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E. N. Tepikina

# DYNAMICS OF CYTOKINE PROFILE PATIENTS WITH JUVENILE IDIOPATHIC ARTHRITIS.

**Abstract**

The results of immunological study of 58 patients with juvenile idiopathic arthritis. It was found in all forms of the disease observed substantial changes of cytokine status in serum and synovial fluid, the degree of severity depends on the variant and the activity of the inflammatory process. Study of cellular immunity was held to a differentiated approach to the choice of therapy, as well as the efficiency of the drugs varied depending on the variant of the disease. The greatest efficiency in the treatment of polyarticular and oligoarticular spreading form was observed in remicade, combination therapy with sandimmun-neoral and methotrexate is preferable to use at oligoarticular persistent form of juvenile idiopathic arthritis.

Key words: juvenile idiopathic arthritis, cytokines, interleukin-17, remicade, methotrexate.

## Introduction

Juvenile idiopathic arthritis (JIA) - one of the most common rheumatic diseases characterized by inflammation of the synovial membrane of joints, destruction of cartilage and bone tissues, the development of extraarticular manifestations of the spectrum, mostly making their debut in children younger than 16 years [3]. In the development of juvenile idiopathic arthritis a significant place given to the activation of T-cell immunity with the subsequent synthesis of inflammatory cytokines [2].

It is shown that Th-17 and produce cytokines involved in the pathogenesis of inflammatory, allergic and autoimmune diseases, as well as protect against extracellular microbes and parasites [4].

Studies conducted in cell cultures and animal experiments confirm the clinical involvement of interleukin-17 in the development of rheumatoid arthritis [11].

Study the mechanisms of development and differentiation of this cytokine in rheumatoid arthritis, revealed the involvement of interleukin-6 in the initiation of production of IL-17 as well as the effect of IL-17 on production of other cytokines [10].

Studying the role of interleukin-17 in juvenile idiopathic arthritis will deepen understanding of the pathogenesis, develop criteria for early diagnosis, effective treatment for the disease.

Currently, the treatment of juvenile idiopathic arthritis using a wide range of antirheumatic (sulfasalazine, gold preparations, methotrexate, sandimmun-neoral, etc.) with general immunosuppressive effect [1].

In addition, in recent years introduced drugs with selective action on the immune system. Infliximab (Remicade) has become one of the first widely used in rheumatological practice [5, 6]. He is a monoclonal antibody to tumor necrosis factor α (TNF-α). Preliminary analysis of the efficiency of anticytokine therapy revealed that the majority of the surveyed patients had significant clinical improvement, reflected in a reduction of pain and swelling of affected joints, duration of morning stiffness, increased range of motion in joints, improve overall health [7, 8, 9].

However, the efficiency of medications used depends on correctly selected treatment regimens. Comprehensive study of immune cells is important for the selection of adequate therapy, since the beginning of the timeliness and adequacy of treatment will determine the length and quality of life.

**The purpose of the study** - to examine indicators of cytokines in children with juvenile idiopathic arthritis, to develop a differentiated approach to prescribing drugs for the treatment of disease.

**Materials and methods**

In complex clinical - immunological study included 58 children aged 2 to 17 years, patients with different variants of juvenile idiopathic arthritis. The control group consisted of 30 apparently healthy children of the same age category. For inclusion in the study with informed consent from children over the age of 14 or parents of children under the age of 14 years.

Clinical studies were conducted on the basis of child kardiorheumatology and cardiac surgery departments of the Samara regional clinical cardiology clinic.

Immunological studies were performed at the Central Scientific Research Laboratory, Samara State Medical University.

Comprehensive clinical examination included a study of data from medical history, clinical indicators, laboratory and instrumental methods. Quantitative determination of lymphocytes and their subpopulations (CD3 +, CD4 +, CD8 +, CD19 +, CD3 + HLADR +) in serum was performed by the standard method of indirect immunofluorescence using monoclonal antibodies ICO-86 and ICO-31 to their surface antigens (NPK "drug", Russia ).

Quantitative indicators of TNF-α, IL-2, IL-4, IL-6, IL-17 in serum of IL-6, IL-17, TNF-α in the synovial fluid of patients with JIA were measured by enzyme-linked immunosorbent assay (ZAO Vector-Best, Russia).

Clinical diagnosis was based on the classification of juvenile idiopathic arthritis EULAR (Durban, 1997, Edmonton, 2001), in accordance with which the children were divided into 3 variants of JIA:

1. oligoarthritis persistent - arthritis, affecting no more than 4 joints throughout the time of disease - 20 (34,4%);

2. oligoarthritis spreads - arthritis, which affects 4 or more joints after first 6 months of illness - 19 people (32,8%);

3. polyarthritis seronegative - arthritis, involving in the inflammatory process 5 or more joints during the first 6 months of disease, RF-negative - 19 people (32,8%).

The average age of patients was 7,1 ± 0,5 years, mean disease duration - 2,8 ± 0,4 years. All children with juvenile idiopathic arthritis were divided into groups depending on the obtained modifying therapy.

Formation of groups was such that in each group were presented to all age categories, the distribution of children was carried out on the floor, laboratory activity and the number of affected joints.

Combination therapy (Sandimmun-neoral + MTX) has been appointed 16 patients (27%), 10 (17%) were on treatment Remicade, 12 (21%) - sulfasalazine, 20 person (35%) were receiving methotrexate monotherapy.

Sulfasalazine treatment started with a dose of 5-10 mg / kg / day, gradually increasing to 30-40 mg / kg / day. Sandimmun-neoral administered at a dose of 1,5 - 3,5 mg / kg / day, methotrexate - 7,5-15 mg/m2/week intramuscularly 1 time per week, infliximab (Remicade) - 6 mg / kg, the first two infusions 2, 4 weeks, then every 8 weeks.

For statistical analysis we used the calculation of average values (M), standard deviations (m). The significance of differences of mean values between two samples was determined by Student's t-test. The critical level of statistical significance (p) was taken to be 0.05.

**Results and discussion**

Indicators of the quantitative distribution of cytokines in different variants of JIA presented in Table 1.

Table 1

The most pronounced changes in cell populations of lymphocytes was accompanied by a version of polyarticular JIA. Changes in the composition of CD-markers for this option were clearly defined the character corresponding to the main pathogenetic mechanisms of disease: an increase in the total number of lymphocytes (CD3 +) and activated forms (CD3 + HLADR +), a significant increase in T-cell-helper (CD4 +), decrease in the activity of T- lymphocytes with cytotoxic activity (CD8 +), moderate increase of B-lymphocytes (CD19 +).

When oligoarthritis pervade most orientation changes of cellular immunity was of the same nature as that of a polyarticular form, only the degree of these changes were less pronounced.

With persistent arthritis also revealed changes in lymphocyte subpopulations, but most indicators are normal.

Analyzing the contents of cytokines in serum, observed a significant increase in proinflammatory IL-2, IL-6, increased levels of IL-17, TNF-α, decrease the concentration of anti-inflammatory IL-4 in all variants of juvenile idiopathic arthritis. More significant percentage of abnormalities observed in the polyarticular form of JIA, which is associated with the disease on the background of the maximum activity of the inflammatory process.

Definite interest to study the contents of individual cytokines (IL-6, IL-17, TNF-α) in synovial fluid of affected joints (Table 2).

Table 2.

Indicators of cytokines in synovial fluid is much higher than those in the serum of patients with JIA. The highest content in blood serum and synovial fluid was observed in IL-6 (12,1 ± 0,1, 369,9 ± 0,3 respectively). In addition, revealed high levels of IL-17 and TNF-α.

Thus, for children with juvenile idiopathic arthritis characterized by increased concentrations of proinflammatory cytokines in serum and in synovial fluid, which can be used for early diagnosis of the disease. In this case, the indicators differed significantly from the control group and depended on the variant of the disease and the degree of inflammatory activity. Reduction of interleukin-4 may indicate a decrease in anti-inflammatory activity in patients with JIA.

Clinical efficacy was assessed on the dynamics of the basic immunological parameters - the immunological system improvements, in which no or low efficacy - improved less than 25% from baseline, a satisfactory effect - improvement of 50% (IIU50), good effect - improvement of up to 75% (IIU 75), an excellent effect - improvement of more than 75% (IIU 100) (Table 3).

Table 3

In the analysis of the data revealed that the efficiency of basic drugs varies depending on the version of the JIA. In persistent oligoarthritis high immunological parameters were obtained with combination therapy sandimmun-neoral and methotrexate - IIU 100 - 36%. Remicade in the treatment of patients with oligoarticular pervasive and polyarticular JIA option at the most rapid immunological parameters - 55% and 51.3% respectively. Shows the efficiency of combination therapy Sandimmun-neoral and methotrexate compared with methotrexate monotherapy. In the application of sulfasalazine found the lowest rates for all forms of the disease.

Summarizing the findings can be stated that the optimal choice of the base of the drug should be made for a specific form of JIA. The results of the comparative immunological tests

have shown that early differential assignment of basic drugs is highly effective and contributes to the stabilization of the autoimmune process.

**Conclusion**

1. In all forms of JIA marked change in cytokine and cellular status in the serum. The intensity of these changes depends on the variant and the degree of inflammatory activity.

2. The concentration of cytokines in synovial fluid is much higher than in the serum of patients with JIA.

3. Most effective for the treatment of polyarticular and oligoarticular pervasive form has Remicade in the treatment of persistent oligoarticular form option is preferable to use the combination therapy Sandimmun-neoral and methotrexate.

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Table 1. Ratio lymphocyte subpopulations and cytokines, depending on the version of the JIA.

| Variant of the disease | Indicators of cytokine profile, pg / ml | | | | | Indicators of lymphocyte subpopulations, pg / ml | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | IL-2 | IL-4 | IL-6 | IL-17 | TNF-α | CD3+ | CD19+ | CD3 HLA DR+ | CD4+ | CD8+ |
| Control group | 20± 0,3 | 6± 0,4* | 2,5± 0,2 | 0,5± 0,1* | 0,3± 0,4 | 1,7± 0,05 | 0,5± 0,1* | 15± 0,5* | 1,1± 0,1* | 0,9± 0,4 |
| Oligoarthritis persistent | 18,3± 0,5* | 3,7± 0,3* | 4,54± 0,5 | 0,5± 0,3 | 0,4± 0,1 | 2,4± 0,3 | 0,5± 0,1 | 15± 0,09* | 1,2± 0,05* | 0,9± 0,2 |
| Oligoarthritis spreads | 19,2± 0,1 | 2,4± 0,1 | 7,2± 0,3 | 0,78± 0,1* | 0,58± 0,05 | 3,2± 0,2 | 0,6± 0,2 | 16± 0,2* | 1,5± 0,06 | 0,7± 0,6* |
| Polyarthritis seronegative | 21,3± 0,05 | 0,9± 0,1 | 24,6± 0,1 | 0,99± 0,2 | 0,72± 0,04* | 3,2± 0,4 | 0,81± 0,5* | 16,8± 0,1 | 1,9± 0,1 | 0,3± 0,5 |

*Note* .* - statistically significant differences from control at $p < 0.05$

Table 2. Levels of IL-6, IL-17, TNF-α in serum and synovial fluid of patients with JIA.

| Name of cytokines | Indicators of cytokines in serum, pg / ml | Indicators of cytokines in synovial fluid, pg / ml |
|---|---|---|
| IL-6 | 12,1±0,1 | 369,9±0,3 |
| IL-17 | 2,27±0,05 | 48,55±0,1 |
| TNF-α | 0,67±0,3 | 8,45±0,05 |

*Note* .* - statistically significant differences from control at p <0.05

Table 3. Dynamics IIU criteria depending on the drug and the disease variant in children with juvenile idiopathic arthritis

| Preparation | Variant of the disease | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Oligoarthritis persistent | | | Oligoarthritis spreads | | | Polyarthritis seronegative | | |
| | IIU 50, % | IIU 75, % | IIU 100,% | IIU 50, % | IIU 75, % | IIU 100,% | IIU 50, % | IIU 75, % | IIU 100,% |
| Sulfasalazine | 31 | 42 | 27 | 48 | 29 | 23 | 57 | 32 | 11 |
| Sandimmun-neoral + methotrexate | 25 | 39 | 36 | 10 | 41 | 49 | 21,8 | 35,7 | 42,5 |
| Methotrexate | 27 | 40 | 33 | 39 | 36 | 25 | 45 | 28 | 27 |
| Remicade | - | - | - | 14 | 31 | 55 | 11 | 37,7 | 51,3 |

**About the authors**
Tepikina Ekaterina - intramural graduate faculty of the Department of Pediatrics, SEI HPE Samara State Medical University, Department of Health and Social Development of Russian Federation
Home address: 443084, Samara, Novo-Vokzalnaya street, 231, 78
e-mail: semenukova @ rambler. ru
Mob. 8-917-119-30-67