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Interleukin-17 and the COVID-19 cytokine storm: Cases reports

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ABSTRACT: We report two cases of patients with COVID-19. Clinical and biological features of the two patients confirm severe form of COVID-19 associated with cytokine storm. High levels of IL-6 and IL-17 were found. Unfortunately the patients died because of the multi-organ failure secondary to the cytokine storm. Cytokine storm is a systemic inflammatory syndrome which leads to aberrant release of cytokines. IL-6 is the most frequently reported cytokine to be increased in COVID-19 patients. Naïve T CD4+ cells in the presence of TGF β and IL-6 will differentiate into T helper 17 cells responsible for secreting IL-17A and IL-17F, which target macrophages, dendritic cells, endothelial cells, and fibroblasts to increase the production of cytokines. IL-6 and IL-17 have been shown to play a role in increasing risk of airway disease**.** They synergistically promote viral persistence by protecting virus-infected cells from apoptosis**.** Immune hyperactivation in cytokine storm amplified levels of cytokines that will have systemic effects and cause collateral damage to vital organ systems. Immunotherapy can play a crucial role in COVID-19 managing. Tocilizumab an anti-IL6 receptor antibody was used with clinical improvement. The possibility of inhibiting IL17 as therapy for COVID-19 should be also considered.

KEYWORDS: COVID-19, Cytokine storm, IL-6, IL-17, Case report.

1 INTRODUCTION

Since the World Health Organization declares coronavirus disease 2019 (COVID-19) a global pandemic in MARCH 2020; this disease has seriously challenged medical health systems. COVID-19 is an infectious disease caused by a newly discovered coronavirus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The clinical manifestations of COVID-19 ranged from mild non-specific symptoms to severe pneumonia with organ function damage. It's mainly included fever, cough, myalgia, fatigue, or dyspnea. In the later stages of the disease, dyspnea may gradually develop into acute respiratory distress syndrome (ARDS) or multiple organ failure [1]. Clinical data have suggested that a cytokine storm (CS) is occurring and is associated with COVID 19 severity and death [2]. C S is a life-threatening systemic inflammatory syndrome involving immune cells hyperactivation and high levels of circulating cytokines [3]. Researches have identified high circulating levels of interleukin-6 in patients with severe COVID-19 [4] and Tocilizumab treatment that blocking IL-6 receptors was used with significant clinical

improvement [5]. There are currently no curative or preventive therapies for COVID-19, highlighting the need to block other cytokines that could reduce the incidence of COVID-19 severity and death is essential. In this article we will report two observations of two patients with a severe form of COVID-19 associated with CS where besides IL-6, IL-17 is also found at very high levels

2 CASE 1

The first case concerns a 28-years women at 21weeks of gestation (Gravida 3, Para 2) with no comorbidity factors or notable medical history. Her pregnancy had been without complications. The patient initially tested positive for COVID-19 by quantitative real time polymerase chain reaction (qRT-PCR), from a nasopharyngeal swab. She was managed as an outpatient for two days until the development of worsening dyspnea and hypoxia. At this time, she was transferred and admitted to the university hospital for higher-level care. Before hospitalization, the patient received azithromycin 500 mg daily in the first day of diagnostic followed by 250 mg daily, 1000 mg of vitamin C, 45 mg of zinc twice daily and 25000 UI of vitamin D. On admission to the intensive care unit, the patient presented with an acute respiratory distress syndrome, fever at 38° C and with oxygen saturation of 73% at ambient air. She received oxygen therapy with a high concentration mask and the saturation was increased to 93%. Complete blood cell count showed elevated white blood cell count (WBC), (13.31×10^3/µl), elevated neutrophil (10.38×10^3/µl) and anemia. Laboratory tests have also shown hypoalbuminemia, elevated C-reactive protein (CRP) and hyperfibrinogenemia (Table 1).

Table 1. Laboratory values of the case 1

Biological parameter	At admission	Second day	Third day	Fifth day	Sixth day	Range
RBC	-	-	$3.27\ 10^6$	$3.01\ 10^6$	$2.87\ 10^6$	3,50-5,50 10^6/ µl
HB	-	-	9.6	8.6	8.3	12-16 g/dl
WBC	-	-	$13.31\ 10^3$	$23.39\ 10^3$	$9.53\ 10^3$	4-10 10^3 / µl
Neutrophils	-	-	$11.38\ 10^3$	$19.44\ 10^3$	$7.34\ 10^3$	1.50-7 10^3/ µl
Lymphocytes	-	-	$1.21\ 10^3$	$2.62\ 10^3$	$1.16\ 10^3$	1,5 10^3/ µl
Plaquetes	-	-	$436\ 10^3$	$500\ 10^3$	$277\ 10^3$	150-400 10^3/ µl
Ferritinemia	130	165	201	218	-	15-200 ng/ml
Serum creatinine	5.8	5.2	5.6	10.7	40.9	5.7-11,1 mg/l
Uremia	0.09	0.09	0.12	0.27	0.78	0.13-0.43 g/l
ASAT	22	18	17	11	25	5-34 UI/l
ALAT	10	9	7	8	7	0-55 UI/l
CRP	269	174	150	208	159	0-5 mg/l
Albuminemia	-	25	26	25	22	35-52 g/l
Prothrombin	-	-	85	71	57	70-140%
APTT	-	-	30.7	35	72.5	23-33 Sec
Fibrinogen	-	-	8.20	6.58	5.67	2-4 g/l
Procalcitonin	0.33	0.28	-	1,62	16.58	<0.5 ng/ml
Troponin	-	-	4.6	54.1	432	0-15 ng/l

RBC: red blood cell count, HB: haemoglobin, WBC: white blood cell count, APTT: activated partial thromboplastin time, ASAT: aspartate aminotransferase, ALAT: alanine aminotransferase

No liver or renal dysfunctions were observed. The patient was treated with antibiotics (azithromycin, ceftriaxone). Besides, she received methylprednisolone 80 mg twice daily and Enoxaparin 100 IU/kg/12h. Due to persistent hypoxemia and hypercapnia at the third day of hospitalisation the patient was intubated and protective ventilation initiated. Laboratory tests showed worsening anemia, a greater increase in white blood cell counts, elevated platelet count, hypoalbuminemia, elevated C-reactive protein, hyperfibrinogenemia a slight increase in ferritinemia and rising activated partial thromboplastin time (APTT). She deteriorated into multisystem organ failure, comprising acute respiratory, acute kidney injury and acute coagulopathy with markedly elevated inflammatory markers concerning for cytokine storm. We measured plasma levels of 7 cytokines involved in the immune reponse; IL-2, IL-4, IL-6, IL-10, IL-17A, TNF-α and IFN-γ (Table 2). High levels of IL-6 and IL-

17A were found (Il-6=23.46 pg/mL and Il-17=161.51 pg/mL). Unfortunately, the patient died the same day because of the multi-organ failure secondary to the cytokine storm.

Table 2. Plasma Levels of the cytokines of the two patients

	IL-17A (pg/mL)	IFN-γ (pg/mL)	TNF-α (pg/mL)	IL-10 (pg/mL)	IL-6 (pg/mL)	IL-4 (pg/mL)	IL-2 (pg/mL)
Case 1	161,51	5,00	7,98	11,97	23,46	4,15	8,67
Case 2	69,86	15,34	15,72	25,48	119,87	28,03	11,69

3 CASE 2

A 77-year-old man with a past medical history of diabetes and hypertension presented to the university Hospital with a fever of 38.5°C, fatigue, dyspnea, and oxygen saturation of 86% on ambient air. The patient was admitted to the hospital and a nasopharyngeal swab was performed and the patient was tested for SARS-CoV-2 by qRT-PCR, which proved to be positive. On admission, laboratory tests reveal elevated WBC (12.74×10^3/µl), elevated neutrophils (10.37×10^3/ µl), elevated CRP (289 mg/l), hyperfibrinogenemia (8.69 g/l) increase in ferritinemia (3780 ng/ml) elevated hypersensitive troponin (132 ng/l) ASAT at 49 IU/L (Table 3).

Table 3. Laboratory values of the case 2

Biological parameter	At admission	Second day	Third day	Range
RBC	-	$4.42\ 10^6$	$4.40\ 10^6$	3,50-5,50 10^6/µl
HB	-	14.5	14.5	12-16 g/dl
WBC	-	$12.74\ 10^3$	$28.39\ 10^3$	4-10 10^3/µl
Neutrophils	-	$10.37\ 10^3$	$26.64\ 10^3$	1.50-7 10^3/ µl
Lymphocytes	-	$1.71\ 10^3$	$1.01\ 10^3$	1.5 10^3/ µl
Plaquetes	-	$428\ 10^3$	$306\ 10^3$	150-400 10^3/ µl
Ferritinemia	-	3780	3800	15-200 ng/ml
Serum creatinine	-	10.8	38.6	5.7-11.1 mg/l
uremia	-	0.66	1.50	0.13-0.43
ASAT	42	49	406	5-34 UI/l
ALAT	24	26	114	0-55 UI/l
CRP	353	289	189	0-5 mg/l
Albuminemia	-	30	30	35-52 g/l
Prothrombin	-	86	47	70-140%
APTT	-	30.7	38.7	23-33 Sec
Fibrinogen	-	8.69	2.32	2-4 g/l
Procalcitonin	-	0.29	-	<0.5 ng/ml
Troponin	53	132	-	0-15 ng/l
D-Dimère	-	-	1.57	<0.28 mg/l

RBC: red blood cell count, HB: haemoglobin, WBC: white blood cell count, APTT: activated partial thromboplastin time, ASAT: aspartate aminotransferase, ALAT: alanine aminotransferase

The patient was started on the following treatment regimen: high concentration of oxygen (the saturation was increased to 96%), azithromycin 500 mg first day followed by 250 mg daily, hydroxychloroquine sulfate 400 mg/12 h the first day followed by 200 mg/ 12 h, enoxaparin sodium 80 mg/12 h and dexamethasone 4 mg/12 h. The patient was also given vitamin C, zinc, and vitamin D supplements. On the 3th day after admission, the patient suffered from severe respiratory distress, his hypoxemia and dyspnea worsened, so he was immediately given invasive ventilation. Laboratory tests shown elevated CRP (189 mg/l), increase in ferritinemia (3800 ng-ml), elevated serum creatinine 38.6 mg/l and uremia at 1.5 g/l. liver tests showed that aspartate aminotransferase and alanine aminotransferase were 12 and 2 times upper level of normal and progressive deterioration in coagulation parameters (table 3). The patient was suspected to have a cytokine storm. Indeed, high levels of IL-6 and IL-17A were found (IL 6=119.87 pg/mL and IL 17=69.86 pg/mL) (Table 2). A single dose of Tocilizumab 400 mg was

administrated. Clinical, respiratory conditions and oxygen saturation are expected to start to improve 24 h after tocilizumab administration. However, no improvement was observed. The patient developed concomitant acute limb ischemia and acute myocardial infraction. He died 24 hours later.

4 DISCUSSION

CS is a systemic inflammatory syndrome which leads to aberrant release of cytokines. This syndrome is triggers by various therapies, cancers, autoimmune diseases and pathogens. A host of pathogens have been described as cause of CS including severe acute respiratory syndrome Coronavirus (SARS Cov) [6] and Middle East respiratory syndrome Coronavirus (MERS Cov). Patients usually present with fever, fatigue, anorexia, headache, rash, diarrhea, arthralgia, myalgia, and neuropsychiatric findings. Severe cases progress to multiorgan failure such as acute respiratory distress syndrome (ARDS), renal failure, and acute liver injury. Some cases can progress rapidly to disseminated intravascular coagulation with vascular occlusion. Here ARDS is not commonly due to the viral load but due to the exuberant immune response, and results in CS. The laboratory findings in the CS are variable and comprise elevated CRP, hypertriglyceridemia, various blood-count abnormalities, such as leukocytosis, leukopenia, anemia, thrombocytopenia, elevated ferritin and D-dimer levels. Patients have also elevated serum levels of proinflammatory cytokines such as IL-1B IL-6 IL-8 IL-17 GM-CSF G-CSF. IL-6 plays a central role in CS and is the most frequently reported cytokine to be increased in COVID-19 patients [7]. IL-6 is a pleiotropic cytokine with redundant functional activity. It's mainly produced, in inflammation by macrophages, T cells, and endothelial cells and it's expresses to participate in host defence by activating acute-phase reactions, immune responses and hematopoiesis. However, immune hyperactivation in CS amplified levels of cytokines that will have systemic effects and cause collateral damage to vital organ systems. Indeed, IL-6 rapidly stimulates the synthesis of acute phase proteins such as CRP, serum amyloid protein A, and antitrypsin. It's directly induces vascular hyperpermeability, thereby resulting in tissue damage and induces tissue factor expression on the cell surface of monocytes and triggers a coagulation cascade, leading to activation of thrombin and formation of fibrin clots. IL-6 inhibits the cytotoxic activity of Natural killer cells by reducing perforin and granzyme B. Naïve T CD4+ cells in the presence of transforming growth factor β (TGF β) and IL-6, will differentiate into T helper 17 cells responsible for secreting IL-17A and IL-17F, which target macrophages, dendritic cells, endothelial cells, and fibroblasts to increase the production of IL-1, IL-6, and TNF. IL 17 has several other functions including the production of chemokines such as IL-8, monocyte chemoattractant protein-1 (MCP-1), the production of the hematopoietic cytokine that stimulates the expansion of myeloid lineages.

Studies have demonstrated the involvement of proinflammatory cytokines in several respiratory system diseases. In particular, IL-6 and IL-17 have been shown to play a role in increasing risk of airway disease [8]. Elevated IL-17 is also observed in MERS-CoV and SARS-CoV patients [9]. Given the role that IL-6 plays in CS targeting this cytokine therapeutically in response to COVID-19 infection has demonstrated encouraging results.

The first observation reported the case of a young pregnant woman with no significant pathological history or risk factors who developed a severe form of COVID-19 with a cytokine storm, high levels of Il-6 and Il-17 and a multi organ failure that died before the introduction of Tocizulimab. Pregnancy increases the risk for severe illness with COVID-19, because of the physiologic immune suppression. Indeed, it's a risk factor for death, pneumonia and intensive care unit admission in SARS-CoV-2-infected women of reproductive age compared to non-pregnant women [10]. The second observation described in this work was that of a man who had risk factors of comorbidities (diabetes and hypertension) and developed a severe form of COVID-19 associated with a cytokine storm, high levels of Il-6 and Il-17 which caused multi organ failure (hepatitis, renal and respiratory) despite administration of Tocilizimab the patient's condition was complicated by arterial ischemia in the lower limb and death.

The early treatment of CS is essential to avoid irreversible tissue damage; special attention for blocking other cytokine that could reduce COVID-19 impact appears as a promising therapeutic. In the two described observations high levels of IL17 are noted, blocking IL-17 to manage COVID-19 patients could be a therapeutic strategy. IL-6 and IL-17 synergistically promote viral persistence by protecting virus-infected cells from apoptosis [11]. Further investigations are needed to determine if we will have to target IL-17 alone or in combination with IL-6.

5 CONCLUSION

To date, there is neither a reliable safe vaccine nor an effective safe treatment modality, specifically designed to treat COVID-19 and it will take several years to develop specific drugs, immunotherapy can play a crucial role in COVID-19 management. The inhibition of the IL-17 as therapy should be taken into consideration. There are several antibody-based TH17 blockades available. However, further clinical research is mandatory in this regard.

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