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Factors of Interleukin-6 Signaling in COVID-19 Patients with Lung Damage of Varying Degrees: A Pilot Study

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Specific features of IL-6 signal transduction were studied in 89 patients with lung damage of varying degrees during the first COVID-19 pandemic wave. The levels of IL-6 signaling components (IL-6, sIL-6R, and sgp130) and highly sensitive C-reactive protein (hsCRP) were examined in patients with intact lungs (CT-0), mild (CT-1), moderate (CT-2), moderate to severe (CT-3), and severe (CT-4) lung damage. Seventy patients were re-examined 3-7 months after discharge from the hospital. The IL-6 and hsCRP levels increased several times with severing lung damage severity. In patients with CT-3, sIL6-R increased statistically significantly and remained high in CT-4 patients. sgp130 levels were lower in CT-1 and CT-2 patients and higher in CT-3 and CT-4 patients compared to CT-0 patients. We revealed a positive correlation between IL-6 and hsCRP levels in CT-1, CT-2, and CT-3 patients. In CT-3 patients, sIL-6R levels positively correlated with IL-6 concentration. The studied parameters decreased considerably in all patients 3-7 months after discharge. It can be suggested that IL-6 classic-signaling is predominant in CT-1 and CT-2, while trans-signaling prevails in CT-3. Disorders in regulatory mechanisms of IL-6 signaling occur in CT-4, which prevents physiological elimination of IL-6 hyperactivity. The results obtained are preliminary and require a broader study.

Key Words: *COVID-19; IL-6; soluble IL-6 receptor (sIL-6R); sgp130; C-reactive protein*

COVID-19 infection caused by SARS-CoV-2 coronavirus is dangerous due to leading to death complications such as severe pneumonia, acute respiratory distress syndrome, and cardiovascular disorders (primarily thrombotic) [1,2]. COVID-19 pneumonia is determined by the development of pathological changes in lung tissue, alveolar and vascular walls [2,3]. By damaging pulmonary tissues, SARS-CoV-2 induces a systemic inflammatory response [4]. The levels of proinflammatory cytokines, including IL-6, are an important parameter characterizing the intensity of inflammatory response. In COVID-19 patients, high levels of IL-6 correlate with unfavourable outcomes and high death rate [5,6]. Variety of IL-6 effects stems from two signaling pathways, namely, classic-signaling and trans-signaling. In classic pathway IL-6 forms a complex with its plasma membrane receptor (IL-6R). In trans-signaling complex is formed with a soluble receptor (sIL-6R). It has been generally accepted that effects of IL-6 promoting pathological processes are predominantly associated with trans-signaling, while its anti-inflammatory activity is realized via classic pathway [7]. Hence, classic-signaling and trans-signaling make oppositely directed contributions to pathological processes during the disease progression.

In humans, IL-6 trans-signaling is regulated by special mechanism. The circulating soluble glycoprotein sgp130 binds to the IL-6/sIL-6R complex thereby inhibiting trans-signaling [8]. Thus, the ratio between IL-6, sIL-6R, and sgp130 concentrations determines initiation of classic- or trans-signaling, which can significantly modify IL-6 effects and IL-6-associated pathologies.

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The aim of this pilot study was to examine the specific features of IL-6 signaling in patients with lung damage of varying degrees during the first COVID-19 pandemic wave.

MATERIALS AND METHODS

The study included 89 patients (46 men (52%) and 43 women (48%)) aged 24-90 years (mean age 60±15 years) hospitalized at the COVID center of the E. I. Chazov National Medical Research Center of Cardiology from April to June 2020. All patients signed an informed consent for the collection of biological samples for banking approved by the Ethics Committee (Protocol No. 249 of September 30, 2019). Blood samples from 70 patients were retested 3-7 months after discharge.

The severity of COVID-19 was assessed by computer tomography (CT) using scale developed in Russia during pandemic: no signs of viral pneumonia (CT-0); mild pneumonia with ground glass opacities, <25% lung damage (CT-1); moderate pneumonia, 25-50% lung damage (CT-2); moderate to severe pneumonia, 50-75% lung damage (CT-4); and severe pneumonia, >75% lung damage (CT-4).

Blood samples were collected on days 2-4 of hospitalization and during outpatient follow-up examinations. Before the study, samples of citrate plasma and serum were stored at -80°C in a Biological Material Bank.

The levels of IL-6, sgp130, and sIL-6R were determined by ELISA using R&D Systems kits. High sensitive C-reactive protein (hsCRP) was measured in serum using a reagent kit for quantitative immunoturbidimetric determination of CRP (Abbott Laboratories).

Statistical analysis was performed in SPSS Statistics 23 software (IBM). For parameters with non-normal distribution, the results are presented as Me (Q1; Q3). When comparing groups, nonparametric Mann–Whitney test was used for independent samples and Wilcoxon's test was used for linked samples. The relationship between test parameters was evaluated with Spearman's rank correlation coefficient. The results were considered statistically significant at $p<0.05$.

RESULTS

The patients were assigned into 5 groups according to CT evaluation of lung damage: CT-0 (17 patients), CT-1, (16 patients), CT-2, (21 patients), CT-3 (25 patients), and CT-4 (10 patients).

The concentrations of IL-6 and hsCRP increased with severity of lung damage. The highest IL-6 levels were in CT-3 and CT-4 groups. Compared to CT-0 patients, in CT-3 and CT-4 groups, IL-6 levels were increased by 7 and 22 times, respectively, while hsCRP levels were elevated by 13 and 19 times, respectively (Table 1).

In all patients, IL-6 plasma concentrations correlated with hsCRP levels. A positive correlation between IL-6 and hsCRP was established in CT-1, CT-2, and CT-3 patients (Table 2).

The concentration of sIL-6R increased with lung damage severity, but not as rapidly as IL-6 levels. sIL-6R is as a component of IL-6 buffer system and is constantly present in the blood at concentrations markedly higher than those of IL-6 [9], therefore sIL-6R increase is not pronounced. sIL-6R levels were almost the same in CT-1 and CT-0 patients and slightly higher in CT-2 group. Increase in sIL-6R concentration was statistically significant in patients with moderate to severe lung injury (CT-3) compared to CRT-0 and CT-1 patients. In patients with severe lung injury (CT-4), sIL-6R level remained high (Table 1). A positive correlation between sIL-6R and IL-6 levels was established in all patients and CT-3 patients (Table 2). Higher levels of sIL-6 and IL-6 were also observed by other authors [10] in patients with severe COVID-19 in comparison with mild and moderate patients. However, these authors did not reveal any correlation between IL-6 and sIL-6R. The possible reason for these results could be the peculiarities of the group formation in

TABLE 1. The Levels of hsCRP and IL-6 Signaling Components in Patients with COVID-19 with Lung Damage of Varying Degrees (Me (Q1; Q3))

Parameter	CT-0 (n=17)	CT-1 (n=16)	CT-2 (n=21)	CT-3 (n=25)	CT-4 (n=10)
hsCRP, mg/liter	5.50 (1.00; 25.70)	11.90 (2.75; 37.15)	44.90 (18.60; 87.60)	69.50*+ (33.57; 122.15)	106.20*+o (28.85; 146.05)
IL-6, pg/ml	7.87 (2.74; 16.73)	10.47 (5.44; 30.12)	20.13* (17.20; 41.57)	65.90*+o (35.94; 111.10)	173.70*+o (46.01; 333.14)
sIL-6R, ng/ml	46.09 (36.35; 55.25)	46.49 (33.61; 55.34)	48.88 (43.61; 72.02)	57.93*+ (48.97; 72.38)	60.34 (37.74; 76.13)
sgp130, ng/ml	277.66 (243.45; 337.03)	254.96 (239.58; 265.45)	247.25 (229.16; 293.42)	267.23 (240.87; 310.67)	335.92 (266.78; 375.04)

Note. $p<0.05$ in comparison with *CT-0, +CT-1, oCT-2.

TABLE 2. Correlations between IL-6, its Signaling Components, and hsCRP in Patients with COVID-19 Lung Damage of Varying Degrees (Spearman's rank correlation coefficient)

Parameter	CT-0 (*n*=17)	CT-1 (*n*=16)	CT-2 (*n*=21)	CT-3 (*n*=25)	CT-4 (*n*=10)	All patients (*n*=89)
hsCRP	—	*r*=0.568, *p*=0.027	*r*=0.684, *p*=0.001	*r*=0.603, *p*=0.002	—	*r*=0.536, *p*<0.001
sIL-6R	—	—	—	*r*=0.886, *p*=0.019	—	*r*=0.864, *p*<0.001

Note. The sign "—" indicates the absence of statistical significance.

TABLE 3. Levels of IL-6, sIL-6R, and sgp130 in COVID-19 Patients at Admission and 3-7 Months after Discharge

Parameter	At admission (*n*=89)	3-7 months after discharge (*n*=70)
IL-6, pg/ml	28.72 (13.15; 68.01)	2.86 (1.87; 4.52)*
sIL-6R, ng/ml	53.30 (42.05; 72.30)	36.85 (29.87; 46.45)*
sgp130, ng/ml	263.02 (241.70; 307.30)	247.25 (229.16; 293.42)*

Note. *p<0.05 in comparison with the level at admission.

the study [10]: the authors compared patients of hospital ward (moderate form), intensive care unit (severe form), and treated at home (mild form).

sgp130 concentration in CT-1 and CT-2 patients was lower than in CT-0 group, increased in CT-3 patients, and reached the maximum level in CT-4 patients (Table 1). At the same time, we revealed no relationship between IL-6 and sgp130 levels in any of the studied groups.

In all patients, the levels of IL-6, spg130, and sIL-6R markedly decreased 3-7 month after discharge from the hospital (Table 3).

The effects of IL-6 manifested by classic signaling are highly important for the maintenance of immune homeostasis. IL-6 is synthesized at the initial stages of inflammation and is the main inducer of CRP synthesis and secretion in liver [11,12]. Hepatocytes are one of the few cell types expressing the membrane IL-6R through which IL-6 classic-signaling occurs [13]. The positive correlation between IL-6 and hsCRP identified in the present study, as well as the decrease in sgp130 levels in CT-1 and CT-2 patients, allow us to suggest that the IL-6 classic-signaling predominates and IL-6 trans-signaling actively inhibited in mild and moderate forms of pneumonia. This is consistent with the suggestion that IL-6 classic-signaling dominates in moderate COVID-19 [14].

sIL-6R is generated predominantly by proteolytic cleavage of membrane IL-6R and to a lesser extent by alternative splicing [15]. The receptor realizes IL-6 trans-signaling. Proteolytic cleavage of membrane IL-6R reduces the amount of IL-6R on cell surface, which lessens cell ability to be activated via IL-6 classic-signaling. Proteolysis of IL-6R controls the balance between IL-6 classic- and trans-signaling [16].

High levels of sIL-6R associated with increased concentration of IL-6 and elevated sgp130 levels in CT-3 patients suggest active participation of IL-6 trans-signaling in the disease progression. This suggestion agrees with the results of other researchers: high levels of IL-6, sIL-6R, and sgp130 indicate markedly increased risk of severe COVID-19 [17].

Despite supposed decrease in IL-6 classical-signaling, hsCRP levels increased with pronounced lung damage (CT-3 and CT-4). Probably, in addition to

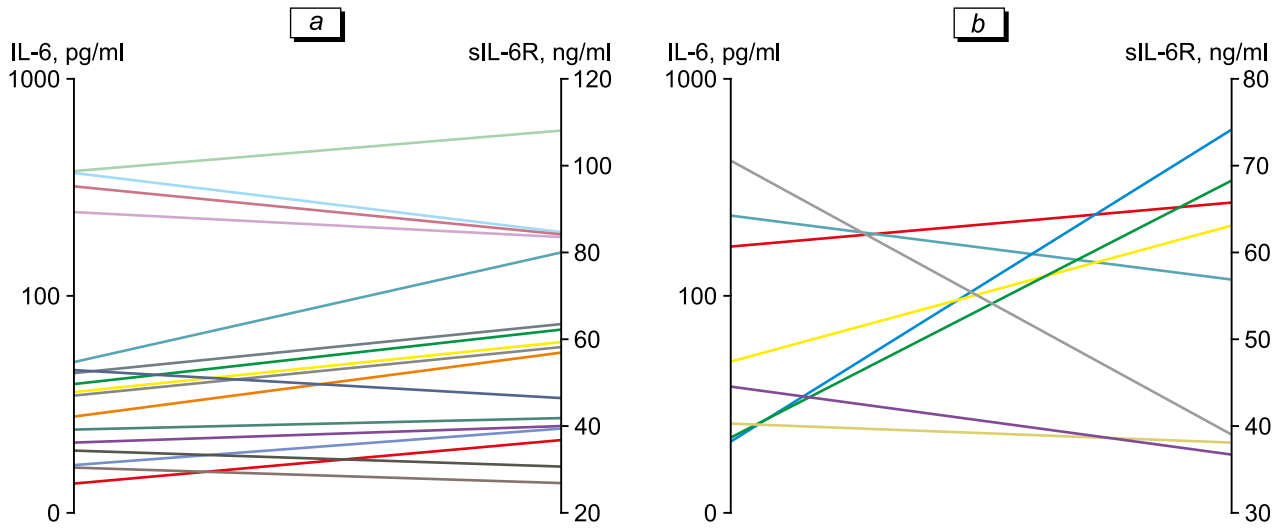

Fig. 1. Individual paired levels of IL-6 and sIL-6R in CT-3 (*a*) and CT-4 (*b*) patients.

classic-signaling, other mechanisms (including IL-6 trans-signaling) are responsible for CRP production in case of severe lung damage.

Interestingly, that the difference in sIL-6R levels was statistically insignificant in CT-4 patients compared to CT-3 but IL-6 and sgp130 levels were higher. In addition, high IL-6 concentrations did not always coincide with high sIL-6R, in contrast to CT-3 (Fig. 1). Therefore, no correlation was revealed between IL-6 and sIL-6R in CT-4 patients. Changes in IL-6/sIL-6R ratio observed at severe stage of the disease reflect impairments in regulatory mechanisms of IL-6 signaling.

Thus, the protective IL-6 classic-signaling prevails in mild and moderate forms of pneumonia induced by SARS-CoV-2, while activation of the IL-6 trans-signaling contributes to the progression of the disease in moderate to severe and severe forms. It should be noted that the results obtained are preliminary and require a broader research.

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Conflict of interest. The authors have no conflicts of interest to declare.

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