboulis EJ et al. [24], in which COVID-19 and sepsis patients had a mean ferritin level of 954 ng/ml (508.4 - 5394). Thus, ferritin seems to be the most distinctive marker for MAS. In addition, in our study ferritin concentration is found to be an independent risk factor for death.

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Monoclonal antibodies against the IL-6 receptor, including tocilizumab and sarilumab, have a pronounced immunosuppressive effect and have been used in the treatment of COVID-19. Data on the effectiveness of treatment with tocilizumab in COVID-19 patients is ambiguous [12,25-27]. There is a general opinion that tocilizumab may be effective in severe forms of COVID-19. It is considered that such therapy is appropriate and can reduce the mortality rate and the need to switch patients to forced ventilation with intubation. However, recent studies [6,28] indicate that tocilizumab is not highly effective in CSS treatment. Thus, reports of the potential harmfulness of tocilizumab should be taken with caution, although it could, to some extent, explain the lack of effectiveness of the therapy in some studies in heterogeneous COVID-19 populations.

The most important finding in our research is the demonstration that the clinical effect of tocilizumab is significantly different in the CSS subtypes investigated. In MAS patients, treatment with tocilizumab reduced mortality and the need for intubation by approximately half. In contrast, in the CRS group, the use of tocilizumab almost doubled the risk of intubation and death. MAS appears fairly rare and its prevalence in our study was 10.1%. It is believed that the presence of MAS in COVID-19 patients worsens the disease course and results in a higher mortality rate. In classic autoimmune MAS, mortality can be up to 100% if not treated appropriately [3]. Thus, only timely diagnosis and early treatment (including tocilizumab) can reduce mortality in patients with COVID-19 and MAS.

Our study revealed a higher median body temperature, more frequent presence of hepato- and splenomegaly, severe hyperferritinemia, hypofibrinogenemia, and pancytopenia i.e. anaemia, leukopenia, and lymphocytopenia and higher D-dimers in patients with MAS. This agrees with findings in MAS resulting from other causes, mostly from rheumatoid diseases [29-31]. As it might be difficult to differentiate MAS from sepsis, especially if the viral infection is a trigger for MAS, it is essential to determine procalcitonin and ferritin levels to distinguish patients with MAS from ones with sepsis. Thus, an appropriate differential diagnosis of MAS seems to be of crucial importance for tocilizumab therapy.

The approach to the prescription of tocilizumab should be differentiated. It is common knowledge that in the case of COVID-19 and sepsis it shouldn't be administered [17]. In our opinion, it should be used cautiously in patients with CRS. We observed the development of sepsis in two patients after treatment with this medication. The target group for tocilizumab treatment should be patients meeting the MAS criteria, which is consistent with the MAS treatment protocol from the Histiocyte Society [13]. Patients with MAS have an extremely high probability of a fatal outcome of the disease without the use of tocilizumab.

It is difficult to draw conclusions from the results because of the limited number of observations, especially in MAS patients: only 10 patients receiving and 10 patients not receiving tocilizumab. The availability of only such a small number of these patients is explained by the rare occurrence of MAS. Therefore, from our point of view, tocilizumab therapy in the case of COVID-19 should be prescribed selectively and considering the CSS subtype that the patient has developed. Further research is necessary to definitively address the effectiveness and appropriateness of its use.

1. Conclusions

In patients with COVID-19 and macrophage activation syndrome, tocilizumab contributes to reduced (p=0.013) mortality, RR = 0.46 (95% CI 0.25-0.86). In contrast, in patients with cytokine release syndrome, it is associated with increased (p=0.043) mortality, RR = 2.1 (95% CI 1.1-4.2).

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