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DEPARTMENT OF INTERNAL DISEASES, PHARMACOLOGY AND CLINICAL PHARMACOLOGY, PEDIATRICS, EPIDEMIOLOGY, INFECTIOUS AND SKIN DISEASES

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Macrophage activation syndrome in childhood - analysis of clinical and laboratory characteristics, evaluation of the diagnostic approach and therapeutic efficacy

SUMMARY

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The PhD thesis consists of 123 standard typewritten pages and is illustrated with 37 figures and 8 tables. The bibliographic reference contains 203 literature sources, from which 2 are by Bulgarian authors and 201 are by foreign medical teams.

The PhD thesis is aimed at public presentation after discussion at the Faculty Council of the Department of Internal Medicine, Pharmacology and Clinical Pharmacology, Pediatrics, Epidemiology, Infectious and Skin Diseases at Sofia University "Kliment Ohridski", Sofia.

The public presentation of the PhD thesis will take place on .....from…….h in ….....
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at an open meeting of the Faculty Council of the Department of „Internal Medicine, Pharmacology and Clinical Pharmacology, Pediatrics, Epidemiology, Infectious and Skin Diseases at Sofia University "Kliment Ohridski", Sofia.

CONTENTS

List of abbreviations

MAS - macrophage activation syndrome
JIA - juvenile idiopathic arthritis
ESR - erythrocyte sedimentation rate
HFLH - hemophagocytic lymphohistiocytosis
DNA - deoxyribonucleic acid
RNA - ribonucleic acid
ASAT - aspartate aminotransferase
ALAT - alanine aminotransferase
LDH - lactate dehydrogenase
CNS - central nervous system
DIC - disseminated intravascular coagulation
IL - interleukin
TNFα - tumor necrosis factor α
γIFN - γ interferon
NK - natural killer
EBV - Epstein-Barr virus
CMV- cytomegalovirus
PCR - polymerase chain reaction
VLDL - Very low density lipoprotein
HDL - high density lipoprotein
PRINTO - Pediatric Rheumatology International Trials Organization
EULAR - European League Against Rheumatism
IVIG - Intravenous immunoglobulin
IgG - immunoglobulin G
ATG - Anti-thymocyte globulin

I. INTRODUCTION

Haemophagocytic lymphohistiocytosis is a complex pathological condition characterized by a hyperinflammatory response of the human body to various stimuli, which in most cases leads to severe and often fatal consequences. This syndrome may be due to homozygous or heterozygous mutations in the genes of the perforin-dependent pathway of cytolysis, thus affecting both the innate (via NK cells) and the acquired immune response (via CD8 + T lymphocytes). This includes variants of familial haemophagocytic lymphohistiocytosis. However, this condition can also develop as idiopathic and / or secondary haemophagocytic lymphohistiocytosis, within a number of other diseases - most often autoimmune and malignant diseases, as well as a number of infections. The accepted term that describes this complex pathological process is the macrophage activation syndrome (MAS). At the heart of MAS is a disrupted relationship between macrophages and T - lymphocytes, which leads to uncontrolled proliferation of overactivated macrophages, cytotoxic T-lymphocytes, NK - cells that secrete excessive amounts of proinflammatory cytokines. This condition is known as a cytokine storm. It is the basis of subsequent generalized organ dysfunction, often fatal.

Clinical manifestations of macrophage activation syndrome include the most common febrile reaction, lymphadenomegaly, hepatosplenomegaly and rash syndrome - all signs resembling a septic condition, which is why the development of sepsis is most often discussed in the differential diagnostic plan. Laboratory tests most often reveal pancytopenia, transaminase activity, hyperferritinemia and the development of disseminated intravascular coagulation syndrome. As can be seen, neither the clinical symptoms nor the laboratory changes are specific to MAS. In this regard, working groups have been set up with the aim of developing diagnostic criteria for the disease to facilitate the correct diagnosis. Currently, the diagnosis of MAS is based on a febrile condition, hyperferritinemia above 684 ng / ml in combination with high values of AST / above 48 U / l / and triglycerides / above 1.76 mmol / l / and low platelet counts / below 181 x 109 (l) and fibrinogen (less than 3.6 g). However, a number of other clinical and laboratory parameters are relevant to the disease and are helpful in making the diagnosis.

As macrophage activation syndrome is a serious condition with possible severe consequences, it is of interest to a number of researchers. We are working both to clarify the detailed mechanisms in the pathogenesis of the disease and to set the most accurate diagnostic criteria, using the clinical manifestations of MAS and analysis of altered laboratory markers.

The diagnosis of macrophage activation syndrome requires the initiation of timely treatment aimed at suppressing the hyperinflammatory condition and placing it under permanent control. In this regard, high-dose corticosteroid regimens and cyclosporine use play a major role in treatment regimens. In addition, attempts have been made to treat the condition with other drugs - intravenous immunoglobulins, antithymocyte globulin, etoposide, rituximab, with

varying degrees of success. New and modern therapeutic strategies developed in recent years, including the use of so-called biological agents, find their place in the treatment of MAS. These are mainly molecules aimed at inactivating major cytokines directly involved in the pathogenesis of MAS, such as IL-1 and IL-6. The data from the studies conducted so far from the application of biological therapy to affect MAS give very encouraging results.

Macrophage activation syndrome occurs in both adults and children. The main feature of this pathological condition is that when it occurs, it is usually severe and often fatal. The lack of clear clinical features and characteristic laboratory changes make it even more challenging. It should be noted that there is no safe and generally accepted therapeutic regimen that guarantees a good outcome of the disease. This gives grounds to study the problem in more detail, to analyze the maximum number of patients in order to develop the most accurate diagnostic criteria and apply the most effective therapeutic strategies. The earlier the diagnosis is made and the faster and more timely the treatment is started, the greater the chance of a good outcome of the macrophage activation syndrome.

II. PURPOSE AND TASKS

The aim of this dissertation is to describe in detail the pediatric patients with macrophage activation syndrome in Bulgaria; to analyze the established clinical and laboratory changes; to evaluate the applied diagnostic approach and the effect of the performed treatment.To achieve this goal it is necessary to perform the following tasks:

To analyze the data of all children with MAS according to the following parameters:

1. To make a distribution by sex and age.
2. To try to identify the triggers of the underlying disease.
3. To present the clinical characteristics of the syndrome
4. To consider in detail and evaluate the changes in laboratory parameters in macrophage activation syndrome
5. To analyze the effect of the applied medications to control clinical changes in macrophage activation syndrome
6. To analyze the effect of the applied drugs on the changes in laboratory parameters
7. To propose an up-to-date diagnostic approach
8. Propose an effective therapeutic strategy

III. MATERIALS AND METHODS

The present PhD thesis includes and analyzes the data of 20 children with macrophage activation syndrome. They were diagnosed, treated and followed up in the following hospital units: Acibadem City Clinic Tokuda Hospital Pediatric Clinic - Sofia, University Multidisciplinary Hospital for Active Treatment of Pediatrics “Ivan Mitov ”- Pediatric Rheumatology Clinic Sofia, University Multidisciplinary Hospital for Active Treatment“ St. Georgi ”, Pediatric Clinic -

Plovdiv and University Multidisciplinary Hospital for Active Treatment, Pediatric Clinic - Varna.

All children included in the present study were diagnosed and treated for macrophage activation syndrome for the period from 2013 to 2019. The indicators that are analyzed are divided into the following groups:

1. Distribution by sex and age

2. Distribution according to the triggers of the macrophage activation syndrome – assessment of clinical and laboratory symptoms of the underlying underlying disease; microbiological and immunological tests to detect infectious agents such as MAS triggers.

3. Assessment of clinical symptoms of MAS - all patients had a history and physical status was studied in accordance with the generally accepted propaedeutic rules in pediatric practice. This assessment was performed on each child repeatedly - on admission to hospital, during the hospital stay, as well as in the post-hospital period. The main clinical parameters that are the subject of this study are:

3.1. Fever - initial manifestation, type of temperature curve;

3.2. Rash - initial manifestation, type, location of rash lesions

3.3. Lymphadenomegaly - initial manifestation, localization of enlarged lymph nodes

nodes, palpation characteristic.

3.4. Presence of hepatomegaly

3.5. Presence of splenomegaly

3.6. Manifestations of the central nervous system - disorientation, irritability, seizures, coma

4. Evaluation of paraclinical indicators

4.1. Clinical laboratory indicators

4.1.1. Haematological parameters - complete blood count - leukocyte count, platelet count, hemoglobin, differential formula.

4.1.2. Markers of inflammation - erythrocyte sedimentation rate, C-reactive protein, procalcitonin

4.1.3. Biochemical indicators

4.1.3.1. - transaminases, cytosolic enzymes - ASAT, ALAT, LDH

4.1.3.2. . - total protein, albumin

4.1.3.3. - cholesterol, triglycerides

4.1.4. Ferritin

4.1.5. Coagulation markers

4.1.5.1. fibrinogen

4.1.5.2. D-dimers

4.2. Microbiological tests

4.2.1. Uroculture

4.2.2. Blood culture

4.2.3. Immunological tests for infectious agents - IgM antibodies to: Mycoplasma pneumoniae, Epstein - Barr virus capsid antigen, Pavovirus B19

4.2.3. Genetic analysis - PCR / polymerase chain reaction / for EBV in lymphocytes

4.3. Methods of diagnostic imaging

4.3.1. Conventional chest X-ray

4.3.2. CNS magnetic resonance imaging

4.3.3. Ultrasound examination of abdominal organs, heart structures and pleura

4.4. Bone marrow puncture - assessment of existing hemophagositis

All patients were diagnosed with macrophage activation syndrome according to the current diagnostic criteria for this EULAR / ACR disease from 2016.

- Fever
- Ferritin> 684 ng / ml and at least two of the following:
- Platelets $<181x10^9$ / l
- ASAT> 48 IU / l
- Triglycerides> 1.76 mmol / l
- Fibrinogen <3.6 g/l

All these clinical and laboratory parameters were evaluated repeatedly in patients - both at diagnosis and in the subsequent period of treatment. All laboratory and imaging studies were performed in certified laboratories, using automatic hematology and biochemical analyzers, without the possibility of manual correction of the data obtained.

After the diagnosis of macrophage activation syndrome, according to the above criteria, treatment was started in all patients. The initial choice of drug is a corticosteroid - methylprednisolone in pulse doses - 30 mg / kg / day for three consecutive days. Depending on the effect on disease control, some patients have been treated with other drugs:

- Cyclosporine
- Intravenous immunoglobulins
- Biological agent - anti interleukin 1 - antibody - kineret
- Etoposide

Patients are divided into subgroups depending on the treatment regimens - high-dose corticosteroid, in combination or not with cyclosporine, the addition of a third drug. The doses of these drugs and dosing regimens will be discussed in detail in the "Results" section. In a separate presentation, the experience of the use of biological therapy with anti-IL-1-receptor antagonist in two of the children with MAS was reported.

In all children, the time interval for monitoring certain laboratory parameters, which are important diagnostic markers in the disease, was assessed:

1. Serum ferritin
2. Platelet count
3. Aspartate aminotransferase
4. Fibrinogen
5. Triglycerides

An attempt was made to assess the effect of the treatment on the change in serum ferritin levels and on the days required to monitor the children's clinical status and changes in their laboratory parameters.

Special attention is paid to the diagnostic value of serum ferritin.

This is a laboratory indicator that increases in other diseases, but it is believed that values above 500-600 ng/ml differentiate the syndrome of macrophage activation from them. For this reason, the current study included a group of children with other diseases that have a pronounced clinical and laboratory inflammatory syndrome:

- Septic arthritis
- Systemic lupus erythematosus
- Kawasaki disease
- Bacterial pneumonia
- Parapneumonic pleural effusion
- Acute purulent otitis media
- Acute adenoiditis
- Acute bacterial tonsillitis
- Acute pyelonephritis

These patients, who are 21 in number, were diagnosed and treated at Acibadem City Clinic Tokuda Hospital, Pediatric Clinic, Sofia. In them, the diagnosis of the underlying disease is made on the basis of generally accepted diagnostic requirements and on the basis of relevant paraclinical studies that support the diagnosis. Serum ferritin was tested in all patients at the beginning of the underlying disease. These values were compared with its values in patients with macrophage activation syndrome at diagnosis, the type and strength of the correlation and its significance were assessed.

In an attempt to find the most accurate and objective diagnostic criteria for macrophage activation syndrome, the author's teams working in this field tested the sensitivity and specificity of the ferritin/ESR ratio. The data obtained show that it is a very good diagnostic marker for MAS. In this regard, it was estimated that the diagnostic value of the ferritin / ESR ratio would be assessed in the two groups of patients included in the present study - those with a primary diagnosis of MAS and those in the control group of children with other major non-MAS diseases; the sensitivity and specificity of the ferritin / ESR ratio in the context of MAS were studied.

All results are presented in the form of tables and / or graphs, accompanied by relevant detailed explanations of the studied parameters.

Statistical methods

Macrophage activation syndrome is an extremely rare disease that is difficult to diagnose. On this occasion, the group of patients studied is small. An attempt was made to gather the maximum number of children with this diagnosis and to analyze as accurately as possible the results of their clinical and laboratory tests. In this regard, a package for statistical data processing by a small group of

patients was used and the analyzes were performed by a professional specialist in statistics and medical data processing.

Statistical processing was performed with SPSS 13.0. A descriptive analysis was performed with the help of groupings by one or several features, summarizing indicators - relative share, arithmetic mean, median.

A diagnostic analysis was also performed to assess the presence of statistically significant effects by testing statistical hypotheses about the presence of a specific relationship in variables measured on weak scales. This analysis was performed using the chi-square method. "Accurate" empirical indicators of Fisher (Fisher exact test) were calculated to decide on the tested effect.

ROC analysis was used to assess the specificity and sensitivity of an alternative diagnosis, including serum ferritin and ESR, presented as a ferritin/ESR ratio.

An analysis of the difference in mean values was performed, and the statistical significance of the differences was tested using models for testing the mean difference (ANOVA, t-test). The results of these models are detected with applied non-parametric analogues, and the aim is to eliminate possible influence from non-compliance with the requirements of the parametric models and at the same time to use the strongest, parametric statistical method.

Levels based on assumptions about the distribution of the tested traits are used to assess the level of significance of certain empirical characteristics. The significance level for the significance level is 0.05, unless another value is explicitly stated. The corresponding estimates for the significance of a certain empirical characteristic of the above-described tests are compared with this limit value of 0.05. If it is less than 0.05, the test effect is considered statistically significant, if it is greater than 0.05, the test effect is considered statistically insignificant.

IV. RESULTS

In the present dissertation the results of 20 children with macrophage activation syndrome are presented and analyzed. The currently valid diagnostic criteria of PRINTO / EULAR from 2016 were used to make the diagnosis.

Diagnostic criteria for SMA, PRINTO 2016
Fever and serum ferritin> 684 ng/ml
Platelets $\leq 181 \times 10^9$ /l
Aspartate aminotransferase> 48 U/l
Triglycerides> 1.76 mmol/l
Fibrinogen $\leq$ 3.60 g/l

Table 1 Diagnostic criteria for MAS, PRINTO / EULAR from 2016.

1. Distribution by sex, age, triggers and clinical symptoms of macrophage activation syndrome

The distribution by gender is as follows: 45% of children are girls / n = 9 /, and 55% / n = 11 / are boys

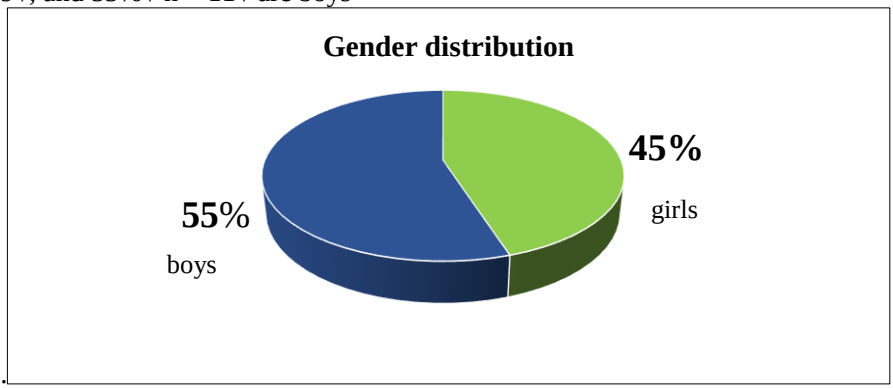

Fig. 1 Distribution by sex

In the age distribution, it was found that the youngest child diagnosed with macrophage activation syndrome was 11 months old and the oldest was 16 years old. The average age is 7.4 years; 40% / n = 8 / of the children are aged from 10 to 16 years, 25% / n = 5 / of the children are between 4 and 9 years old, and 35% / n = 7 / of the children are under 3 years old age.

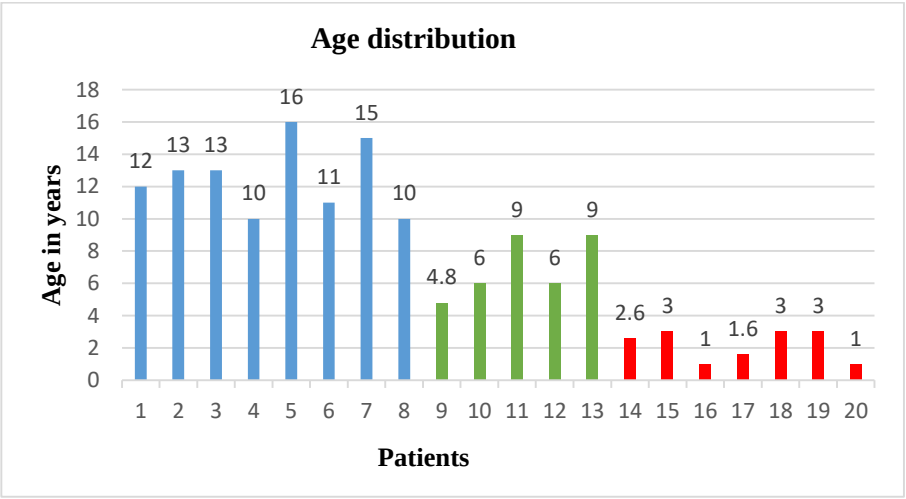

Fig. 2 Distribution by age in years

The diagnosis of macrophage activation syndrome raises the question of the triggers of this severe condition. The analysis of the patients included in the present study revealed the following results: in 75% / n = 15 / of the children SMA occurs within juvenile idiopathic arthritis, in 5% / n = 1 / dermatomyositis was found, and in the remaining 20% Infectious triggers were identified as follows: 10% / n = 2 / Epshtein-Barr virus infection, 5% / n = 1 / Mycoplasma pneumoniae infection, 5% / n = 1 / Parvovirus B19 infection.

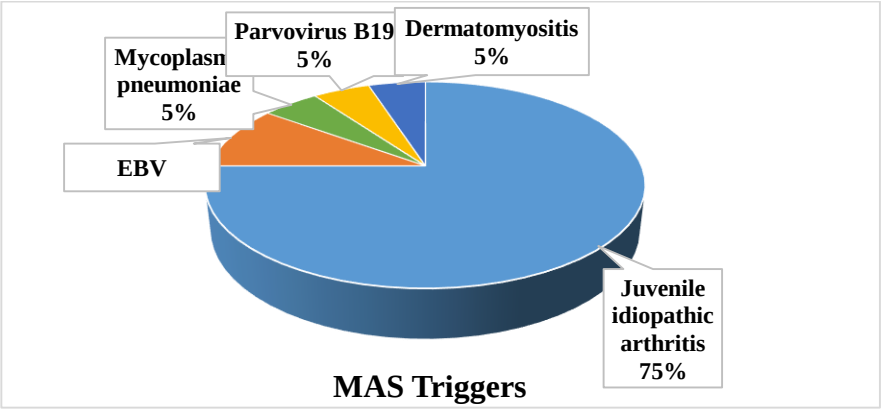

Fig. 3 Trigger for macrophageal activation syndrome

The processing of data from clinical manifestations in the diagnosis of macrophage activation syndrome in the studied children revealed the following results: with fever are 100% / n = 20 / of children, with hepatomegaly - 100% / n = 20 /, with rashes are 85% / n = 17 /, with peripheral lymphadenomegaly - 65% / n = 13 /, with splenomegaly - 65% / n = 13 /, with manifestations of the CNS - 15% / n = 3 /, with pericarditis - 15 % / n = 3 / and pleurisy - 15% / n = 3 /

In 60% (n = 12) of the patients, fever, rash, lymphadenomegaly and hepatomegaly were present at the time of diagnosis.

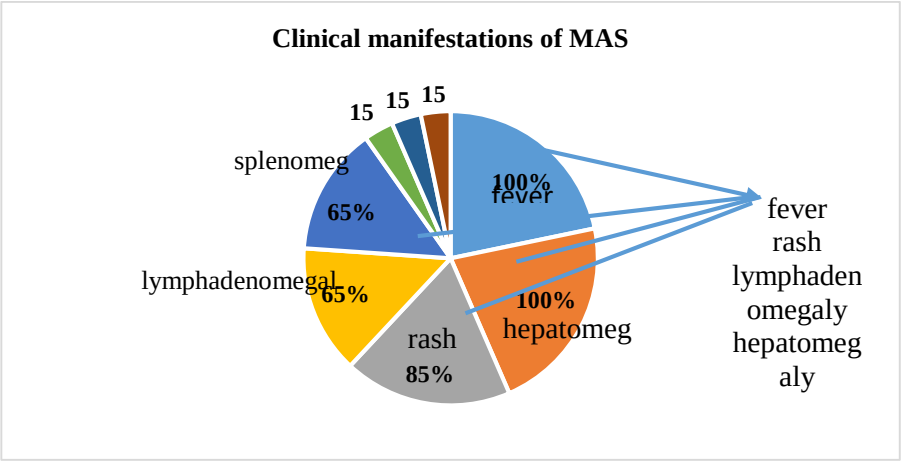

Fig. 4 Clinical manifestations of MAS – percentage

The presentation of clinical symptoms is as follows:

- The fever is remittent and was established at the diagnosis of MAS in all 20 children;
- The rash syndrome is presented with different rash lesions in different patients - from generalized erythema, erythematous macules, papules, petechiae, ecchymoses and suffusions;
- Peripheral lymphadenomegaly is from generalized to isolated bilateral involvement of the cervical lymph nodes, axillary and / or inguinal lymph nodes;
- CNS manifestations are found in three of the children - in the form of quantitative changes in consciousness, seizures, and in one of these children by conducting magnetic resonance imaging of the CNS was found and cerebral hemorrhage;
- Pericardial involvement was found in three of the children by echocardiography in the form of small pericardial effusions;
- Pleural involvement was found in three of the patients again by ultrasound in the form of small pleural effusions, not requiring invasive therapeutic procedures to affect them.

2. Presentation of laboratory test results

2.1. Analysis of results of indicators of inflammatory activity and some parameters of complete blood count that are relevant to MAS

The ESR study at the diagnosis of SMA shows measured values from 5 mm / h to 60 mm / h; the average value is 25 mm / h; most children have ESR 20 mm / h; in 60% of patients ESR is in reference values, and in 40% it is accelerated.

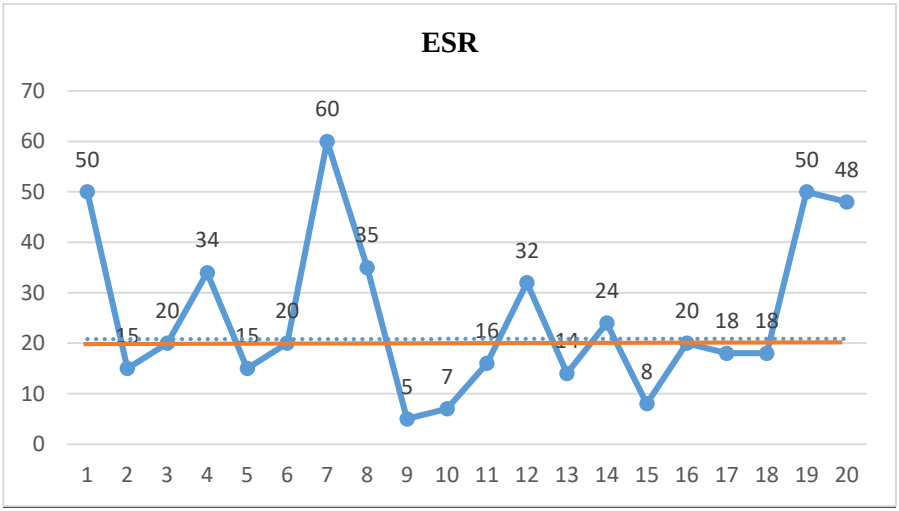

Fig. 5 ESR values in mm / h at diagnosis

In the study of C-reactive protein, the lowest measured value was 5.99 mg / l and the highest was 266.1 mg / l; the arithmetic mean is 78.4 mg / l; median - 53.6 mg / l. In all 20 children (100%) C-reactive protein is elevated.

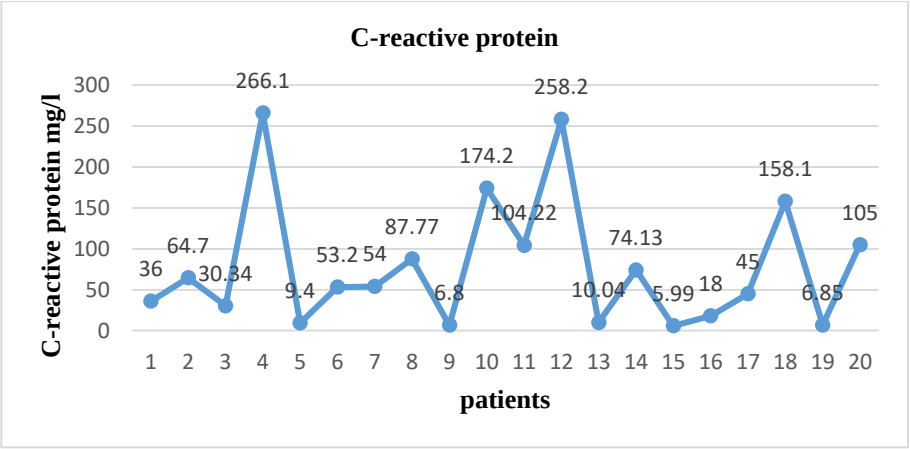

Fig. 6 C-reactive protein values

In the study of hemoglobin it was found that the lowest measured value is 74 g / l, the highest is 155 g / l; the arithmetic mean is 104.5 g / l; median 103.5 g / l. In percentage terms, the results show that 15% of children have normal hemoglobin, 75% have hemoglobin below normal and only 10% have hemoglobin content above the reference upper limit.

Procalcitonin was tested in 6 of the patients. From the analysis of the obtained data it turned out that in all children this marker is in increased values / at reference values 0-0.05 ng / ml /. The lowest measured value is 0.14 ng / ml and the highest is 27.26 ng / ml. The arithmetic mean for procalcitonin is 7.05 ng / ml. A more detailed examination of the changes in this group of patients is as follows:

- Provoking factor

- 3 of the children develop MAS based on juvenile idiopathic arthritis - their procalcitonin is in the range of 0.31 ng / ml to 27.26 ng / ml;

- in the other 3 children the triggers are infectious agents / EBV and Parvovirus B19 / - in them procalcitonin is in values from 0.14 ng / ml to 12.38 ng / ml.

- Ferritin

- its values vary from 695.5 ng / ml to 15,061 ng / ml, but in most children it is in the range of 1400 ng / ml to 2500 ng / ml.

- in 5 of these children there is a recovery of ferritin values below 150 ng / ml; normalization of serum ferritin was not observed in only one of the children.

- The recovery period for ferritin below 150 ng / ml varies from 20 to 186 days, but most often it occurs within 20-21-25 days.

- Conducted treatment

- 3 of the children were treated with methylprednisolone only 30 mg / kg / day - one child with three pulse doses, two of the children with 4 pulse doses;

- Cyclosporine was added for the other 3 children, and kineret was used for one of them.

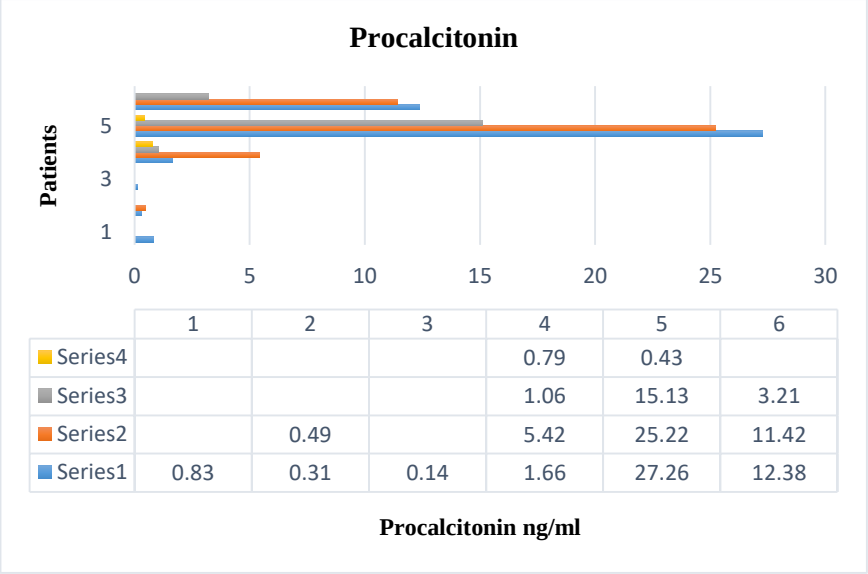

Fig. 7 Values of procalcitonin in ng / ml in patients with MAS

The results of the leukocyte count study show that the lowest measured value is 2.5 x 109 / l, and the highest - 30.9 x 109 / l; the arithmetic mean is 10.5 x 109 / l; in the study of the cumulative percentage it turned out that 40% of children have a leukocyte count below 4.77 x 109 / l. In 25% of children the leukocytes are in normal values, in 35% they are in values below 4.5 x 109 / l, and in 40% they are above the upper reference limit.

In the study of the differential formula it was found that in 80% of children neutrophilia is observed, in 10% neutrophils are in normal values. The arithmetic mean of the neutrophil count was 65.7%, with a median of 70.9% of the total leukocyte count.

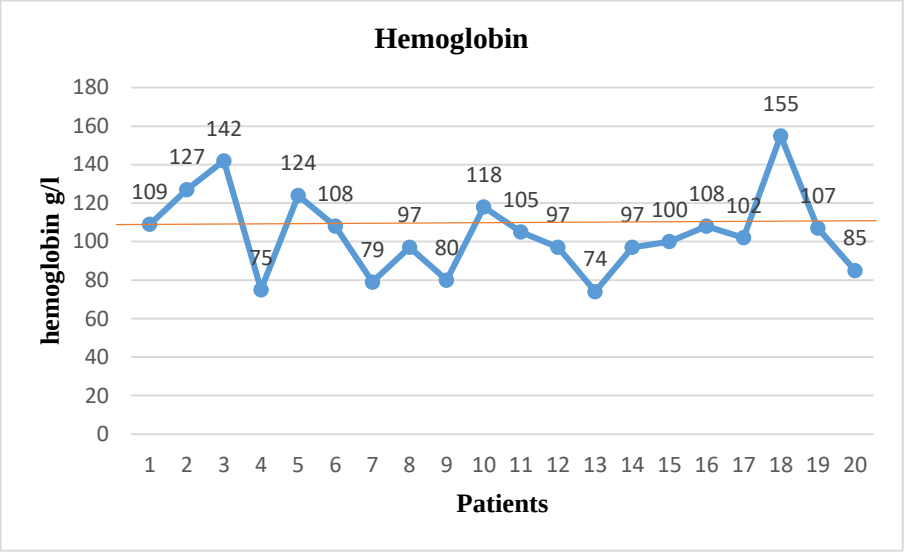

Fig. 8 Hemoglobin values in g / l

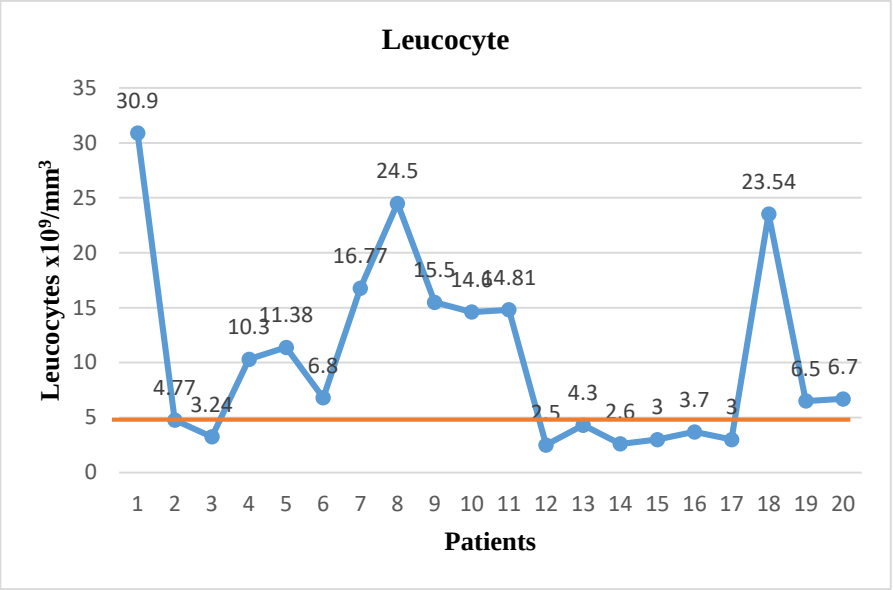

Fig. 9 Leukocyte values x 10^9 / l

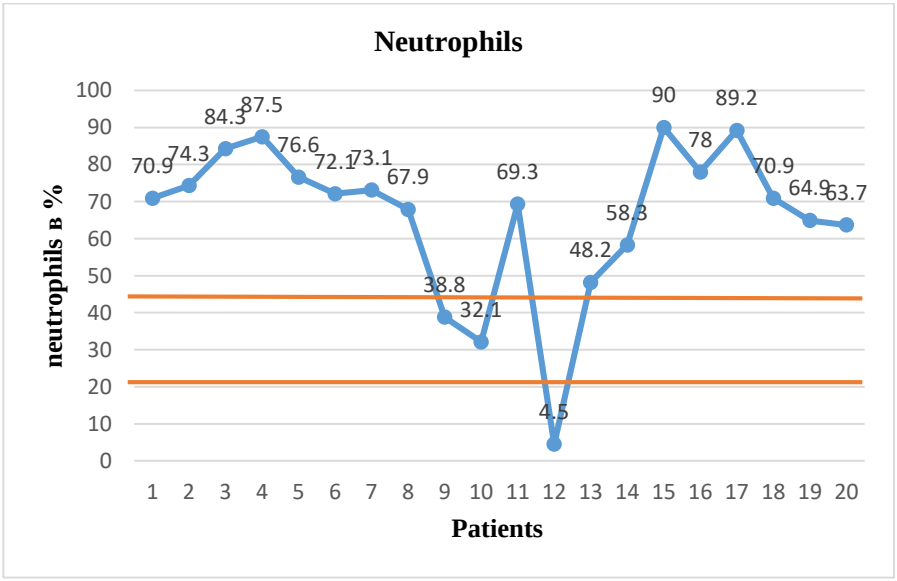

Fig. 10 Neutrophil count - percentage of total leukocyte count

In the study of platelet count, the lowest value was 23 x 10^9 / l and the highest 358 x 10^9 / l; the arithmetic mean of this indicator is 147 x 10^9 / l; the median is 133 x 10^9 / l.

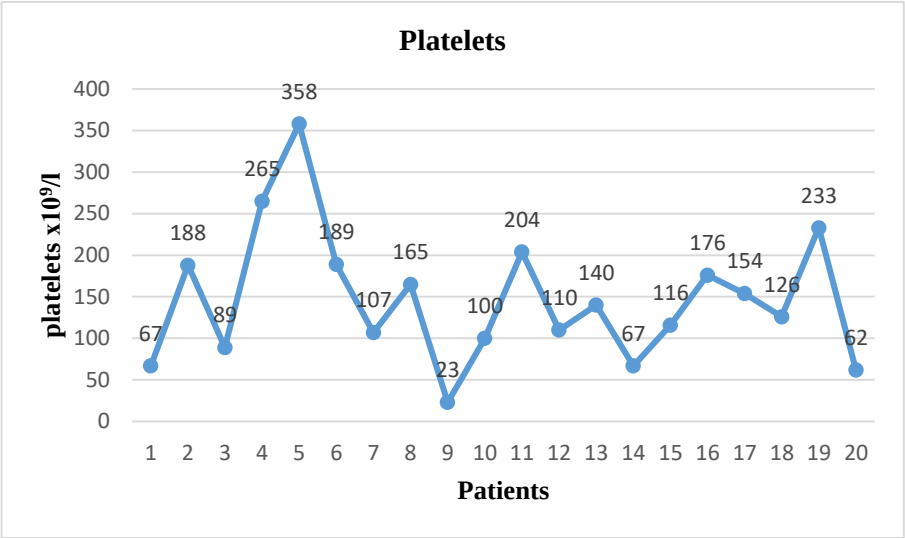

Fig. 11 Platelet count x 10^9 /l. As a normal value is reported that over 181x10^9/ l.

Platelet count is one of the diagnostic criteria, therefore a platelet count above 181 x 109 / l was reported as normal in the present study. In this regard, when diagnosed with SMA, only 30% of children had platelets above this value, and 70% of children were reported with low platelet counts (below 181 x 10^9 / l). The study of the cumulative platelet percentage found that 70% of children had platelets from 23 x 10^9 / l to 176 x 10^9 / l, and 75% had platelets from 23 x 10^9 / l to 188 x 10^9 / l.

2.2. Analysis of results from the study of biochemical indicators

The present study presents the results of the study of some biochemical indicators that are relevant to the diagnosis of macrophage activation syndrome.

The following results were obtained during the processing of the results of the study of AST, ALT and LDH:

- ASAT - in 90% / n = 18 / of the children the values of ASAT are above the norm and only in 10% of the patients / n = 2 / ASAT is in reference values for the age. The lowest measured value is 15 U / l and the highest is 958 U / l; the arithmetic mean of this indicator is 316 U / l and the median is 291 U / l.

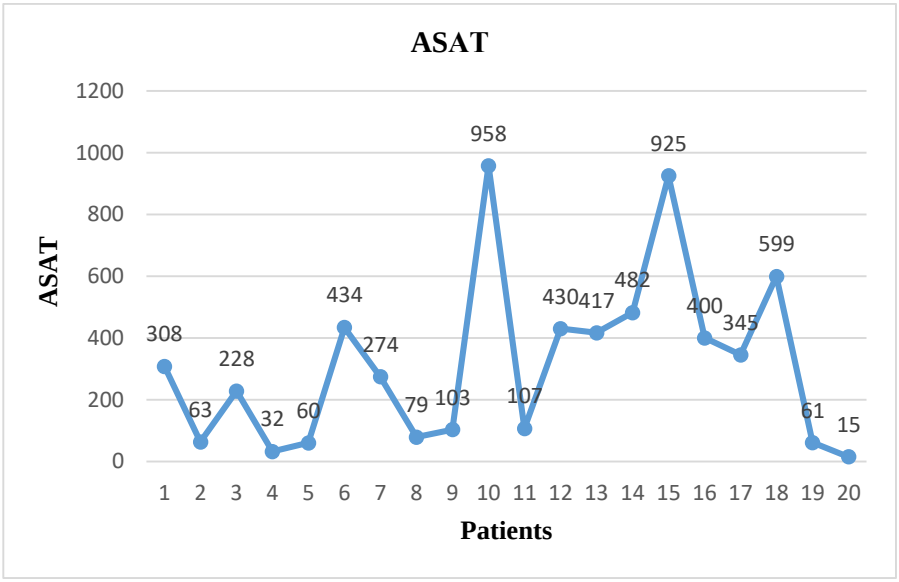

Fig. 12 ASAT values in U / l - 48 U / l is taken as the upper reference value

As ASAT is one of the diagnostic criteria for MAS, values above 48 U / l have been reported as elevated, therefore in patients included in the present study this value is taken as the upper reference limit above which the result is considered elevated.

- ALAT - in the study of ALT 70% / n = 14 / of the children have elevated values, 30% / n = 14 / have normal ALT values; the lowest measured value is 7 U / l and the highest is 380 U / l; the arithmetic mean for this indicator is 151.6 U / l and the median is 140.3 U / l.

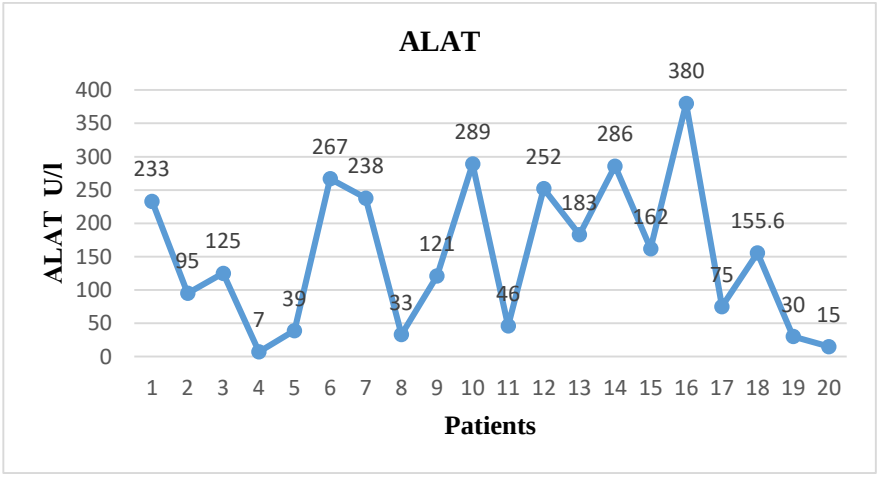

Fig. 13 ALAT values in U / l

➢ LDH - in the study of this indicator it turns out that 95% / n = 19 / of children have elevated values and only 5% / n = 1 / have LDH within the norm. The lowest measured value is 454 U / l and the highest is 9452 U / l; The arithmetic mean is 2019.6 U / l and the median is 1058.0 U / l. Monitoring the cumulative percentage for this indicator shows that 57% of children have LDH values in the range of 454 U / l to 1064 U / l, and 78% of patients have values of 454 U / l to 2651 U / l.

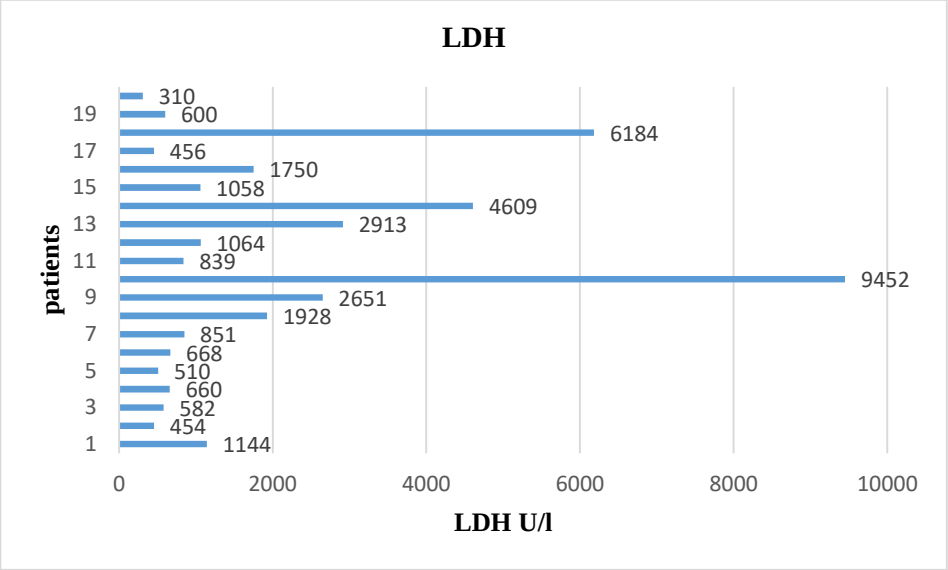

Fig. 14 LDH values in U / l

➢ Triglycerides - triglycerides were studied in 19 of the children and the results are as follows: in 25% / n = 5 / of the children this indicator is in normal values, and in 70% / n = 14 / triglycerides are in elevated values; the lowest measured value is 1.1 mmol / l and the highest is 12.9 mmol / l; the arithmetic mean is 4.3 mmol / l and the median is 3.1 mmol / l. Because triglycerides are a diagnostic criterion, elevated values above 1.76 mmol / l have been reported.

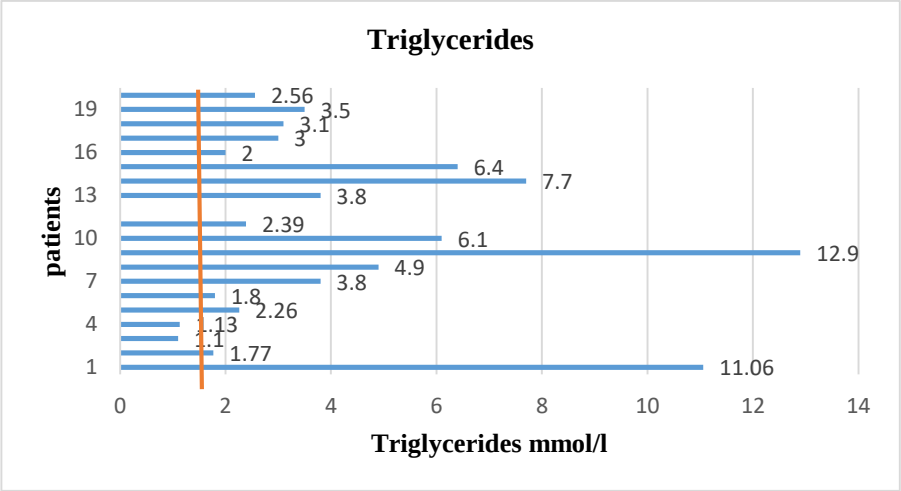

Fig. 15 Triglyceride values in mmol / l

➢ Cholesterol; Bilirubin - total and direct fraction; Total protein and albumin

The study of total cholesterol yielded the following results: 60% / n = 12 / of children have normal values, 5% / n = 1 / have low total cholesterol and 35% / n = 7 / have values above the above reference value. The lowest measured value is 2.94 mmol / l, the highest is 6.64 mmol / l; the arithmetic mean is 5.1 mmol / l and the median is 5.0 mmol / l.

The results of the study of total bilirubin are as follows: 80% / n = 16 / of children have normal values, 15% / n = 3 / have high values and only one child / 5% / total bilirubin is below the lower reference value . The lowest measured value is 3.3 µmol / l, and the highest - 155.3 µmol / l; the arithmetic mean of this indicator is 21 µmol / l and the median is 12.6 µmol / l; when monitoring the cumulative percentage for this indicator, it was found that 90% of the studied children have a total bilirubin of 3.3 µmol / l to 42.5 µmol / l.

Examination of the direct fraction of bilirubin revealed that 95% (n = 19) of the patients had a normal fraction and only 1% (n = 1) had elevated levels. The lowest measured value is 2.3 µmol / l, and the highest - 14.8 µmol / l; the arithmetic mean is 11.2 µmol / l and the median of this indicator is 4.8 µmol / l.

The study of total protein revealed the following results: 40% / n = 8 / of children have total protein in reference values, 55% / n = 11 / of patients have low total protein, 5% / n = 1 / a high value is found. The lowest measured value is 42 g / l and the highest 77 g / l; the arithmetic mean is 55.4 g / l and the median is 52.1 g / l. Monitoring the cumulative percentage for this indicator found that 55% of children had a total protein of 42 g / l to 53.1 g / l.

The results of the serum albumin test are as follows: 85% (n = 17) of the children have hypoalbuminemia and only 15% (n = 3) have normal albumin. The

lowest measured value for this indicator is 19.1 g / l, and the highest - 43.11 g / l; the arithmetic mean is 29.6 g / l and the median of this indicator is 27 g / l. Monitoring of the cumulative percentage for serum albumin shows that 80% of children have values ranging from 19.1 g/l to 35.19 g/l.

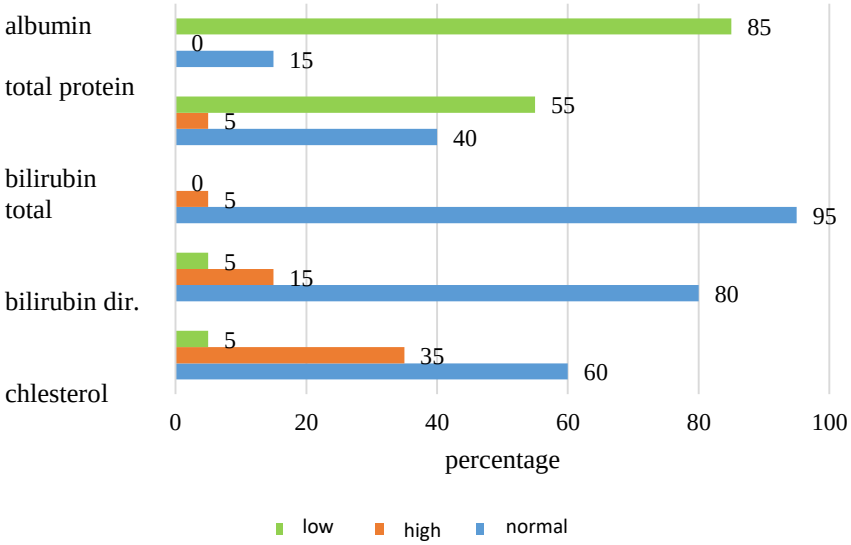

Fig. 16 Percentage ratio of normal, high and low values of the indicated indicators - albumn, total protein, bilirubin - direct and total fraction, cholesterol

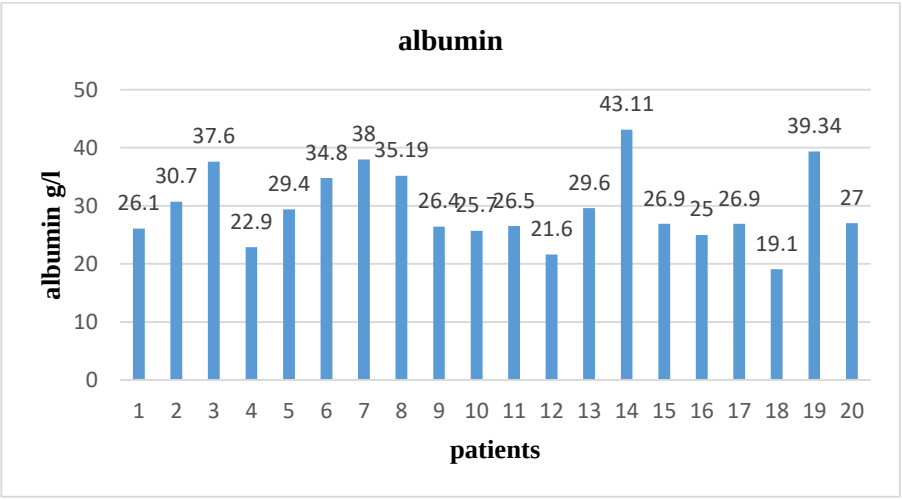

Fig. 17 Serum albumin in g / l

➢ Fibrinogen - this indicator is one of the diagnostic criteria. In this regard, values lower than 3.6 g / l are considered pathological. The study of this parameter in our patients showed the following results: 90% / n = 18 / of children have low values / below 3.6 g / l / and only 10% / n = 2 / fibrinogen is normal. The lowest measured value is 0.42 g / l and the highest is 3.9 g/l; the arithmetic mean for this indicator is 2.3 g / l and the median is 2.5g/ l.

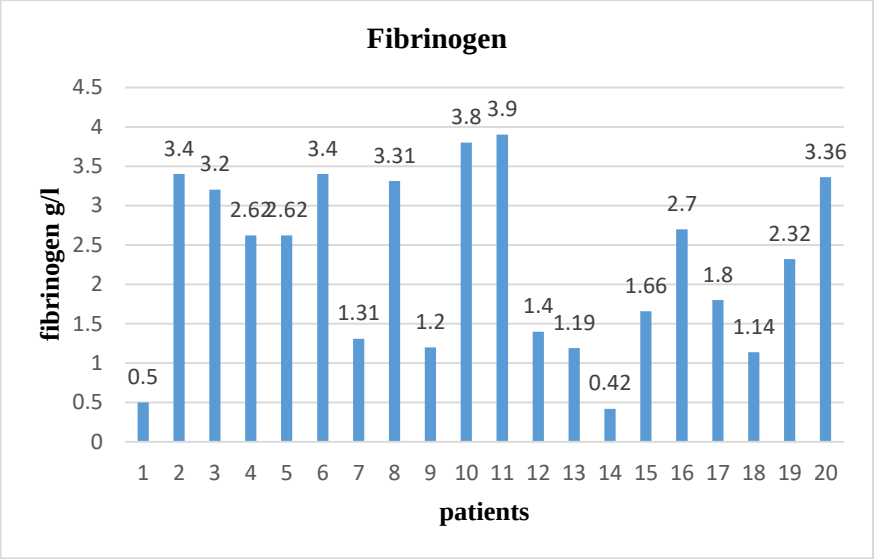

Fig. 18 Fibrinogen values in g / l

➢ D-dimers - the study of this indicator showed that in 100% of patients it is in values above the reference and in some of them it is in extremely high values. The lowest measured value for this indicator is 1.9 μg / ml, and the highest is over 35.2 μg / ml; the arithmetic mean is 12.7 μg / ml and the median is 8.3 μg / ml. Following the cumulative percentage of this indicator, it was found that 50% of children with D-dimers from 1.9 to 7.8 μg / ml, and 75% of patients with values from 1.9 to 16.32 μg / ml.

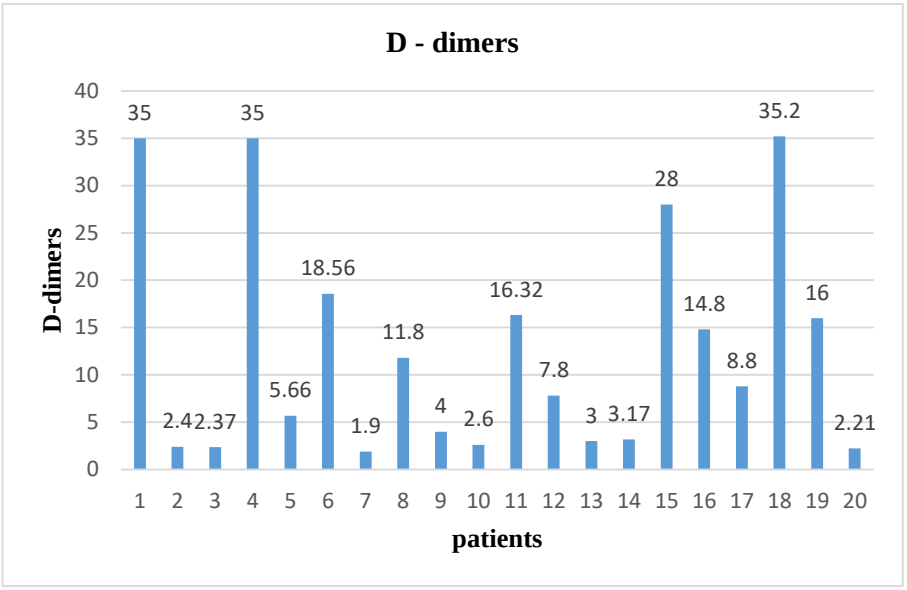

Fig. 19 D-dimer values

- Ferritin - this indicator is a key marker in the diagnosis of macrophage activation syndrome and is very important in terms of monitoring the effect of therapy and the possible stabilization of the patient's condition. For this reason, it is considered in several aspects, which will be presented in the "Results" section.

The study of serum ferritin in the diagnosis of macrophage activation syndrome in the patients included in the present study showed that in all 20 children / ie. at 100% / high values have been established. As it is a diagnostic laboratory indicator, values above 684 ng / ml are considered elevated according to PRINTO criteria. The lowest measured value is 695.5 ng / ml and the highest 17,120 ng / ml; the arithmetic mean is 5099.9 ng / ml, with the initial ferritin values ranging from 2000 ng / ml to 4965.9 ng / ml in most patients.

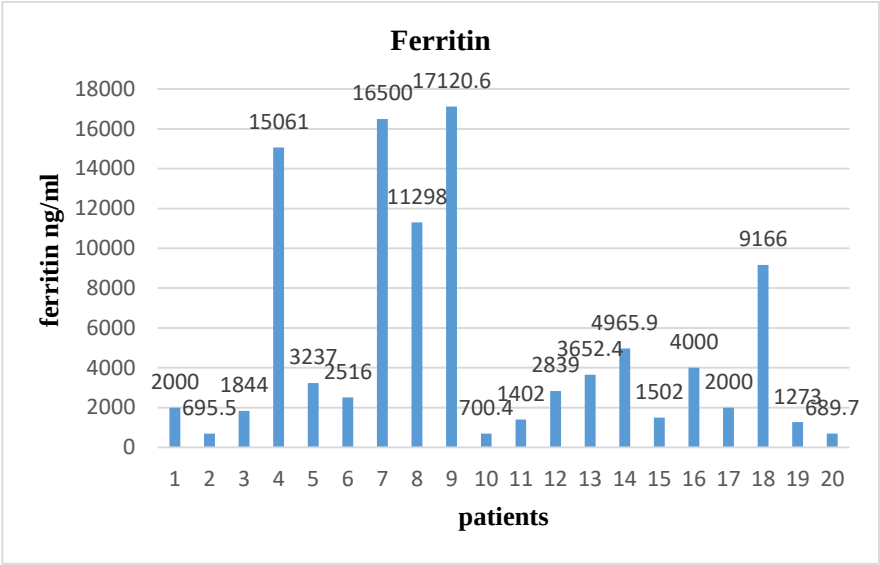

Fig. 20 Ferritin values in ng / ml at diagnosis of MAS

Due to the importance of this indicator, it is traced over time in all children. The minimum follow-up period is 20 days and the maximum is 186 days; the arithmetic mean of the days of follow-up of serum ferritin was 69.17.

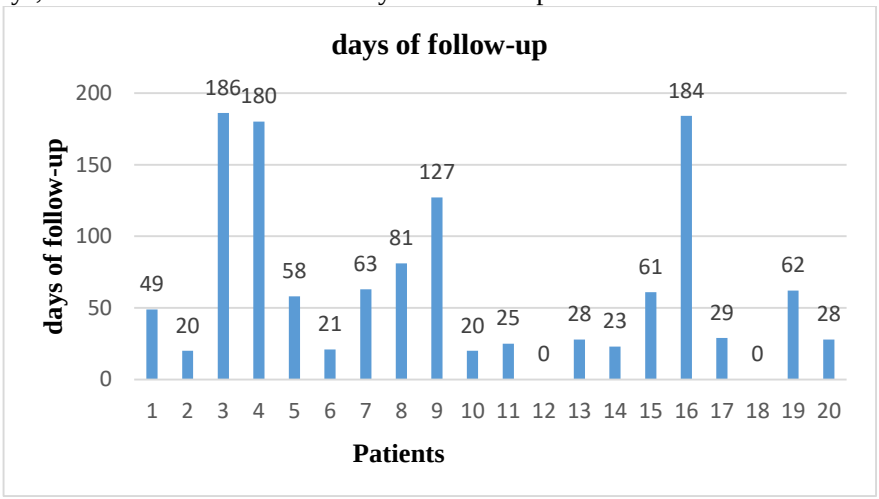

Fig. 21 Number of days of follow-up of serum ferritin

Follow-up of patients has been discontinued with the following options:

• restoration of normal serum ferritin levels and stabilization of the general condition,

• stabilization of the general situation with high values of this indicator, but within significantly lower than initially established;

• The third possibility to stop the follow-up is the occurrence of lethal outcome - in the current study there are two children with exitus lethalis, therefore they are excluded from the processing of the indicator "follow-up days" and in the graph are marked with "zero" .

When processing the data of all patients, it was found that the initial values of serum ferritin, no matter how high they are, have no bearing on the rate of recovery of this indicator. Patients with an initial ferritin value significantly above 4000 - 5000 ng / ml had a shorter follow-up time than patients with an initial value around or below 2000 ng / ml.

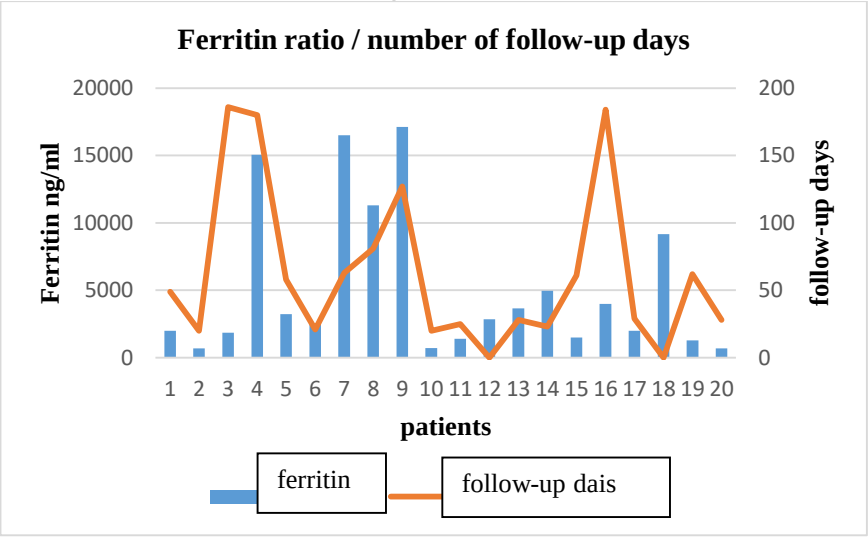

Fig. 22 Ferritin ratio at diagnosis of SMA and days of follow-up

For the entire follow-up period in individual patients, it was reported in how many of them there was a normalization of ferritin levels and in how many it remained elevated, but not in extremely high values at the time of diagnosis. The results show that in 40% of children ferritin falls below 150 ng / ml, which is the generally accepted upper reference limit for this indicator, and in 60% of patients remains above 150 ng / ml by the follow-up deadline for each patient.

Indicator	Number of patients	%
Normalization of ferritin (<=150 ng/ml)	8	40%
No normalization	12	60%
Total	20	100%

Table 2 Serum ferritin values at the follow-up date for each patient

According to some recent publications, not only the individual values of serum ferritin levels but also the ferritin / ESR ratio are becoming increasingly important. According to the researchers, at values above 21.5 the sensitivity is 82% and the specificity is 78%, and at values above 80 it is 100% sensitive and specific for macrophage activation syndrome. It was considered appropriate to calculate the ferritin ratio in ng / ml to ESR in mm / h for the patients included in the present study. The results are as follows: the lowest value is 12.9, but it is only for one child; the next largest value is 25.46; the highest value is 3424.1; the arithmetic mean is 335.9. If we exclude the single very high value of this ratio /3424.1/ this arithmetic mean value is obtained 175.5. A closer look at the data for this ratio shows that 75% of children / n = 15 / have values above 80 when diagnosed with SMA; in 20% of the children / n = 4 / it is in values between 25.46 and 46.3 and only in 5% / n = 1 / the value is 12.9.

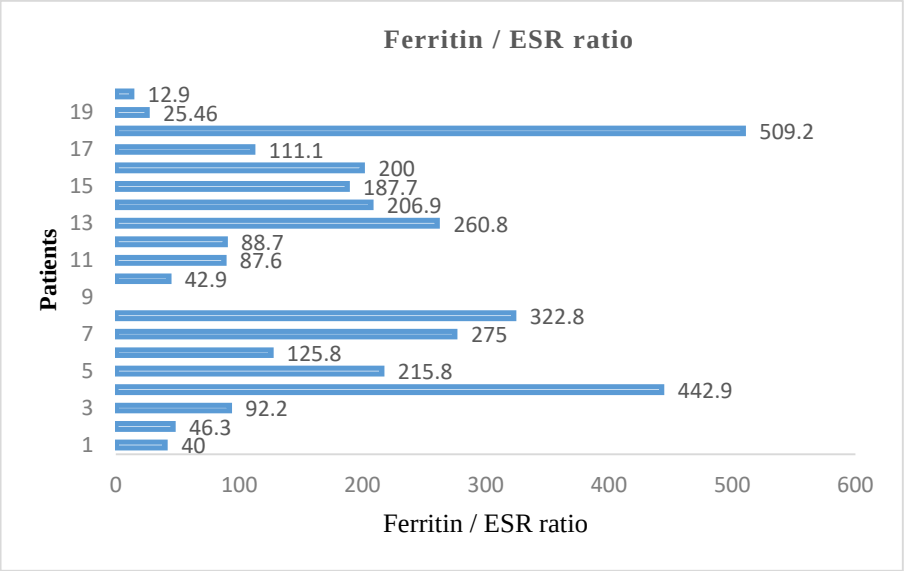

Fig. 23 Ferritin/ESR ratio at diagnosis of MAS/ferritin in ng/ml, ESR in mm / h /

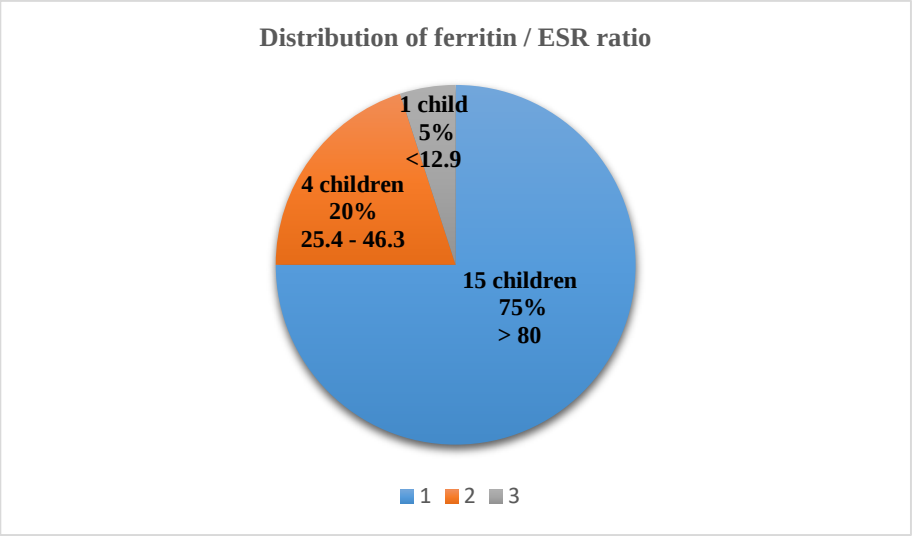

Fig. 24 Distribution of the ferritin / ESR ratio among children with MAS at diagnosis

The present study included a control group of patients who have other major diseases with a pronounced clinical and laboratory inflammatory syndrome. Serum ferritin levels were examined and compared with children with macrophage activation syndrome. The results of these data are analyzed below in the presentation. Here, this control group of patients will be considered in the context of the ferritin / ESR ratio. From the processing of the obtained data it was found that the value of this ratio is in the range from 1.51 as the lowest result to 15.26 as the highest result. The arithmetic mean is 5.61.

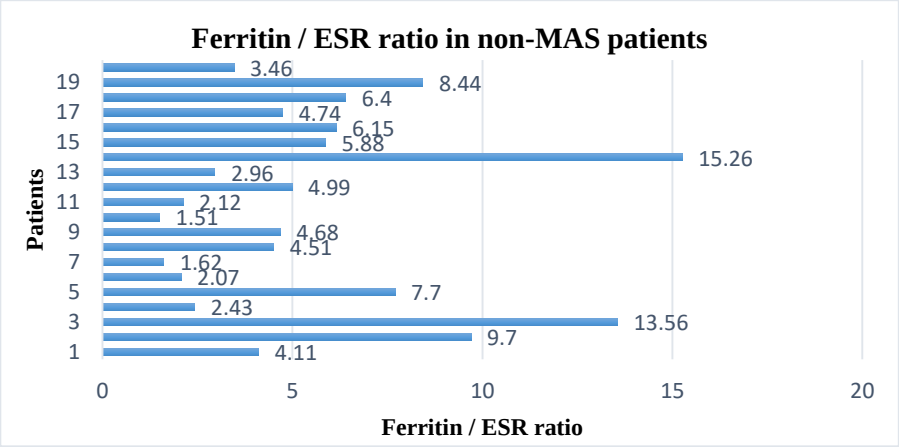

Fig. 25 Ferritin / ESR ratio in a control group of patients with other major diseases

The sensitivity and specificity of the ferritin / ESR ratio in patients with macrophage activation syndrome were determined by statistical analysis methods. The results of data processing of children with SMA and those of the control group of patients with non-SMA diagnoses show that at a ferritin / ESR ratio equal to or higher than 11.3 the test has 100% sensitivity and 100% specificity. The strength of these results is confirmed by conducting ROC analysis.

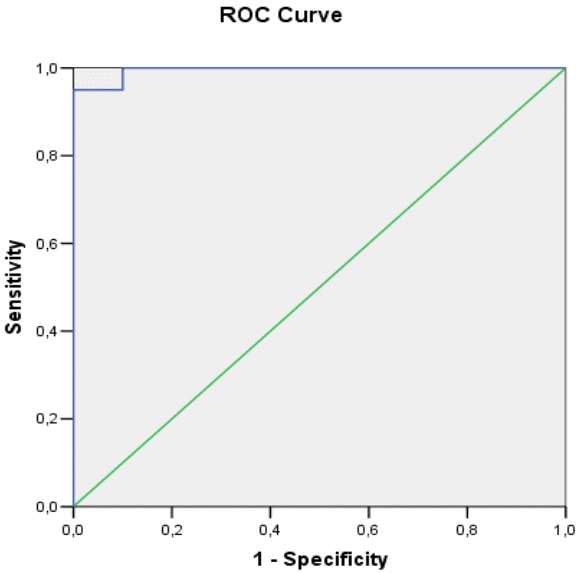

Fig.26 ROC analysis for sensitivity and specificity of the ferritin / ESR ratio in the context of macrophage activation syndrome in children

Because in addition to ferritin as laboratory diagnostic criteria for macrophage activation syndrome, it is customary to take into account the values of platelets, ASAT, fibrinogen and triglycerides. For tach it is also tracked for what period of time / in days / they recover from changes in normal values.

• Platelets - the minimum period for recovery of platelets above $181x10^9$ / l is 2 days, the maximum - 40 days; the arithmetic mean of this indicator is 9.25 days.

• ASAT - the minimum term for recovery of ASAT below 48 U / l is 3 days, and the maximum is 60 days; the arithmetic mean is 11.9 days.

• Fibrinogen - the minimum recovery period for fibrinogen above 3.6 g / l is 2 days, and the maximum is 20 days; the arithmetic mean for this indicator is 10.63 days.

• Triglycerides - the minimum recovery period for triglycerides below 1.76 mmol / l is 2 days and the maximum is 60 days; the arithmetic mean for this indicator is 17 days.

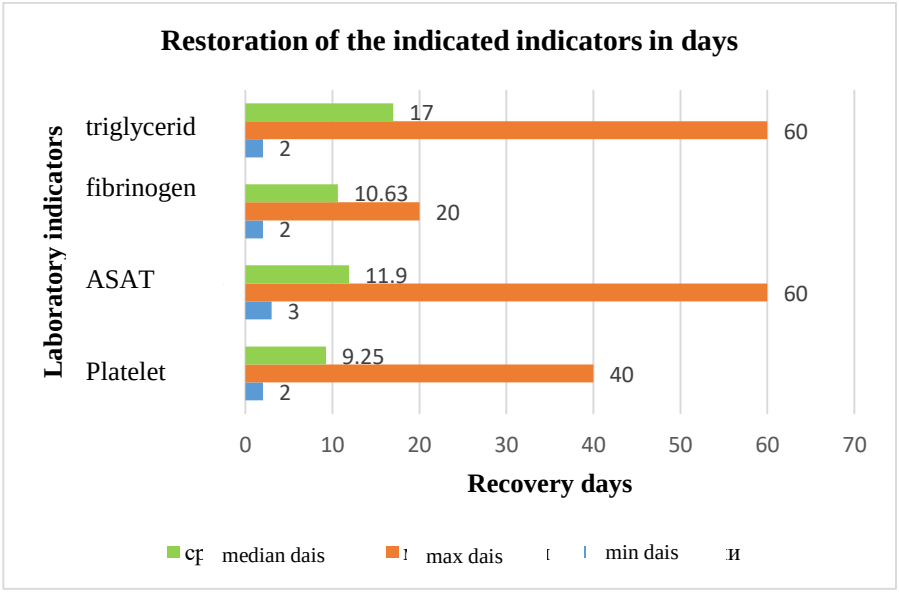

Fig. 27 Days to restore platelet count, ASAT, fibrinogen and triglycerides to values declared pathological in the context of MAS

2.3. Bone marrow aspirate

In 7 of the children / 35% / a bone marrow puncture was performed. The results show that only one child has changes in the bone marrow corresponding to hemophagocytosis. No pathological bone marrow changes were found in all other patients who underwent myelogram. These 7 children who underwent a bone marrow puncture also have the following additional results:

- Initial values of ferritin - the average value of the initial ferritin is 6853.38 ng / ml; the lowest value is 690.7 ng / ml and the highest is over 16,500 ng / ml; in a child with established hemophagocytosis in the bone marrow, the value of the initial ferritin is 15 061 ng / ml.

- Of the children in this group, only one recovers normal ferritin (less than 150 ng / ml) for the follow-up period for MAS;

- The term for monitoring the children in this group is on average 56.14 days; the shortest period is 25 days and the longest is 63 days.

3. Presentation of results from the processing of data for treatment

3.1. High-dose corticosteroid and cyclosporine treatment

The data from the treatment performed in all 20 children included in the present study were processed. Statistical analysis of the available sample sometimes yields low significance results, but this is due to the small number of available pediatric patients diagnosed with macrophage activation syndrome and not the lack of real significance of the results obtained; for this reason, the tendency to change the result obtained is discussed in the event of a larger group of patients.

In all children included in the present dissertation, at the time of diagnosis of MAS, treatment with a high-dose corticosteroid was started - 30 mg / kg / day; in some patients due to non-response to the clinical course of the disease and / or retention of changes in laboratory parameters, especially hyperferritinemia, treatment with cyclosporine is included.

The distribution of patient groups in terms of therapy is as follows:

• In 55% of children (n = 11) the treatment was presented only with methylprednisolone at a dose of 30 mg / kg / day intravenously

• A combination of methylprednisolone at a dose of 30 mg / kg / day intravenously and cyclosporine at a dose of 2-5 mg / kg / day orally was performed in 45% of children (n = 9).

• In 55% of the children (n = 11) more than three (from four to eight) pulses of methylprednisolone were administered at a dose of 30 mg / kg / day; in 9 of these 11 children, in addition to methylprednisolone, cyclosporine was added

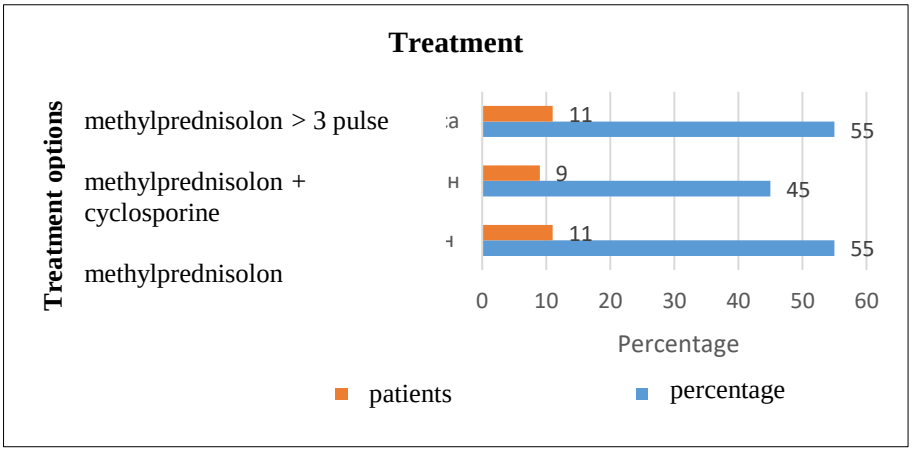

Fig. 28 Treatment options for children with MAS

Children who received only methylprednisolone in a pulse dose / 30 mg / kg / day / distribution was as follows:

- in six of them, three high-dose corticosteroid applications were performed,

- in five of the children 4 corticosteroid applications in a pulse dose were performed;
Regarding the change in ferritin values in this group, 4 of the children recovered from normal ferritin (less than 150 ng / ml); in five of the children the ferritin was between 150 ng / ml and 719 ng / ml and in two children it was fatal.

	Ferritin < 150 ng / ml	**Ferritin 151 ng/ml - 719 ng/ml**	**death**
3 pulse KS	2	4	0
4 pulse KS	2	1	2
Total children	4	5	2

Table 3 Distribution of children in the group who received only methylprednisolone in a pulse dose of 30 mg / kg / day - from 3 to 4 applications and the ratio of changes in serum ferritin

In the group of children / n = 9 / who received, in addition to methylprednisolone in high-dose regimen and cyclosporine, the following was found:
- 3 high-dose corticosteroid applications were performed in three of them,
- for all other 6 children the pulses are of four / n = 1 /, five / n = 4 / and 8 / n = 1 / applications.

Five of the patients in this group did not experience normalization of ferritin, and four of them fell below 150 ng / ml at the end of the follow-up period, but it is important to note that three of these 4 children underwent additional treatment with a third drug / intravenous immunoglobulins, etoposide, kineret /.

	Ferritin < 150 ng/ml	**Ferritin 151 ng/ml - 900 ng/ml**
3 pulse CS + neoral	1	2
4 pulse CS + neoral	1 /+ kineret/	0
5 pulse CS + neoral	1 /+ etoposide /	3
8 pulse KS + neoral	1 /+ IVIG/	0
Total children	4	5

Table 4 Distribution of children in the group receiving methylprednisolone at a pulse dose of 30 mg / kg / day - three to eight applications in combination with cyclosporine and presentation of the relationship to the change in serum ferritin values

In the statistical treatment between the two groups of patients - those who received only methylprednisolone in high-dose regimen and those who received in addition to methylprednisolone in pulse dose and cyclosporine were

monitored on two indicators - normalization of ferritin / below 150 ng / ml / and number of days to monitor ferritin. The results obtained are the following:

- in 4 of the children / 36% / who received only methylprednisolone in a pulse dose and in 4/44% / of the children in the group with methylprednisolone 30 mg / kg / day in combination with cyclosporine normalization of serum ferritin below 150 ng was achieved / ml;

- in 7 of the children /63.63%/ in the group only with methylprednisolone 30 mg / kg / day and in 5 of the children / 55% / in the group methylprednisolone 30 mg / kg / day in combination with cyclosporine no normalization of ferritin was achieved, t .e. it is above 150 ng / ml.

Based on statistical comparisons between the two groups of patients, the different therapeutic approaches did not have a significant effect on the achievement of normal serum ferritin (below 150 ng / ml), but the days to achieve normal ferritin were influenced by whether cyclosporine was administered.

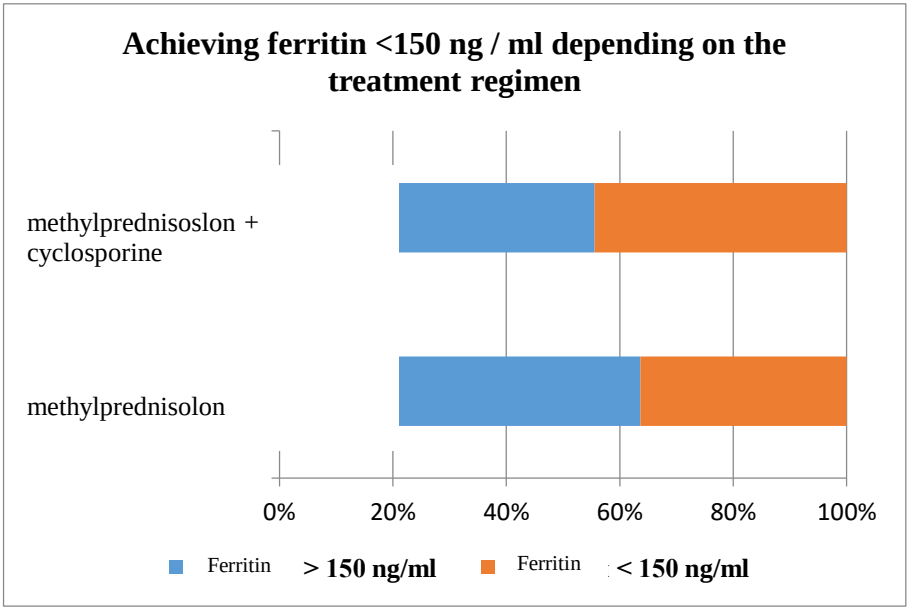

Fig. 29 Achieving ferritin below 150 ng / ml in children with MAS depending on the type of treatment regimen

As serum ferritin is one of the most important diagnostic criteria for macrophage activation syndrome, it is estimated that patients in the present study will also be considered at 684 ng / ml, which is considered to be above the limit. hyperferritinemia in the context of MAS. Children were again divided into two main groups - those who received only methylprednisolone in a pulse dose and those who received a combination of methylprednisolone in a

pulse dose and cyclosporine. The following is established during the data processing:

• in 15 of all children (75%) a decrease in serum ferritin below 684 ng / ml was achieved; of these, 7 received only methylprednisolone 30 mg / kg / day and 8 received a combination of methylprednisolone 30 mg / kg / day and cyclosporine;

• in 5 of the children (25%) the level of serum ferritin remained above 684 ng / ml, as 4 of them received only methylprednisolone at a dose of 30 mg / kg / day and one of them falls into the group of methylprednisolone 30 mg / kg / day and cyclosporine.

Data processing within the groups found that 89% of children in the methylprednisolone and cyclosporine groups had a decrease in serum ferritin below 684 ng / ml, while in the methylprednisolone group only 30 mg / kg / day this stabilization occurred occurs in 63.6% of children. From a formal point of view, no statistically significant effect is observed, but this is due to the small group of patients who are available for data processing; it is important to note, however, that there is a tendency for the positive effect of the addition of cyclosporine to methylprednisolone treatment at a dose of 30 mg / kg / day; in the event of a larger group of patients, this effect would become a statistically significant value /p<0.05/

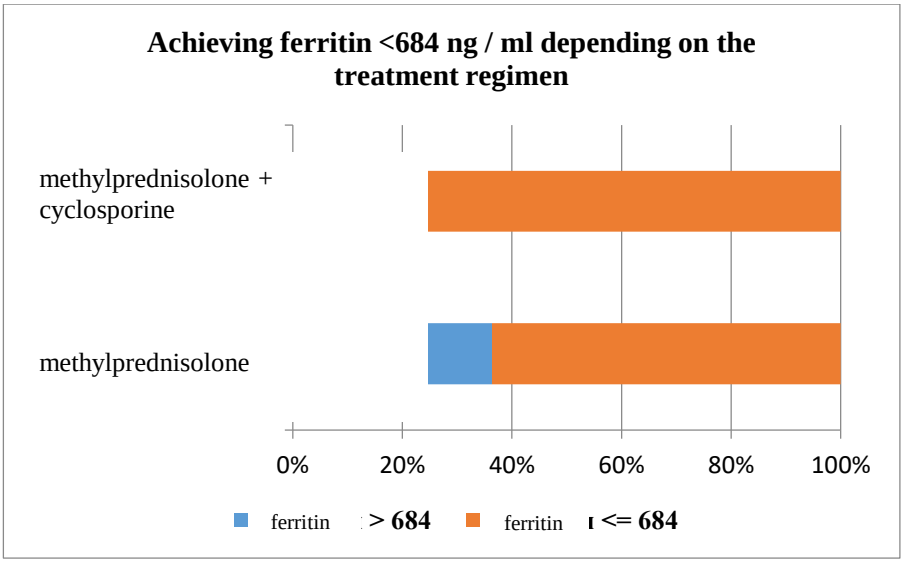

Fig. 30 Achieving ferritin below 684 ng / ml in patients with MAS depending on the type of treatment regimen / p 0,194 /

Patients with MAS were divided into two groups according to the number of courses of high-dose methylprednisolone - those who received only

three pulse doses and those who received more than 3 applications of methylprednisolone at a dose of 30 mg / kg / day. A comparative analysis was performed for the effect of this therapeutic model on the fall of serum ferritin below 684 ng / ml. It was found that in the first group of children who received only three pulses of methylprednisolone at a dose of 30 mg / kg / day at 89% / n = 8 / ferritin was achieved below 684 ng / ml and at 11% / n = 1 / remains above 684 ng / ml. In the second group of children who received more than three starting doses of methylprednisolone, the distribution was as follows: 64% (n = 7) achieved ferritin below 684 ng / ml and 36% (n = 4) remained above 684 ng / ml . This comparative analysis showed that the administration of more than three pulses of methylprednisolone alone did not affect the normalization of serum ferritin.

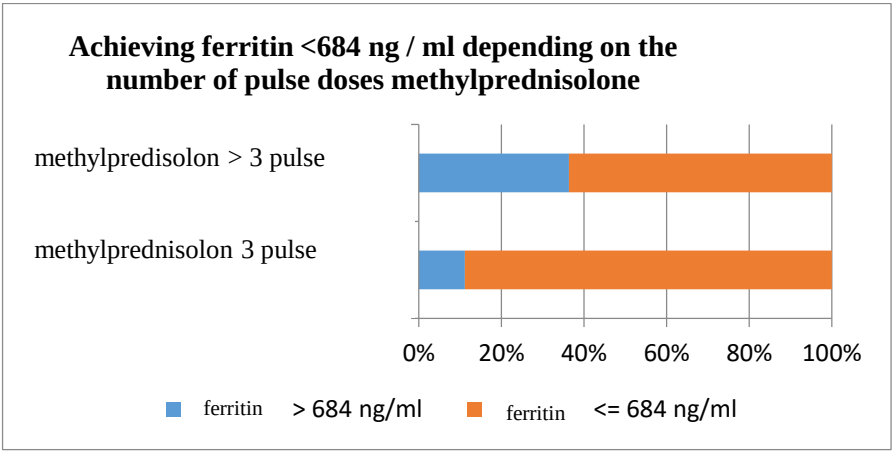

Fig. 31 Achieving serum ferritin below 684 ng / ml depending on the number of pulse doses methylprednisolone / p 0,194 /

In this regard, an attempt was made to evaluate the effect of adding cyclosporine to high-dose corticosteroid therapy in another way. It was found that the patients who received three applications of methylprednisolone 30 mg / l, mkg / day are a total of 9 in number / 45% of all patients /. In 8 of them (40% of all patients) there was a decrease in serum ferritin below 684 ng / ml. The remaining 60% of patients continued to receive more high-dose corticosteroid pulses and / or cyclosporine was added to the treatment.

The change in serum ferritin levels was assessed in patients who continued corticosteroid therapy after the third administration of methylprednisolone 30 mg / kg / day and those who received cyclosporine. Data processing revealed that a negative mean was obtained after the addition of cyclosporine, which means that the addition of cyclosporine resulted in a statistically significant decrease in serum ferritin, while in the group remaining

with methylprednisolone only after the third pulse this mean has a positive sign, which means that the decrease in ferritin is insignificant.

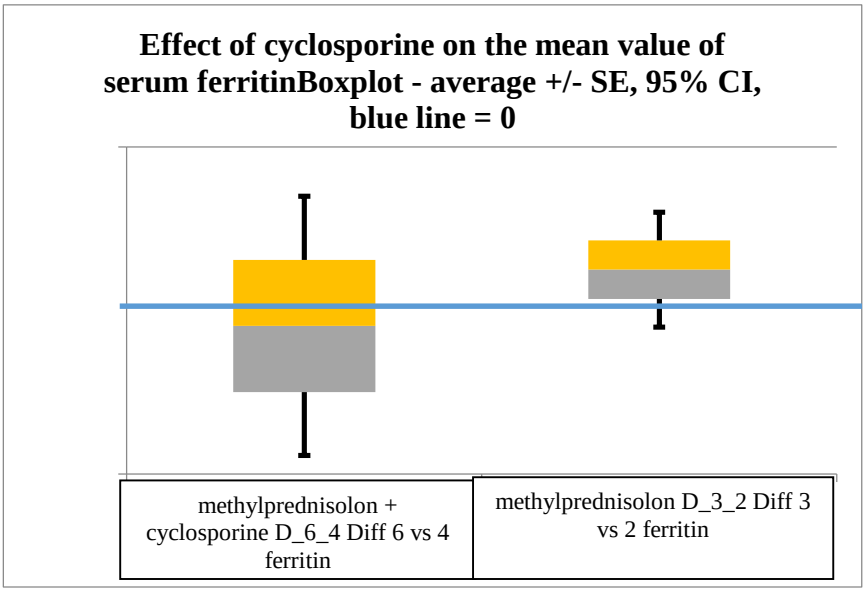

Fig. 32 Effect of the addition of cyclosporine to the methylprednisolone 30 mg / kg / day regimen in relation to the decrease in serum ferritin levels

Patients in the methylprednisolone group of 30 mg / kg / day in combination with cyclosporine also showed a statistically significantly higher number of follow-up days ($p = 0.04$) than those who received methylprednisolone alone at a dose of 30 mg. kg / day. The reason for this can be explained by the fact that cyclosporine is added in more severe cases where there is no effect from the initial application of high-dose corticosteroid and therefore requires a longer period to restore general condition and altered laboratory parameters.

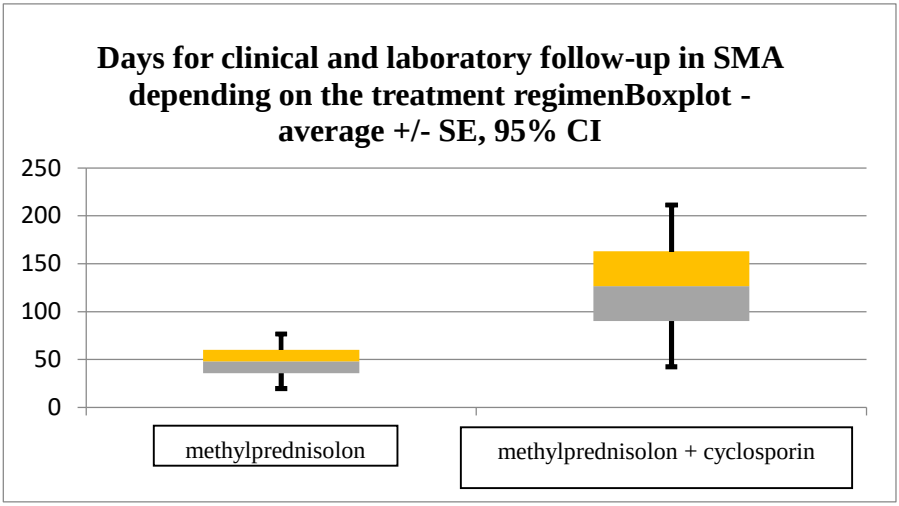

Fig. 33 Days of clinical and laboratory follow-up of children with MAS depending on the type of treatment regimen

The aim of the present study was to make a comparative analysis of the data of patients who develop MAS provoked by an infectious agent and those who develop MAS within the systemic form of juvenile idiopathic arthritis. The results are as follows:

- There are 4 patients / trigger infectious agent / 20% /. Their ferritin values range from the lower limit for SMA 684 ng / ml to 15,061 ng / ml, an average of 4,747.75 ng / ml; in patients with MAS within the systemic form of JIA, this mean value for ferritin is 5211.53 ng / ml.
- in three of the patients with infectious provoked MAS normal ferritin is restored / below 150 ng / ml /, in one child it is 497 ng / ml - remains above 150 ng / ml, but below the laboratory norm for MAS / 684 ng / ml /. In patients with MAS within the systemic form of JIA 6 ferritin is normalized below 150 ng / ml, in 6 of the children it is between 150 ng / ml and 684 ng / ml and only in two children it remains above 684 ng / ml.
- the period for recovery of normal ferritin values is 20 - 25 days / in two of the children / up to 180 - 186 days in the other two patients with infectious provoked MAS; on average this period is 102.75 days, while in the group of children with SMA on the background of the systemic form of JIA this average time interval is 60.14 days.
- Regarding the treatment, the data show that two of the children in the group of infectious provoked SMA needed three pulse doses of corticosteroid, after which normalization of the general condition and the changed laboratory parameters was registered, and in the other two children respectively three in one and four pulses with corticosteroid in the other, cyclosporine was added, and in addition to this combination therapy was added

IVIG in one and biological therapy with anti-IL-1 receptor antagonist in the other child. In the group with MAS within JIA in 5 of the children had to use a third drug / IVIG, kineret, etoposide, roactemra /.

3.2. *Treatment of macrophage activation syndrome with biological agents – anakinra*

Diagnosis of macrophage activation syndrome requires rapid and timely initiation of treatment. It is generally accepted worldwide that initiation therapy should be with a high-dose intravenous course of corticosteroids alone or in combination with cyclosporine. However, the use of so-called biological agents, which are usually molecules targeting key cytokines that are key from a pathogenetic point of view, is becoming increasingly important. In the context of macrophage activation syndrome, such a drug is an anti-IL 1-receptor antagonist (anti-Interleukin-1-receptor antagonist) - anakinra (kineret).

Two of the children in the current study received anti-IL 1 receptor antagonist therapy. From the point of view of the statistical processing of the data from this treatment, it is clear that due to the small number of patients to whom it has been administered, no conclusion can be drawn with statistical significance; however, it was considered important to describe the data for these two children, as this type of therapy is applied for the first time in Bulgaria to pediatric patients. For greater clarity and differentiation of the results of the two children, for the sake of brevity in the statement below will appear as "Patient №1" and "Patient №2".

- *Gender, age, triggers and clinical symptoms*

Patient №1 and patient №2 are female;

Patient №1 is 13 years old and patient №2 is 15 years old

Mycoplasma pneumoniae infection has been identified as a provoking factor for macrophage activation syndrome in patient №1. In patient №2, SMA develops within the systemic form of juvenile idiopathic arthritis.

In both children the clinical symptoms are represented by fever, rash syndrome, generalized lymphadenomegaly, hepatomegaly and splenomegaly.

- Data from laboratory tests

- Some indicators of complete blood count and indicators of inflammatory activity

In patient №1 hemoglobin 142 g / l, leukocytes 3.24x109 / l, neutrophils - 84.3%, platelets 89x109 / l;

In patient №2 hemoglobin 108 g / l, leukocytes 3.7x109 / l, neutrophils - 78%, platelets 176x109 / l;

In both children the ESR is 20 mm / h; C-reactive protein - 30 mg / l in patient №1 and 18 mg / l in patient №2;

- Biochemical indicators

In patient №1 ACAT 228 U / l, ALAT 125 U / l, LDH 582 U / l, triglycerides - 1.1 mmol / l, total protein - 63.7 g / l, albumin - 37.6 g / l, fibrinogen - 3.2 g / l, D-dimers 2.37 µg / ml.
In patient №2 ACAT 400 U / l, ALAT 380 U / l, LDH 1750 U / l, triglycerides - 2 mmol / l, total protein - 46 g / l, albumin - 25 g / l, fibrinogen - 2, 7 g / l, D-dimers 14.8 µg / ml.

- Ferritin

In patient №1, the initial value of serum ferritin was 1844 ng / ml and it was the highest of all subsequent ferritin samples tested for the follow-up period;
In patient №2, the initial value of serum ferritin was 4000 ng / ml; subsequently, for the follow-up period, ferritin levels reach 10 500 ng / ml;

- *Treatment*

Both children were diagnosed with macrophage activation syndrome based on these clinical and laboratory data. Treatment was started with:

- high-dose intravenous corticosteroid - 30 mg / kg / day - a total of 4 pulses were performed in patient №1 and 5 pulses in patient №2;

- Cyclosporine - cyclosporine was added to both children - 2 mg / kg / day orally

- In patient №2, IVIG was administered at a dose of 2 g / kg

- *Tracking of changed laboratory parameters*

The change of the above laboratory parameters, which are included as diagnostic criteria for macrophage activation syndrome according to PRINTO / EULAR from 2016, was monitored.

- Platelet count

In patient №1 the lowest value for this indicator is $55x10^9$ / l; recovery of platelet count over $181x10^9$ / l occurs on the 18th day;
In patient №2 the lowest value for this indicator is $104x10^9$ / l; recovery of platelet count over $181x10^9$ / l occurs on the 10th day;

- ASAT

In patient №1 the highest value for this indicator is 228 U / l; recovery of ASAT below 48 U / l occurs on the 6th day;
In patient №2 the highest value for this indicator is 420 U / l; recovery of ASAT below 48 U / l occurs on the 15th day;

- Fibrinogen

In patient №1 the lowest value of this indicator is 2.4 g / l; recovery of this indicator above 3.6 g / l occurs on the 20th day;
In patient №2 the lowest value of this indicator is 3.1 g / l; recovery of this indicator above 3.6 g / l occurs on the 14th day;

- Triglycerides

In patient №1 the highest value of this indicator is 2.8 mmol / l; recovery of this indicator below 1.76 mmol / l occurs on the 18th day;
In patient №2 the highest value of this indicator is 3.58 mmol / l; recovery of this indicator below 1.76 mmol / l occurs on the 60th day;

- Ferritin

Patient №1 and patient №2 stabilized the clinical condition and normalized most of the altered laboratory parameters as described above; however, in both children, 2 ½ months after the diagnosis of SMA, high levels of serum ferritin persisted against the background of continued corticosteroid therapy at a dose of 1.5 mg / kg / day in combination with cyclosporine 2 mg / kg / day; this condition is defined as a subclinical syndrome of macrophage activation;

It is estimated that both girls will be treated with a biological agent - anti-IL 1-receptor antagonist / kineret / at a dose of 100 mg / day s.c. Follow-up revealed that serum ferritin levels (below 150 ng / ml) returned to normal 3 months after initiation of biologic therapy, allowing ciclosporin treatment to be discontinued and reduced and ultimately discontinued. with a corticosteroid.

indicator	**Patient №1**	**Patient №2**
Gender	girl	girl
Age	13	15
Trigger	Mycoplasma pneumoniae	Juvenile idiopathic arthritis - systemic form
Clinical symptoms:		
- Fever	+	+
- Rash	+	+
- Lymphadenomegaly	+	+
- Hepatomegaly	+	+
- Splenomegaly	+	+
Laboratory indicators		
- CBC		
Hemoglobin g / l	142	108
Leukocytes x109 / l	3,24	3,7
neutrophils%	84,3	78
platelets x109 / l	89	176
- ESR mm / h	20	20
- C-reactive protein g / l	30.34	18
Biochemical indicators		
- ASAT U / l	228	400
- ALAT U / l	125	380
- LDH U / l	582	1750
- Triglycerides mmol / l	1.1	2
- Total protein g / l	63,7	46
- Albumin g / l	37,6	25
- Fibrinogen g / l	3,2	2,7
- D-dimers µg / ml	2,37	14,8

Ferritin ng/ml	1844	4000
Treatment - Corticosteroid 30 mg / kg / day - Cyclosporine 2 mg / kg / day - IVIG - Biological agent / Kineret 100 mg / day /	 4 pulse + - +	 5 pulse + + +
Follow up Recovery in days of: Platelets over $181x10^9$ / l ASAT below 48 U / l Fibrinogen above 3.6 g / l Triglycerides mmol / l Ferritin ng / ml	 18 6 20 18 186	 10 15 14 60 184

Table 5 Comparative presentation of clinical, laboratory parameters, treatment and recovery data on days of altered laboratory criteria for MAS in both children treated with anti-IL-1 receptor antagonist therapy

4. Comparative evaluation of serum ferritin levels in SMA and other diseases with high inflammatory activity

In the present scientific study, an attempt has been made to assess the significance of serum ferritin in the diagnosis of macrophage activation syndrome. In this regard, a group of patients with other diseases is formed, usually with a pronounced laboratory syndrome of inflammation, in which the level of serum ferritin is examined. The obtained values were compared with the values of ferritin in the group of children diagnosed with macrophage activation syndrome.

The control group included 21 children with the following diseases: pneumonia - 4 children, pleurisy and pneumonia - 4 children, acute pyelonephritis - 3 children, acute tonsillitis - 2 children, acute adenoiditis and acute purulent otitis media - 1 child, Kawasaki disease - 3 children , systemic lupus erythematosus - 2 children, septic arthritis - 2 children. In all children in this group, the underlying disease was diagnosed on the basis of clinical symptoms, laboratory and paraclinical criteria accepted as relevant at present. In all children, without exception, high inflammatory activity was found by laboratory indicators. Serum ferritin levels in all these children were studied. The obtained results show that the lowest measured value of ferritin is 154.6 ng / ml, and the highest - 514.1 ng / ml. The distribution by nosological units is as follows:

Diagnosis	Ferritin ng/ml
pneumonia	493.8
pneumonia	340.8
pneumonia	470.9
pneumonia	384.4
Pleurisy + pneumonia	291
Pleurisy + pneumonia	281.1
Pleurisy + pneumonia	263.9
Pleurisy + pneumonia	308.8
Acute pyelonephritis	154.6
Acute pyelonephritis	212
Acute pyelonephritis	261
Acute tonsillitis	243.1
Acute tonsillitis	399.8
Acute otitis media and adenoiditis	189.9
Septic arthritis	549
Septic arthritis	489.8
Kawasaki disease	246.5
Kawasaki disease	243.1
Kawasaki disease	234.1
Systemic lupus erythematosus	514.1
Systemic lupus erythematosus	305.4
Septic arthritis	549
Septic arthritis	489.8

Table 6 Values of serum ferritin in ng / ml at different nosological units in the control group

The ferritin values in this control group of patients were compared with the initial ferritin values of the children diagnosed with macrophage activation syndrome. The results of this comparative analysis show that there is a significant difference in the mean value between the two groups /$p<0.05$/. This value is 15.57, i.e. with so many times the value of ferritin in the group of patients with MAS is higher than its value in the control group of patients. The difference is so large and significant that the values of the control group of patients are not well visualized on the indicated graph.

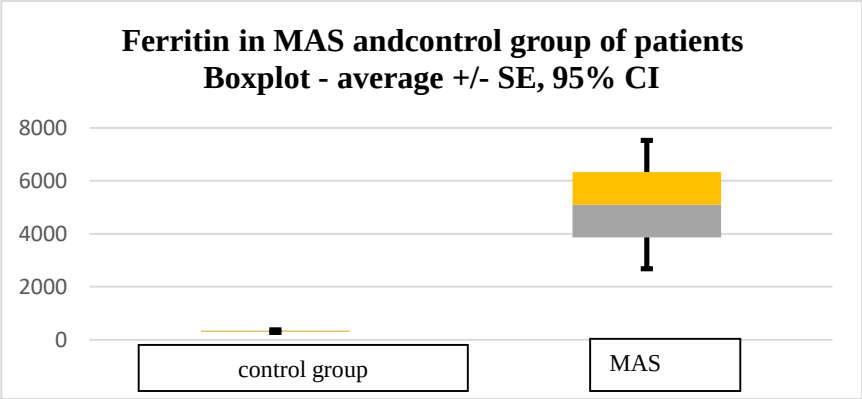

Fig. 34 Comparative analysis between ferritin values in patients with MAS and control group / children with other non-MAS diseases /. The difference is large and significant / p <0.001 /

5. Assessment of the change in the values of laboratory parameters that are included in the diagnostic criteria for macrophage activation syndrome

According to the currently valid PRINTO / EULAR diagnostic criteria from 2016, the laboratory parameters that are taken into account when diagnosing SMA are: ferritin, platelet count, ACAT, triglycerides and fibrinogen. The following analysis was performed in the patients included in the present study - it was estimated what percentage of children start the disease with normal values of the respective indicator and how this percentage changes at the end of the disease. The following results were obtained:

- *Platelet count*

65% of children / n = 13 / at diagnosis have a platelet count above 181 x 10^9/ l;

90% of children (n = 18) at the end of follow-up had a platelet count above 181 x 10^9/ l. / p = 0.05 /

- *ASAT*

35% of children (n = 7) at diagnosis have ASAT within the SMA reference value

85% of the children / n = 17 / at the end of the follow-up have normal values of ASAT / p = 0.04 /

- *Triglycerides*

45% of patients (n = 9) have triglycerides within the normal value at diagnosis

90% of patients (n = 18) had normal triglyceride levels at the end of follow-up; / p = 0.002 /

- *Fibrinogen*

55% of children (n = 11) start the disease with normal fibrinogen levels

90% of the children (n = 18) had normal fibrinogen at the end of the MAS follow-up. / p = 0.01 /

- *Ferritin*

100% of children have elevated serum ferritin levels - well above 684 ng / ml

75% (n = 15) with ferritin below 684 ng / ml at the end of the MAS follow-up

Taking into account the indicated laboratory parameters, it was estimated what proportion of the patients started the disease with simultaneously changed laboratory parameters and what proportion remained with such changes at the end of the follow-up. The results are as follows:

- 65% of children / n = 13 / at the diagnosis of macrophage activation syndrome have changed simultaneously ferritin, platelet count, ACAT, triglycerides and fibrinogen; in the remaining 35% of children these indicators change in the course of the disease; / p = 0.06 /

This analysis shows that slightly more than half of children with macrophage activation syndrome meet all the criteria for diagnosis at the same time. Monitoring during the course of the disease shows that these laboratory parameters change and correspond to those specified by PRINTO / EULAR, but in a time period that is different for each child.

V. FINAL ANNOUNSMENT

With the present dissertation an attempt is made to analyze and summarize the available clinical and laboratory data related to the syndrome of macrophage activation in childhood. This condition, which occurs most often as a complication of other underlying diseases, deserves to be investigated, as it begins suddenly, is severe, difficult to respond to, and highly life-threatening. The analysis of the presented data shows that the most common trigger is the systemic form of juvenile arthritis, but it turns out that MAS can develop in the course of some infections / mycoplasma, Epstein-Barr virus and Parvovirus B19 / or other autoimmune diseases. Clinical symptoms are usually due to fever in combination with rash, lymphadenomegaly and hepatosplenomegaly, present in varying degrees. Of the changes in laboratory parameters, hyperferritinemia is of the greatest diagnostic importance. In addition to the laboratory parameters included as diagnostic criteria, special attention should be paid to changes in the values of LDH, total protein and serum albumin, as well as D-dimers. Diagnosis of MAS requires timely initiation of treatment. Usual initial therapy includes a high-dose corticosteroid in combination or not with cyclosporine. In the absence of sufficient therapeutic effect, additional treatment regimens with second-line drugs should be considered; In severe cases, which are difficult and incomplete to respond to "standard treatment" with corticosteroids and cyclosporine, it is appropriate to use treatment with biological agents (anti-IL-1 antagonists). World and in particular

our, albeit small, experience with the application of biological therapy shows that the successful treatment of this serious disease is likely to develop in this direction.

VI. CONCLUSIONS

Based on the results obtained from the processing of patient data included in this dissertation, the following conclusions can be made:

1. Macrophage activation syndrome in childhood affects all age groups. There is no sexual predilection in the disease, there is a slight predominance of males.

2. The disease most often develops against the background of a systemic form of juvenile idiopathic arthritis. A number of infectious agents / Mycoplasma pneumoniae, Epshtein-Barr virus, Parvovirus B19 / are also part of the triggers of MAS.

3. Patients with infectious provoked MAS do not differ significantly from those who develop MAS in the course of systemic JIA in terms of initial values of serum ferritin, the likelihood of its normalization and the type of treatment regimen. It is only established that children with infectious provoked MAS are followed for twice as long until they stabilize their condition, which implies slower recovery and the need for longer treatment.

4. The most common clinical manifestations of MAS are fever, rash, hepatomegaly and lymphadenomegaly. These, as well as other symptoms (splenomegaly, CNS manifestations, haemorrhagic diathesis, pericardial and pleural involvement) can develop at any stage of the disease.

5. Patients with pediatric macrophage activation syndrome have moderate laboratory inflammatory syndrome and mild to moderate anemic syndrome.

6. In almost all patients there are changes in the laboratory parameters perceived as diagnostic criteria: elevated levels of ferritin, ASAT, triglycerides and low levels of platelets and fibrinogen. To these indicators it is appropriate to add the increased values of LDH, D-dimers and the low values of total protein and serum albumin, as they are found in 100% of sick children.

7. Serum ferritin is a laboratory marker of great importance. It has a special weight both in diagnosing SMA and in monitoring the course of the disease and the effect of treatment. Values above 600 ng / ml are in favor of macrophage activation syndrome.

8. The ferritin / ESR ratio is an extremely useful, effective and rapid method for differentiating MAS from other diseases with similar clinical symptoms and laboratory changes. According to the data obtained at a value equal to or greater than 11.3, the test has 100% sensitivity and 100% specificity for MAS.

9. The follow-up of the changed laboratory parameters, accepted as diagnostic criteria, shows that the latest normalization / stabilization of serum ferritin / approximately 2 months after the onset of MAS /; for other indicators / platelet count, AST, triglycerides, fibrinogen / this interval is significantly shorter and varies on average from 9 to 17 days.

10. Therapy with high-dose corticosteroid / methylprednisolone 30 mg / kg / day / is mandatory in patients with MAS. In most cases, the addition of cyclosporine leads to stabilization of the condition and to a decrease in serum ferritin values below the accepted diagnostic limit of 684 ng / ml.

11. The addition of a biological agent - anti-IL-1-receptor antagonist leads to the achievement of complete clinical and laboratory control of the disease and allows the gradual cessation of treatment.

VII. **CONTRIBUTIONS**

Original Contributions

1. For the first time in Bulgaria the data of patients diagnosed with macrophage activation syndrome in childhood are described and summarized

2. Data on the age and sex distribution of the disease shall be provided and the provoking factors for SMA shall be identified.

3. The data on the clinical manifestations of MAS in children are analyzed

4. The data for the changes in the laboratory indicators in the children diagnosed with SMA are analyzed and provided and their diagnostic significance is determined.

5. The changes in ferritin values in MAS and non-MAS patients are compared and analyzed.

6. The therapeutic regimens used for the treatment of macrophage activation syndrome in childhood are analyzed

Applied Contributions

1. It is proposed to use the ferritin / ESR ratio as a fast and effective method for differentiating MAS from non-MAS patients
2. Describe and analyze the effect of the application of biological therapy with anti-IL-1-receptor antagonists

Confirmatory Contributions

1. Changes have been demonstrated in all laboratory parameters currently accepted by PRINTO / EULAR as diagnostic criteria for MAS
2. Changes were also shown in the values of LDH, D-dimers, total protein and albumin, which also have a high diagnostic value in the context of MAS
3. The therapeutic regimen comprising a high-dose corticosteroid / methylprednisolone 30 mg / kg / day or another corticosteroid drug in an equivalent dose / in combination or not with cyclosporine has no significant alternative at this stage. In the future, great hopes are placed on the use of biological agents in the treatment of MAS

VIII. PUBLICATIONS AND PARTICIPATIONS IN SCIENTIFIC FORUMS WITH COMMUNICATIONS RELATED TO THE TOPIC OF THE DISSERTATION

1. Macrophage activation in childhood - main characteristics, diagnostic and therapeutic approach. K. Lisichki, V. Kenderova. Pediatrics, 2019, 4, 7-10.
2. Allergy to corticosteroids in macrophage activation syndrome - two clinical cases and literature review. K. Lisichki, V. Kenderova. Pediatrics, 2020, 2, 40-44.
3. Macrophage activation syndrome in childhood. K. Lisichki, V. Kenderova. Practical Pediatrics, 2020, 5, 6-9.
4. Macrophage activation syndrome provoked by Mycoplasma pneumoniae infection in childhood. Clinical case. K. Lisichki, V. Kenderova. Practical Pediatrics, 2020, 5, 10-12.
5. Macrophage Activation Syndrome: A Report of a Case Triggered by Mycoplasma Pneumoniae Infection in Childhood. Lisichki K, Kenderova V, Ganeva M, Stefanov St. Journal of Biogeneric Science and Research https://dx.doi.org/10.46718/JBGSR.2020.05.000136

Participation in congresses and conferences

1. Macrophage activation syndrome in pediatric rheumatology. K. Foxes. XIII Congress of Pediatrics, Nessebar, 28-31.05.2015
2. Macrophage activation syndrome in the systemic form of adolescent idiopathic arthritis. K. Foxes. Conference on Emergency Pediatrics, Fifth National Conference on Emergency Pediatrics, Hissarya, 23-25.10.2015.
3. Macrophage activation syndrome - many causes, one person. K. Foxes. 5th Veliko Tarnovo National Conference for Pediatricians, Neonatologists and General Practitioners "From Symptom to Diagnosis - Clinical Cases", Veliko Tarnovo, March 15-17, 2019.

4. Macrophage activation syndrome - current issues. K. Foxes. V National Pediatric Conference "Prevention, Diagnosis and Therapy in Children and Adolescents. The impossible yesterday, the possible today. Nessebar, 24-27.1.09.2020