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Clinical and immunological features in children with multisystem inflammatory syndrome associated with SARS-CoV-2

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Abstract. *Background and aim of the research:* MIS-C is characterized by intense immune activation and increased production of cytokines. The aim of our study was to analyze the changes of cellular and humoral immune responses in children with MIS-C, depending on the severity of the disease. *Methods:* Clinical, hematological and immunological parameters in children with severe and extremely severe MIS-C were compared. The study included a total of 50 patients divided into 3 groups: 20 children with extremely severe MIS-C requiring ICU care (MIS-C ICU "+"); 15 children with severe MIS-C but not requiring ICU care (MIS-C ICU "-"); and a control group of 15 children who had previously had COVID-19 and didn't have MIS-C (MIS-C "-"). *Results:* In patients with MIS-C requiring ICU care, heart and liver damage, hematological changes, and the development of severe complications such as edematous syndrome, polyserositis, DIC, and cardiogenic shock were statistically more common. Both groups of children with MIS-C had CD3+ T-cell lymphopenia and a decrease in CD95 expression. In the group of children with MIS-C requiring ICU care, a significant increase in the relative numbers of B-lymphocytes, CD3-HLA-DR+ and CD25 and a decrease of NK-cells was observed. *Conclusions:* Conditions requiring ICU care in children with MIS-C are associated with a more profound immune dysregulation, as evidenced by our data. (www.actabiomedica.it)

Key words: MIS-C, children, ICU, immunophenotyping, cytokines.

Introduction

SARS-CoV-2-associated multisystem inflammatory syndrome (MIS-C) occurs in children 4-6 weeks after the infection (1). The disease causes multiple organ failure manifested by gastrointestinal, cardiovascular, hematological, mucocutaneous, neurological and respiratory symptoms (2). Some patients suffer from a more severe course of the disease and need intensive care unit (ICU) care (3). According to various studies, 50-80% of children with MIS-C require ICU care (4). In Kazakhstan, since August 1, 2020, a total of 96 children with MIS-C have been registered; 52% of them required ICU care (5). According to many authors, the development of MIS-C is associated with high immune activation (6), but it is not known how high this activation gets and whether it is related to the severity of the disease.

At present, the crucial task is to determine the most significant surface antigens of the immunocompetent cells, both in the normal immune response and in pathology. MIS-C is characterized by signs of intense immune activation with increased production of cytokines (7). The first immunological studies of MIS-C described NK cell cytopenia (8). Another study reported depletion of effector CD8+ T cells and natural killer (NK) cells (9). These studies suggest that persistent hyperinflammation may be associated with

depletion of NK cells followed by depletion of CD8+ T cells. Also, CD8+ T cells play a crucial role in the pathology associated with viral infection (10). However, in the case of NK cell depletion, an increase of CD8+ T cells may be the cause of the immunopathology observed in patients with MIS-C (11,12).

The aim of our study was to analyze the changes of cellular and humoral immune responses in children with MIS-C, depending on the severity of the pathology. Considering that in our and most foreign studies, more than 50% children had extremely severe cases of MIS-C and required ICU care, we decided to compare the immune response and clinical characteristics of children with MIS-C who needed ICU care and those who didn't.

Materials and methods

A total of 50 patients participated in the study; 35 of them were diagnosed with MIS-C and were undergoing inpatient treatment in 11 children's medical organizations in Kazakhstan between June 5, 2021 and November 1, 2021. All the patients received their MIS-C diagnosis from an interdisciplinary board according to the case definition criteria established by the WHO and CDC.

The patients were divided into 3 groups: 20 children with extremely severe course of MIS-C requiring ICU care (MIS-C ICU "+"); 15 children with severe MIS-C but not requiring ICU care (MIS-C ICU "-"); and a control group of 15 children who had previously had COVID-19 and didn't have MIS-C (MIS-C "-").

Informed consent to participate in the study was obtained from the guardians of the children with MIS-C and the control group (children who had had COVID-19). Blood samples for immunological studies in patients with MIS-C were taken in the first week of hospitalization. In the control group, blood samples were obtained through outpatient clinics after IgG antibodies to SARS-CoV-2 were detected. Delivery of biological material met all the necessary criteria.

Analysis of clinical data

We collected the following data: gender, age, co-morbidities, epidemiological data on SARS-CoV-2, general clinical laboratory studies (complete blood count, erythrocyte sedimentation rate (ESR), biochemical studies - alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein, albumin, creatinine, urea), inflammatory markers (ferritin, procalcitonin, C-reactive protein (CRP)). Manifestations of symptoms and syndromes of MIS-C (fever, changes in skin, events of the central nervous system and gastrointestinal tract, respiratory and cardiovascular disorders, acute renal failure, edema, pain syndrome, conjunctivitis, shock and disseminated intravascular coagulation syndrome (DIC)) were evaluated in all the patients.

Immunophenotyping

The main lymphocyte subpopulations were determined using laser flow cytometry on a FACSCanto II flow cytometer (Becton Dickinson, USA). The following antibody panel was used in the study: CD3, CD4, CD8, CD16+56, CD19, HLA-DR, CD25, CD95, CD279. The study was carried out according to the manual for the reagent kit.

Concentration of total immunoglobulins

The level of immunoglobulins was determined using an ARCHITECT chemiluminescent microparticle immunoassay analyzer (USA). Reagent kits from Abbott Laboratories, USA were used according to the manual.

Level of cytokines

The concentration of cytokines was determined by enzyme immunoassay using reagent kits from Vector BEST (Russia) according to the manual. The following cytokines were determined: IL-1β, IL-2, IL-6, IL-10, FNO. The results were obtained using a Stat Fax-2100 analyzer (USA).

Antibodies to SARS-CoV-2 IgM and IgG

Antibodies to the receptor binding domain (RBD) of the SARS-CoV-2 spike glycoprotein IgM and IgG were determined by enzyme immunoassay using reagent kits from Vector BEST (Russia).

Ethics

This study was approved by the local ethics committee of the Kazakh National Medical University named after Asfendiyarov (Decision No. 1147, dated 06/01/2022).

Statistical analysis

Statistical analysis was carried out using StatTech v.2.8.8. Quantitative variables were assessed for normal distribution using the Shapiro-Wilk test.

Quantitative variables with normal distribution were expressed as mean (M) and standard deviation (SD), 95% confidence interval (95% CI). In non-normal distribution, quantitative data were described with the median (Me) and the lower and upper quartiles (Q1 – Q3). Categorical data were described using absolute values and percentages.

Comparison of quantitative variables with normal distribution of two groups, provided the variances were equal, was performed using the Student's t-test; whereas in the non normal distribution, the Mann-Whitney U test was applied.

Comparison of quantitative variables with normal distribution of three or more groups was performed using the one-way ANOVA test; post hoc comparisons were done using the Tukey test (if the variances were equal) or the Games-Howell test (if the variances were unequal). In non-normal distribution, the comparison of three or more groups was performed using the Kruskal-Wallis test; Dunn's test with Holm adjustment was used as a post-hoc test.

The graphical presentation of the data was designed using Prism 8 (GraphPad software).

Results

During the study period, 38 children with MIS-C were identified. The guardians of 35 patients agreed to participate in the study. Children for the control group were selected after the detection of IgG antibodies to SARS-CoV-2 from among the first 15 who agreed to participate in the study.

The average age in the MIS-C ICU "+" group was 5 ± 3 years, while in the MIS-C ICU "-" and MIS-C "-" groups children were older (10 ± 5 years). There was no statistically significant difference among the groups with respect to sex. Symptoms of COVID-19 and respiratory infections 2 months prior to the sampling were observed significantly more often in children with MIS-C who didn't require ICU care and in children with no MIS-C compared to children from the MIS-C ICU "+" group (Table-1). Demographic, clinical and laboratory data are presented in Table 1.

In MIS-C ICU "+" children myocarditis, pericarditis, pleurisy, liver damage, edema syndrome, DIC, and shock were significantly more common compared to the MIS-C ICU "-" group. Moreover, in the MIS-C ICU "+" group there were twice as many organs and systems involved in the pathological process as in the MIS-C ICU "-" group. In hematological parameters, MIS-C ICU "+" patients had more pronounced leukocytosis, higher procalcitonin, ALaT, ASAT, urea and lower levels of hemoglobin, platelets and total protein compared to the MIS-C ICU "-" group, while there were no significant differences in ESR, CRP and ferritin between the two groups.

Analysis of administered treatment revealed that children from the MIS-C ICU "+" group more often needed inotropic support drugs, diuretics, transfusion of fresh frozen plasma, red blood cells and albumin, oxygen therapy and mechanical ventilation compared to children from the MIS-C ICU "-" group. The average hospital stay for the MIS-C ICU "+" group was 18 days, while that for the MIS-C ICU "-" group was 8 days. 32 (91.4%) children were discharged with improvement, 3 children (8.6%) died (Table 1).

Immunophenotyping of children in both groups with MIS-C showed a decrease in the relative number of CD3+ T-lymphocytes below the reference range, while the number of CD3+ T-lymphocytes in the control group without MIS-C was within the normal range (Figure 1a). The median of CD4+ T-lymphocytes relative values in both groups with MIS-C was also below the reference range but did not differ statistically significantly from the MIS-C "-" group (Figure 1b). There was a considerable difference in the relative values of cytotoxic cells of the innate and adaptive immune system: in the MIS-C ICU "+" group, cytotoxic CD8+ T-lymphocytes were within the normal range,

Table 1. Demographic, clinical and laboratory characteristics of the group.

Parameters	MIS-C ICU«+» (n-20)	MIS-C ICU«-» (n-15)	MIS-C«-» (n-15)	*P-value
Demographics				
Age (M ± SD)	5 ± 3	10 ± 5	10 ± 5	**MIS-C ICU «+» vs MIS-C ICU«-» p=0.001 MIS-C ICU «+» vs MIS-C «-» p=0.001**
Sex (boys/girls)	10/10 (50/50%)	12/3 (80/20%)	9/6 (60/40%)	0.191
Co-morbidities	9 (45%)	3 (20%)	2 (13.3%)	0.084
Symptoms of Covid-19/ ARVI before sampling	2 (10%)	6 (46.7%)	6 (46.7%)	**MIS-C ICU «+» vs MIS-C ICU«-» p=0.042 MIS-C ICU «+» vs MIS-C «-» p=0.042**
Incidence of clinical manifestations				
Parameters	**MIS-C ICU «+» (n-20)**	**MIS-C ICU «-» (n-15)**		***P-value**
Fever	19 (95%)	15 (100%)		1.0
Duration of fever (in days) Me (IQR)	7 (5-8)	7 (6-8)		0.958
Rash	15 (75%)	11 (73.3%)		1.0
Neurological symptoms (headache, meningeal symptoms, hyperesthesia, seizures)	12 (60%)	6 (40%)		0.315
Gastrointestinal events (vomiting, diarrhea, abdominal pain)	19 (86.4%)	11 (84.6%)		1.0
Myocarditis	11 (55%)	1 (6.7%)		**0.004**
Coronary involvement	0 (0%)	1 (6.7%)		0.429
Pericarditis	11 (55%)	1 (6.7%)		**0.004**
Pneumonia	17 (85%)	8 (53.3%)		0.062
Pleurisy	5 (33.3)	16 (80%)		**0.013**
Pain syndrome (abdominal pain, myalgia, arthralgia, chest pain, sore throat)	15 (75%)	13 (86.7%)		0.675
Edema syndrome (swelling of the face, limbs, scrotum)	14 (70%)	5 (33.3%)		**0.044**
Conjunctivitis	8 (40%)	12 (80%)		**0.037**
Liver damage	16 (80%)	3 (20%)		**<0.001**
Lymphadenopathy	6 (30%)	3 (20%)		0.7
Acute renal failure	9 (45%)	2 (12.3%)		0.069
DIC syndrome	9 (45%)	1 (6.7%)		**0.022**
Cardiogenic shock	11 (55%)	0 (0%)		**<0.001**
Thrombosis	3 (15%)	0 (0%)		0.244
The number of organs affected (Me; IQR)	6 (4-7)	3 (2-4)		**0.002**
Death	3 (15%)	0 (0%)		0.244
Quantitative change in laboratory test values				
Leukocytes (M ± SD) [× 10^9 /L]	24 ± 13	16 ± 8		**0.05**
Lymphocytes % (Me; IQR)	10 (6 – 15)	10 (6 – 12)		0.627

Parameters	MIS-C ICU «+» (n-20)	MIS-C ICU «-» (n-15)	*P-value
Neutrophils % (M ± SD)	83 ± 8	83 ± 8	0.93
Hemoglobin (Me; IQR) [g/dl]	84 (76 – 106)	112 (102 – 122)	**0.004**
Platelets (Me; IQR) [× 10^9 /L]	94 (34 – 158)	190 (156 – 232)	**0.01**
ESR (M ± SD) мм/час	40 ± 19	34 ± 19	0.33
CRP (Me; IQR) [mg/L]	81 (22 – 130)	106 (93 – 122)	0.089
Ferritin (Me; IQR) [ng/ml]	661 (370 – 944)	400 (306 – 626)	0.243
Procalcitonin (Me;IQR) [ng/ml]	14 (4 – 23)	3 (0 – 10)	**0.031**
Creatinine (Me; IQR) [μmol/l]	82 (53 – 188)	50 (38 – 77)	0.099
Urea (Me; IQR) [mmol/l]	9 (6 – 21)	5 (4 – 6)	**0.021**
ALT (Me; IQR) [U/L]	69 (48 – 150)	25 (14 – 30)	**0.004**
AST (Me; IQR) [U/L]	98 (73 – 138)	32 (19 – 49)	<**0.001**
Total protein (M ± SD) [g/L]	50 ± 5	58 ± 9	**0.003**
Albumin (M ± SD) [g/L]	27 ± 6	31 ± 8	0.132
Therapy			
Intravenous immunoglobulin	19 (95.5%)	13 (86.7%)	0.565
Corticosteroids	17 (89.5%)	14 (93.3%)	1.0
Biological immunomodulatory drugs	1 (5%)	0 (0%)	1.0
Anticoagulants	14 (70%)	12 (80%)	0.7
Acetylsalicylic acid	4 (20%)	3 (20%)	1.0
Antibiotics	20 (100%)	15 (100%)	1.0
Dobutamine	11 (55%)	0 (0%)	<**0.001**
Diuretics	18 (90%)	8 (53.3%)	**0.022**
Red cell transfusion	8 (40%)	0 (0%)	**0.006**
Transfusion of fresh frozen plasma	9 (45%)	1 (6.7%)	**0.022**
Albumen	13 (65%)	4 (26.7%)	**0.041**
Oxygen	12 (60%)	0 (0%)	<**0.001**
Artificial lung ventilation	6 (30%)	0 (0%)	**0.027**
Day of inpatient care at the time of sampling (in days) Me (IQR)	7 (5-9)	5 (4-7)	0.233
Duration of ICU care (in days, Me;IQR)	8 (5-12)	-	-
Duration of hospital stay (in days, Me;IQR)	18 (13-33)	10 (10-13)	**0.001**

* Statistically significant values are highlighted

while in the MIS-C ICU "-" and MIS-C "-" groups, there was a significant increase in the amount of these cells (Figure 1c). Also, in the MIS-C ICU "+" group, there was a marked decrease in the relative and absolute numbers of NK cells, while in the other two groups, the average value of NK cells was within the normal range (Figure 1d). The median of the immune regulatory index (IRI) in the MIS-C ICU "+" group was within the reference range, whereas in the MIS-C ICU "-" and MIS-C "-"groups, the CD4/CD8 ratio was below normal due to an increase in cytotoxic T cells (Figure 1e).

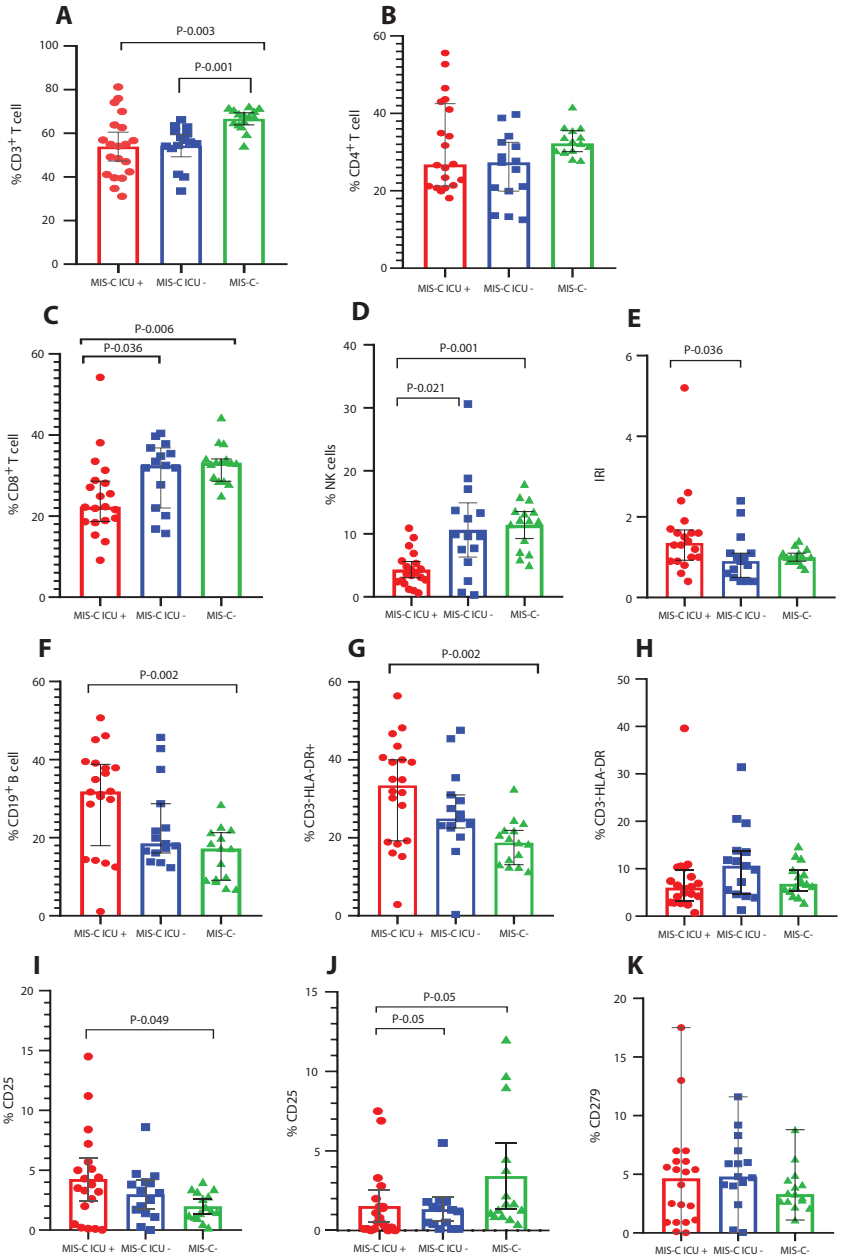

Figure 1. Changes of cellular immune response parameters in all the studied groups.

a) Absolute white blood cell count; the Kruskal-Wallis test was used to determine statistically significant differences;
b) Relative number of lymphocytes; Fisher's F-test was used to determine statistically significant differences;
c) Relative number of CD3+ T-lymphocytes; Welch's F-test was used to determine statistically significant differences;
d) Relative number of CD4+ T-lymphocytes;
e) Relative number of CD8+ T-lymphocytes; the Kruskal-Wallis test was used to determine statistically significant differences;
f) Relative number of NK cells; Welch's F-test was used to determine statistically significant differences;
g) Immunoregulatory index; the Kruskal-Wallis test was used to determine statistically significant differences;
h) The relative number of CD19+ B-lymphocytes; the Kruskal-Wallis test was used to determine statistically significant differences;
i) The relative number of CD3-HLA-DR+ B-lymphocytes; the Kruskal-Wallis test was used to determine statistically significant differences;
j) Relative number of CD3+HLA-DR+ T-lymphocytes;
k) Relative amount of CD25; Welch's F-test was used to determine statistically significant differences;
l) Relative number of CD95; Fisher's F-test was used to determine statistically significant differences;
m) Relative number of CD279.

The relative number of CD19+ B cells in the MIS-C ICU "+" group was almost twice as high as the corresponding rates in the MIS-C ICU "-" group and in the MIS-C "-" group (Figure 1f). The same changes in the humoral immune response were observed when analyzing the surface markers of B-lymphocyte activation CD3-HLA-DR+: they were statistically higher in the MIS-C ICU "+" group compared to the control group without MIS (Figure 1g). HLA-DR is a marker of both late and long-term cell activation. HLA-DR positive lymphocytes circulate in the blood for a long time, and the expression of this marker best reflects the activation of the cells [13]. Examination of the CD3+HLA-DR+ on T-lymphocytes showed no statistically different difference in either group (Figure 1h).

Expression of the early activation marker CD25 was determined in all the groups. This marker was significantly higher in the MIS-C ICU "+" group compared to the MIS-C "-" group (Figure 1i).

CD95 (Fas/APO-1) is able to trigger apoptosis in the cell after interacting with its ligand (FasL). Fas is a typical death receptor that is constitutively expressed in most tissues. Upon CD95 ligation, the association of the FADD adapter molecule (MORT1) and the caspase-8/10 regulator c-FLIP results in the formation of a death-inducing signaling complex (DISC) (14). CD95 is weakly expressed on the membranes of resting T cells, but it increases 10-fold after activation. At the same time, FasL is expressed only by activated T-lymphocytes (15). Ramenghi U. et al. assign CD95 a critical role in protection against autoimmunity in human and animal models (16). In our work, we observed a weak expression of CD95 on T-lymphocytes in both groups with MIS-C compared to the control group with no MIS-C after infection (Figure 1j).

CD279, or PD-1 (Programmed cell death-1), is a transmembrane protein belonging to the CD28/CTLA-4/ICOS family of regulatory proteins found on the surface of various lymphocyte subpopulations. In the case of T cells, PD-1 plays an important role in downregulating the effector functions of T helpers and cytotoxic T lymphocytes by blocking the signal from the T cell receptor (17). Our study compared CD279 in the three groups and found no significant differences (Figure 1k).

For charts a, d, e, g, h, j, m, the upper boundary of the box represents the median; the whiskers represent the interquartile ranges (25th,75th procile value).

For charts b, c, f, i, k, l, the upper boundary of the boxes is the mean value; the whiskers represent the 95% confidence interval.

The values of total serum immunoglobulins A, M, G, E were within the reference ranges in all three groups. All the children in our study had antibodies to SARS-CoV-2 IgG, but there were no statistically significant differences in the level of antibodies among the compared groups.

Determination of the levels of 5 cytokines in the blood serum provided the following data. IL-2 was significantly higher in both groups with MIS-C compared to the control group (Figure 2b). IL-6, IL-10, and FNO levels were statistically significantly higher in the MIS-C ICU "+" group compared to the MIS-C "-" group (Figure 2 c, d, e). No significant differences were found in IL-1β levels among the three compared groups (Figure 2a). The correlation of the number of elevated interleukins and the number of affected organs in children with MIS-C showed a moderate positive relationship (P-0.011) (Figure 2f).

For charts a, b, c, d, the upper boundary of the box represents the median; the whiskers represent the interquartile ranges (25th, 75th procilles).

For chart e, the upper boundary of the boxes represent the mean; the whiskers represent the 95% confidence interval.

In order to broaden our understanding of immune diseases in MIS-C children, we compared our findings with those of other authors (8, 18, 19, 20, 21, 22) (Table 2).

The studies we were able to find to compare with our study did not include a large number of children which may have contributed to some of the discrepancies in the results. The immunological profile of patients with MIS-C is characterized by NK and T cell cytopenia with a decrease in CD4 and CD8 lymphocytes. In our study and the work of Gowin E et all. the majority of children with MIS-C had an increased relative number of B-lymphocytes, while in other studies, B-cell lymphopenia was more common.

Although the average expression of the activation marker CD3+HLA-DR+ on T-lymphocytes in

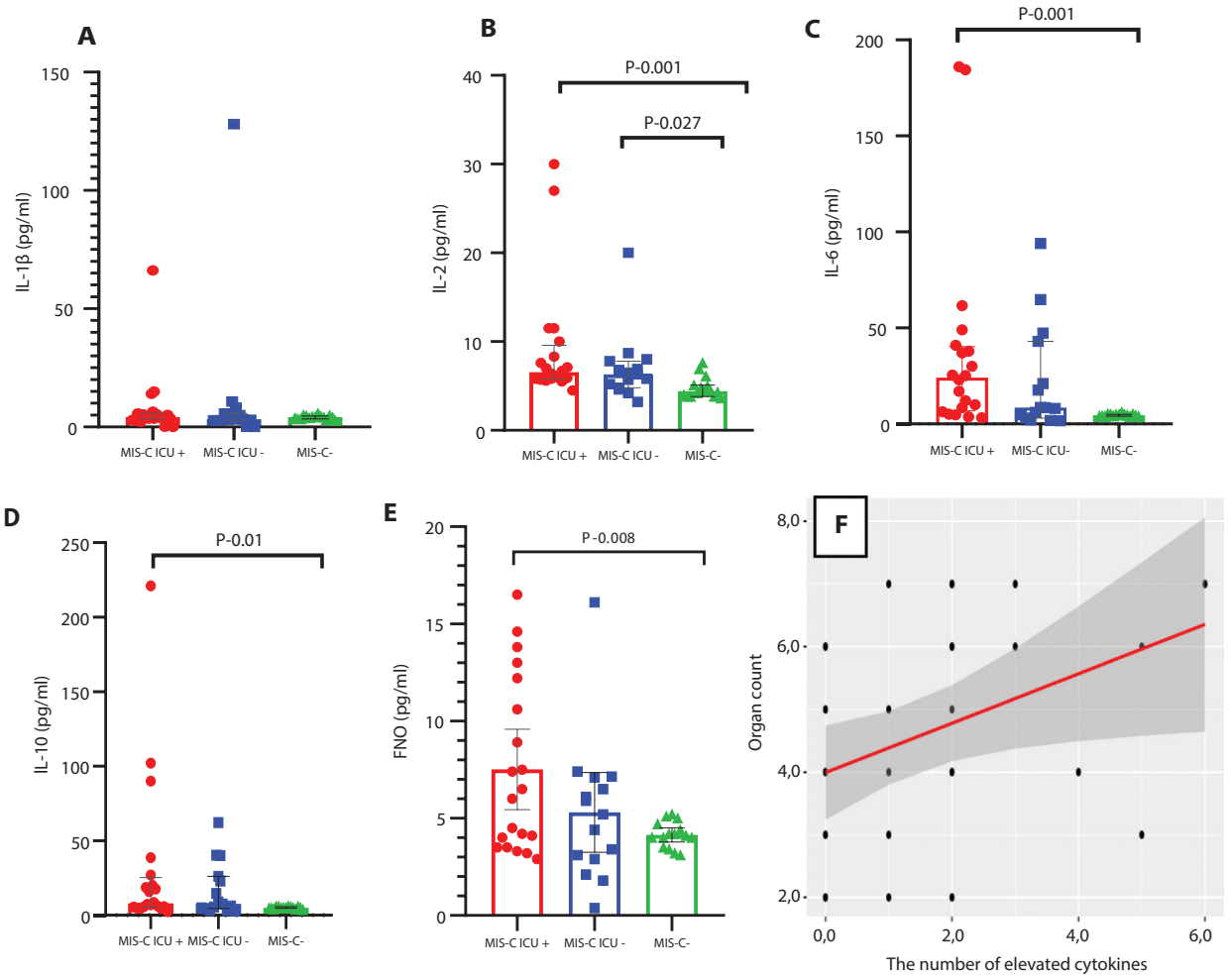

Figure 2. Changes in cytokine parameters in all the studied groups

a) IL-1β level;
b) IL-2 level; the Kruskal-Wallis test was used to determine statistically significant differences;
c) IL-6 level; Kruskal-Wallis test was used to determine statistically significant differences;
d) IL-10 level; Kruskal-Wallis test was used to determine statistically significant differences;
e) FNO level; Welch's F-test was used to determine statistically significant differences;
f) The direction and strength of the correlation between the two quantitative variables were estimated using Spearman's correlation coefficient.

our study was within the normal range, in 57.2% of children this indicator was above the reference range values. In 70.3% of patients in our study, an increase in the expression of CD3-HLA-DR+ on B cells was observed. Gowin E et all. also determined this marker of activation in children with MIS-C, on T and B-lymphocytes, which in most children was reduced on T-lymphocytes and increased on B-lymphocytes.

Children with MIS-C had elevations in several cytokines, most frequently in IL-6, IL-10, and FNO.

Discussion

This study was aimed at identifing the immunological features of children with MIS-C requiring ICU care and other MIS-C patient depending on the severity of the disease.

MIS-C is slightly more common in boys than girls in systematic reviews (7). In our study there were more boys (63%) than girls (37%). However, when patients were grouped by ICU admission, the ratio of boys and

Table 2. Immunological changes in children with MIS-C.

Parameters	Results of own research (n-35)	Okarska-Napierała M. et all. (18) (n-32)	Gowin E et all. (19) (n-7)	Laura A. Vella et all. (20) (n-9)	Lee PY et all. (8) (n-18)	Moreews M et all. (21) (n-21)	Carter MJ et all. (22) (n-23)
Decreased CD3 T-lymphocytes	71.4% (25)	87.5% (28)	85.7% (6)	100% (9)		47.6% (10)	69.6% (16)
Decreased CD4 T-lymphocytes	54.3% (19)	71.9% (23)	42.9% (3)	66.7% (6)	44.4% (8)	47.6% (10)	69.6% (16)
Decreased CD8 T-lymphocytes	54.3% (19)	84.4% (27)	71.4% (5)	66.7% (6)	44.4% (8)	61.9% (13)	87% (20)
Decreased NK cells	57.2% (20)	62.5% (20)	42.8% (3)	100% (5/5)	44.4% (8)	75% (12)	39.1% (9)
Decreased/ increased B-lymphocytes	↓2.9% (1)/ ↑57.2% (20)	↓59.4% (19)	↓4.2% (1) / ↑42.9% (3)	↓66.7% (6)	↓50% (9) / ↑16.6% (3)	↓6.3% (1) / ↑12.5% (2)	↓39.1% (9)
Decreased/ increased CD3+HLA-DR+	↓ 2.9% (4) / ↑57.2% (20)		↓70.3% (4) / ↑28.6% (2)				
Decreased CD3-HLA-DR+	71.4% (25)		71.4% (5)				
Decreased IL-1β	11.4% (4%)				11% (2)		15/15 (100%)
Decreased IL-2	14.3% (5%)				5.5% (1)		
Decreased IL-6	71.4% (24)				55.5% (10%)	88.9% (24/29)	100% (15/15)
Decreased IL-10	20% (7)				83.3% (15)	38% (11/29)	93.3% (14/15)
Decreased FNO	42.9% (15)				11% (2)	62% (18/29)	86.7% (13/15)

girls in the MIS-C ICU "+" group was 50/50%, while in the MIS-C ICU "-" group it was 80/20%; however, this difference was not statistically significant. Abrams JY. (23) noted in their study of 1080 patients that admission to the ICU was more likely in patients aged 6-12 years compared to patients aged 0-5 years. In our study, the median age of children with MIS-C admitted to ICU was 5 years, while in the non-MIS-C group it was 10 years.

Patients with MIS-C have a wide range of clinical manifestations and show a range of changes in lab tests which sometimes make diagnosing difficult (24). A study by Kıymet E. (25) showed that respiratory and cardiac involvement predominated in children with MIS-C admitted to ICU compared to MIS-C children admitted to other pediatric units. In our study, children in the MIS-C ICU "+" group more frequently experienced heart and liver damage, edematous syndrome, and polyserositis.

A retrospective study in the USA showed that children with MIS-C who had elevated concentrations of C-reactive protein, troponin, ferritin, D-dimer, thrombocytopenia, and lymphopenia were more likely to be admitted to ICU (23). The analysis of laboratory test changes in this study showed that children admitted to ICU had higher levels of procalcitonin, Alat, ASAT, urea, leukocytes and lower levels of hemoglobin, platelets and total protein.

Peripheral blood immunophenotyping in our study showed CD3+ T-cell lymphopenia in both groups of children with MIS-C compared to the children who had previously had SARS-CoV-2 infection

but no MIS-C. Several authors described depletion of NK cells and effector CD8+ T cells in children with MIS-C (9, 21). In our study, NK cell depletion was noted only in the MIS-C ICU+ group. The authors of a previous study (9) concluded that NK cell-dependent depletion of effector CD8+ T cells can improve symptoms of inflammatory disease, while the absence of this depletion can lead to severe and even fatal T-cell immunopathology after a viral infection. We were unable to confirm these findings as we did not observe depletion of CD8+ T cells in any of the groups with MIS-C. While in the MIS-C ICU "+" group the average value of CD8+ was within the reference range, in the MIS-C ICU "-" and MIS-C "-" groups the number of these cells was significantly increased. This decrease of NK cells, followed by depletion of CD8+ T cells, may lead to a persistent inflammatory environment, which in turn may enhance the autoreactivity as a hallmark of MIS-C (9). Our study confirms these findings, as 6 (75%) out of 8 children who had an extremely severe form of disease did not exhibit depletion of CD8+ T cells, whereas depletion of NK cells was present in 7 (87%) out of 8 children.

Other studies have described a decrease in CD8+ T lymphocytes, NK cells, and CD4+ T cells in most patients with MIS-C (8,26). Due to the profound decline in CD8+ T cells, an increase in the CD4+/CD8+ ratio was observed in children with MIS-C (19). In our study, we also observed a decrease below the reference range in the relative number of CD4+ T cells, but there were no statistically significant differences from the group who had previously had SARS-CoV-2 but didn't have MIS-C. The immunoregulatory index in children requiring ICU care was within the reference values, while in children in the other two groups MIS-C ICU "-" and MIS-C "-" it was below the references range due to an increase in CD8+ T-lymphocytes.

Carter, M.J. et al in their study evaluated HLA-DR on T and B cells as an indicator of activation (22). According to their data, HLA-DR on T cells in children with MIS-C was similar to that in healthy people, while HLA-DR on B cells was considerably reduced. In our study, the number of CD3+HLA-DR+ on T cells was within the normal range in all the groups. In the analysis of CD3+HLA-DR+ in 6 (75%) of 8 patients who required mechanical ventilation and/or inotropic support, the expression of this marker was reduced.

Other studies also evaluated B cell populations in children with MIS-C compared to acute COVID-19 and healthy children, but their results differed slightly (27,28). For instance, in a study by Ramaswamy A., no increase in the total number of B cells was noted, however, in patients with MIS-C, plasmablasts and naive B cells were increased, and memory B cells were reduced compared to healthy children (27). In another study, the authors described a decrease in the number of circulating B cells after a while, but found no difference in B cell subsets for MIS-C compared with acute COVID-19 (28). In our work, in children in the MIS-C ICU "-" and MIS-C "-" groups, the relative number of B cells was within the reference range, but in the MIS-C ICU "+" group, the relative number of these cells was increased. The activated CD3-HLA-DR+ B cells were also increased in children in both MIS-C groups, compared to MIS-C "-" children, which indicates a predominant activation in the humoral immunity, especially in severe cases in children with MIS-C. However, there were no statistically significant differences in the level of total serum immunoglobulins and antibodies to SARS-CoV-2 Ig G in the three compared groups.

Since one of the possible mechanisms of immune disorders in MIS-C is chronic antigen exposure causing immune dysfunction or depletion (29), authors (30) examined a marker associated with this depletion. They found that the number of CD279-expressing T cells was significantly higher in MIS-C patients compared to children with COVID-19. In our work, we did not find statistically significant differences in the level of CD279 among the three compared groups.

Bellesi S. in their study observed a significantly higher expression of the apoptosis-associated molecules CD95 and CD279 on both CD4+ and CD8+ T cells in 42 adult patients with COVID-19 compared to healthy controls of the same age (30). However, we did not find studies that determined the expression of CD95 in the pediatric population of patients with COVID-19 and MIS-C. CD95+ depletion has a beneficial effect on autoimmune diseases by slowing inflammation and progression of the process. Decreased

CD95 predisposes to autoimmunity, as described in CD95 blocking experiments in mice (31). A rather unexpected fact in our work was the weak expression of CD95 in both groups of children with MIS-C compared with the non-MIS-C group.

Triggering of the proliferative response of T-lymphocytes is a cascade process in which the expression of the T-cell growth factor interleukin-2 (IL-2) and its receptor (IL-2R) plays a key role (32). This receptor is carried on their membrane by various types of peripheral blood cells: CD4+, CD8+ T cells, NK, B cells, and monocytes. It is activated within 24 hours of TCR/CD3 complex stimulation and remains elevated for several days (33). The receptor plays a key role in the response to IL-2, which leads to the activation of lymphocytes and further production of IL-2. Syrimi E. studied cytokines and chemokines in blood plasma in children with MIS-C. One of the significantly elevated MIS-C biomarkers in children was the soluble IL-2 receptor (sCD25) (34). In our study, we identified the expression of CD25 as an early activation marker that was elevated in the MIS-C ICU "+" group compared to the other two groups.

A multi-author study reported increased levels of IL-1β, IL-2, IL-6, IL-10, TNF, IL-8, IL-18, IFNγ, soluble IL-2R, CCL2, CCL3, CCL4, CXCL8, IFNγ -induced chemokines CXCL9 and CXCL10 and other cytokines in the serum of patients with MIS associated with SARS-CoV-2 compared to children with COVID-19 or healthy controls (35,36). The authors note that an increase in these pro-inflammatory molecules indicates inflammatory responses of myeloid and lymphoid cells. We investigated 5 cytokines and observed statistically significant differences in the levels of IL-2, IL-6, IL-10, TNF between the MIS-C ICU "+" group and the control group, but not between the two groups with MIS-C, which may be due to the fact that many children received systemic glucocorticosteroids and/or intravenous immunoglobulins before the blood samples for the immunological analysis were collected. In the presented results, the dependence between the production of pro-inflammatory cytokines IL-6, TNF and anti-inflammatory cytokine IL-10 was revealed, which indicates the autocrine regulation of the immune system (Figure 2 c, d, e).

Research limitations

Almost all the samples from the patients with MIS-C were obtained after anti-inflammatory treatment had started, and it is likely that this treatment may have affect the serum cytokine levels and correspondingly the results of comparisons between the two MIS-C groups.

Conclusions

MIS-C is a severe pathology manifested by multiple organ damage against the background of a delayed immune response after a patient has suffered COVID-19. According to our studies, a deeper immune dysregulation is characteristic of patients with a critical course of MIS-C, which is confirmed by the results of a comparative analysis among children admitted to ICU and those not requiring ICU care. So, in the group of patients with MIS-C admitted to ICU, increased likelihood of damage to the heart and liver was statistically significant, changes in hematological parameters were more pronounced, and complications such as edematous syndrome, polyserositis, DIC, and cardiogenic shock developed more often. Besides, the number of affected organs was twice as high as that in children with MIS-C not requiring ICU care.

Activation of the immune system in both groups of children with MIS-C was expressed in CD3+ T-cell lymphopenia and decreased expression of CD95. An correlation was also observed between a high likelihood of ICU admission and signs of profound immune suppression, as evidenced by an increase in the relative number of B-lymphocytes, an increase in the expression of CD3-HLA-DR+ activation markers, a decrease in NK-cells, and an increase in CD25 expression in these patients. In the majority of critically ill children, NK-cell cytopenia was observed while CD8-cell counts were preserved, as well as a decrease in the expression of the HLA-DR activation marker on T-cells and an increase in the expression of this marker on B-cells.

Findings of different studies of immunological disorders in MIS-C are sometimes contradictory, and this indicates the need to continue researching these

mechanisms in large groups of patients and using a wide range of immunological markers.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g., consulting services, shareholding, equity interest, patent/license agreement, etc.) that could create a conflict of interest in connection with this study.

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