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CLINICAL SIGNIFICANCE OF AUTOANTIBODIES AND BIOMARKERS OF FIBROSIS IN JUVENILE SCLERODERMA

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Abstract

Juvenile scleroderma is a rare chronic autoimmune condition with the onset of the disease before the age of 16 years, manifested by typical fibro-sclerotic affection of the skin and musculoskeletal system with possible development of Raynaud's syndrome and involvement of internal organs. Clinically, there are two forms of juvenile scleroderma: systemic and limited. The latter is characterized by the absence of internal organ involvement and Raynaud's syndrome.

The article contains the results of the investigation for the presence of autoantibodies and fibrosis markers in 255 children with juvenile scleroderma (among them 35 children with systemic form, 190 children with limited form of the disease). The vast majority of the surveyed patients (84.5%) were children with juvenile limited scleroderma. This fact reflects the predominance of limited forms over systemic disease in childhood. Children with severe juvenile limited scleroderma prevailed. They had diffuse and linear cutaneous forms, which are characterized by involvement of underlying tissue, i.e. subcutaneous tissue, muscles, joints and bones into the pathological process.

Rheumatoid factor, antinuclear factor, anti-double-stranded DNA antibodies, anti-centromere and anti-topoisomerase antibodies, antibodies to type I-IV collagens, increased fibronectin and hyaluronic acid in serum were detected in patients with both forms of the disease, but mostly in patients with systemic scleroderma. The exceptions were antibodies to type I-IV collagens, which reported a higher rate of detection in limited scleroderma. The antibodies to type I and III collagens had a dominant detection frequency in the examined patients. This fact proves the activity of autoimmune inflammatory cutaneous reactions. The antinuclear factor had a leading place in the detection frequency in both groups and the detection percentage amounted to 81% in the systemic form and 45% in the limited form of scleroderma. The second place in the detection frequency belonged to cryoglobulins which were detected in 76.4% of patients with juvenile systemic scleroderma, whereas the cryoglobulins were detected in less than 30% of the patients with limited scleroderma. This reflects the high immunological activity in the systemic form of the disease. Scleroderma specific autoantibodies (anti-centromere and anti-topoisomerase antibodies) were detected less frequently than in adult patients.

The obtained data were agreed with the results of the international multicenter studies, conducted by the method of survey research. At the same time, the study of autoantibodies in a large population of children with scleroderma in a single clinical center has a significant scientific value. The detection of a large variety of autoantibodies and markers of fibrosis in juvenile limited scleroderma is considered to be a reasonable justification for using immunosuppressive therapy in the limited form of scleroderma in children.

Keywords: *juvenile scleroderma, autoantibodies, fibrosis markers.*

Introduction

Juvenile scleroderma is a rare chronic autoimmune condition with the debut of the disease before the age of 16 years, manifested by typical fibro-sclerotic affection of the skin and musculoskeletal system with possible development of Raynaud's syndrome and involvement of internal organs. Juvenile scleroderma is a separate form of a disease only clinically, it is not an independent nosological unit according to the 10th Revision of International Classification of Diseases.

The clinical etiology has not been established. There are three main elements in the pathogenesis of scleroderma: intensive collagen and fibrosis formation, microcirculation disturbance as a result

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of inflammatory changes and spasm of the small arteries, arterioles and capillaries, humoral immunity changes with the production of autoantibodies to connective tissue components.

There are two forms of juvenile scleroderma: limited and systemic. The latter is characterized by internal organ involvement and Raynaud's syndrome.

The variety of clinical manifestations of scleroderma in different periods of childhood and the rare incidence of the disease are, apparently, the main factors that create obstacles in formulating a single standard classification of the condition. Using the classification created for adult patients with systemic scleroderma, the pediatric rheumatologists distinguish the following clinical forms of juvenile systemic scleroderma: diffuse and limited cutaneous scleroderma, as well as overlap syndrome [*Athreya B, 2002; Foeldvari I, 2006*]. The European Pediatric Rheumatology Association has developed the preliminary diagnostic criteria for juvenile systemic scleroderma [*Zulian F et al., 2007a*].

Juvenile limited scleroderma is divided into five clinical groups, according to the preliminary classification criteria adopted in 2004 [*Zulian F, 2008*]. Juvenile scleroderma is characterized by the predominance of the limited forms over the systemic disease, atypical cutaneous syndrome manifested by focal or linear (hemitype) skin damage, as well as lower incidence and severity of visceral lesions as compared to those in adults.

Actual incidence of juvenile scleroderma is not known, because there are objective difficulties in holding epidemiological studies and because of differences in the use of classification criteria. According to the study conducted in Finland, juvenile scleroderma incidence is 0.05 per 100.000 population [*Pelkonen P et al., 1994*], while according to the results obtained in England, it amounts to 0.27 per 100.000 population [*Herrick A et al., 2010*]. It is known that the juvenile limited scleroderma has 5-10 times more incidence than juvenile systemic scleroderma. The incidence of juvenile limited scleroderma in children varies according to different authors from 1 case per 100.000 population to 4.7-20 cases per 100.000 population [*Zulian F, 2008*]. Before the age of 8 years, scleroderma incidence is equal in boys and girls, while the girl incidence is dominant among older children (3:1) [*Zulian F et al., 2007b*]. Being a rare childhood disease, juvenile scleroderma, however, creates a specific medical and social problem, because the outcomes of the disease, such as defects of the musculoskeletal system, chronic functional failure of organs and systems lead to disability of patients. Early administration of basic therapy, such as systemic use of glucocorticoids, immune-suppressants, anti-fibrotic drugs, has a crucial influence on the prognosis of the disease, suppressing its progression [*Li S et al., 2010; Zulian F et al., 2011*].

Most researchers have recognized the coherence of the pathogenetic and pathomorphological mechanisms of systemic and limited sclerodermas, however, according to the 10th Revision of International Classification of Diseases, systemic and limited scleroderma belongs to different categories. Patients with limited scleroderma are seen by dermatologists to receive mainly local treatment without internal use of basic therapy. In pediatric cardio-rheumatological hospitals, there is a concentration of juvenile limited scleroderma patients with severe skin affections and muscle, joint, bone structure involvement, which might have crippling consequences for a child in the absence of basic therapy (Fig.1, 2).

The general use of basic therapy drugs by rheumatologists in recent years for the treatment of scleroderma, prompted to explore a range of antibodies, markers of inflammation and fibrosis in children with juvenile systemic and limited scleroderma in order to justify the relevance of inclusion and administration of systemic immunosuppressive therapy. Indicators, which directly or indirectly were interested in the pathogenesis of scleroderma, were selected for the conduction of present study.

It is known that there are anti-centromere and anti-topoisomerase antibodies in blood serum of both adult and pediatric patients with systemic scleroderma, the presence of which is the serological criterion of the systemic form of the disease. Anti-centromere antibodies are found in 16-30% of adult patients with systemic scleroderma [*Ho T, Reveille J, 2003*] and 5-7.1% of patients with juvenile systemic scleroderma [*Martini G et al., 2006*]. The presence of anti-centromere antibodies in adult patients with systemic scleroderma is associ-

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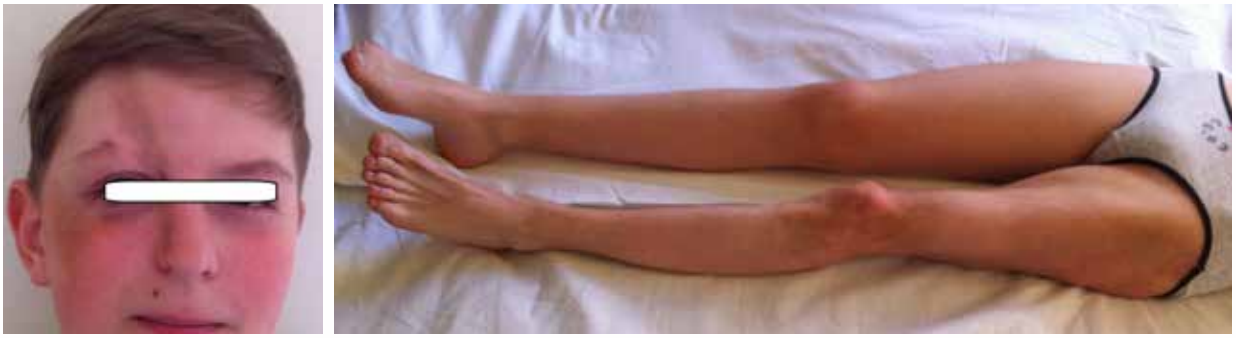

Figure 1. *Varieties of juvenile limited scleroderma:* ***a)*** *Scleroderma presenting on the face called "a blow of the sword",* ***b)*** *linear form with shortening and deformity of the left leg*

ated with the limited cutaneous form, calcification, the CREST syndrome, Raynaud's syndrome, and digital ulcers, as well as with affection of internal organs, specifically esophagus hypotension and pulmonary hypertension. Scl-70 antibodies are found in 21-30% of adult patients and in 23-34% of children with systemic scleroderma [*Martini G et al., 2006; Herrick A et al., 2010*]. The presence of Scl-70 antibodies correlates with diffuse cutaneous form and with the development of interstitial pulmonary fibrosis [*Kayser C, Fritzler M, 2015*]. A prolonged cohort study showed that patients with anti-centromere antibodies have a better 10-year survival rate as compared with patients with systemic scleroderma who have Scl-70 antibodies [*Ferri C et al., 2002*]. Anti-centromere antibodies and Scl-70 are mutually exclusive antibodies and can be found together in the same patient with systemic scleroderma in less than 0.5% of cases [*Ho T, Reveille J, 2003*].

The detection rate of these antibodies is different depending on the methods they are determined by: immunodiffusion and enzyme immunoassay methods. Anti-centromere antibodies are characterized by a larger detection potential in white patients as compared to African-Americans, Latinos, Thais. It is also known that the level of Scl-70 antibodies may reduce in parallel with the scleroderma reduction activity. This is even accompanied by their seronegative conversion, while the values of anti-centromere antibodies remain unchanged during the clinical course [*Ho T, Reveille J, 2003; Kayser C, Fritzler M, 2015*]. The mentioned facts seem to greatly affect the results of studies conducted in various rheumatological centers.

Antinuclear antibodies, rheumatoid factor, antinuclear factor, anti-double-stranded DNA antibodies and anti-histone antibodies were detected not only in the systemic [*Kayser C, Fritzler M, 2015*], but also in the limited form of scleroderma [*Falanga V et al., 1987; Takehara K, Sato S, 2005; Arkachaisri T et al., 2008; Arkachaisri T, Pino S, 2008*]. Thus, according to some authors, the antinuclear factor is detected in 46-80%, anti-double-stranded DNA antibodies – in 50%, anti-histone antibodies – in 47%, rheumatoid factor – in 26% of adult patients with limited scleroderma [*Chung L et al., 2006*]. There are some data stating that antinuclear factor is detected in 1/3 of the patients with linear form of limited scleroderma and directly correlates with joint lesions [*Gilliam A, Gilliam A, 2012*].

Cryoglobulins are a heterogeneous group of immunoglobulins, characterized by the ability to abnormal precipitation or gel formation at temperatures below 37°C. Patients with rheumatic conditions often have mixed cryoglobulins, composed of Class M and rheumatoid factor immunoglobulins, Class G polyclonal immunoglobulins and fibronectin. The level of cryoglobulins correlates with clinical and laboratory activity of scleroderma and may be used as an easy-to-do, cheap and accessible test for detection of laboratory activity of the disease.

Mechanisms of collagen and fibrosis production in scleroderma are very complex. Excessive accumulation of connective tissue components occurs due to the fibroblast overproduction, which are activated by hypoxia, reactive oxygen species, biochemical and mechanical signals coming from the extracellular matrix, in parallel with the damaged mechanisms of natural collagen degradation. The extracellular matrix of skin, tendon and bone

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tissue is composed mainly of type I collagen, and of type III collagen to a lesser extent. Type II collagen is mostly located in the articular cartilage, collagen IV – in basal cell membranes. Some researchers detect type I and IV collagen molecule autoantibodies in systemic scleroderma [*Kayser C, Fritzler M, 2015*].

As the result of monocyte overproduction, an intensive secretion of monokines, such as fibronectin and interleukin-1 are stimulated. Fibronectin is a chemotactic factor for fibroblasts, and interleukin-1 can stimulate the proliferation of collagen cells. Fibronectin is a high molecular weight glycoprotein which performs numerous functions for the organization of the extracellular matrix and cellular interactions. It has a high affinity to the native and denatured collagen. The intensive processes of collagen and fibrosis production due to the increased synthesis of proteoglycans, glycosaminoglycans (which include fibronectin) and collagen are one of the main elements in the pathogenesis of scleroderma. There are investigations that show an increased level of fibronectin in scleroderma and the possibility of its use as a biomarker of fibrosis along with hyaluronic acid and other substances the extracellular matrix contains [*Varga J et al., 2012*]. Hyaluronic acid is a type of glycosaminoglycans, which is a chain of repeating disaccharide units containing glucosamine-glucuronic acid and N-acetylglucosamine. Hyaluronic acid belongs to the innate immune system. It is an important component of articular cartilage, skin and is involved in the regeneration of tissue, migration and proliferation of cells and interacts with its CD44 primary surface receptor. According to the literature data, patients with scleroderma have increased hyaluronic acid level in the blood serum [*Yoshizaki A et al., 2008; Varga J et al., 2012*].

Material and methods

Totally 225 patients with juvenile scleroderma who were under supervision at the specialized rheumatology department of I.M. Sechenov First MSMU Children's Clinical Hospital for a five-year period from 2006-2011 were examined. The surveyed group consisted of children from 3 to 17 years of age, including 162 girls and 63 boys.

The diagnostics of systemic scleroderma was performed using the preliminary diagnostic criteria [*Zulian F et al., 2007a*]. The patients with limited scleroderma were divided into groups based on the clinical forms of the disease in accordance with the preliminary classification criteria [*Zulian F, 2008*]. Thirty five children (I group) were diagnosed with juvenile systemic scleroderma, among them 32 children were with diffuse form and 3 with limited cutaneous form. II group consisted of 190 children with juvenile limited scleroderma, among them 14 patients were with limited patchy form of scleroderma, 89 patients with the linear form, 77 children with diffuse patchy form, 3 patients with pansclerotic form, and 7 children with mixed form of scleroderma (Table 1).

As the table shows, the vast majority of the surveyed patients (84.5%) were children with limited scleroderma. This fact reflects the predominance of limited forms over systemic disease in childhood. Children with severe juvenile limited scleroderma prevailed in II group. They had diffuse and linear cutaneous forms, which are characterized by involvement of underlying tissue, i.e. subcutaneous tissue, muscles, joints and bones into the pathological process.

At the time of examination the patients with different clinical forms of the disease had dissimilar length of clinical activity. Only 76 children (13.7%) of all the patients did not receive basic therapy.

The conducted investigation included the following laboratory tests: determination of antinuclear factor, cryoglobulins, antibodies to collagens of I-IV types, rheumatoid factor, anti-topoisomerase (Scl-70) and anti-centromere B antibodies, double-stranded DNA antibodies, fibronectin, glucuronic acid in blood serum.

Levels of Scl-70 and anti-centromere antibodies were measured in the serum of 1/3 patients by double radial immunodiffusion testing. The other patients underwent enzyme immunoassay test using kit "Anti-Scl-70 Orgentec", (Germany). The absence of antibodies was considered as the reference value. Antinuclear factor was determined by indirect immunofluorescence. In the test with cryostat sections of liver and kidney of rats the reference titer was >1:40, with HEp it was 2>1:160. The rheumatoid factor was determined by ELISA test with the normal values of <20 *IU/mL*, and anti-DNA was determined by ELISA test with the reference value of <20 *IU/mL*.

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Unconjugated concentration of fibronectin in serum was measured by ELISA testing with the use of assay kit "Fibronectin Technoclone", (Austria) on "Multiscan" analysis medical system (Microplate photometer "Thermo Fisher Multiscan™ FC", Germany. Normal values of unconjugated fibronectin are 70 to 148 *mg/ml* according to the manufacturer's recommendations.

The concentration of hyaluronic acid in blood serum was measured by ELISA testing using "Hyaluronic Acid (HA) Testkit Corgenix", (USA). The level of hyaluronic acid in children was considered to be high when its concentration exceeded 30 *ng/ml* in accordance with the recommendations of the manufacturer.

The presence of cryoglobulins in blood serum was determined with "Solar" PV 1251C (Solar, Belarus) spectrophotometer at a wavelength of 500 *nm* in accordance with the optical fluid density difference in a buffer solution (pH 8.6), incubated for 1 hour at the temperature of 4°C and then at 37°C [*Konstantinova A, 1999*]. The normal values are up to 0.06 optical density.

Determination of type I-IV collagen antibodies in blood serum was performed with ELISA method using "IMTEK" medical sets (Russia), by dilution of serum at 1:50. The results were evaluated on "Multiscan" ("Thermo Fisher Multiscan™ FC", Germany) device at a wavelength of 450 *nm*. The test was considered positive if the negative control value exceeded.

To interpret the results of the research the concept of "positive" was introduced for this test. The results were thought to be "positive" if they showed exceeding reference values in the tests with quantitative expression, presence of Scl-70 and anti-centromere antibodies in blood serum, antinuclear factor titer of 1:80 or higher.

Statistical analysis of the results was performed with the use of Statistica 6.0 software. The quantitative indices were presented as mean values ± standard deviation and range of values. Quality indices were presented as an absolute number of observations and proportion (in %) of the total number. The validity of differences in the compared values was determined by Student's t-test for interval variables. The differences were considered statistically significant at $p<0.05$.

Results and discussion

The absolute value (fluctuation range) of antinuclear factor, cryoglobulins, type I-IV collagen antibodies, rheumatoid factor, DNA antibodies, fibronectin and hyaluronic acid levels in serum, as well as the percentage of positive results for the above indices and Scl-70 and anti-centromere antibodies in I and II group of patients are presented in table 2.

The analysis of the data suggests that patients in both groups were "positive" in all the required tests. At the same time, the absolute percentage of positive values was higher in the group with juvenile systemic scleroderma for all the indices except collagen antibodies (differences are not statistically valid) (Fig. 2).

The cohort study of patients with juvenile scleroderma showed that antinuclear factor was detected in 81.5% of patients with systemic scleroderma, and in 45% of patients with limited scleroderma. The obtained data are agreed with the results of the international multicenter studies of children, in which the proportion of antinuclear antibodies detection in children with systemic scleroderma ranges from 81% to 97% [*Foeldvari I, 2012*] and it amounts to 42.3% in patients with limited scleroderma [*Zulian F, 2012*].

According to the literature data, there is no connection between antinuclear factor detection and any clinical form of juvenile limited scleroderma [*Gilliam A, Gilliam A, 2012*]. Cryoglobulins were

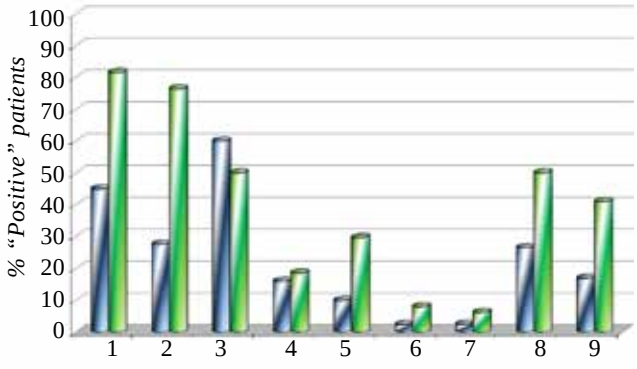

***Figure 2**. Detection rate of autoantibodies and fibrosis markers in juvenile systemic and limited scleroderma*

***Notes:** **1** – antinuclear factor, **2** – cryoglobulins, **3** – anti-collagen, **4** – rheumatoid factor, **5** – Scl-70 – topoisomerase I, **6** – anti-centromere antibodies, **7** – anti-DNA, **8** – fibronectin, **9** – hyaluronic acid, Juvenile limited (■) and Juvenile systemic (■) scleroderma.*

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Table 1

Distribution of examined patients according to the clinical forms of the disease

Type of scleroderma	Clinical form of the disease	Number of patients			
		Suffering	Men	Women	Total
Juvenile systemic	Diffuse cutaneous scleroderma	32	1	34	35
	Limited cutaneous scleroderma	3			
Juvenile limited	Limited patchy form	14	62	128	190
	superficial	4			
	deep	10			
	Linear form of	89			
	body and extremities	58			
	face	31			
	Diffuse Patchy form (including hemitype*)	77 (31*)			
	Panssclerotic form	3			
	Mixed form	7			

Table 2

Values of laboratory test indices in patients with juvenile systemic and limited scleroderma

Indices	I Group (n=28)			II Group (n=190)		
	The range of absolute values	Mean values (M±m)	% positive	The range of absolute values	Mean values (M±m)	% positive
Antinuclear factor* (titer)	1:80-1:1250	_	81.5	1:80-1:640	_	45
Cryoglobilins* (optical density unit)	0.048-0.405	0.127±0.102	76.4	0.037-0.216	0.051±0.037	27.6
Collagen antibodies (*mkg/ml*) I type*	0.199-0.514	0.363±0.087	62	0.297-0.893	0.405±0.112	66
Collagen antibodies (*mkg/ml*) II type*	0.215-0.693	0.460±0.142	33	0.330-1.129	0.551±0.161	52
Collagen antibodies (*mkg/ml*) III type*	0.252-0.615	0.379±0.097	79	0.287-0.681	0.370±0.108	72
Collagen antibodies (*mkg/ml*) IV type	0.152-0.362	0.252±0.060	46	0.151-0.577	0.300±0.085	51
Rheumatoid factor* (*IU/ml*)	8-140	80.6±52.2	18.6	11-125	82.4±60.3	16
Anti Scl-70*	-	-	29.7	-	-	10.5
Anti-centromere B antibodies*	-	-	8	-	-	2.4
ds-DNA*(*IU/ml*)	0-164	46.1±40.5	6.2	0-62	34.5±36.2	2,5
Fibronectin in serum* (*mg/ml*)	72-564	163.2±94.4	50	68-264	124.8±41.9	26.5
Hyaluronic acid* (*ng/ml*)	2.4-64.6	32.7±12.35	41	5.4-68.4	15.7±16.7	17

Note: ** – differences between I and II groups are not statistically valid (p>0.05)*

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the second most frequently detected element in juvenile systemic scleroderma and it was detected in 76.4% of patients, while its detection rate was less than 30% in patients with juvenile limited scleroderma, which indicates the high immunological clinical activity in the systemic form of scleroderma. Increased level of cryoglobulins in juvenile limited scleroderma confirms the involvement of immune mechanisms in the pathogenesis of this clinical form. There were patients with a positive rheumatoid factor in both groups with the same percentage of "positivity", which was 18.6% in systemic scleroderma and 16% in limited scleroderma. The results of rheumatoid factor detection in present study are the same as those in international studies [*Martini G et al., 2006; Zulian F et al., 2006*], in which the rheumatoid factor was detected in 17% of children with systemic and in 16% – with limited scleroderma. Besides, the authors suggest a significant positive correlation between the detection rate of the rheumatoid factor and joint affection in scleroderma. It is also known that the percentage of the rheumatoid factor detection in scleroderma is higher (23%) in adult patients [*Foeldvari I, 2006*].

Scl-70 and anti-centromere scleroderma-like antibodies were detected in both groups of patients. So, Scl-70 was found in 29.7% and anti-centromere antibodies – in 8% of patients with systemic scleroderma, and limited scleroderma – in 10.5% and 2.4%, respectively. The number of limited scleroderma patients with Scl-70 was significantly greater in present study as compared with the foreign source data mentioning 2-3% [*Martini G et al., 2006; Zulian F et al., 2006*]. This can be explained by the fact that there are more children with linear and diffuse forms of the disease among the surveyed patients with limited scleroderma. Hemitype lesions were found in 31 children with the involvement of musculoskeletal structures, contractures of the large joints, severe scleroatrophic limb deformities into the pathological process. So far, the pediatric rheumatologists have not recognized the presence of the systemic form of the disease in the mentioned categories of patients in the absence of visceritis and Raynaud's syndrome [*Zulian F, 2012*].

DNA antibodies were found in a small number of patients in both groups, specifically in 6.2% of children with systemic and in 2.5% of children with the limited form. This coincides with the data of other authors [*Foeldvari I, 2006; Gilliam A, Gilliam A, 2012*]. However, studies of DNA antibodies in adult patients with scleroderma have shown that the presence of these antibodies predisposes to the development of systemic forms of scleroderma, diffuse cutaneous scleroderma. The presence of DNA antibodies in the examined patients with juvenile limited scleroderma is an additional indicator of immunological aggressiveness of the disease, which makes the inclusion of basic therapy reasonable.

Increased fibronectin and hyaluronic acid in blood serum had a higher detection rate in systemic form of the disease, whereas the "positive" results in children with limited scleroderma were identified in 41% and 17%, respectively.

The detection rate of type I-IV collagen antibodies in patients from different clinical groups is presented in figure 3.

Collagen antibodies were the only group of antibodies, which had a higher percentage of detection rate in juvenile limited scleroderma cases than in juvenile systemic form. It is believed that the reason for this may be the significant extent and depth of the affected skin, muscles, tendons, periarticular tissue, which occurred in the patients with limited scleroderma from the surveyed group. The detection rate of type I and III collagen antibodies was dominant in the surveyed patients, which confirms the activity of autoimmune inflammatory reactions in the skin.

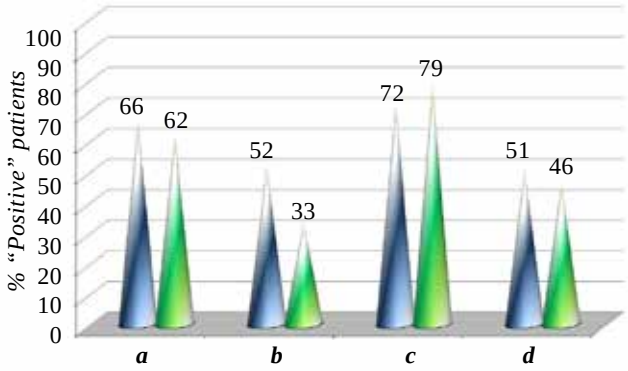

Figure 3. *Detection rate of antibodies to type I-IV collagens in patients with juvenile systemic and limited scleroderma*

Notes: ***a****- Anti-collagen I* ***b****-Anti-collagen II,* ***c****-Anti-collagen III,* ***d****-Anti-collagen IV, Juvenile limited (■) and Juvenile systemic (■) sclerodermas.*

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Conclusion

Thus, the detection of autoantibodies and biomarkers of fibrosis in both juvenile systemic and limited sclerodermas indicates the common characteristics of pro-fibrous and autoimmune mechanisms of pathological process development. Immunological disorders and increased levels of fibronectin and hyaluronic acid in patients with different clinical forms, prior to and after basic therapy inclusion, have a great clinical significance. In systemic scleroderma cases clinicians rarely doubt the wisdom of including corticosteroids and cytotoxic drugs in the therapy. In contrast, the treatment of limited forms of juvenile scleroderma is believed to have successful outcomes with the topical use of immunosuppressive agents. The detection of autoantibodies and markers of fibrosis involved in the pathogenesis of scleroderma also determines the target points for the treatment of juvenile limited scleroderma with inclusion of antifibrotic agents and immunosuppressants in the basic therapy.

It should be emphasized that the chronic nature of scleroderma, the prevalence of linear and diffuse forms of the disease in children, complicated by the development of specific irreversible fibrosclerotic defects of the musculoskeletal system, encourage pediatric dermatologists and rheumatologists to elaborate a unified tactics for the treatment of patients with juvenile limited scleroderma relying on effective methods of evaluating the clinical activity and the disease progression. The spectrum autoantibodies and markers of fibrosis in juvenile systemic and limited scleroderma have been studied in order to justify the relevance of basic therapy inclusion in limited scleroderma.

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