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Original article

Long-term outcomes and immune profiling in children with multisystem inflammatory syndrome (MIS-C)

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Abstract. *Background and aim:* Existing follow-up data after MIS-C is limited. Purpose of the study: to investigate the long-term consequences in children who have undergone MIS-C. *Methods:* The retrospective study included 93 children. The identified changes were divided into the following periods: occurred within first 6 months, 1 year, 2 years, and more than 2 years after MIS-C. Besides, 31 children underwent prospective immunophenotyping of peripheral blood and the determination of cytokines during the acute period of the disease and after discharge. *Results:* Outpatient monitoring events included pneumonia (9.6%), somatic disorder syndrome (11.8%), visual impairment (7.5%), anemia (8.6%), reactive arthritis (2.2%), weight changes (2.2%), and MIS-C recurrence (2.2%). A study of the cardiovascular system showed a statistically significant decrease in the frequency of the right and left heart dilatation, left ventricular dysfunction, pericarditis, pulmonary arterial hypertension, coronaritis, mitral regurgitation. But at the same time an increase in pulmonary and tricuspid valve regurgitation and arrhythmias compared with the acute period was detected. Most of the changes took place within first year of observation. Immune profiling showed reconstitution of CD3, CD4 T-lymphocytes, NK-cells, maintenance of a high relative value of CD8, reduction of CD19+ B-cells, expression of CD3-HLA-DR+, CD25, CD279, CD95. *Conclusions*: After the history of MIS-C, children in the long-term follow-up had various somatic disorders and disease recurrence. Most patients (64.1%) showed subclinical signs of myocardial involvement within first year of observation. Low expression of CD95 may justify a certain role in the pathogenesis of the disease. (www.actabiomedica.it)

Key words: MIS-C, follow-up observation, echocardiography, electrocardiogram, immunophenotyping, CD95, CD279

Introduction

Multisystem inflammatory syndrome (MIS-C) is a severe complication of SARS-CoV-2 infection in children (1). The disease has a severe course with multiorgan involvement and a high risk of hospitalization in the intensive care unit (ICU), due to the cardiac dysfunction (2). Valve insufficiency, left ventricular dysfunction, coronary artery lesions, and pericardial effusion were the most common cardiac abnormalities found on echocardiography in children with MIS-C (3). Cardiovascular system involvement has a multifactorial mechanism, including direct damage to cardiomyocytes resulting from SARS-CoV-2 viral

invasion, as well as immune response dysregulation leading to microvascular dysfunction and endothelial damage (4).

Immune changes in patients with MIS-C are characterized by a decrease in the subpopulation of T cells (CD4, CD8), NK cells, and B lymphocytes, with subsequent reconstitution over several months (5,6). However, there are relatively few studies in the available literature devoted to the dynamic changes in the immune profile following the disease. Moreover, none of them determined the expression of CD95 and CD279 markers, which are responsible for immune regulation, tolerance, and autoreactivity (7,8).

The clinical manifestations of MIS-C are currently well studied and described by many authors (9,10,11). Recently, more attention has been paid to observing these patients in follow-up with the aim of identifying possible complications after the disease, as existing data on the long-term consequences of MIS-C in patients is limited.

Thus, the aim of our study was to conduct long-term observation of children after MIS-C, monitoring the states of the cardiovascular and immune systems.

Materials and methods

Retrospective study

The study included 93 children with a history of MIS-C from 17 regions of the Republic of Kazakhstan, included in the national registry, during the period from August 20, 2020 to January 1, 2023. The clinical picture and laboratory data during hospitalization of some patients (n = 89) have been previously published (11). The diagnosis of MIS-C was confirmed by an interdisciplinary team of specialists in accordance with the criteria established by WHO and CDC.

We monitored the follow-up of all children after MIS-C through the electronic system Damumed, which included a retrospective analysis of hospitalization records for diseases that were not observed before MIS-C, records of complaints and records of instrumental examinations of the cardiovascular system. The data from 58 children echocardiograms (ECHO) and 59 children electrocardiograms (ECG) in the acute period of the disease and during outpatient observation were compared. The identified changes during the follow-up were divided into the following periods: occurred in the first 6 months, 1 year, 2 years, and more than 2 years after.

Prospective study

Dynamic immunological analysis was conducted on 31 out of 93 children. Blood samples for immunophenotyping included: 31 children during the first week of hospitalization, 10 patients 3 months after discharge and 31 patients at 6 months after hospitalization. Blood samples for serological analysis of SARS-CoV-2 IgM and IgG antibodies and interleukins were checked twice during hospitalization and at 6 months after discharge. Delivery of biological material met all necessary criteria. Informed consent was obtained from the guardians of children with MIS-C.

Immunophenotyping

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

Level of cytokines

The concentration of cytokines IL-1β, IL-2, IL-6, IL-10, FNO was determined by enzyme immunoassay (reagent kits Vector BEST Russia) according to the manual on 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 revealed by enzyme immunoassay using reagent kits from Vector BEST (Russia).

Ethics

This study was approved by the Institutional Review Board of S.D. Asfendiyarov Kazakh National Medical University (IRB No. 1147 dated 01.06.2021, with subsequent review on 26.06.2022). No personal or private data was collected, and the data collection procedure adhered to the principles outlined in the Declaration of Helsinki regarding human subjects. Participants had the option to withdraw from the study at any time. Those willing to participate were provided with an informed consent form that outlined all the ethical considerations of the study.

Statistical analysis

Statistical analysis was performed using StatTech v. 2.8.8. Categorical data were described by absolute and relative values. Quantitative indicators were evaluated for normal distribution using the Shapiro-Wilk test. The chi-square test or Fisher's exact test was applied for categorical variables.

Comparison of binary indicators characterizing two related populations was performed using the McNemar's test. When comparing quantitative indicators in two related groups whose distribution did not differ from normal, the paired Student's t-test was used, otherwise the Wilcoxon rank-sum test was used.

Graphical representation of the data was performed by Prism 8 software (GraphPad).

Results

Outpatient monitoring of patients with MIS-C

From August 1, 2020 till January 1, 2023, 99 patients with MIS-C were registered in the Republic of Kazakhstan (Figure 1). The mean age made up 6 years. There were 65 boys (69.9%) and 28 girls (31.1%). Six patients passed away, and 93 children in satisfactory condition were discharged home for outpatient follow-up (Figure 1).

In Figure 1, it can be seen that in our study, the duration of follow-up observation was more than 2 years in 43 (46.4%) children, from 1 year to 2 years in 42 (45.1%) children, from 6 months to 1 year in 6 (6.4%) children, and up to 6 months in 2 (2.1%) children.

During the entire follow-up period, 25 (27.9%) children were hospitalized and records of symptoms related to different organs or conditions that were not

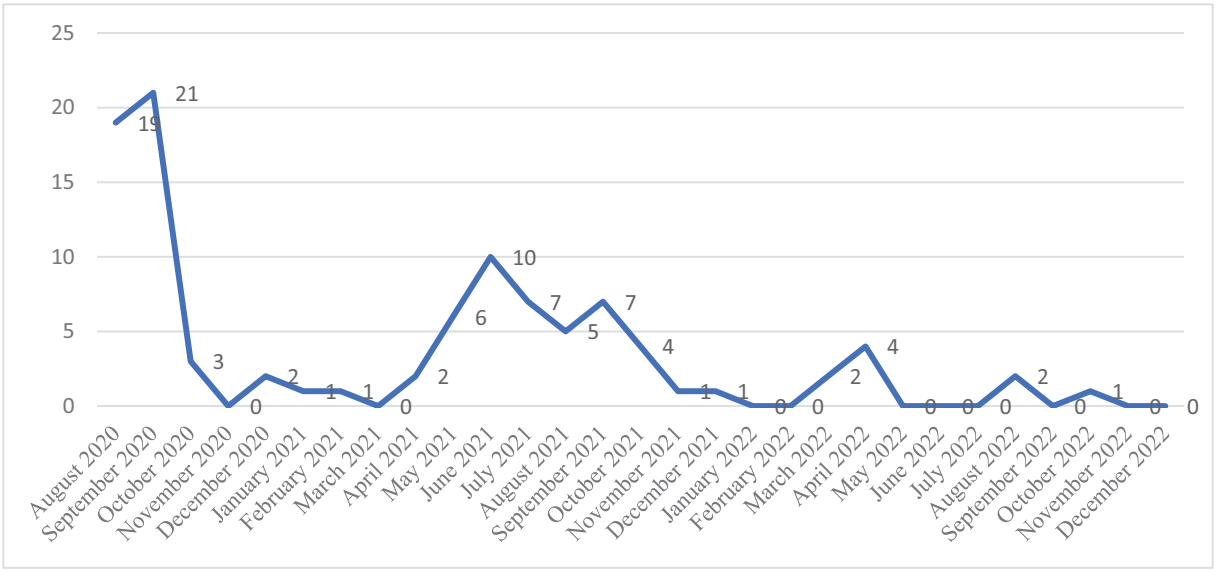

Figure 1. Registered cases of children with MIS-C in the Republic of Kazakhstan.

Table 1. Major pathological conditions developed after recovering from MIS-C.

Symptoms	N	%
Neurological symptoms	7	7.5
prolonged headaches	5	5.4
dizziness	2	2.2
seizures	2	2.2
Respiratory system involvement	13	14
cough	13	14
shortness of breath	8	8.6
Gastrointestinal symptoms	8	8.6
abdominal pain	4	4.3
changes in stool	4	4.3
Liver involvement	17	18.3
elevated transaminases	2	2.2
hepatomegaly (on abdominal ultrasound) (N-26)	15	57.7
Cardiovascular symptoms	9	9.7
chest pain	4	4.3
low blood pressure	1	1.1
fainting	2	2.2
myocarditis	2	2.2
Hematological changes	10	10.8
anemia	10	10.8
thrombocytopenia	2	2.2
Kidney involvement	1	1.1
Joint involvement	6	6.5
arthralgia	6	6.5
arthritis	1	1.1
Visual impairment	7	7.5
Hearing impairment	1	1.1
Allergic rash	9	9.7
Obesity	2	2.2
Weight loss	2	2.2

noted before MIS-C were found in 23 (25%) children Table 1 shows the changes observed in organs and systems after recovering from the disease.

During further observation and examination, various diagnoses were established for the aforementioned children (Table 2).

In 2 (2.2%) children (under the age of 2) after examination, a diagnosis of MIS-C recurrence was established, which developed 3 weeks after the initial illness. Patients were hospitalized in the ICU with complaints of fever, diarrhea, vomiting, cough and increased breathing. The clinical picture included multi-organ

Table 2. Structure of diseases in children after recovering from MIS-C.

Diagnoses	N	%
Somatic disorders syndrome	11	11.8
Pneumonia	9	9.7
Bronchitis	4	4.3
Anemia	8	8.6
Myopia	4	4.3
Astigmatism	2	2.2
Partial atrophy of the optic nerve	1	1.1
Reactive arthritis	2	2.2
Atopic dermatitis	3	3.3
Bilateral conductive and neurosensory hearing loss	1	1.1
Recurrence of MIS-C	2	2.2

involvement and changes in laboratory parameters such as anemia, thrombocytopenia, elevated inflammatory markers (CRP, ferritin, procalcitonin, IL-6, ESR), and cardiovascular system dysfunction. One child was discharged with improvement 35 days after hospitalization. The second child developed acute kidney injury with anuria and multiple organ failure due to the recurrence of MIS-C. Due to the progression of pathological symptoms, the child died 41 days after hospitalization.

Among 10 children (10.8%), mild or moderate anemia persisted. Among them, 2 children (2.2%) had anemia combined with thrombocytopenia. These were the children with MIS-C recurrence.

We compared the results of follow-up observation depending on onset disease period (Figure 2). Thus, we see that most of the diseases occurred in the first year after MIS-C.

Analysis of cardiovascular system status in children who have experienced MIS-C based on dynamic changes in echocardiography and electrocardiography

During outpatient monitoring, 58 children underwent ECHO and 59 children - ECG. Figure 3 shows the scope of cardiovascular examinations in children depending on the periods of follow-up observation.

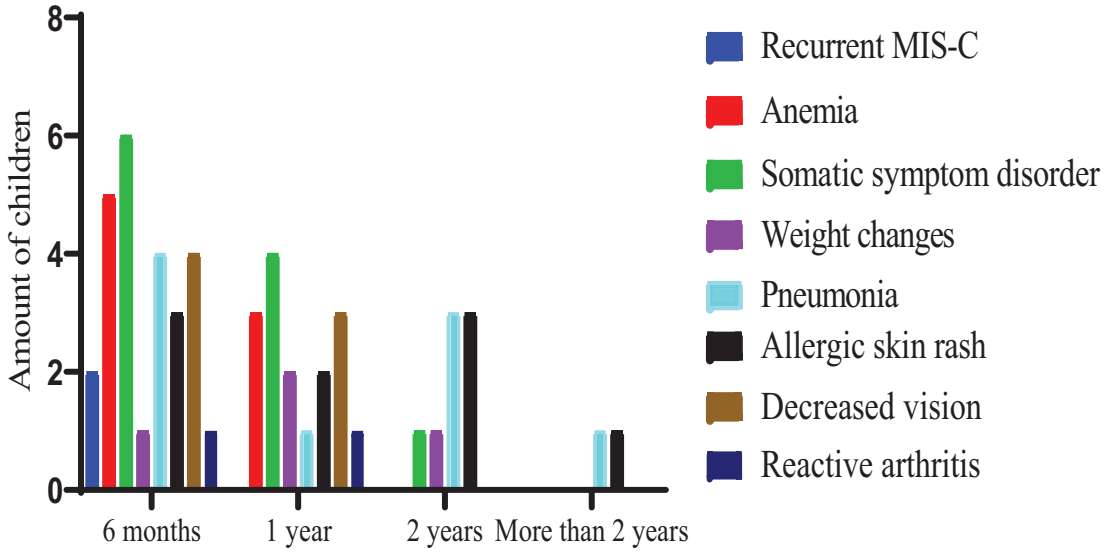

Figure 2. A comparative analysis of post-MIS-C illnesses by observation periods.

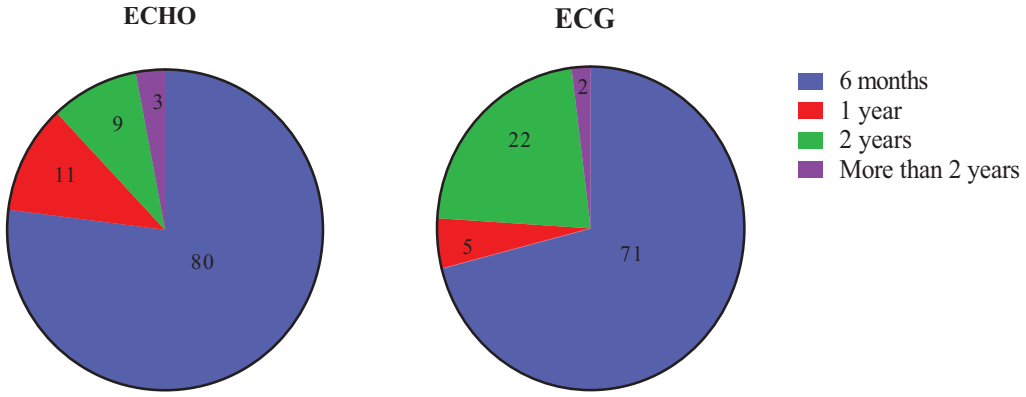

Figure 3. Coverage of children under outpatient ECHO and EGG monitoring examinations over time.

Given that the majority of children with MIS-C have cardiovascular system involvement (2), we conducted a comparative analysis of the dynamic changes on ECHO and ECG in children during the acute phase of the disease and outpatient monitoring (Table 3).

In children after MIS-C, statistically significant reductions in such changes on echocardiography as dilatation of the right and left heart chambers, decreased left ventricular ejection fraction (LVEF), pericarditis, pulmonary arterial hypertension, and valve leaflet thickening were observed during outpatient follow-up. However, one child had persistent pulmonary hypertension 3 weeks after discharge, although it was not significant (27 mmHg). Coronary artery involvement was observed in six (6.5%) children with MIS-C. Moderate coronary artery dilatation persisted in only one child after 2 years of outpatient follow-up (proximal descending aorta Z-score -2.5, proximal right coronary artery Z-score -1.97).

Valve regurgitation was observed in half of the children with MIS-C during both inpatient and outpatient evaluations. After recovery, the frequency of mitral regurgitation (MR) and aortic regurgitation (AR) decreased, but the frequency of pulmonary

Table 3. Analysis of dynamic changes on ECHO and ECG in children with MIS-C.

Changes	Admission data	Outpatient data	P- value
ECHO	n-58		
Reduction of left ventricular ejection fraction.	17 (28.8%)	2 (3.4%)	**< 0.001**
Pericarditis	17 (28.8%)	4 (6.9%)	**< 0.001**
Dilation of the right chambers of the hear	12 (20.7%)	4 (6.9%)	**0.046**
Dilation of the left chambers of the heart	13 (22.4%)	5 (8.4%)	**0.046**
Pulmonary arterial hypertension	12 (20.7%)	1 (1.7%)	**0.002**
Coronary artery disease	6 (10.3%)	1 (1.7%)	**0.025**
Mitral valve regurgitation	30 (50.8%)	20 (34.5%)	**0.05**
Aortic valve regurgitation	8 (13.6%)	5 (8.5%)	0.257
Pulmonary valve regurgitation	5 (8.6%)	11 (19%)	**0.05**
Tricuspid valve regurgitation	13 (22%)	29 (49.2%)	**0.002**
Valve leaflet thickening	5 (8.5%)	1 (1.7%)	**0.046**
ECG	n-59		
Arrhythmia	9 (15.3%)	15 (25.4%)	0.083
Ventricular repolarization disturbance	11 (18.6%)	2 (3.4%)	**0.013**
Incomplete right bundle branch block Гиса	19 (32.2%)	14 (23.7%)	0.137
Slowed atrioventricular conduction	4 (6.6%)	0 (0%)	**0.046**
Prolonged PR interval	2 (7.4%)	0 (0%)	0.157

* The statistically significant values are marked in bold. The comparison of the indicators was performed using the McNemar test.

(PR) and tricuspid regurgitation (TR) increased two-fold (Table 1). All valve regurgitations were mild or moderate.

Analysis of EGG changes at the outpatient level showed a statistically significant decrease in the frequency of ventricular repolarization processes disturbances (abnormal ST segment or T wave) and atrioventricular conduction slowing, but there was preservation of incomplete right bundle branch block (IRBBB) in the majority of children, and an increase in the frequency of arrhythmias compared to the acute phase of the disease (Table 1).

We compared the results of the cardiovascular system check depending on the period of occurred changes (Figure 4). At first year of follow-up, echocardiograms were performed in 55 (91%) children (Figure 3), and despite statistically significant reductions in changes, not all children showed complete recovery of cardiovascular function. A decrease in left ventricular ejection fraction was observed in 1 (1.7%) deceased child from MIS-C recurrence. Mild pulmonary hypertension persisted in 1 (1.7%) child in the first month after discharge. Right and left heart dilatation persisted in 4 (6.9%) children, valve leaflet thickening in 1 (1.7%) child, and moderate coronary artery dilation in 1 (1.7%) child. Heart valve regurgitation was present in 22 (41.5%) children during the first year of follow-up.

Comparative analysis of immunological parameters dynamic changes

Immune parameters were measured at three time points. During hospitalization (point A) in 31 patients, 3 months after discharge (point B) in 10 children, and 6 months after discharge (point C) in 31 patients. The results obtained are presented in Table 4.

According to the results of immunophenotyping, a reconstitution of CD3+, CD4+ T-lymphocytes, and NK-cells relative quantity to reference values was

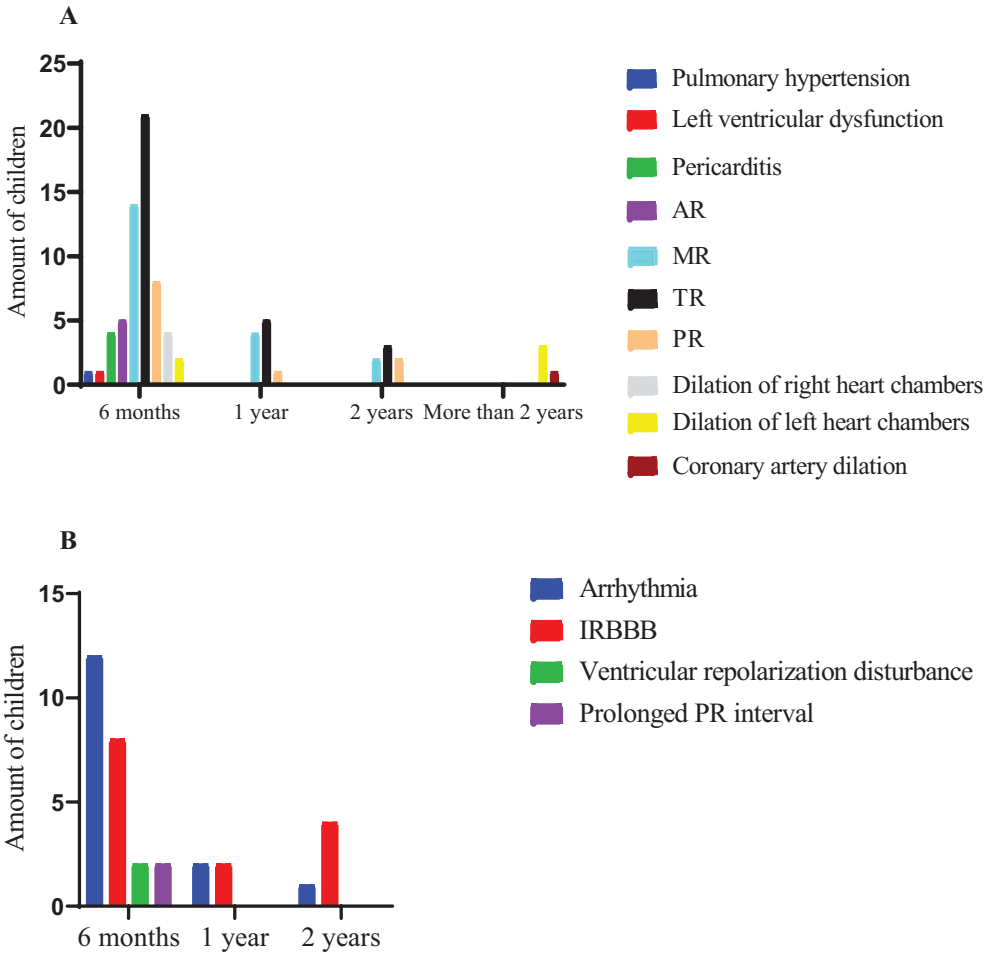

Figure 4. Dynamics of detected changes on ECHO (a) and ECG (b) in children after previous MIS-C, by periods of examination.

observed at points B and C compared to point A. Meanwhile, the relative value of CD8+ T-lymphocytes remained elevated at all time points.

During MIS-C patients hospitalization, there was an increase in the relative amount of mature CD19+ B-cells above reference values with a statistically significant decrease at 3 and 6 months after discharge. Similar changes in humoral immune response when analyzing the surface markers B-lymphocytes activation, CD3-HLA-DR+ were detected. The expression of CD25 receptor for IL-2, CD25, was higher than the normative values in the acute period of the disease, with normalization after discharge.

We also determined the expression of immune regulation markers and tolerance. A statistically significant decrease in CD279 expression was revealed at 3 and 6 months after discharge compared to the acute period. However, the expression of CD95 was below reference values at all time points.

Levels of 5 cytokines in serum were measured during hospitalization and at 6 months after discharge. Such significant changes were detected as a decrease in IL-6 and IL-10 levels (Figure 5 - c,d). No significant changes in levels of IL-1, IL-2, and TNF were observed in children with MIS-C during the observation period (Figure 5 - a,b,e). However, an increase in several cytokines was demonstrated in 4 children at 6 months after recovery. IL-1, IL-6, and TNF were elevated in one child, and the parents reported a three-day fever before the blood sample was tested. Two

Table 4. Immune profiling dynamics in children with MIS-C.

Indicator	Point A (n-31)	Point B (n-10)	Point C (n-31)	P- value
CD3 T – lymphocytes % (M ± SD)	54 ± 12	66 ± 7	65 ± 8	**0.02** **<0.001**
CD4 T – lymphocytes % (Me; IQR)	26 (21-34)	30 (26-34)	31 (26-36)	0.073 **0.017**
CD8 T – lymphocytes % (M ± SD)	28 ± 9	35 ± 9	33 ± 8	**0.05** **0.013**
NK – cells % (Me; IQR)	5 (3-10)	15 (8-18)	11(9-17)	**0.05** **<0.001**
CD19 B – lymphocytes % (Me; IQR)	29 (17-38)	16 (14-18)	15 (11-18)	**0.006** **<0.001**
CD3+HLA-DR+% (Me; IQR)	7 (5-11)	7 (3-9)	7 (5-12)	0.821 0.906
CD3-HLA-DR+% (Me; IQR)	30 (23-40)	18 (15-19)	19 (14-23)	**0.006** **<0.001**
CD25% (M ± SD)	4.1 ± 2.9	2.7 ± 1.8	2.4 ± 2.6	0.169 **0.023**
CD279% (Me; IQR)	5.4 (3.7-6.6)	2.4 (2.3-2.6)	1.8 (1.1-2.7)	**0.049** **0.001**
CD95% (M ± SD)	1.1 ± 1.6	1.2 ± 0.7	2.1 ± 2.6	0.131 0.18

*Significant values are highlighted. In the "P-value" columns, the first row corresponds to the P-value between points A-B, and the second row corresponds to the P-value between points A-C. Paired Student's t-test was used for normally distributed variables, and the Wilcoxon test was used for variables with non-normal distribution.

children had elevated levels of IL-1 and IL-6, but parents did not report fever or poor health in the children. One child had only elevated IL-6, and the parents also reported a fever on the day before blood collection.

Discussion

This study is dedicated to the comprehensive dynamic observation of children after experiencing MIS-C. The demographic and age data of our patients correspond to consistent with the current available literature (12).

In our study, during the follow-up observation, 25% of the children required hospitalization due to various somatic diseases. Sezer M. et al. (3) reported an 8-month follow-up of 123 patients with MIS-C, where re-hospitalization after discharge was required in only 6.2% of children. We believe that this difference is due to the fact that our study covered a longer period of 2.5 years. The most common reasons for hospitalization in the work of Sezer M. et al. (3) were recurrent abdominal pain (14.2%), cardiac disorders (14.2%), pulmonary symptoms (8%), fever (7.1%), psychoneurological disorders (6.2%), and arterial hypertension (3.5%). These matches ours, as the most common post-infection syndrome observed was somatic disorders (11.8%), while the frequency of pulmonary pathology in the form of pneumonia was 9.6%.

Penner et al. observed children with MIS-C for six months and found visual disturbances or saccades in 16.6% of cases (13). Additionally, 7.5% of their patients experienced deterioration of vision ranging from mild myopia to partial optic nerve atrophy. The same study identified three different types of rashes in three patients: hypopigmented, maculopapular, and

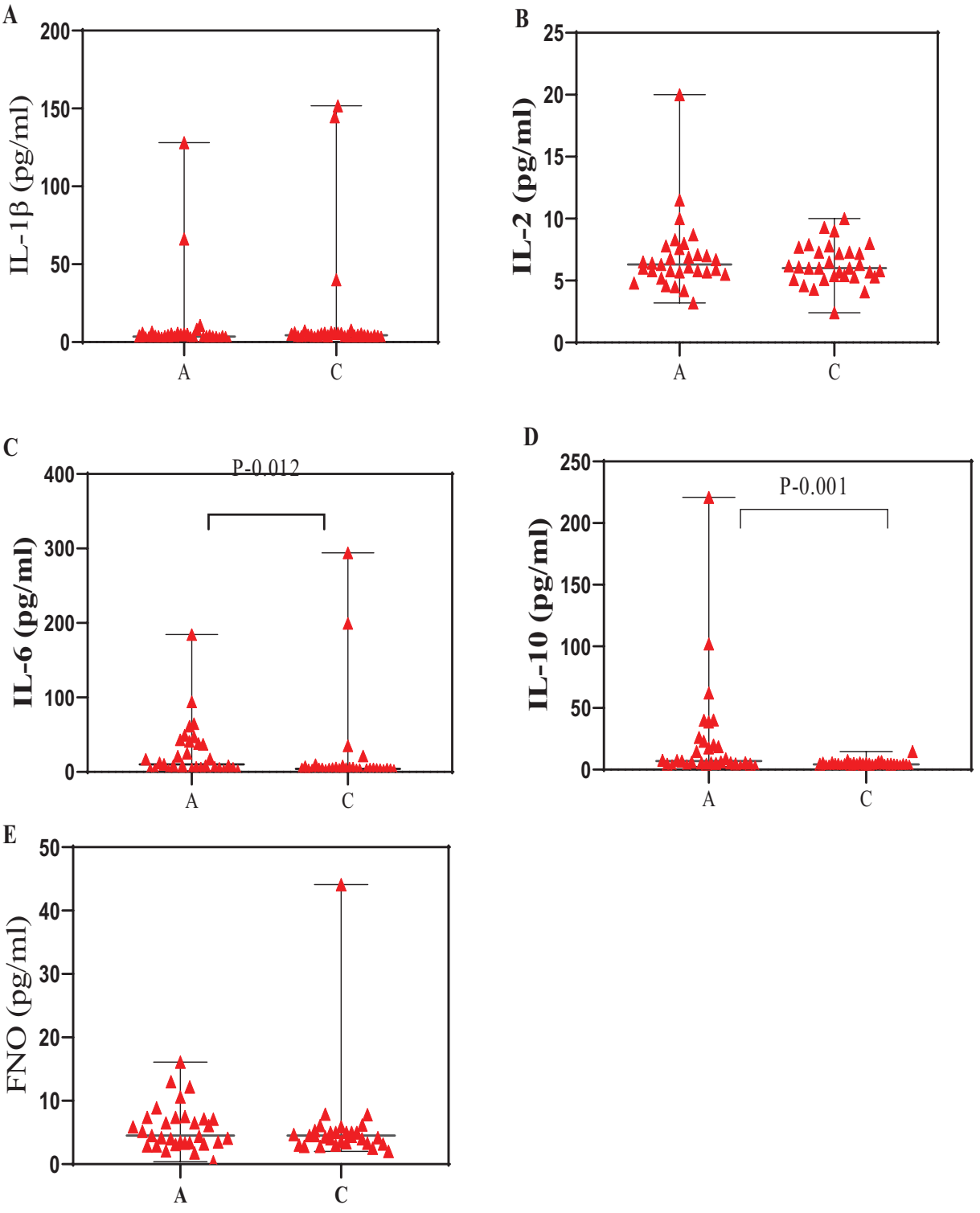

Figure 5. Dynamic changes in cytokine parameters in children with MIS-C. For diagrams a,b,c,d,e, the upper boundary of the rectangle represents the median, and the whiskers represent the interquartile range (25th-75th percentile values). The Wilcoxon rank-sum test was used to determine statistically significant changes in all parameters.

dermographism. In our study, cutaneous manifestations were observed in nine children (9.7%). Three of them were diagnosed with atopic dermatitis.

Pawar et al. reported a case of recurrent MIS-C four weeks after discharge (14). We also described two cases of MIS-C recurrence occurring 3-4 weeks after discharge. One case resulted in a fatal outcome.

In the available literature, there were no reports of joint complications after recovering from MIS-C. However, in our study, 6 children (6.5%) reported occasional joint pains, and reactive arthritis was diagnosed in 2 children (2.2%) among them.

Cardiovascular system involvement is the leading cause of severe illness in children with MIS-C. In the study by Chakraborty et al. (15), 33.8% of patients had systolic dysfunction upon admission, which improved to 11.3% upon discharge, with complete resolution within 2 weeks. In 28.8% of our patients during the acute phase of the disease, there was a decrease in LVEF with an improvement of this parameter to 2.2% during outpatient monitoring. Right and left heart chamber dilation was observed in 20.7% and 22.4% of cases, respectively, during the acute phase, and decreased to 6.9% and 8.4%, respectively, during outpatient monitoring. Pericarditis was present in 28.8% of cases during the acute phase with subsequent reduction to 6.9%. Pulmonary arterial hypertension was present in 20.7% of cases and decreased to 1.7%. Valve thickening was observed in 8.5% of cases and decreased to 1.7% during outpatient follow-up examination.

Coronary anomalies in children with MIS-C, in contrast to Kawasaki disease, typically disappear within several months after discharge (16). However, in the study by Chakraborty A (15), 1 (1.9%) out of 9 (17.9%) children still had coronary artery aneurysms after one year of follow-up. Our study confirms these findings, as coronary artery involvement was observed in 10.3% during the acute phase of MIS-C and decreased to 1.7% over time, with only 1 child experiencing it by 2 years of follow-up. In the same study, the authors noted that clinically significant myocardial dysfunction was present in almost every patient with anomalous LVFW and was closely associated with the MR presence in most children. In this study, at the time of discharge, 15.1% of patients had anomalous LVFW, while significant MR persisted in 30.2% of children. Thus, the authors concluded that resolution of MR lags behind resolution of systolic dysfunction. Upon analysis of our data, we identified regurgitation on all heart valves, with mitral regurgitation (MR) being the most commonly encountered (50.8%) during the acute phase of the disease and persisting in 34.5% of cases during outpatient follow-up. It was also interesting to note a twofold increase in the frequency of TR and LR during follow-up. Although all regurgitations were mild to moderate, this further supports the hypothesis that MIS-C can cause subtle, prolonged heart damage that requires more time to recover (15,17).

In the study by Valverde et al. (18), ECG abnormalities were detected in 35.3% of patients at admission, with normalization at discharge in 72.4%. The observed deviations included changes in ventricular repolarization, present in 22%, prolonged PR interval in 6.3%, interventricular conduction delay in 3.8%, and atrioventricular block in 2.1% of children. These changes were also present in our patients. During ambulatory monitoring, a decrease in the frequency of ventricular repolarization abnormalities was observed, from 18.6% to 3.4%. Atrioventricular conduction slowing during hospitalization was present in 6.6% of children with complete resolution during outpatient follow-up. However, we also found an increase in the frequency of arrhythmias from 15.3% to 25.4%, which also indicates a more prolonged recovery of the heart after the illness.

In our previous work, we described the immune profile of 35 patients with MIS-C in the acute phase of the disease (19). In this study, we continued a dynamic study of immune changes only in 31 children, as three children died during hospitalization, and one child died after a recurrence of MIS-C. According to some studies, MIS-C is characterized by cytopenias of CD3, CD4, CD8, CD19 lymphocytes, and NK-cells, which recover after several months of treatment (5,20,21). Immunological parameters in children during the acute period of the disease in our study were as follows: a decrease in CD3, CD4 T-lymphocytes, NK-cells, but an increase in active B-lymphocytes and cytotoxic CD8 T-cells, as well as the expression of CD25 and activation markers of B-lymphocytes CD3-HLA-DR+. All parameters were reconstituted at two outpatient follow-up points, except for CD8

T-lymphocytes, which remained above normal, indicating a predisposition to autoreactivity.

CD279 is a key regulator of immune tolerance to autoreactive T cells and controls autoimmune diseases (22,23). If we did not find statistically significant differences in CD279 expression among patients with MIS-C during hospitalization, as well as in the control group of children who had SARS-CoV-2 infection without MIS-C (19), then after 3 and 6 months after discharge, a decrease in the expression of this marker (1.8% at 6 months compared to 5.4% during the acute phase, P=0.001) was observed in the main group, which may indicate its possible role in the pathogenesis of this disease.

The CD95 (Fas) receptor belongs to the TNFR superfamily and is best known for its ability to induce cell death in CD95-sensitive cells. In this context, CD95-induced apoptosis plays an important role in maintaining immune homeostasis and tolerance, as well as in terminating immune responses (24). The CD95 receptor on B-cells is important for the regulation of autoimmunity, as dysfunction of Fas on B-cells leads to uncontrolled production of autoantibodies and autoimmunity (25). The interactions of PD-1 with its ligands PD-L1 and PD-L2 inhibit the effector functions of antigen-specific T-cells (26). Bellesi S. (27) observed significantly higher expression of CD95 and CD279 in 42 adult COVID-19 patients compared to a control group of healthy individuals. In a previous study, we compared the expression of CD95 in patients with MIS-C and a group without MIS-C. In the control group, CD95 expression was statistically significantly higher (P-0.05) than in the main group (19). Interestingly, weak expression of CD95 was present in 90% of children at 3 months of outpatient follow-up (1.2% compared to 1.1% during the acute phase) and in 96.8% of children at 6 months of follow-up (2.1%). Currently, there are no published studies dedicated to the expression of CD95 and CD279 in children with MIS-C. However, in a recent study (21) of children with MIS-C, the authors also noted a prolonged increase in the population of double-negative T-cells (DNT) (CD3+, CD4-CD8-). Analysis of these cells showed that the majority of them were γδ T-cells.

Studying the role of CD95 and CD279 receptors in the pathogenesis of MIS-C is considered important and relevant. As the role of CD95 in the disease pathogenesis is currently ambiguous, it may be a subject of further research.

Research limitations

The study has several limitations. As the data was collected from electronic medical records, some data may have been missed. Due to the retrospective design of the study, it was not possible to standardize outpatient monitoring of children. In addition, not all children had echocardiography and electrocardiogram data available during analysis of outpatient records, and there were differences in the timing of these evaluations, which could have led to data distortion in some cases. Almost all samples from MIS-C patients during illness were obtained after starting systemic glucocorticosteroids and intravenous immunoglobulins treatment, and it is quite possible that this treatment could have affected cytokine levels in serum in both MIS-C groups.

Conclusions

Our study shows that despite MIS-C being a severe disease involving multiple organs, most children did not experience severe consequences after clinical recovery. However, after a child has recovered from MIS-C, there is a risk of recurrence, which in our study was 2.2%. Other possible complications included somatic disorders (11.8%), anemia (8.6%), decreased vision (7.5%), reactive arthritis (2.2%). Coronary artery dilation decreased six-fold after two years of observation and was present only in one patient (1.7%). On follow-up echocardiograms in the first year of observation, there was a statistically significant decrease in left ventricular dysfunction, pericarditis, pulmonary arterial hypertension, right and left heart chamber dilation, mitral regurgitation, and valve thickening. However, ambulatory monitoring showed an increase of TR (P=0.002) and PR (P=0.046), also, not all children had a complete restoration of the functions of the cardiovascular system. Thus, an increase in left ventricular ejection was observed in 1 (1.7%) child who died

from a relapse of MIS-C. In 1 (1.7%), insignificant pulmonary hypertension persisted in the 1st month after discharge. Dilatation of the right and left parts of the heart persisted in 4 (6.9%) children, the use of valves in 1 (1.7%) child, as well as in 1 (1.7%) child, moderate dilation of the coronary arteries remained. Heart valve regurgitation in the first year of follow-up required 22 (41.5%) children. Thus, 34 (64.1%) children after MIS-C are of great importance as a subclinical disease. Considering this, it may be necessary to optimize outpatient monitoring protocols with mandatory monitoring of the cardiovascular system and to increase the follow-up period for patients who still exhibit signs of subclinical myocardial damage one year after recovering from MIS-C as well as conducting contrast-enhanced cardiac magnetic resonance imaging. Since some patients in our study had decreased vision, we recommend that they receive ophthalmological consultations after discharge, as not all children can assess changes in visual acuity in a timely manner.

Immune profiling showed the restoration of cellular and humoral immune response in children after MIS-C indicators, including an increase in the relative value of CD3+, CD4+ T- lymphocytes, NK-cells, a decrease in CD19+ B-cells and CD3-HLA-DR+ expression. However, CD8+ T cells were elevated at all study time points. Our work showed a possible role in the pathogenesis of the disease of CD95 and CD279 receptors. To confirm this role, studies including a larger number of patients are necessary. We believe that the prerequisites for future work could be further determination of CD95 expression and its ligand CD95L, double-negative γδ T-cells, as well as analysis of mutations in genes responsible for various stages of FAS-dependent apoptosis.

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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References

1. Noval Rivas M, Porritt RA, Cheng MH, Bahar I, Arditi M. Multisystem inflammatory syndrome in children and long COVID: the SARS-CoV-2 viral superantigen hypothesis. Front Immunol. 2022 Jul 7;13:941009. doi: 10.3389/fimmu.2022.941009.
2. Das N, Hill R, Trivedi M, et al. Longitudinal assessment of cardiac function following multisystem inflammatory syndrome in children associated with COVID-19. Pediatr Cardiol. 2023 Mar;44(3):607-617. doi: 10.1007/s00246-022-02972-3.
3. Sezer M, Çelikel E, Tekin ZE, et al. Multisystem inflammatory syndrome in children: clinical presentation, management, and short- and long-term outcomes. Clin Rheumatol. 2022 Dec;41(12):3807-3816. doi: 10.1007/s10067-022-06350-5.
4. Lin J, Harahsheh AS, Raghuveer G, et al. Emerging insights into the pathophysiology of multisystem inflammatory syndrome associated with COVID-19 in children. Can J Cardiol. 2023 Jan 7:S0828-282X(23)00004-1. doi: 10.1016/j.cjca.2023.01.002.
5. Rajamanickam A, Nathella PK, Venkataraman A, et al. Unique cellular immune signatures of multisystem inflammatory syndrome in children. PLoS Pathog. 2022 Nov 2;18(11):e1010915. doi: 10.1371/journal.ppat.1010915.
6. Carter MJ, Fish M, Jennings A, et al. Peripheral immunophenotypes in children with multisystem inflammatory syndrome associated with SARS-CoV-2 infection. Nat Med. 2020 Nov;26(11):1701-1707. doi: 10.1038/s41591-020-1054-6.
7. Ricci-Vitiani L, Conticello C, Zeuner A, De Maria R. CD95/CD95L interactions and their role in autoimmunity. APOPTOSIS. 2000;5(5):419–24.
8. Jiang TT, Martinov T, Xin L, et al. Programmed Death-1 culls peripheral accumulation of high-affinity autoreactive CD4 T cells to protect against autoimmunity. Cell Rep. 2016 Nov 8;17(7):1783-1794. doi: 10.1016/j.celrep.2016.10.042.
9. Patel JM. Multisystem Inflammatory Syndrome in Children (MIS-C). Curr Allergy Asthma Rep. 2022 May;22(5):53-60. doi: 10.1007/s11882-022-01031-4.
10. Tong T, Yao X, Lin Z, et al. Similarities and differences between MIS-C and KD: a systematic review

and meta-analysis. Pediatr Rheumatol Online J. 2022 Dec 5;20(1):112. doi: 10.1186/s12969-022-00771-x.
11. Jaxybayeva I, Boranbayeva R, Abdrakhmanova S, et al. Comparative analysis of clinical and laboratory data in children with multisystem inflammatory syndrome associated with SARS-CoV-2 in the Republic of Kazakhstan. Mediterr J Hematol Infect Dis. 2022 Sep 1;14(1):e2022064. doi: 10.4084/MJHID.2022.064.
12. Irfan O, Muttalib F, Tang K, Jiang L, Lassi ZS, Bhutta Z. Clinical characteristics, treatment and outcomes of paediatric COVID-19: a systematic review and meta-analysis. Arch Dis Child. 2021 Feb 16;106(5):440–8. doi: 10.1136 /archdischild-2020-321385.
13. Penner J, Abdel-Mannan O, Grant K, et al. 6-month multidisciplinary follow-up and outcomes of patients with paediatric inflammatory multisystem syndrome (PIMS-TS) at a UK tertiary paediatric hospital: a retrospective cohort study. Lancet Child Adolesc Heal. 2021 Jul;5(7):473–82. doi: 10.1016/S2352-4642(21)00138-3.
14. Pawar RS, Tarkasband VA, Patil RK, Naik AV. Second episode of multisystem inflammatory syndrome in children: relapse, rebound, or recurrence? Pediatr Infect Dis J. 2021 Nov 1;40(11):e452. doi: 10.1097/INF.0000000000003249.
15. Chakraborty A, Johnson JN, Spagnoli J, et al. Long-term cardiovascular outcomes of multisystem inflammatory syndrome in children associated with COVID-19 Using an Institution Based Algorithm. Pediatr Cardiol. 2023 Feb;44(2):367-380. doi: 10.1007/s00246-022-03020-w.
16. Capone CA, Misra N, Ganigara M, et al. Six month follow-up of patients with multi-system inflammatory syndrome in children. Pediatrics. 2021 Oct;148(4):e2021050973. doi: 10.1542/peds.2021-050973.
17. Sperotto F, Friedman KG, Son MBF, VanderPluym CJ, Newburger JW, Dionne A. Cardiac manifestations in SARS-CoV-2-associated multisystem inflammatory syndrome in children: a comprehensive review and proposed clinical approach. Eur J Pediatr. 2021 Feb;180(2):307-322. doi: 10.1007/s00431-020-03766-6.
18. Valverde I, Singh Y, Sanchez-de-Toledo J, et al. Acute cardiovascular manifestations in 286 children with multisystem inflammatory syndrome associated with COVID-19 Infection in Europe. Circulation. 2021 Jan 5;143(1):21–32. doi: 10.1161/CIRCULATIONAHA.120.050065.
19. Jaxybayeva I, Boranbayeva R, Bulegenova M, Urazalieva N. Clinical and immunological features in children with multisystem inflammatory syndrome associated with SARS-CoV-2. Acta Biomed. 2023 Apr 24;94(2):e2023016. doi: 10.23750/abm.v94i2.13777.
20. Okarska-Napierała M, Mańdziuk J, Feleszko W, et al. Recurrent assessment of lymphocyte subsets in 32 patients with multisystem inflammatory syndrome in children (MIS-C). Pediatr Allergy Immunol. 2021 Nov 11;32(8):1857–65. doi: 10.1111/pai.13611.
21. Liu Y, Gao Y, Hao H, Hou T. CD279 mediates the homeostasis and survival of regulatory T cells by enhancing T cell and macrophage interactions. FEBS Open Bio. 2020 Jun;10(6):1162-1170. doi: 10.1002/2211-5463.12865.
22. Paulsen M, Janssen O. Pro- and anti-apoptotic CD95 signaling in T cells. Cell Commun Signal. 2011 Apr 8;9:7. doi: 10.1186/1478-811X-9-7.
23. Francisco LM, Sage PT, Sharpe AH. The PD-1 pathway in tolerance and autoimmunity. Immunol Rev. 2010 Jul;236:219-42. doi: 10.1111/j.1600-065X.2010.00923.x.
24. Paulsen M, Janssen O. Pro- and anti-apoptotic CD95 signaling in T cells. Cell Commun Signal. 2011 Apr 8;9:7. doi: 10.1186/1478-811X-9-7.
25. Koncz G, Hueber AO. The Fas/CD95 receptor regulates the death of autoreactive B cells and the selection of antigen-specific B cells. Front Immunol. 2012 Jul 25;3:207. doi: 10.3389/fimmu.2012.00207.
26. Fife BT, Pauken KE. The role of the PD-1 pathway in autoimmunity and peripheral tolerance. Ann N Y Acad Sci. 2011 Jan;1217:45-59. doi: 10.1111/j.1749-6632.2010.05919.x.
27. Bellesi S, Metafuni E, Hohaus S, et al. Increased CD95 (Fas) and PD-1 expression in peripheral blood T lymphocytes in COVID-19 patients. Br J Haematol. 2020 Oct;191(2):207-211. doi: 10.1111/bjh.17034.

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