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Predicting the Severity of COVID-19 Pneumonia in Children

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Abstract: Today, the relationship between cytokines in blood serum and the pathogenesis of the disease during COVID-19 in children has not yet been fully clarified. Research shows that the course of the disease in children is more satisfactory than in adults. However, the clinical profiles and pathophysiology of COVID-19 in children remain unclear. The aim of our study was to determine the level of cytokines in children with COVID-19 pneumonia and to study their role in predicting the severity of the disease. For this purpose, 75 children under the age of 18 were included. The patients included in the study were divided into 2 groups according to the degree of severity: 49 (65.3%) moderately severe patients with COVID-19 pneumonia were included in group I, and 26 (34.7%) patients with severe COVID-19 pneumonia were included in group II. Clinical, instrumental and laboratory examinations, determination of cytokines IL-1β, IL-6, IL-18, vitamin D, fibrinogen, ferritin and D-dimer levels were included in the examination methods of patients. Fever (66 cases (88.0%)) and cough (74 cases (98.7%)) were the most frequently reported cases. Laboratory examinations of blood in children with COVID-19 (PCR) showed a tendency to lymphocytosis and thrombocytopenia, an increase in ESR, CRP, ferritin, D-dimer, fibrinogen, and cytokines levels. In comparison between groups, the levels of IL-1β, IL-6, IL-18 increased in group II compared to group I. Determination of pro-inflammatory cytokines IL-1β, IL-6, IL-18 in children with COVID-19 (PCR), depending on the severity of the disease, is appropriate for early diagnosis and prediction of the course of the disease.

Keywords: Children, COVID-19, Cytokine Profile, Prognosis

1. Introduction

For the first time, at the end of 2019, a new SARS-CoV-2 infection was announced in Wuhan, China. [1, 2]. It turned out that the new virus infection - COVID-19 can cause clinical manifestations from a mild form of acute respiratory infection to severe acute respiratory syndrome, accompanied by damage to other organs and systems. The decision of the WHO has declared a pandemic since March 2020 [3]. The fact that COVID-19 is a global threat to health and society in the world has created the basis for its comprehensive study.

Scientific studies show that children of all ages, like adults, are at risk of being infected with the coronavirus, and even play an important role in the transmission of the virus [4, 5]. According to many researchers, coronavirus infection in children is mild, even asymptomatic. However, a severe course of the disease is not excluded [6-8]. According to researchers, children are a potential source for the spread of COVID-19 infection, and the fact that the disease is asymptomatic and mild in them leads to the assessment of the epidemiological importance of the child population in the spread of the new coronavirus infection [9, 10]. However, in children there is still little information about the biological features of this infection, and there is a need for more research.

Changes in the cytokine system in the pathogenesis of COVID-19 infection have been noted by many authors [11-13]. Cytokines are biologically active substances that are vital in the regulation of immunological and inflammatory responses. After infection, the virus can multiply in the mucosa of the upper respiratory tract, causing a acute increase in the level of cytokines (cytokine storm). However, despite the existence of a number of scientific studies on the study of the level of cytokines in the blood serum during COVID-19 in children, there are still unclear features in their mechanism of action.

The prognosis of the pathological process is of great importance both from the medical and economic point of view. At a time when the body's resources are exhausted, and the disease puts high demands on the organism, the doctor needs maximum knowledge about the patient, among which the evaluation of the prognosis of the disease is one of the important factors. In each case, the assessment of the prognosis can facilitate the choice of treatment tactics by assigning the patient to a high or low risk group. The prediction models found in the medical literature led us to investigate the predictors of disease progression and build a mathematical model that allows us to predict the condition based on this information [14, 15]. The study was to determine the changes in the cytokine system in children with COVID-19 pneumonia and to identify prognostic criteria that evaluate the development of the disease.

2. Materials and Methods

The contingent of the study was made up of examination results of 75 children under 18 years of age who were diagnosed with COVID-19 pneumonia and received inpatient treatment at Children's Infectious Diseases Hospital No. 7 in 2021. Patients included in the study (37 (49.3%) boys + 38 (50.7%) girls) were divided into 2 groups according to the degree of severity: Group I included 49 (65.3%) patients with moderately severe COVID-19 pneumonia, II the group included 26 (34.7%) severely evaluated patients with COVID-19 pneumonia. Typical multisystem inflammatory syndrome (MIS-C) and death were not recorded in the patients included in this study. Examination methods of patients include anamnestic and epidemiological data (learned from the history of the disease and parents). At the same time, clinical, instrumental and laboratory examinations were carried out (general and biochemical blood analysis, vitamin D, inflammatory markers - ferritin, fibrinogen, D-dimer). Examinations were carried out during the acute period of the disease. A typical diagnosis of COVID-19 was established by polymerase chain reaction (PCR) of a nasopharyngeal swab according to protocol. The SARS-CoV-2 virus RNA test was positive in the pathological material taken from the nasopharynx of all investigated patients, and unilateral or bilateral pneumonia was determined in the X-ray examination of their lungs.

2.1. Exclusion Criteria

Congenital heart defects, bronchial asthma, autoimmune disorders, oncological diseases, primary or acquired immune deficiency, chronic diseases are excluded.

In order to assess the levels of circulating cytokines (Il-1, IL-6, Il-18) in blood serum, reagent kits from the company "Vektor Best" (Russian Federation) were used by the enzyme-linked immunosorbent assay (IFA) method. Measurements were carried out on the "Stat Fax 303+" device.

2.2. Statistical Processing

Statistical data processing was carried out using the methods of variation (U-Mann-Whitney), discriminant (Pearson's Chi-square), correlation (Rho-Spearman), dispersion (ANOVA test, F-Fisher and F-S-Fisher-Snedekor tests) tests, and also using ROC analysis, multiple logistic regression analysis (Backward Wald). All statistical calculations were carried out in MS EXCEL-2019 and IBM Statistics SPSS-26 programs. The null hypothesis was rejected at $p < 0.050$.

3. Results

The analysis of the epidemiological anamnesis showed that the vast majority of cases of the disease described in children were related to their contact with family members and/or other sick children with COVID-19. This clearly shows human-to-human transmission. Most of the COVID-19 patients were urban residents. Thus, 63 (84.0%) of the patients were urban residents, and 12 (16.0%) were from the region. This is explained by the fact that cities are the main centers of the spread of COVID-19, accounting for 90% of the recorded cases of the disease. The brunt of this crisis is felt in cities, as most of them have an overburdened healthcare system.

Patients' breathing was counted in the hospital, SpO2 level was determined with the "Pulse Oximeter CMS50C" device. Laboratory examinations: general and biochemical analyzes of blood were performed, coagulogram results were analyzed. During the x-ray examination of the lungs in the patients were observed infiltrative shadows of different sizes. All examined patients had a moderate or severe course of the disease. During our study, the clinical picture of the disease was typical for the studied pathology. Thus, the main leading symptoms in patients confirmed to be positive for COVID-19 (PCR) were fever and cough. Rarely, muscle pains, loss of sense of smell and taste, headaches have been observed in older children. From the total sample, fever in 66 patients (88.0%), cough in 74 patients (98.7%), dyspnoe in 13 patients (17.3%), loss of sense of smell and taste in 5 patients (6.7%), headache in 7 patients (9.3%), muscle pain in 13 patients (17.3%), vomiting in 14 patients (18.7%), diarrhea was observed in 7 patients (9.3%). Sluggishness was observed in 21 patients (80.0%), cyanosis in 8 patients (30.8%), muscle hypotonia in 16 patients (61.5%). Sp02 - 97.7±0.2 in group I; Sp02-92.5±0.7 ($p<0.001$) was recorded in group II. In the radiological examination, 33 (67.3%) children in group I had one-sided pneumonia, 16 (32.7%) children had bilateral pneumonia, 16 (61.5%) children in group II had one-sided pneumonia, and 10 (38.5%) children had bilateral pneumonia (P=0.615).

During the study, the clinical manifestations recorded in the patients who tested positive for COVID-19 (PCR test) are described in the table 1.

Table 1. *Clinical symptoms of confirmed patients with positive COVID-19 (PCR test).*

	I group n=49	II group n=26	Px2	Pu
	absolute number, %	absolute number, %		
Temperature	45 (91,8%)	21 (80,8%)	0,001	0,001
Cough	48 (98%)	26 (100%)	0,463	0,466
Loss of sense of smell and taste	2 (4,1%)	3 (11,5%)	0,218	0,221
Headache	3 (6,1%)	4 (15,4%)	0,189	0,192
Muscle pain	6 (12,2%)	7 (26,9%)	0,110	0,112
Dyspnea	3 (6,1%)	10 (38,5%)	0,001	0,001
Vomiting	5 (10,2%)	9 (34,6%)	<0,010	<0,010
Diarrhea	1 (2,0 %)	6 (23,1%)	0,003	0,003
Sluggishness	–	21 (80,8%)	–	<0,001
Cyanosis	–	8 (30,8)	–	<0,001
Muscle hypotonia	–	16 (61,5%)	–	<0,001
Pathological course of pregnancy in the anamnesis	2 (4,1%)	10 (38,5%)	<0,001	<0,001

Note: The statistical significance of the difference between the indicators of the groups:
Pχ2- according to the Chi-square Pearson criterion
P_U – according to the Mann-Whitney criterion

Egarding laboratory characteristics, a statistical analysis of the hemogram in children with COVID-19 pneumonia was performed. Some parameters were evaluated in blood serum. Table 2 shows the data of blood clinical examination in patients with positive SARS-CoV-2 (PCR test).

Table 2. *Statistical analysis of hemogram in children with COVID-19 positive pneumonia (PCR test).*

Indicators	I group n=49	II group n=26	p_F	p_U
Erythrocytes q/l	4,53±0,07 (3,43-5,55)	4,10±0,13 (2,86-5,39)	0,001	0,006
Hemoqlobin Hb q/l	12,2±0,2 (9,5-15,2)	11,6±0,4 (9-17,1)	0,100	0,041
Leukocytes q/l	7,08±0,34 (3,1-17,3)	9,97±0,84 (3,41-23,3)	0,001	0,001
Lymphocytes %	47,9±2,4 (11- 83,4)	46,0±4,0 (8,3-80,3)	0,109	0,859
Monocytes %	9,2±0,6 (2-20)	9,2±1,0 (2-22)	0,628	0,692
Neutrophils %	45,2±2,2 (14,5-75,5)	40,3±4,7 (14,5-75,5)	0,173	0,076
Trombocytes q/l	278,6±14,7 (48-521)	294,8±28,2 (121-796)	0,432	1,000
ESR mm/hour	16,5±1,5 (3-48)	19,7±2,9 (5-65)	0,001	0,555

Note: The statistical significance of the difference between the indicators of the groups:
P_F – according to the F-Fisher criterion
P_U- according to the U-Mann-Whitney criterion

The analysis of the blood in the conducted studies showed that the indicators of peripheral blood in patients confirmed positive for COVID-19 (PCR) are not informative in predicting the disease. But most of these laboratory indicators are non-specific and reflect inflammation caused by the virus.

We observed a difference in some indicators of biochemical analyzes in patients confirmed to be positive for COVID-19 (PCR). Table 3 shows the results of biochemical analyzes in patients with positive COVID-19 (PCR).

Table 3. *Statistical analysis of biochemical indicators in COVID-19 (PCR) positive patients (M±m).*

	I group n=49	II group n=26	Referans	P_F	P_U
CRP mq/l	44 (7,4±1,7) (0,16-57,31)	24 (8,9±3,1) (0,6-69)	0-5	0,646	0,969
ALT U/L	34 (38,0±5,5) (7,9-196)	22 (31,1±3,3) (13,4-68,9)	< 31	0,355	0,551
AST U/L	35 (40,4±2,8) (16,3-99,2)	22 (45,6±7,2) (13,9-169)	< 40	0,444	0,915
Creatine mg/dl	33 (39,0±6,0) (0,22-86,2)	21 (40,0±8,1) (0,15-90,2)	44-48	0,885	0,993
Protein U/l	20 (69,3±1,5) (44,1-74,2)	13 (66,5±2,9) (51-77,8)	65-85	0,356	0,927
Albumin q/l	34 (4,21±0,07) (3,1-4,91)	21 (4,05±0132) (3-5,43)	3,5-5,2	0,244	0,205
Glucosa mmol/l	24 (5,38±0,28) (3,63-11)	15 (4,65±0,26) (3,36-6,2)	4,44-6,66	0,080	0,088

Note: The statistical significance of the difference between the indicators of the groups:
pF – According to the F-Fisher criterion
p_U - According to the U-Mann-Whitney criterion

As can be seen from the table, CRP in the blood increased in both groups during the acute period of the disease. Thus, CRP was 7.4±1.7 mg/l in patients with positive COVID-19 (PCR) group I, and this indicator was 8.9±3.1 mg/l in group II patients, but it is not statistically honest (p=0.969). As can be seen from the table, the analysis of indicators of other biochemical analyzes showed that these indicators were within the reference level.

Thus, hematological indicators of blood changed in different ways during the acute period of the disease. We think that this is related to the hematological effect of cytokines, which are increased during inflammation.

The comparison of the average indicators of inflammatory markers - ferritin, D-dimer, fibrinogen in the blood serum of patients confirmed positive for COVID-19 (PCR) is shown in table 4.

Table 4. *Statistical analysis of ferritin, D-dimer, fibrinogen in COVID-19 (PCR) positive patients (M±m).*

	I group n=49	II group n=26	Pu
Ferritin, ng/mL	196,6 ± 16,8 (52,4 – 510)	268,6±42,6 (66,7-1013)	0,319
Fibrinogen, q/l	327,3± 12,0 (179 - 489)	368,8±22,9 (164 - 582)	0,148
D-dimer, Ug/ml	1037,6±378,4 (50-10000)	1504,7±553,8 (50-10000)	0,562

An increase in ferritin, D-dimer, and fibrinogen was observed in both groups of patients. Ferritin was 196.6 ± 16.8 ng/ml in group I, 268.6 ± 42.6 ng/ml in group II (p=0.319), fibrinogen was 327.3 ± 12.0 g/l in group I, and 368.8 ± 22.9 g/l in group II (p= 0.148). D-dimer concentration (in 57 (76.0%) patients) fluctuated in the upper limits of reference norms in both groups. Thus, in the acute period of the disease, this indicator was 1504.7±553.8 Ug/mL in group II, and D-dimer concentration in group I was 1037.6±378.4 Ug/mL (p=0.562). In our previous studies, average indicators of vitamin D were observed to decrease in children with COVID-19 pneumonia [16].

In our study, when we measured the level of pro-inflammatory cytokines IL-1β, IL-6, and IL-18, it was clear from the results that their level increased in children with COVID-19 pneumonia. The level of IL-1β in blood serum in group II was 2.97±0.86 pg/ml (average 0.04-16.8 pg/ml), and in group I was 1.24±0.37 pg/ml (average was 0.02-12.6 pg/ml) (p=0.044). The concentration of IL-6 in blood serum was 4.79 ± 0.62 pg/ml in group II (average 0.3-13.7 pg/ml), in group I 3.54 ± 0.43 pg/ml (0,2-12.5 pg/ml) (p=0.048). The level of IL-18 in blood serum was 396.1±25.2 pg/ml (146.5-891 pg/ml) in group I, 469.2±34.2 pg/ml in group II (average 258-973 pg/ml) (p=0.041). The level of IL-1β, IL-6, IL-18 in SARS-CoV-2 patients is shown in table 5.

Table 5. *Statistical analysis of IL-1β,İL-6,İL-18 in COVID-19 (PCR) positive patients (M±m).*

	I group n=49	II group n=26	P_U
IL-1β, pg/ml	1,24± 0,37 (0,02 – 12,6)	2,97±0,86 (0,04-16,8)	0,044
IL-6, pg/ml	3,54± 0,43 (0,2 - 12,5)	4,79±0,62 (0,3-13,7)	0,048
IL-18, pg/ml	396,1±25,2 (146,5-891)	469,2±34,2 (258-973)	0,041

In the next stage of the study, we performed ROC (receiver operating characteristic) analysis of these indicators to evaluate the diagnostic significance of these indicators during the COVID-19. Specificity and sensitivity of each indicator were evaluated and ROC-curve was constructed.

Figure 1 Shows the informativeness of cytokines in the diagnosis of severity in COVID-19 patients.

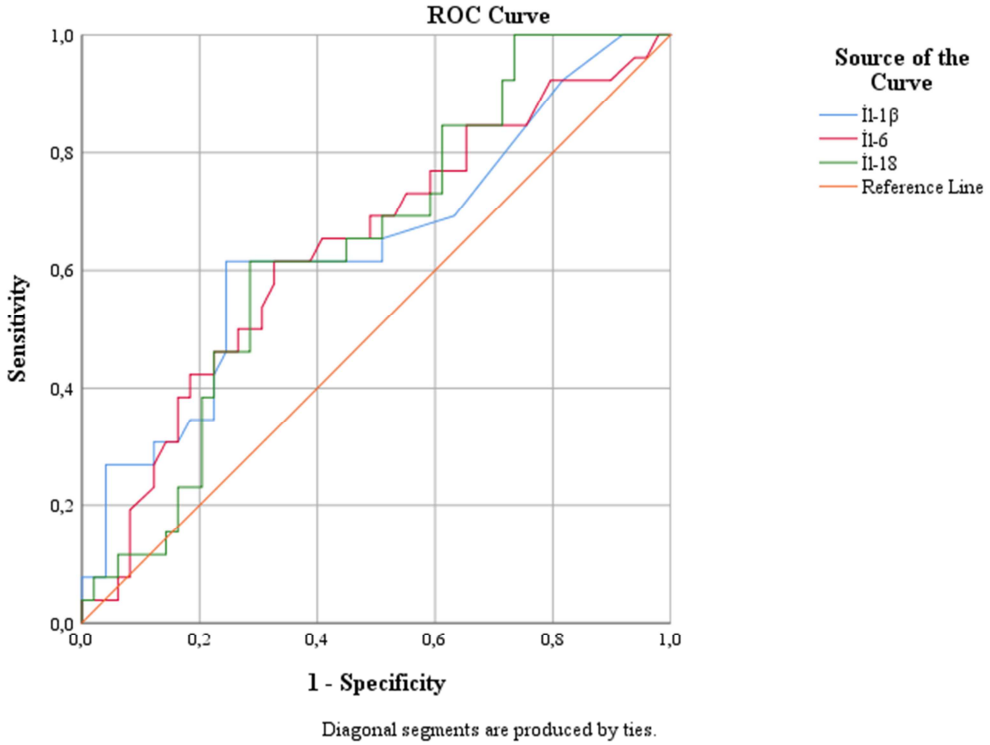


Figure 1. *Results of ROC-analysis in the diagnosis of severity in patients with COVID-19.*

Table 6. *Results of ROC-analysis in the diagnosis of severity in patients with COVID-19.*

Area Under the Curve					
Test Result Variable (s)	**Area**	**Std. Error**	**Asymptotic Sig.**	**Asymptotic 95% Confidence Interval**	
				Lower Bound	**Upper Bound**
İl-1β	0,641	0,070	0,045	0,505	0,778
İl-6	0,639	0,068	0,048	0,506	0,773
İl-18	0,644	0,065	0,041	0,518	0,771

According to the ROC analysis, depending on the degree of severity, all indicators have statistically significant diagnostic value. The area of the ROC curve for the IL-1β indicator is 0.641±0.070; 95% CI: 0.505-0.778: statistically significant with p=0.045. The area of the ROC curve for the IL-6 indicator is 0.639±0.068; 95% CI: 0.506 - 0.773: p=0.048 can be considered diagnostically significant. The area of the ROC curve of the IL-18 indicator is 0.644±0.065; 95% CI: 0.518 - 0.771: p=0.041 can be considered statistically significant.

In the next stage of the research, the goal was to evaluate the points farthest from the reference line (cut-off point) in the coordinates of the ROC-curves, where the total value of specificity and sensitivity is the largest. The calculation was performed on statistically reliable indicators in ROC-analysis. The informativeness of the indicators studied at the cut-off point during COVID-19 is shown in table 6.

Table 7 *Informativeness of the indicators studied at the cut-off point in COVID-19.*

Statistical parameters	**İL-1β**	**İL-6**	**İL-18**
Cut off point	≥ 1	≥ 3,9	≥ 457
Sensitivity Sn%	61,5±9,5	61,5±9,5	61,5±9,5
Specificity Sp%	75,5±6,1	67,3±6,7	71,4±6,5
GDV %	70,7±5,3	65,3±5,5	68,0±5,4
pPV%	57,1±9,4	50,0±8,8	53,3±9,1
nPV%	78,7±6,0	76,7±6,4	77,8±6,2

Note. Sn- sensitivity; Sp – specificity; GDV- general overall diagnostic value; pPV (nPV) – positive (neqativ) predictive values

The cut off point of IL-1β concentration in blood is ≥1 pg/ml. At this point, the sensitivity (Se) is 61.5±9.5%, the specificity (Sp) is 75.5±6.1%, and the predictive effect due to positive and negative values is 57.1±9.4, 78.7±6.0, respectively is rated as sufficient. Cut off point for IL-6 ≥ 3.9 pg/ml, Sn=61.5±9.5%, Sp=67.3±6.7%; positive and negative values 50.0±8.8; 76.7±6.4. Cut off point for IL-18 ≥ 457pg/ml, Sn=61.5±9.5%, Sp=71.4±6.5%; positive and negative values 53.3±9.1; 77.8±6,2. As can be seen from the table, IL-1β, IL-18 are sufficiently evaluated for the accuracy of the positive result from the indicators. During the general diagnostic evaluation, the GDV % of IL-1β was 70,7±5,3, IL-6 was 65,3±5,5, and IL-18 was 68,0±5,4 which indicates the high diagnostic value of these examined indicators. In our previous studies, we showed that the cut-off point for vitamin D is ≥ 21 pg/ml, Sn=50.0±9.8%, Sp=81.6±5.5%; positive and negative values are 59.1±10.5; 75.5±5.9, the GDV% was 70,7±5,3.

In the next stage of the research, the Efficiency influence of factor (EIF) was evaluated with the help of ANOVA dispersion (FS-Fisher Snedekor) analysis.

Table 8. *The strength of the influence of some studied factors in the diagnosis of the severity of COVID-19 positive patients.*

İndicators	**Cut-off point**	**EIF (95% CI)**	**P**
İL-1β pg/ml	>1	13,3 (8,6-18,0)	0,001
İL-6 pg/ml	>3,9	7,7 (2,7-12,7)	0,016
İL-18 pg/ml	>457	10,3 (5,4-15,1)	0,005

As can be seen from Table 8, IL-1β has a higher rate of impact on the course of the disease; EIF = 13.3; 95% CI -8.6-18.7; p=0.001. The indicators of influence of other factors were as follows: IL-6 - EIF = 7.7; 95% CI- 2.7-12.7; p=0.016; IL-18 - EIF = 10.3; 95% CI- 5.4-15.1; p=0.005. A change in the concentration of cytokines indicates the manifestation of the inflammatory process, the possibility of aggravation of the damage in the lungs. Vitamin D EIF = 10.9; 95% CI- 6.1-15.8; p=0.004.

Correlation relations between the clinical and laboratory indicators were studied.

Table 9. *Results of correlation analysis in the patients with COVID-19.*

		Condition	**T**	**SpO2**	**Dyspnea**	**Vomiting**	**Diarrhea**	**Pathological course of pregnancy**
Condition	P	1,000	0,389	-0,713	0,407	0,298	0,344	0,446
	P		0,001	0,000	0,000	0,009	0,003	0,000
T	P	0,389	1,000	-0,319	0,154	0,126	0,287	0,123
	P	0,001		0,005	0,186	0,281	0,012	0,293
SpO2	P	-0,713	-0,319	1,000	-0,459	-0,233	-0,357	-0,265
	P	0,000	0,005		0,000	0,045	0,002	0,021
Dyspnea	P	0,407	0,154	-0,459	1,000	-0,039	0,095	0,281
	P	0,000	0,186	0,000		0,743	0,416	0,015
Vomiting	P	0,298	0,126	-0,233	-0,039	1,000	0,670	0,071
	P	0,009	0,281	0,045	0,743		0,000	0,545
Diarrhea	P	0,344	0,287	-0,357	0,095	0,670	1,000	0,110
	P	0,003	0,012	0,002	0,416	0,000		0,347
Pathological course of pregnancy	P	0,446	0,123	-0,265	0,281	0,071	0,110	1,000
	P	0,000	0,293	0,021	0,015	0,545	0,347	
WBC	P	0,403	0,168	-0,303	0,283	0,098	0,136	0,282

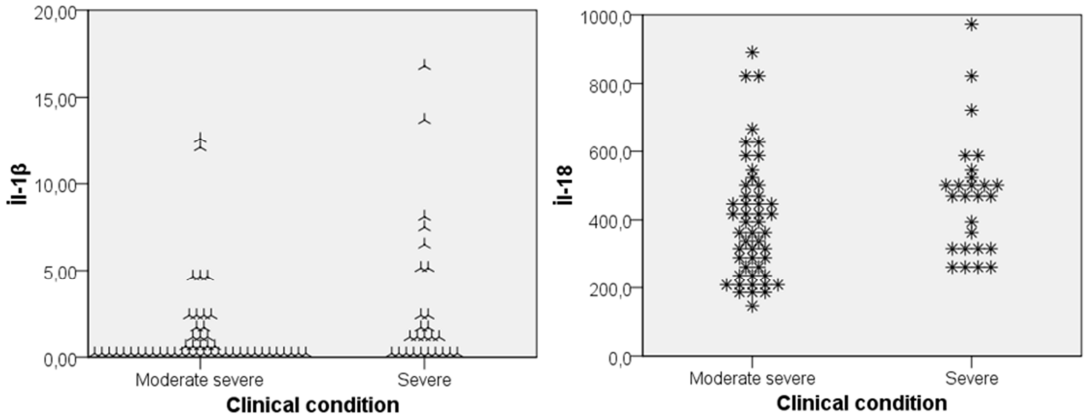
		Condition	T	SpO2	Dyspnea	Vomiting	Diarrhea	Pathological course of pregnancy
	P	0,000	0,150	0,008	0,014	0,403	0,246	0,014
RBC	P	-0,319	-0,140	0,426	-0,129	-0,161	-0,313	-0,090
	P	0,005	0,230	0,000	0,269	0,167	0,006	0,443
HGB	P	-0,238	-0,114	0,372	-0,029	-0,074	-0,249	0,096
	P	0,040	0,329	0,001	0,803	0,531	0,031	0,414
İl-1β	P	0,234	0,206	-0,097	0,099	-0,064	0,012	0,193
	P	0,043	0,076	0,408	0,398	0,588	0,921	0,097
İl-6	P	0,230	0,060	-0,117	-0,048	-0,040	-0,048	0,078
	P	0,047	0,609	0,317	0,682	0,731	0,685	0,505
İl-18	P	0,238	0,111	-0,103	-0,101	0,058	0,032	0,134
	P	0,040	0,345	0,377	0,389	0,618	0,787	0,250
Vit. D	P	-0,227	0,003	0,094	-0,366	0,098	0,050	-0,297
	P	0,051	0,978	0,420	0,001	0,403	0,672	0,010

Table 9. *Continued.*

		WBC	RBC	HGB	İl-1β	İl-6	İl-18	Vit. D
Condition	P	0,403	-0,319	-0,238	0,234	0,230	0,238	-0,227
	P	0,000	0,005	0,040	0,043	0,047	0,040	0,051
T	P	0,168	-0,140	-0,114	0,206	0,060	0,111	0,003
	P	0,150	0,230	0,329	0,076	0,609	0,345	0,978
SpO2	P	-0,303	0,426	0,372	-0,097	-0,117	-0,103	0,094
	P	0,008	0,000	0,001	0,408	0,317	0,377	0,420
Dyspnea	P	0,283	-0,129	-0,029	0,099	-0,048	-0,101	-0,366
	P	0,014	0,269	0,803	0,398	0,682	0,389	0,001
Vomiting	P	0,098	-0,161	-0,074	-0,064	-0,040	0,058	0,098
	P	0,403	0,167	0,531	0,588	0,731	0,618	0,403
Diarrhea	P	0,136	-0,313	-0,249	0,012	-0,048	0,032	0,050
	P	0,246	0,006	0,031	0,921	0,685	0,787	0,672
Pathological course of pregnancy	P	0,282	-0,090	0,096	0,193	0,078	0,134	-0,297
	P	0,014	0,443	0,414	0,097	0,505	0,250	0,010
WBC	P	1,000	-0,142	-0,054	0,205	0,089	-0,036	0,041
	P		0,225	0,644	0,078	0,449	0,758	0,726
RBC	P	-0,142	1,000	0,819	0,044	0,079	-0,096	-0,022
	P	0,225		0,000	0,709	0,503	0,415	0,851
HGB	P	-0,054	0,819	1,000	0,039	0,008	-0,127	-0,247
	P	0,644	0,000		0,741	0,948	0,276	0,033
İl-1β	P	0,205	0,044	0,039	1,000	0,084	0,297	-0,142
	P	0,078	0,709	0,741		0,475	0,010	0,225
İl-6	P	0,089	0,079	0,008	0,084	1,000	0,341	0,170
	P	0,449	0,503	0,948	0,475		0,003	0,146
İl-18	P	-0,036	-0,096	-0,127	0,297	0,341	1,000	0,133
	P	0,758	0,415	0,276	0,010	0,003		0,254
Vit. D	P	0,041	-0,022	-0,247	-0,142	0,170	0,133	1,000
	P	0,726	0,851	0,033	0,225	0,146	0,254	

Note: ρ – correlation coefficient (ρ-Spearman criterion)
p – statistical significance of the correlation coefficient

The relationships between the patient's clinical condition and cytokines, Vit. D.



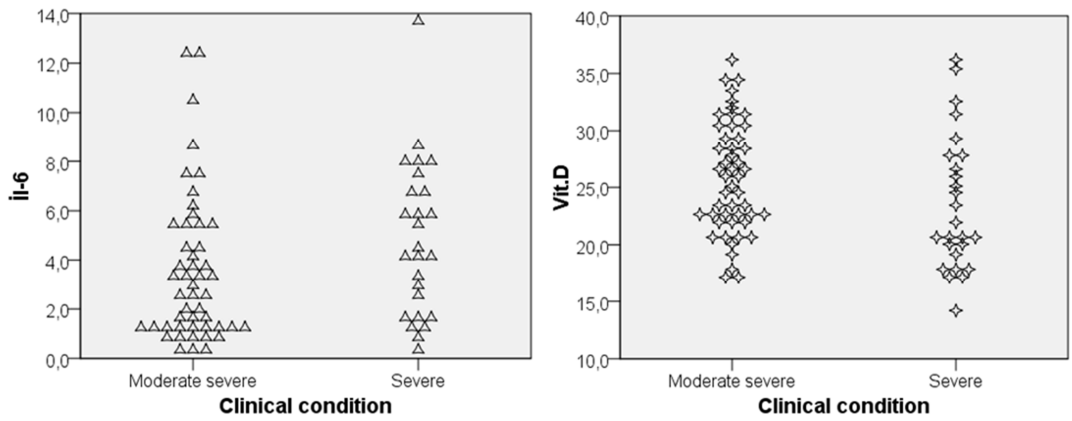


Figure 2. *Correlations between cytokines, Vit. D and severity of disease in children with COVID-19.*

In order to predict the severity of the disease in patients, a logistic regression analysis was performed on clinical and laboratory indicators that were statistically different from each other as a result of previous studies. Patients with moderately severe course of the disease were included in the regression model as "0", and severe patients as "1".

Logistic Regression

Table 10. *Case Processing Summary.*

Unweighted Cases		N	Percent
Selected Cases	Included in Analysis	75	100,0
	Missing Cases	0	0,0
	Total	75	100,0
Unselected Cases		0	0,0
Total		75	100,0

Table 11. *Dependent Variable Encoding.*

Original Value	Internal Value
Moderate severe	0
Severe	1

In the first step of the binary classification table, all patients were included in the "0" group.

Table 12. *Classification Table.*

Observed			Predicted		
			Severity		Percentage Correct
			Moderate severe	Severe	
Step 0	Severity	Moderate severe	49	0	100,0
		Severe	26	0	0,0
	Overall Percentage				65,3

Logistic regression analysis was performed using two algorithms: forward and backward methods. The best results were obtained in a stepwise backward elimination Wald model.

In the first step, 13 indicators were entered into the model: T, SpO2, dyspnoe, vomiting, diarrhea, the pathological course of pregnancy, WBC, RBC, HGB, IL-1β, IL-6, IL-18, vitamin D. After a 9-step calculation procedure, 6 indicators were removed from the model, which were correlated with each other and did not statistically worsen the result: T, shortness of breath, diarrhea, RBC, HGB, IL-6.

The regression model was built on 7 indicators that improved the result statistically.

Table 13. *Variables in the Equation.*

		B	S. E.	Wald	Df	Sig.	Exp (B)
Step 9	SpO2	-3,541	1,794	3,895	1	0,048	0,029
	Vomiting	14,486	8,094	3,203	1	0,073	1955312,480
	Pathological course of the pregnancy	6,665	4,217	2,498	1	0,114	784,636
	WBC	1,339	0,809	2,737	1	0,098	3,816
	İl-1β	1,130	0,622	3,298	1	0,069	3,094
	İl-18	0,024	0,014	3,177	1	0,075	1,024
	Vit. D	-1,062	0,606	3,064	1	0,080	0,346
	Constant	334,288	170,207	3,857	1	0,050	1,512E+145

The received logistic regression model made it possible to determine that the patient's condition was severe with 92.3% sensitivity and 98.0% specificity. The overall prognostic value was 96.0%.

Table 14. *Classification Table.*

Observed			Predicted		
			Severity		Percentage Correct
			Moderate severe	Severe	
Step 9	Severity	Moderate severe	48	1	98,0
		Severe	2	24	92,3
	Overall Percentage				96,0

Thus, the logistic regression model was characterized by the following formula.

Severity of disease = 334,288 + 3,541 x SpO2 + 14,486 x Vomiting + 6,665 x pathological course of pregnancy + 1,339 x WBC + 1,130 x İl-1β + 0,024 x İl-18 – 1,062 x Vit.D

Here, the case with clinical signs is included in the report with the number "1", and the case without it with the number "0". If the result of the regression model is less than 0.5, the condition is considered moderate, otherwise severe.

The received formula was posted at https://amu.edu.az/page/1327/elm for easy use by practicing doctors and researchers, and was kept open for notes and discussions.

4. Discussion

Thus, pro-inflammatory cytokines increased in the patients we observed during the acute period of the COVID-19 disease. COVID-19 is an inflammatory disease. Pro-inflammatory cytokines, which have a role in the pathogenesis of inflammatory diseases and influence their course, play a role in the proliferation, differentiation, and activation of immune system cells. The study of the mentioned pro-inflammatory cytokines in the blood serum shows that they have high fidelity for the diagnosis and prediction of the course of COVID-19. To date, a limited information is known about the cytokine profile in children infected with SARS-CoV-2. It is known that IL-1β, IL-6 is one of the pyrogenic cytokines and they cause an increase in acute phase proteins. Affecting the hypothalamic and pituitary center, IL-1β is the cause of fever, lack of appetite, sleep disorders, lethargy and other symptoms. It is consistent with the literature data showing the expression of IL-1β in the pathogenesis of COVID-19. Hala K. S. et al., examining the cytokine profile in children with SARS-CoV-2 infection, showed increased levels of circulating serum IL-1β early in the course of the disease, while other key pro-inflammatory cytokines such as IL-6 showed only slightly elevated levels. Ulhaq Syambani, Hala K. Ş., Qian G. (2021), Wu Huan and many researchers found that IL-6 was elevated in the normal range or average concentration in children with confirmed positive COVID-19 (PCR) while Sun D noted that it increased [17-20]. IL-6 increases the permeability of the vascular walls, increases the expression of adhesion molecules, as a result of which some biologically active substances, prostaglandins are secreted and cause exacerbation of the inflammatory process. IL-18 increases the activity of T-lymphocytes and NK cells during viral infections, stimulates the differentiation of T-helper in the direction of Th1 by creating interaction between macrophages and lymphocytes. As a result, IL-18 regulates the interrelationships between cellular and humoral immunity in respiratory diseases. The role of IL-18 in the pathogenesis of COVID-19 is reflected in the literature [21, 22].

Thus, in response to the inflammatory process in COVID-19 infection, the level of IL-1β, IL-6, and IL-18 significantly increased, which indicates the active level of the inflammatory response in the organism. This suggests that in sick children, cytokines are produced to resist viral invasion and attempt to suppress the inflammatory response in the early phase of SARS-CoV-2. Wenjie Lu (2021) and colleagues came to similar conclusions [23]. However, further research is needed.

5. Conclusion

1) Depending on the severity of the disease, pro-inflammatory cytokines IL-1β, IL-6, IL-18 can be biomarkers for early diagnosis and prediction of the course of the disease in COVID-19 (PCR) positive children. These findings support the need for future studies to predict the severity of the disease in SARS0CoV-2 infection.
2) The received logistic regression model made it possible to determine that the patient's condition was severe with 92.3% sensitivity and 98.0% specificity. The overall prognostic value was 96.0%.

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