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Th-17 Phenotype of Juvenile Idiopathic Arthritis

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Urgency. *Juvenile idiopathic arthritis (JIA) remains one of the most urgent issues of pediatric rheumatology due to incapacitation at early stages of the disease. It has been established that the first years of arthritis are key in the aspect of progression of the pathological process. Despite significant progress in treating JIA with genetically engineered biopharmaceuticals, some patients do not respond to such a therapy and thus aggravate the disease course.* ***The study was aimed at*** *improving diagnosis and treatment of children with JIA by means of analyzing the main immunological factors Th17, suggesting methods of differentiating lymphocytes and developing recommendations on assessing prognosis of the disease's course and outcomes.* ***Methods.*** *The study included 108 2-18-years-old children with various forms of JIA. The control group was comprised of 18 conventionally healthy 6-17-years-old children with unburdened heredity regarding autoimmune diseases and without any clinical symptoms of diseases. The total T helper population and subpopulations thereof (naïve Th cells and Th memory cells, double-positive Th cells), subpopulations of Th17 cells and anti-inflammatory cytokines (IL 1β, IL 6, IL 17A and TNF α) in the peripheral blood were analyzed in all the patients, including the control group.* ***Results.*** *The levels of Th17 memory cells and anti-inflammatory cytokines (IL 1β, IL 6 and IL 17A) were significantly higher in children with enthesitis-associated arthritis (HLA B27+ juvenile ankylosing spondyloarthritis) and systemic JIA. The level of Th17 cells and high blood level of IL 17A affect development of an active disease; simultaneous increase in IL 6 results in higher risk of osteochondral destruction in children with JIA.* ***Conclusion.*** *According to the immunological data, there are at least 2 JIA phenotypes: Th17-dependent and Th17-independent. Th17-dependent JIA is characterized by the most adverse course of the disease and high risk of osteoarticular destruction, which is why children require earlier prescription of genetically engineered biopharmaceuticals. By contrast, Th17-independent phenotype features a more favorable disease outcome and low risk of osteoarticular destruction.*
Keywords: *juvenile idiopathic arthritis, arthritis, Th17 cells, IL 17A, IL 1β, IL 6, TNF α, cytokines, enthesitis-associated arthritis, HLA B27, systemic arthritis.*

RELEVANCE

Notion "phenotype" refers to the total of internal and external organism traits acquired as a result of the ontogenesis. Term "phenotype" was coined by Danish scientist Wilhelm Johannsen in 1909 together with the concept of genotype to differentiate the hereditary background from the way it manifests itself. The total of genetic and environment factors determines profusion and the variety of the phenotype: the more versatile and sensitive the phenotype is, the greater its difference from the genotype is and the more various manifestations it has.

According to the contemporary views, juvenile idiopathic arthritis (JIA) remains one of the most widespread children diseases, its structure includes all forms of arthritis; it is characterized as a chronic inflammatory disease of joints with an unknown etiology usually occurring under the age of 16 and lasting more than 6 weeks unless there is some other articular pathology. [1].

JIA is considered to be a term uniting the heterogenic group of chronic articular diseases with different etiopathogenesis and immunogenetic origin, different nosology and an ambiguous prognosis [2]. The key mechanism influencing the disease development in children is an antigen-induced autoimmune inflammatory process [3, 4].

Identification of a new Th subpopulation (Th17) in 2005 г. (Harrington, Langrish, Park et al.) extended the knowledge on the pathogenesis of most inflammatory and contagious diseases [5-8]. Th17 cells have been intensively studied in recent years; this has resulted in the identification of origin sources, as well as ways for differentiation and demonstration of functional peculiarities. While studying the process of Th17 differentiation in human blood samples *in vitro*, E.V. Acosta-Rodrigues et al. [8] found out that activated monocytes and circulating dendritic cells producing a large number of IL 1β and IL 6 are sufficient for Th17 differentiation. When antibodies neutralizing these cytokines were added, Th17 differentiation was blocked. The researchers analyzed the Th17 subpopulation to prove their difference from Th1 and Th2 as well as from other immune system cells. Membrane chemokine receptors were chosen as molecules for identifying of the Th subtype as they are the instrument for Th memory cells characterization due to the capacity to differentiate one Th type from another with a high degree of certainty. These assumptions provided a possibility to show that the CCR6 and CCR4 expression identifies a homogeneous memory T-cell population in the human body which is capable of producing IL 17 but not interferon γ. At the same time, CCR6 is a more specific chemokine receptor as it is expressed only on the surface of Th17 cells while CCR4 is present on Th1 lymphocytes as well [8, 9].

IL 17 was identified in 1993 by P. Rouvier et al. long time before the actual discovery of Th17 cells, and was initially referred to as CTLA-8 (cytotoxic T lymphocyte-associated antigen 8). IL 17 cytokine family includes 6 members: 17A, 17B, 17C, 17D, 17E (or IL 25) and 17F. Different members of the IL 17 family probably have diverse biological functions but Th17 cells are produced by two members of the family only — IL 17A and IL 17F, but IL 17A is more efficient in a biological way [10].

The main function of IL 17A is activation of granulopoiesis out of progenitor cells in human bone marrow. Hyperexpression of IL 17A leads to significant increase in the amount of neutrophils in peripheral blood and increase of neutrophil progenitors in the spleen. Granulopoietic response is important for control of immune protection from extracellular pathogens including bacteria and fungi. IL 17A is characterized as a cytokine inducing a polypeptide mediator, demonstrates its anti-inflammatory functions and hematopoietic functions due to the ability to stimulate and to release secondary cytokines and chemokines having a biological influence onto different cell types [6, 10, 11].

Th17 can influence the osteoclast biology by expressing RANKL (receptor activator of nuclear factor κ B ligand) and tumor necrosis factor (TNF α), which directly induce osteoclastogenesis. There is also an indirect way of this process induction — IL 17A favours RANKL expression with synovial fibroblasts and osteoblasts through the activation of synovial macrophages leading to a secretion of osteoclastogenic factors such as TNF α and IL 1β. This Th17-mediated induction of osteoclastogenesis may represent an important cell mechanism in the pathogenesis of bone destruction at autoimmune arthritis [11, 12].

Th17 and IL 17A impact into JIA development has not been studied in full yet. Thus, it is still unclear how Th17 influences the course and the outcome of the disease in children with various JIA courses as well as the capacity of modern genetically engineered biopharmaceuticals administered to children to inhibit Th17 differentiation out of the naïve Th cells.

Research objective: Improvement of diagnosis and treatment of JIA in children through study of the main immunological factors of Th17 pathway of lymphocyte differentiation with the development of recommendations to evaluate the prognosis of disease course and outcome.

Patients

The research included children from the age group of 2-18 years with various forms of JIA. The diagnosis and the form of the disease were determined according to the criteria of the International League of Associations for Rheumatology (ILAR, Durban, Edmonton, 2001; second revision 2004) [13]. All children were divided into subgroups: with active and passive disease forms. Active disease was identified as a presence of one or more joint with inflammatory signs in the form of edema/effusion, soreness, and functional limitation during treatment. Passive disease was characterized by the absence of all clinical and laboratory signs of joint inflammation and uveitis coinciding with zero activity on the visual analog scale during at least 1 year of treatment (tb. 1) [2]. Research was randomized and controlled by design. The criteria for exclusion from the research were presence of juvenile psoriatic arthritis and the SEA syndrome in children.

The control group included 18 conventionally healthy children without compromised family history of autoimmune diseases and any clinical signs of diseases.

Table 1. ***Distribution of children suffering from juvenile idiopathic arthritis (JIA) depending on the JIA course and disease activity***

JIA course / Activity	Oligoarthritis		Polyarthritis		ERA		Systemic		Total	
	abs.	%	abs.	%	abs.	%	abs.	%	abs.	%
Active	23	21.6	26	24.4	26	24.4	12	11.3	87	81.5
Passive	9	7.9	4	3.5	7	6.2	1	0.9	21	18.5
Total	32	29.6	30	27.8	33	30.6	13	12.0	108	100.0

Note. ERA — enthesitis-related arthritis.

Methods

All patients underwent radiographic and ultrasound examination of the osteoarticular system. Some patients underwent ultrasound densitometry. The important advantage of this examination in comparison with radiographic densitometry is a complete absence of radiation exposure of the patient and, correspondingly, absence of contradictions to its conduction.

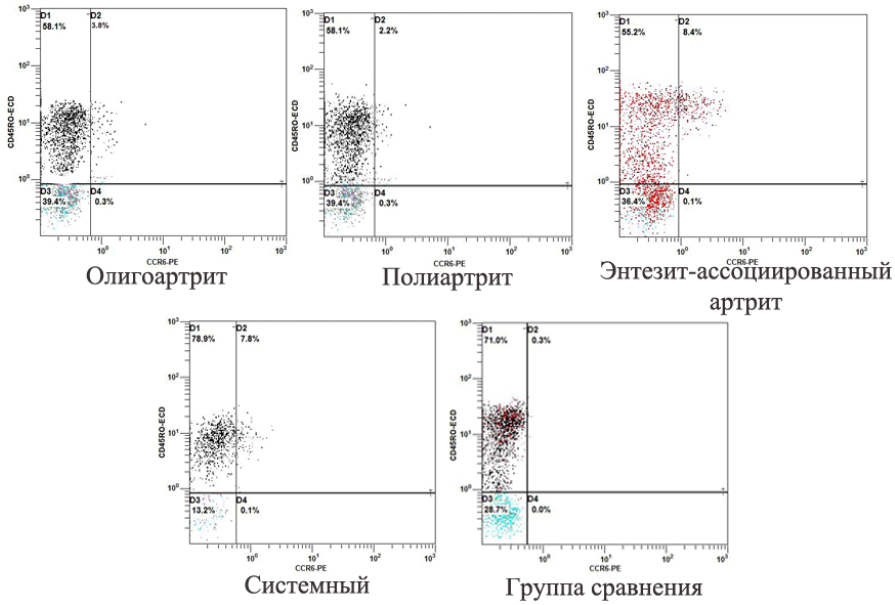
To evaluate how active the disease was and to assess the treatment efficiency, different scales developed specifically for use in pediatric rheumatologic practice were used — ACR_{pedi} (American Collegia of Rheumatologists criteria) and JADAS-71_{CRP} (criteria of active disease identification in children suffering from JIA).

Quantitative identification of lymphocytes and their subpopulations *in vitro* was performed via indirect immunofluorescence with the help of flow cytometry and use of monoclonal antibodies to their surface antigens (CD). The main and the control groups underwent immunological examination including determination of the total number of $CD4^+$ (monomeric transmembrane glycoprotein expressing largely on T-helpers) T-cells as well as identification of differentiating antigen CD45 with RA isoforms — the marker of naïve TH-cells ($CD4^+CD45RO^-CD45RA^+$) and the RO expressed on Th memory cells ($CD4^+CD45RO^+CD45RA^-$). The identified transition form of Th-cells has both isoforms — double-positive Th-cells ($CD4^+CD45RO^+CD45RA^+$). To identify the Th17 population a membrane chemokine receptor CCR6 (CD196) was measured which is usually expressed onto the surface of these cells and marks them, thus enabling to reveal the subpopulation of naive Th17-cells ($CCR6^+$RA) and Th17 memory cells ($CCR6^+$RO).

To determine the number of T-helper cells (CD4 +) and their subpopulations in the peripheral blood taken from the cubital vein in vacutainers with K_2EDTA, monoclonal antibodies CD45RA-FITC, CD45RO-ECD, CD4-PC5 (all reagents – Beckman Soulter, USA) were used, as well as monoclonal antibodies to the membrane chemokine receptor CCR6 (PE conjugated anti-

human CD196, Clon: R6H1, eBioscience, San Diego, CA, USA). Lysis of erythrocytes was performed with reagent OptiLyse C (Beckman Coulter, USA). Analysis of samples was carried out in multi-color protocol using the no-clean technology on a flow cytometer NAVIOS (Beckman Coulter, USA). The number of T-helper cells was expressed in percentage of the total number of lymphocytes while the number of naive and memory cells was calculated in relation to the pool of CD4 + cells. All reagents were used according to the instructions of the manufacturer (Fig. 1).

Fig. 1. Examples of histograms of Th17 memory cells distribution in patients with different forms of JIA



Oligoarthritis	Polyarthritis	Enthesitis-related arthritis
Systemic arthritis	Control group	

Quantitative determination of cytokines IL 1β, 6, 17, and TNF α in the blood serum of the JIA group and the control group was performed by means of enzyme multiplied immunoassay (EIA-ELISA) using a kit of reagents for IL 1β, IL 6 and TNF α produced by "Vektor-BEST", ZAO (Novosibirsk), for IL-17A — eBioscience (San-Diego, USA).

All children participating in the research and their parents were provided with full information on methods and objectives, as well as the associated risks. Written informed consent for the participation was received from all parents or individuals acting as parents, as well as from over-15 children. It has been explained that before the medical intervention the patient or the legal representative of the patient has a right to reject such intervention.

Statistical analysis of the results of the research was carried out using SPSS IBM package version 19.0 and Statistica 10. To compare the data samples the analysis of contingency tables was applied, where the Pearson chi-square ($\chi 2$) for the analysis of nominal variables defined by the table of contingency of the type N × M was appraised. Double-sided Fisher's test was used for the 2 × 2 tables. The non-parametric Mann-Whitney U-Test was used to compare the medians of the two samples, in case the distribution of at least one of them was significantly different from a normal one. Determination of dependence of the average values of Th populations and their subpopulations, naive Th17 cells (CCR6 + RA +), Th17 memory cells (CCR6 + RO +), as well as IL 1β, IL 6, IL 17 and TNF α on the ordinal value was identified by constructing three ordinal regressions with the determination of value $\chi 2$. In order to analyze risk factors of persistence of symptoms of JIA, a method of logistic regression was applied with the construction of a decision tree and ROC-curve.

A hierarchical cluster analysis was used to identify JIA phenotypes. This type of analysis allows us to classify multi-dimensional observations without making a priori assumptions about the data set and without imposing restrictions on the representation of the researched objects. It gives an opportunity to analyze the values of different types of data. To evaluate the differences between the selected clusters in the process of analysis, a stepwise discriminant analysis was used.

RESEARCH OUTCOMES

108 children corresponded to the criteria for inclusion in the main group (average age 10.5 ± 3.8 years). Depending on the articular lesions, the children were differentiated as follows: oligoarthritis – 32 patients (29.6%); arthritis seronegative for the rheumatoid factor – 30 patients (27.8%); enthesitis-related arthritis (EAS) – 33 children (30.6%) with juvenile ankylosing spondylarthritis, positive for HLA B27; the number of children with systemic JIA course amounted to 13 (12.0%).

Comparative evaluation of the general population of CD4 + cells did not reveal differences between the main group and the control group (p = 0.088), the level of these cells was within the normal range. The subgroups of children with active and passive arthritis did not show any statistically significant differences for the overall population of CD4 + cells (p ⍰ 0.05; tb. 2).

Table 2. ***Comparative characteristics of general T-helper population and their subpopulations***

JIA form	$CD4^+$, %			$CD45^+RO$, %			$CD45^+RA$, %			$CD45^+RO^+RA^+$, %		
	M ± SD	SE	Min–Max	M ± SD	SE	Min–Max	M ± SD	SE	Min–Max	M ± SD	SE	Min–Max
Active oligoarthritis	40.6 ± 8.9	1.9	29. 0–64.0	31.8 ± 12.8	2.7	8.9–53.5	62.9 ± 12.9	2.7	42.8–89.0	4.1 ± 2.7	0.6	1.2–10.3
Passive oligoarthritis	40.2 ± 5.4	1.8	32.0–47.0	30.6 ± 8.1	2.7	18.1–40.7	65.3 ± 7.7	2.6	54.9–75.8	3.9 ± 1.3	0.4	1.7–6.1
Active polyarthritis	39.3 ± 7.2	1.4	23.0–50.0	30.9 ± 8.5	1.7	10.0–49.0	61.7 ± 12.3	2.4	36.8–88.4	4.8 ± 2.6	1.5	0.2–9.8
Passive polyarthritis	36.3 ± 8.3	4.1	28.0–46.0	31.6 ± 9.5	4.8	20.3–43.4	59.2 ± 8.5	4.2	53.6–71.8	4.7 ± 2.3	2.1	2.4–11.9
Active ERA	40.9 ± 7.1	1.4	21.0–54.0	38.8 ± 11.9	2.3	9.5–52.0	53.8 ± 14.1	2.8	29.9–82.7	4.2 ± 2.9	1.8	1.7–9.3
Passive ERA	40.7 ± 6.6	2.5	33.0–54.0	38.8 ± 13.3	5.0	21.8–61.0	54.6 ± 12.6	4.8	38.0–70.9	3.9 ± 2.1	0.8	1.3–7.5
Active and systemic form	41.6 ± 10.2	2.8	27.0–58.0	38.3 ± 5.8	1.6	29.7–50.5	54.5 ± 6.6	1.8	44.5–65.5	4.0 ± 2.6	0.7	2.3–10.4
Control group	39.3 ± 6.0	1.4	30.0–50.0	38.4 ± 7.8	1.8	23.4–47.0	54.4 ± 8.7	2.0	43.2–73.8	3.8 ± 1.7	0.4	1.0–7.7

Note. JIA — juvenile idiopathic arthritis, ERA - enthesitis-related arthritis.

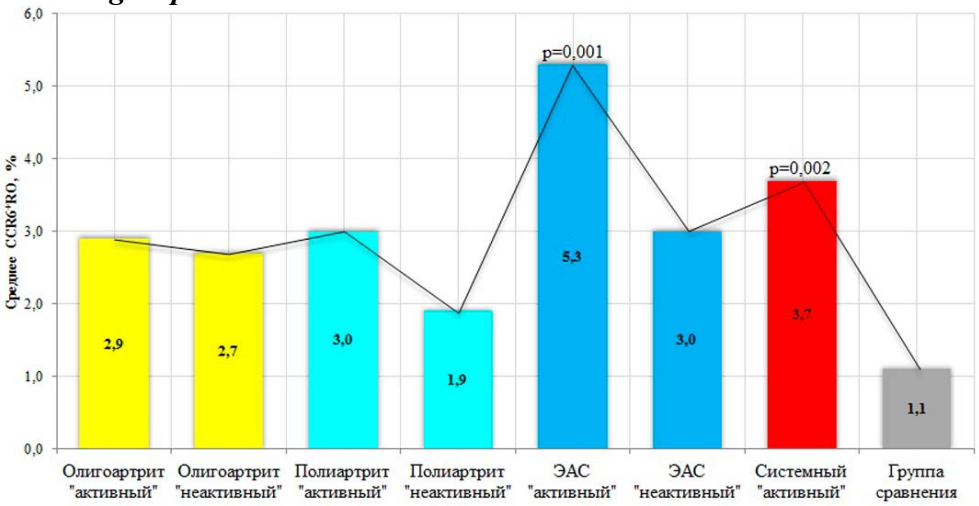
The smallest number of Th memory cells and the largest number of naive Th cells was observed in patients with oligo- and polyarthritis regardless of the process activity (p = 0.022 and p = 0.005, respectively), whereas in the event of enthesitis-related arthritis and a systemic form of JIA there were no statistically significant differences (p □ 0.05). The level of double-positive Th cells (CD45RO + CD45RA +) in the peripheral blood did not differ at various JIA courses in the event of either active or passive form of the disease (p □ 0.05; tb. 2).

Comparative evaluation of the level of the Th17 marker on naive Th cells – CCR6 + RA – revealed no statistically significant differences between children with JIA and the control group (p □ 0.05). Statistics for the level of Th17 memory cells (CCR6 + RO) was significantly different at all JIA courses when compared to the control group (p = 0.001). The highest level of these cells was detected in children with active HLA B27-associated arthritis (p = 0.001) and systemic arthritis (p = 0.002; Fig. 2).

Levels of cytokines IL 1β, 6, 17A and TNF α were measured in the blood serum of 90 patients from the main group and 15 children from the control group. IL-1β levels in children with both active and passive JIA were significantly higher when compared with the control group (p =

0,038). A particularly high IL-1β level was observed in patients with active HLA B27-associated arthritis (p = 0.007; Fig. 3).

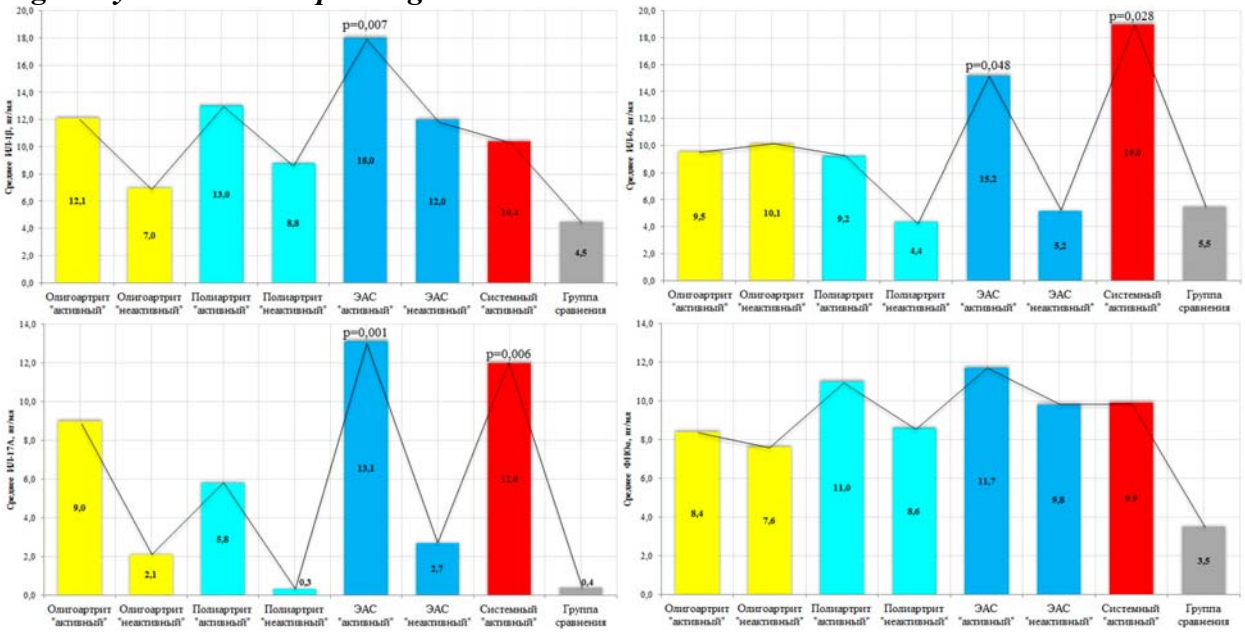
Fig. 2. ***Moderate concentration of Th memory cells in the blood of children suffering from JIA and in the control group***



Note. ERA — Enthesitis-related arthritis.

Average value of $CCR6^{+}RO^{+}$								
	Active oligoarthritis	Passive oligoarthritis	Active polyarthritis	Passive polyarthritis	Active ERA	Passive ERA	Active and systemic form	Control group

Fig. 3. ***Cytokine level depending on the JIA course***



Note. ERA — Enthesitis-related arthritis.

The concentration of IL 6 in the serum of children with JIA regardless of arthritis forms and its activity was significantly higher than in the control group (p = 0.005). The concentration of IL 6 in the blood serum of children with JIA, regardless of arthritis forms and its manifestation, was significantly higher than in the control group (p = 0.005). The highest level of IL 6 was observed

in patients with systemic arthritis (p = 0.028) and in children with enthesitis-related arthritis (p = 0.048; Fig. 3).

Comparative analysis of the IL 17A level in the blood serum revealed statistically significant differences between subgroups of children with JIA and the control group (p = 0.001), but the highest IL 17A values were observed in children with active ERA (p = 0.001) and systemic JIA (p = 0.006; Fig. 3).

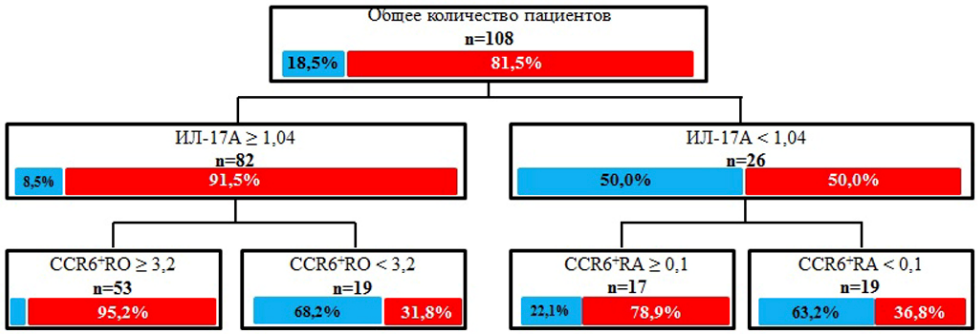
TNF α levels in the blood serum of almost all JIA subgroups was higher than in the control group (p = 0.005). No significant differences between the groups of children with JIA and inside the groups were detected (p □ 0.05; Fig. 3).

Evaluation of the total number of CD4 + cells (Th) and their subpopulations in the peripheral blood revealed a direct link between the Th general population and naive Th cells (CD45RA +) and a negative correlation with Th memory cells (CD45RO +). Further evaluation of these parameters revealed a negative correlation between the CD45RO + level and CD45RA + level, as well as CD45RA + with double-positive Th cells (CD45RO + CD45RA +).

In order to evaluate the impact of Th17 cells and the main pro-inflammatory cytokines on the main clinical and laboratory parameters more accurately, a method of linear correlation with the construction of a multiple regression equation was used, where p < 0.05 was considered to be a statistically significant value.

Both boys and girls revealed a positive correlation between Th17 memory cells (CCR6 + RO) and the overall level of Th memory cells (R2 = 0,178; p = 0,03) and a negative correlation with naive Th cells (R2 = 0,285; p = 0.0001). Further evaluation of links identified a strong positive correlation between IL 1β and IL 6 (R2 = 0,324; p = 0.001), and correlation of IL 1β with TNF α (R2 = 0,229; p = 0.001).

Fig. 4. ***Scheme of evaluation of a relative risk of disease relapse depending on the levels of CCR6$^+$RO, CCR6$^+$RA u IL 17A***



№	Правило	Число наблюдений	Риск рецидива, %	p
1	ИЛ-17А ≥ 1,04 и CCR6+RO ≥ 3,2	53	95,2%	0,003
2	ИЛ-17А ≥ 1,04 и CCR6+RO < 3,2	19	31,8%	0,049
3	ИЛ-17А < 1,04 и CCR6+RA ≥ 0,1	17	78,9%	0,033
4	ИЛ-17А < 1,04 и CCR6+RA < 0,1	19	36,8%	0,06

Remission – red colour

Relapse – blue colour

Total number of patients
n=108
ИЛ-17А /IL-17A

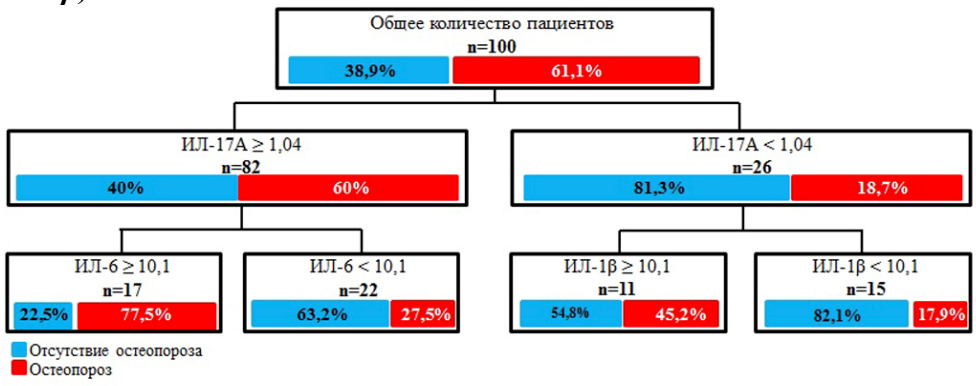
No.	Rule	Number of observations	Relapse risk, %	p
1	IL-17A ≥ 1.04 and CCR6+RO ≥ 3.,2	53	95.2%	0.003
2	IL-17A ≥ 1.04 and CCR6+RO < 3.2	19	31.8%	0.049
3	IL-17A < 1.04 and CCR6+RA ≥ 0.1	17	78.9%	0.033
4	IL-17A < 1.04 and CCR6+RA < 0.1	19	36.8%	0.06

Evaluation of relative risk of active arthritis and remission in children with JIA, depending on the level of Th17 cells and the main cytokines was performed using a decision tree both in the mixed group of boys and girls, as well as in the subgroups with different JIA forms with further interpretation of the obtained results. Regression analysis model showed that the combination of two research factors has a significant diagnostic value. Thus, it was found that when the value of IL 17A in the blood serum is above 1.04 pg / ml, the risk of disease relapse increases by 91,5% (OR = 2,755; 95% CI – 0,884-8,588; p = 0.04), and, when combined with increased CCR6 + RO in the peripheral blood above the level of 3.2%, the risk increases by 95,2% (OR = 3,030; 95% CI – 0,867-10,590; p = 0.003; Fig. 4).

Use of the ROC-analysis for prediction of active arthritis with the construction of the characteristic curve showed a good predictive ability of model "relapse – the level of CCR6 + RO" (AUC 0,656; p = 0.05; CI – 0,544-0,768) and "relapse – the level of IL 17A" (AUC 0,745; p = 0.0001; CI – 0,625-0,866), although the greatest specification and sensitivity were demonstrated by model "relapse – IL 17A".

When evaluating the influence of the main immunological parameters on the radiographic results, it has been found that in the event of the aggregate levels of IL 17A above 1.04 pg / ml and IL 6 above 10.1 pg / ml in the blood serum, the risk of osteoporosis development increases by 77,5% (OR 2,902 ; 95% CI – 0,584-14,421; p = 0.008), and providing a value of IL 6 in blood serum is less than 10.1 pg / ml, the risk of osteoporosis is dramatically reduced – 27,5% (OR 0,394; 95% CI – 0,097-1,598; p = 0.037). Evaluation of the aggregate levels of IL 17A and IL 1β showed no statistically significant dependence on the risk of osteoporosis development (Fig. 5).

Fig. 5. ***Scheme of evaluation of a relative risk of osteoporosis development depending on the levels of IL 1β, IL 6 and IL 17A***



№	Правило	Число наблюдений	Риск развития остеопороза, %	p
1	ИЛ-17А ≥ 1,04 и ИЛ-6 ≥ 10,1	17	77,5%	0,008
2	ИЛ-17А ≥ 1,04 и ИЛ-6 < 10,1	22	27,5%	0,037
3	ИЛ-17А < 1,04 и ИЛ-1β ≥ 10,1	11	45,2%	0,1
4	ИЛ-17А < 1,04 и ИЛ-1β < 10,1	15	17,9%	0,01

Absence of osteoporosis – blue colour
Osteoporosis – red colour

Total number of patients n=100
(ИЛ-1β for IL-1β; ИЛ-6 for IL-6; ИЛ-17А for IL-17A)

No.	Rule	Number of observations	Osteoporosis development risk, %	p
1	IL-17A ≥ 1.04 and IL-6 ≥ 10.1	17	77.5%	0.008
2	IL-17A ≥ 1.04 and IL-6 < 10.1	22	27.5%	0.037
3	IL-17A < 1.04 and IL-1β ≥ 10.1	11	45.2%	0.1
4	IL-17A < 1.04 and IL-1β < 10.1	15	17.9%	0.01

Use of the ROC-analysis for prediction of osteoporosis with the construction of the characteristic curve showed a good predictive ability of model "osteoporosis - the level of IL 6" (AUC 0,678; p = 0.045; CI – 0,485-0,732). Models "osteoporosis and levels of IL 17A" (AUC 0,608; p = 0.069; CI – 0,491-0,725) and "Osteoporosis - IL 1β" (AUC 0,584; p = 0.157; CI – 0,469-0,699) showed a poor predictive ability at separate consideration of each indicator.
The final stage of the study comprised the selection of clusters using hierarchical cluster analysis followed by a discriminant analysis. Selected clusters allow characterization of peculiarities of the JIA dynamics in combination with certain immunological parameters.

Table 3. ***Cluster analysis results***

Variables included in the cluster analysis	JIA clusters			p
	Cluster I	Cluster II	Cluster III	
Age	10.9 ± 4.2	11.8 ± 2.5	9.1 ± 3.8	0.2
Sex	12 males / 20 females	22 males / 6 females	26 females / 14 males	0.15
Age of disease onset	5.1 ± 2.1	6.6 ± 1.7	7.8 ± 2.0	0.35
Disease duration	3.6 ± 2.8	4.1 ± 3.3	3.9 ± 2.2	0.31
ACR_{pedi}	□ 50	□ 70	50−70	**0.037**
$JADAS\text{-}71_{CRP}$	3.4 ± 0.8	□ 12.4	4.15 ± 0.6	**0.041**
Osteoporosis *	10	55	12	**0.038**
Bone defect*	1	16	2	**0.05**
Cystoid deformation *	0	10	5	**0.042**
Erosion*	0	1	1	0.43
$CCR6^{+}RO$, %	2.4 ± 1.04	4.1 ± 2.3	3.2 ± 2.02	**0.004**
IL 1β, pg/ml	3.4 ± 2.8	17.6 ± 12.5	9.9 ± 11.5	**0.0001**
IL 6, pg/ml	5.5 ± 4.8	17.9 ± 15.0	9.04 ± 8.4	**0.0001**
IL 17A, pg/ml	6.5 ± 0.5	12.5 ± 10.4	0.2 ± 0.1	**0.0001**
TNF α, pg/ml	5.1 ± 2.6	12.8 ± 15.3	9.7 ± 9.1	**0.007**

Note. * — values of variables characterize frequency of sign occurrence in each cluster (%).
JIA — juvenile idiopathic arthritis.

The analysis included 100 patients with different courses of JIA. Clustering used 10 binary and 5 quantitative variables that characterize dynamics of the disease and the main clinical and laboratory parameters under study. This analysis resulted in identification of three JIA clusters.
Cluster I comprised 32 patients with JIA: 20 girls and 12 boys (average age – 10,9 ± 4,2 years) with the disease onset in the preschool age and disease duration of 3,6 ± 2,8 years. This group mostly included children with passive disease form without radiographic evidence of bone destruction and low levels of Th17 cells, IL 1β, IL 6, TNF α and increased levels of IL 17A.
Cluster II comprised 28 patients, including children from the group of HLA B27-associated arthritis (16 boys and 6 girls) and 6 boys with systemic JIA course (average age – 11,8 ± 2,5 years) with the disease onset in the late preschool age and disease duration of 4,1 ± 3,3 years. The disease activity was regarded as high (ACRpedi □ 50, JADAS-71CRP □ 12.5). All children included in this cluster had radiological signs in the form of osteoporosis (primarily a systemic

one) and/or bone defect, as well as cystoid deformation. Levels of Th17 memory cells, IL 1β, IL 6, TNF α and IL 17A were significantly higher than the normal values and were the highest against other clusters.
Cluster III included 40 children, mostly girls (26 girls and 14 boys) aged 9,1 ± 3,8 years with the disease onset in the early school years and disease duration of 3,9 ± 2,2 years. The children in this group mostly manifested active polyarthritis with moderate activity (ACRpedi – 50-70, JADAS-71CRP – 4,15 ± 0,6). While evaluating the radiological changes, osteoporosis was observed in 12% of children (no bone destruction signs). Levels of Th17 memory cells, IL 1β, IL 6, TNF α were significantly increased. In contrast to the cluster I children, the level of IL 17A in blood serum was not increased (trace amounts; tb. 3).
The subsequent discriminant analysis indicated statistically significant differences between the clusters formed (tb. 4).

Table 4. ***Results of checking statistically significant differences using the discriminant analysis (Overall result of the discriminant analysis; No. of checked variables in the model: 7; Wilks' lambda: 0.56057; approx. F (12.184) = 5.1463; p < 0.0001)***

	Wilks' lambda	Privat lambda	F-criterion	**p**	Tolerance	1-Tolerance
ACRpedi	0.565346	0.991546	0.392205	0.676689	0.839488	0.160512
JADAS-71_{CRP}	0.571367	0.962113	1.336621	0.300875	0.932455	0.067545
$CCR6^{+}RO$	0.565346	0.911546	4.392206	**0.016689**	0.839488	0.160512
IL 1β	0.615553	0.910671	4.512197	**0.013509**	0.869842	0.130158
IL 6	0.620354	0.903623	4.906190	**0.009450**	0.930809	0.069191
IL 17A	0.640151	0.875679	6.530655	**0.002228**	0.871773	0.128227
TNF α	0.580419	0.895796	4.629103	**0.021711**	0.854988	0.145012

DISCUSSION

This work has studied the peculiarities of Th17 cells and the main pro-inflammatory cytokines influence on the course of JIA. The results of our research show that the JIA in children has a heterogeneous nature and may be represented by different phenotypes. The need to differentiate variants of the disease is justified by the disease control lack in individual patients suffering from JIA. It shall be emphasized that key statistically significant differences between clusters were observed between the studied immunological parameters (CCR6 + RO, IL 1β, IL 6, IL 17A and TNF α), the criteria of disease activity and radiographic examination results. Notably, these were the children with systemic JIA and juvenile ankylosing spondylarthritis who entered cluster II and were marked by the most unfavorable course of the disease with the development of a high degree of disease activity and osteoarticular destruction aggravated by high levels of Th17 memory cells, IL 1β, IL 6, TNF α and IL 17A in the peripheral blood.
On the basis of the studied immunological parameters, we can distinguish at least between Th17-dependent and Th17-independent phenotypes of JIA. Th17-dependent phenotype mainly includes children with systemic JIA and juvenile ankylosing spondylarthritis with high risk of osteoarticular destruction. This phenotype is characterized by a high level of Th17 cells and memory IL 17A in the peripheral blood both for active and passive form of the disease. Th17-independent phenotype mostly includes children with oligo- and polyarthritis, with a low risk of osteoarticular destruction and normal levels of Th17 memory cells and IL-17A in the peripheral blood (Fig. 6).

Fig. 6. ***Th17-dependent and Th17-independent phenotypes of JIA***

Th17-independent		Th17-dependent
Th17-naïve cells	Olygoarthritis	Th17-naïve cells
Th17-memory cells	Polyarthritis	Th17-memory cells

Pro-inflammatory cytokines in blood (TNFα; IL-6; IL1β; Il17A)	Systemic arthritis	
	HLA B27-associated arthritis	
	Pro-inflammatory cytokines in blood (TNFα; IL-6; IL1β; Il17A)	
	Chondro-osseous destruction	

Children suffering from JIA and having a Th17-dependent phenotype are characterized by a more adverse course of the disease, a high risk of osteoarticular destruction and require an earlier GEBP prescription in case of failure of the baseline treatment.
In spite of significant progress in the treatment of JIA with GEBP, in particular, with antagonists TNF α, IL 1β or IL 6, 1/3 patients do not achieve clinical and medically induced remission. Apparently, pathogenesis of the disease in these patients is largely influenced by Th17 cells and their key cytokines IL 17A and IL 17F. Laboratory testing of monoclonal antibodies to IL 17A / F (Secukinumab/Ixekizumab) at such autoimmune diseases as psoriasis, rheumatoid arthritis, Marie-Striinipell disease show promising results, and the main effects are complete blocking of symptoms and sustained remission [14, 15]. On the basis of these data, the most probable next step will be the introduction of these drugs in the registry for the treatment of JIA patients with the development of immunological markers for prognosis of osteoarticular destruction and the risk of evolving into the active disease form.

CONCLUSION

Summing up, the research of Th 17 pathway and its influence on the course of the disease in the group of children suffering from JIA seems to be the most prospective way of research and has an action-oriented interest connected with the strategy for management and treatment of children suffering from JIA.

CONFLICT OF INTEREST
The authors have indicated they have no financial support / conflict of interest relevant to this article to disclose.

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