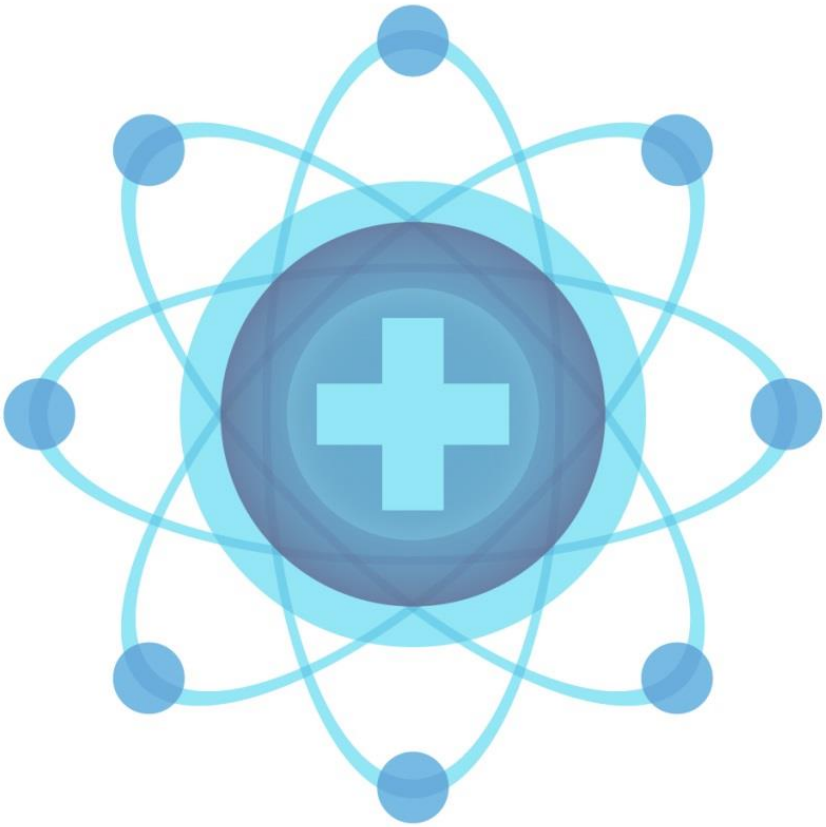

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The role of endothelial dysfunction in the development of immune microthrombovasculitis

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Abstract: comparative assessment of endothelial dysfunction indicators in patients with IMTV depending on the form of the disease and the characteristics of its course.
Material and methods. We examined 140 patients with BMI at the age of 18-62 years. All patients, depending on the clinical manifestations of the disease, were subdivided into 4 groups: 1st - 32 patients with skin, 2nd - 84 patients with skin-articular, 3rd - 12 patients with mixed skin-articular and abdominal and 4th group - 12 patients with generalized skin-articular and renal forms of IMTV. The control group consisted of 20 conditionally healthy individuals. The indices of the vascular-platelet, coagulation link of hemostasis, the anticoagulant system were assessed according to generally accepted methods. Endothelial dysfunction was assessed by the level of endothelin 1 (ET-1), von Willebrand factor (vWF), thrombomodulin and adhesion molecules (sICAM-1)) by enzyme immunoassay. The digital material was processed by the method of variation statistics. Results. The study of the vascular-platelet link of hemostasis in patients with IMTT showed a tendency to develop moderate thrombocytosis and an increase in their thrombogenic activity. The study of three phases of the coagulation link of hemostasis indicated pronounced hypercoagulation in all groups. The dynamics of changes in hemostasiological parameters showed their relationship with the activity and severity of IMTV. A pronounced endothelial dysfunction was revealed, manifested by an increase in the content of ET-1, vWF, sICAM-1, and thrombomodulin in the blood plasma. The severity of these changes is minimal in the cutaneous form of the disease and sharply increases in its generalized form.. **Output:** In patients with IMTV, depending on the form of the disease, dysfunction of endotheliocytes of microvessels develops with inhibition of anticoagulant and increased procoagulant components.

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Keywords: immune microthrombovasculitis, vascular endothelium, endothelin 1, von Willebrand factor, adhesion molecules, thrombomodulin, hemostasis.

Among the general human pathology, there is an increase in the incidence of hemorrhagic diathesis (HD). HD is characterized by widespread distribution, a variety of clinical manifestations, and a high incidence of severe hemorrhagic and thrombohemorrhagic complications, which are often fatal. In the general structure of HD, in terms of the frequency of its occurrence among all populations and age groups of the population, polymorphism and severity of the clinical course, a special place is given to pathologies of the primary hemostasis of an acquired nature, including immune microthrombovasculitis (IMTV) [3, 4, 5, 7, 21] ... The annual incidence of IMTV is 2 people per 10,000 population and there is an increase in the number of patients [6, 9]. The pathogenesis of the disease is associated with overproduction of low molecular weight circulating immune complexes and the deposition of granular IgA deposits in the vascular wall [1, 10, 13]. This leads to the activation of the complement system, an increase in the permeability of the vessels of the microvasculature and with the involvement of the hemostatic system in the process. As a result, the rheological properties of blood are disturbed, platelet aggregation increases, and hypercoagulability syndrome with depression of the fibrinolytic system develops [1, 8, 10, 11]. According to the literature, the leading role in the development of predisposition to this pathology belongs to the polymorphism of genes of proinflammatory cytokines, genes of the angiotensin and endothelial systems [2, 16, 17]. The study of the formation mechanisms of IMTV is a priority in a number of large research centers of the world [12, 14, 19]. In the development of this multifactorial disease, an important place is given to both the individual characteristics of the organism, the influence of exo- and endogenous factors, the intensity of which largely determines the risk of its formation [15, 18, 20].

The expansion of innovative strategies and the introduction of high technologies in our Republic allow a targeted approach to the development of the foundations of evidence-based medicine, including such important aspects as the

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mechanisms of formation, the development of effective methods for predicting the development of a severe course of the disease and the risks of developing complications of IMTV. The introduction of methods for determining endothelial dysfunction made it possible to decipher the main mechanisms of the development of pathological conditions, develop criteria for early and differential diagnosis and improve the tactics of treating various diseases manifested by endothelial dysfunction. However, in the literature, there are sporadic data on the presence of endothelial dysfunction with IMTV.

Purpose of the study: comparative assessment of endothelial dysfunction indicators in patients with IMTV, depending on the form of the disease and the characteristics of its course.

Material and research methods.

The study included 140 patients with an established diagnosis of IMTV. Verification of the BMI diagnosis was carried out according to modern classification criteria. The selection of patients was carried out by the method of random sampling as they approached. The age of the patients with BMI ranged from 18 to 62 years (median age 32.1 ± 3.9 years), mostly young and mature people (86.4%). Analysis of the distribution of patients by age and sex showed that women predominated among the patients, and there were almost 2 times less men.

All patients examined by us, depending on the clinical manifestations of the disease, were subdivided into the following forms of IMTV: 32 (22.8%) patients had a skin form (group 1), 84 (60.0%) patients had a skin-articular form (group 2), 12 (8.6%) had mixed skin-articular and abdominal form (group 3) and 12 (8.6%) had generalized skin-articular and renal form (group 4). The control group consisted of 20 conditionally healthy unrelated persons who did not have a history of hemostasis system pathology, corresponding in terms of sex and age to the examined main group of patients.

The diagnosis and clinical form of the disease were established on the basis of complaints, history of life and disease, clinical symptoms and laboratory data.

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Particular attention was paid to objective data and anamnesis of the disease: periodic appearance of small-point cutaneous hemorrhages, leaving behind hyperpigmentation, pain in the joints, edema, pain in the stomach during the onset of hemorrhages, blood in the urine and feces, general weakness, other symptoms and the reasons with which the patient associates the development of the disease. Upper respiratory tract infections (56.4%) and drug intake (22.1%) were provoking factors for the development of IMTT. 48.6% of patients had a history of allergic diseases, and 16.4% of patients noted the presence of hereditary diseases of the blood coagulation system.

To assess the state of the vascular-platelet link of hemostasis, the number of platelets was counted according to Fonio in peripheral blood smears by the method of phase-contrast microscopy, the aggregation of platelets was studied using the hemolysate-aggregation test in dilutions 10-2, 10-6 according to ZS. Barkagan, B.F. Arkhipov and V.M. Kuchersky (1980), platelet adhesion on fiberglass, blood clot retraction in a test tube according to V.P. Baluda et al. (1980). To study the coagulation link of the hemostasis system, the activated partial thromboplastin time (APTT), prothrombin index (PTI), thrombin time (TB), soluble fibrin-monomer complexes (RFMK), the amount of fibrinogen) were determined on a HumaClot Junior coagulometer (Germany) and anticoagulant system blood by determining the activity of antithrombin III (AT III) and XII-α dependent fibrinolysis according to G.F. Eremin and A.G. Arkhipov (1982), using reagents of NPO RENAM (Russia).

Determination of indicators of endothelial dysfunction (endothelin 1 (ET-1), von Willebrand factor (vWF), thrombomodulin and adhesion molecules (sICAM-1)) was determined on a HumaReader HS (Human) enzyme immunoassay analyzer using commercial ELIZA kits from Human. All statistical analyzes were performed using SAS software. Quantitative variables were presented as mean ± standard deviation. In addition, when analyzing the correlations, the Spearman rank test was used. $P < 0.05$ was considered statistically significant.

Results and its discussion

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Symmetrical petechial hemorrhagic rashes on the legs up to the knee were present in 32.3% of patients in group 1 with the cutaneous form of BMI, in 29% - also petechiae on the hips and buttocks, in 29% - in most parts of the body. In severe cases, maculopapular rash with necrotic changes in the skin in the center and the appearance of small ulcers was observed in 16.1% of patients. At first, rashes appeared on the feet and legs, later they spread higher, the abundance of rashes correlated with the severity of BMI. The duration of the disease in patients with the cutaneous form was the shortest and amounted to 15.0 ± 2.3 days.

In group 2 patients, along with skin lesions in the form of a symmetrical hemorrhagic rash, joint damage was also observed. The articular syndrome was expressed by pain and periarticular swelling, redness, disorder of motor functions, mainly in large joints. Basically, 61.9% of patients had ankle joint damage, in 22.6% of cases - ankle and knee joint damage, in 15.5% of cases other joints were also affected. Symmetrical involvement of the joints was observed; joint deformities with impaired function were not observed. The duration of the disease in patients with skin-articular form averaged 8.7 ± 1.8 months.

In group 3 patients with a mixed skin-articular and abdominal form of the disease, in addition to damage to the skin and joints, damage to the gastrointestinal tract was also observed, resulting from hemorrhage in the intestinal wall and mesentery. Patients complained of vomiting, cramping abdominal pains like intestinal colic, tension and tenderness of the abdomen on palpation, but they could not pinpoint the location of pain. In 25% of patients, vomiting, abdominal pain preceded skin-articular symptoms, which made diagnosis difficult. During esophagoduodenoscopy in patients of this category, petechial rashes were observed in the mucous membranes of the stomach and duodenum 12. In 16.6% of patients, the abdominal form was complicated by intestinal bleeding, simulating acute surgical diseases of the abdominal cavity. The duration of the disease with a mixed skin-articular and abdominal form of IMTV was more than 2 years, proceeded with relapses in case of violation of the diet.

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In group 4 of patients with a mixed skin-articular and renal form of IMTV, there was kidney damage of varying degrees: in 50% of patients there was short-term unstable hematuria up to 2-3 days, in 33.3% - persistent but unexpressed hematuria up to 5-7 days, and 16.7% had pronounced gross hematuria for more than 10.0 ± 0.7 days. Chronic renal failure was established in 16.7% of patients in this group. The duration of the course of this form of IMTV was about 1.5 years.

The study of the vascular-platelet link of hemostasis in patients with IMTV showed a tendency to develop moderate thrombocytosis (from 308 ± 32.3 x 109 / L to 453 ± 56.1 x 109 / L), an increase in thrombocrit from 0.26 ± 0.02% to 0 , 45 ± 0.02%. The study of the functional activity of platelets showed an increase in thrombogenic activity of platelets, manifested by an increase in the adhesive and aggregation properties of platelets by 18.6-42.7%, a shortening of the retraction time of a blood clot by 12.5-25.0%.

Analysis of the plasma link of hemostasis showed a pronounced shortening of the blood coagulation time and APTT, prothrombin time, thrombin time, INR, an increase in the prothrombin index, the amount of fibrinogen, and plasma tolerance to heparin. The study of the three phases of the coagulation link of hemostasis indicated pronounced hypercoagulation in all groups in relation to the control group. The dynamics of changes in hemostasiological parameters showed their relationship with the activity and severity of IMTV.

Currently, the immunocomplex nature of IMTV has been proven, in which aseptic inflammation develops in microvessels with wall destruction, thrombosis and the appearance of purpura of various localization due to the damaging effect of circulating immune complexes and activated components of the complement system [2, 11, 20]. Expression of such pro-inflammatory cytokines as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) leads to an increase in the synthesis of vascular growth factor (VEGF) by endothelial cells during the acute phase of the disease [3, 6, 21] ... In this regard, we investigated the indicators of the functional activity of the endothelium showed the development of its dysfunction (Table 1). Thus,

dysregulation of vascular tone during IMTV is associated with an increase in the production of endothelial peptides, in particular ET-1, the content of which in the blood serum of patients increases statistically significantly increases depending on the form of the disease. If in the cutaneous form of BMI the content of ET-1 exceeds the standard level by 15.8%, then in the cutaneous-articular, abdominal and renal forms this excess was 28.5; 30.2 and 67.1%, respectively, groups, indicating the development of vasoconstriction in patients. It should be said that at low physiological concentrations, ET-1 has an effect on endothelial receptors, causing the release of relaxation factors. At the same time, at high concentrations, it activates smooth muscle cell receptors and causes persistent vasoconstriction.

Table 1

Indicators of endothelial dysfunction in patients with IMTV, M ± m

Groups	Von Willebrand factor, %	Endothelin-1, pg / ml	Thrombomod-lin, pg / ml	sICAM-1, pg / ml
Control group, n = 20	89,89±2,56	4,10 ± 0,24	3,54±0,28	55,69±4,44
ИМТВ				
Skin form, n = 8	133,75±7,46*	4,75±0,54	2,44±0,22**	69,95±4,94*
Skin-articular form, n = 18	145,17±5,60**	5,27±0,36*	2,18±0,12***	86,00±7,04***
Abdominal form, n = 12	188,00±3,29***	5,34±0,53*	1,93±0,17***	110,80±11,66**
Renal form, n = 7	191,41±5,52***	6,85±0,52**	1,56±0,15***	146,83±12,86***

Note: * - the differences between the indices of the control group of the examined and the patients with IMTV are significant (P <0.05), ** - P <0.01 and *** - P <0.001.

Along with this, we also revealed the content of vWF: exceeding the standard values by 48.8; 61.5; 109.1 and 112.9% in groups of patients with skin, skin-articular, abdominal and renal forms of the disease, respectively groups. This factor is a carrier-stabilizer for the procoagulant protein FVIII: C circulating in the serum in the form of a non-covalently bound complex, and is an adhesion protein during hemostasis [11]. Therefore, elevated vWF levels are an indicator of endothelial damage. On the other

hand, the increased formation of collagen in the vascular wall leads to a loss of their elasticity, the ability of the vessels to dilate decreases, which is manifested by the inhibition of anticoagulant and increased procoagulant properties [3, 5]. The pathogenesis is based on the formation of immune complexes and the activation of components of the complement system, which have a damaging effect on the vascular wall [4, 6]. The main initiators of endothelial damage in PSG are cytokines, which are involved in the activation of neutrophils. IL-8, which activates neutrophil epithelial protein (ENA-78), and T-lymphocytes are involved in providing chemotaxis of neutrophils to sites of inflammation [3, 5, 20]. Circulating immune complexes cause vasculitis, which is accompanied by perivascular edema, microcirculation disorders, and hemorrhages. Damage to the vascular wall leads to activation of the hemostasis system: functional activity of platelets, hypercoagulation, a decrease in the level of antithrombin III [3, 5].

According to the literature, with thrombophilia, the content of homocysteine in the blood plasma increases [3, 6]. According to B.I. Kuznik et al. (2012), hyperhomocysteinemia is the leading pathogenetic mechanism of endothelial dysfunction [6]. Its oxidized form activates the formation of free radicals, inhibits the synthesis of antioxidant enzymes in endothelial cells. As a result, there is damage to the endothelial lining of blood vessels, proliferation of smooth muscle cells, activation of platelets and a decrease in the content of heparan sulfate, which is an antithrombin III receptor (AT-III). At the same time, the binding of AT-III to endothelial cells decreases, which disrupts the atrombogenicity of the inner layer of the endothelium [3, 6]. Along with this, the activation of leukocytes, the release of cyto- and chemokines, and the expression of adhesion molecules are noted.

In this regard, we also studied the content of serum sICAM-1. Studies have shown an increase in its content by 26 and 54.4% in cutaneous and skin-articular forms of IMTV. In the generalized form of BMI, the level of sICAM-1 increases more pronounced: in the abdominal form by 99.89%, in the renal form - by 163.7% relative to the values of practically healthy individuals. These indicators were

significantly higher than the values of groups with skin and skin-articular forms of IMTV. Therefore, we can say an increase in the adhesive properties of leukocytes. Under physiological conditions, the endothelial cell does not express adhesion molecules. An increase in the concentration of the latter on the surface of endothelial cells occurs under the action of various damaging factors: an increase in the linear shear stress in a certain part of the artery, the accumulation of oxidized lipids and lipoproteins in the subendothelial space [3, 6]. sICAM-1 of fibroblasts and endothelial cells is induced by inflammatory mediators. The interaction of leukocyte β2-integrin with sICAM-1 accelerates the adhesion of leukocytes and their transendothelial migration.

It should be said that vascular endothelial dysfunction leads to a decrease in the activity of protein C (PC) and protein S (PS), a decrease in the affinity of thrombomodulin for thrombin, leading to a sharp drop in the activity of natural anticoagulants [3]. Thrombomodulin is a receptor for thrombin expressed on endothelial cell membranes. When interacting with thrombin, the resulting thrombomodulin / thrombin complex activates protein C, i.e. thrombomodulin carries out anticoagulant regulation, since active protein C inactivates factors fVa, fVIIIa, fXa and fXIIIa, inhibits the conversion of fibrinogen to fibrin, accelerates thrombin inactivation by antithrombin III.

In this regard, we also studied the content of thrombomodulin in the blood plasma of patients with IMTV. Studies have shown a decrease in its content by 31.1; 38.4; 45.5 and 55.9%, respectively, in the groups with cutaneous, skin-articular, abdominal and renal forms of BMI. It should be said that the concentration of thrombomodulin increases with an increase in the ratio of the surface of the vessels to the volume of blood. This ratio changes more than 1000 times when moving from large vessels to microcirculation. In microcirculation, almost all thrombin is associated with CD141, its clotting activity is suppressed and activation is increased. The concentration of the level of thrombomodulin in plasma indicates damage to the vascular endothelium.

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Thus, the conducted studies have shown dysfunction of the vascular endothelium in patients with IMTV. The severity of these changes is minimal in the cutaneous form of the disease and sharply increases in its generalized form. In patients with a severe form of IMTV, it is indicative of a hypercoagulable syndrome due to dysfunction of endothelial cells of microvessels with inhibition of anticoagulant and an increase in procoagulant components. Our data demonstrate the importance of studying specific inducers of platelet aggregation, indicators of the functional activity of the vascular endothelium in patients with IMTV.

Conflicts of Interest. The authors declare no conflicts of interest.

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References.

1. Berman Yu.O., Davydkin I.L., Krivova S.P. Folic acid in the treatment of patients with hemorrhagic vasculitis with hereditary disorders of homocysteine metabolism // Bulletin of the Samara Scientific Center of the Russian Academy of Sciences. - 2014. - Vol. 16, No. 5 (4). - P. 1384-1388.
2. Gulyaev S.V., Strizhakov L.A., Moiseev S.V., Fomin V.V. From Shenlein-Genoch purpura to IgA-vasculitis; pathogenetic aspects of the disease // Therapeutic archive. - 2018.– No. 10. - P.109-114.
3. Kireeva V.V., Koh N.V., Lifshits G.I., Apartsin K.A. Endothelial dysfunction as a cornerstone of cardiovascular events: molecular and pharmacogenetic aspects // Russian Journal of Cardiology. - 2014.- T.10 (114) .- P.64-68.
4. Kochetova E.V., Svetlova M.S., Koryakova N.V. Purpura Shenlein-Genokh: a textbook // Petrozavodsk: PetrSU Publishing House, 2017.- 23 p.
5. Kudryashova M.A., Podchernyaeva N.S., Frolkova E.V. The value of antigen indices of von Willebrand factor and D-dimer for assessing the activity of Shenlein-Genoch disease in children // Man and medicine: Sat. thesis. 21st nat. Congress.- M., 2014.- P.66-67.
6. Kuznik B.I., Sturov V.G., Maksimova O.G. Hemorrhagic and thrombotic diseases and syndromes in children, Novosibirsk: Nauka, 2012, 456 p.
7. Novikov P.I., Zykova A.S., Moiseev S.V. Modern therapy of systemic vasculitis: achievements and prospects // Therapeutic archive.- 2018.- No. 1.- P.76-85.
8. Raimzhanov A.R., Dzhakypbaev O.A. Hemorrhagic microthrombovasculitis: primary hemostasis and immunological parameters // Clinical hematology oncology. - 2016. - T.9 (1). - P.70–74.
9. Sinyachenko O.V., Gerasimenko V.V., Egudina E.D., Chernyshova O.E., Pomazan D.V. The defeat of the joints with Shenlein's purpura
10. Audemard-Verger A., Pillebout E., Gullevin L. et al. IgA vasculitis (Henoch - Shonlein purpura) in adults: diagnostic and therapeutic aspects // Autoimmun. Rev.– 2015.– Vol.10.– P. 3-7.
11. Babadjanova Sh.A., Islamova Z.S., Kurbanova Z.Ch., Yusufkhojaeva Kh.S., Inoyatova F.Kh. Disturbance of Coagulative Hemostasis among Patients with Henoch-Schonlein Purpura // Indian Journal of Forensic Medicine & Toxicology. - 2020. - Vol. 14 (4). - P. 7438-7442.
12. Garbe E., Andersohn F., Bronder E. et al. Drug-induced immune thrombocytopaenia: results from the Berlin Case-Control Surveillance Study, 2012.
13. Jarasvaraparn C., Lertudomphonwanit C., Pirojsakul K. et al. Henoch-Schönlein without purpura: a case report and review literature // J. Med. Assoc. Thai.- 2016.- Vol. 99 (4) .- P.441-445.
14. Juwon Kim. IL-1β-31 and IL-1Ra polymorphisms associated with increased host susceptibility to immune thrombocytopenia 2017;
15. Khanna G., Sargar K., Baszis K. W. Pediatric vasculitis: recognizing multisystemic manifestations at body imaging // Radiographics. 2015 Vol. 35 (3) P.849-865.

16. López-Mejíasa R., Castañeda S., Genrea F. et al. Genetics of immunoglobulin-A vasculitis (Henoch-Schönlein purpura): An updated review // Autoimmunity Reviews. - 2018. - Vol. 17 (3). - P.301-315.
17. Mahévas M., Fain O., Ebbo M. et al. The temporary use of thrombopoietin-receptor agonists may induce a prolonged remission in adult chronic immune thrombocytopenia. Results of a French observational study // Br J Haematol. 2014. Vol. 165. P.865-869.
18. Marieke H. Heineke, Aranka V. Ballering, Agnès Jamin et al. The era of the immunoglobulin A Fc receptor FcRI; its function and potential as target in disease Immunological Reviews. - 2015. - Vol. 268 (1). P. 123-138. DOI: 10.1111 / imr.12337.
19. Park C.H., Han D.S., Jeong J.Y. et al. The optimal cut-off value of neutrophil-to-lymphocyte ratio for predicting prognosis in adult patients with Henoch-Schönlein purpura // PLoS One. - 2016. - Vol. 11 (4). - E.0153238.
20. Topaloglu R, Sungur A, Baskin E, et al. Vascular endothelial growth factor in Henoch – Schonlein purpura. // J Rheumatol. - 2001. - Vol.28 (10). - P.2269-2273.
21. Vanesa Calvo-Río, José Luis Hernández, Francisco Ortiz-Sanjuán, Javier Loricera et al. Relapses in patients with Henoch – Schönlein purpura. Analysis of 417 patients from a single center // Medicine.- 2016.- Vol.95 (28) .- P.1-8.