КЛИНИЧЕСКИЙ СЛУЧАЙ
CASE REPORT

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THE EXPERIENCE OF USING DARK-FIELD MICROSCOPY TO ASSESS DAMAGE TO THE ENDOTHELIAL GLYCOCALYX IN RHEUMATOID ARTHRITIS

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◆ Rheumatoid arthritis is the most common inflammatory joint disease and one of the most common chronic inflammatory diseases. The leading role in the pathogenesis of rheumatoid arthritis is the damage to the endothelial glycocalyx — a thin dynamic layer of macromolecules located on the surface of the endothelium and consisting of proteoglycans, glycoproteins and glycosaminoglycans, which contributes to the maintenance of rheumatoid arthritis activity. Therefore, early detection of violations of the vasculr endothelium condition, especially the endothelial glycocalyx, will identify a group of patients with a poor prognosis. In this context, the method of dark-field microscopy may be promising. It allows non-invasive and *in vivo* assessment of the thickness of the sublingual endothelial glycocalyx, which will make it possible to search for new unconventional risk factors for the unfavorable course of rheumatoid arthritis and cardiovascular risk in these patients, as well as personalize treatment by developing a complex of preventive and therapeutic measures aimed at restoring endothelial function, reducing the risk of cardiovascular complications, disability and mortality from rheumatoid arthritis. The unique capabilities of this research method are demonstrated by the example of the clinical case.

◆ **Keywords:** rheumatoid arthritis; damage to the endothelial glycocalyx; dark-field microscopy; vasomotor dysfunction; laser Doppler flowmetry.

ОПЫТ ПРИМЕНЕНИЯ ТЕМНОПОЛЬНОЙ МИКРОСКОПИИ ДЛЯ ОЦЕНКИ ПОВРЕЖДЕНИЯ ЭНДОТЕЛИАЛЬНОГО ГЛИКОКАЛИКСА ПРИ РЕВМАТОИДНОМ АРТРИТЕ

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◆ Ревматоидный артрит является наиболее частым воспалительным заболеванием суставов и одним из наиболее распространенных хронических воспалительных заболеваний. Ведущую роль в патогенезе ревматоидного артрита отводят повреждению эндотелиального гликокаликса — тонкого динамического слоя макромолекул, расположенного на поверхности эндотелия и состоящего из протеогликанов, гликопротеинов и гликозаминогликанов, что способствует сохранению активности ревматоидного артрита. Именно

поэтому раннее выявление нарушений состояния сосудистого эндотелия, особенно эндотелиального гликокаликса, позволит идентифицировать группу пациентов с неблагоприятным прогнозом течения ревматоидного артрита. В этом контексте перспективным может оказаться метод темнопольной микроскопии, при помощи которого можно неинвазивно и прижизненно оценивать толщину подъязычного эндотелиального гликокаликса, осуществлять поиск новых нетрадиционных факторов риска неблагоприятного течения ревматоидного артрита и кардиоваскулярного риска у данных пациентов, а также персонализировать лечение, разработав комплекс профилактических и лечебных мероприятий, направленных на восстановление эндотелиальной функции, снижение риска сердечно-сосудистых осложнений, инвалидности и смертности от ревматоидного артрита. Уникальные возможности указанного метода исследования продемонстрированы на примере клинического случая.

◆ **Ключевые слова:** ревматоидный артрит; повреждение эндотелиального гликокаликса; темнопольная микроскопия; вазомоторная дисфункция; лазерная допплеровская флоуметрия.

Rheumatoid arthritis (RA) is a very common inflammatory joint disease [1] and is widely considered as one of the most prevalent chronic disease of its kind [2]. It imposes a major burden both for a patient and society as a whole, significantly increasing the disability and mortality rates in a population [2]. In recent years, the incidence of RA in the Russian Federation has increased, as confirmed by recent epidemiological studies [3]. Despite the decrease in overall mortality due to the application of modern anti-inflammatory therapy strategies [4], the continued investigation of RA remains relevant. This is due to the presence of risk factors for an unfavorable course of RA [5]. Another reason is the high risk of cardiovascular complications [4, 6], the identification of which would enable timely changes in the approaches employed to manage such patients [7].

The key link in the pathogenesis of RA is endothelial dysfunction [8], which is an imbalance between the production of vasodilating, angioprotective, and antiproliferative factors, on the one hand, and vasoconstrictive, prothrombotic, and proliferative factors, on the other hand [9]. A special role in the functioning of the endothelium is assigned to the endothelial glycocalyx (eGC), which is a thin, dynamic layer of macromolecules located on the endothelial surface and consisting of proteoglycans, glycoproteins, and glycosaminoglycans [10]. According to a recent study, the eGC is the major regulator of endothelial function and serves as a biological barrier, which is the first to be damaged during the development of autoimmune inflammatory and cardiovascular diseases [11]. Damage to the glycocalyx causes a disorder of the barrier function of blood vessels, increasing their permeability; in turn, this induces protein extravasation and tissue edema, changes the shear stress, enhances the adhesion of platelets and leukocytes to the vascular wall, changes blood flow, facilitates a prothrombotic effect, and triggers oxidative stress [10, 11]. These pathological processes contribute to the preservation of RA activity; therefore, the early detection of disorders of the vascular endothelium, especially the endothelial glycocalyx, enables the identification of poor prognosis in some patients. In turn, this can help in the design of personalized treatment by developing preventive and therapeutic measures aimed at restoring the endothelial function, reducing the risk of cardiovascular vascular complications, disability, and mortality resulting from RA. In this context, the methods of Doppler flowmetry and dark-field microscopy may be promising options. The use of both techniques can help in conducting in vivo and non-invasive assessment of the structure and function of the vascular endothelium and in searching for new, unconventional risk factors for an unfavorable course of RA.

Despite the fragility and instability of the glycocalyx, its assessment can also be used as a tool for diagnostics and monitoring of diseases [10]. One of the methods for visualizing the glycocalyx is dark-field microscopy, which allows the non-invasive and *in vivo* assessment of the sublingual eGC [12, 13]. The method is based on the measurement of the boundary area of eGC perfusion, that is, the degree of immersion of erythrocytes in the thickness of the vascular wall. In the future, the thickness of the glycocalyx can be calculated mathematically [12, 14]. This method can be a potential marker for the stratification of cardiovascular risks and the verification of pathological changes in the vascular bed [15]. For example, in a study of patients with cardiovascular diseases, this

was used as an auxiliary indicator for assessing the severity of coronary atherosclerosis [16]. In another study, dark-field microscopy was employed to identify an unfavorable prognostic value in case of a family history of cardiovascular diseases [17]. A correlation was also found between the borderline region of eGC perfusion and the level of syndecan-1 in the blood serum, thus indicating glycocalyx destruction [18].

Despite the fact that there are currently no data on the use of dark-field microscopy in RA, the results of scientific research and the opinions of Russian and international scientists [10, 15] enable the conduct of studies using dark-field microscop, thereby clarifying its place in assessing the state of the endothelium in RA patients. It is advisable to compare dark-field microscopy with the conventional method of laser Doppler flowmetry (LDF) for several reasons; it is a faster method for assessing vascular endothelium with a lower risk of errors, it is characterized by good implementability and tolerability by patients, and because it has good reproducibility of measurements, which do not depend on the field of study and the time intervals between measurements [19].

LDF is one of the preferred methods of screening for endothelial disorders, and it can be used to assess quickly and non-invasively the vasomotor function of the endothelium, which is the main argument when choosing this method [20]. In 2011, LDF was included in the list of recommended methods for assessing the state of the endothelium in humans [21]. According to Russian and international authors, this method is actively used to assess endothelial dysfunction in RA patients, as the presence of significant disorders of skin microcirculation has been proven during both the basal measurement of microcirculation [22] and after functional tests [23]. This method can also be used in the correlation of RA with acute phase indicators [24] and the degree of RA activity [25]. In some studies, a correlation was revealed between an increase in cardiovascular risk and coronary perfusion as well as the degree of impaired blood flow in the microvasculature during LDF [25, 26]. Thus, the assessment of peripheral circulation in LDF can be extrapolated to general microvascular function, including coronary blood flow, to predict the risk of cardiovascular complications.

This work aimed to demonstrate — using a clinical case as an example — the relationship of markers of endothelial glycocalyx damage with other indicators of endothelial dysfunction in RA, depending on the disease activity.

Patient M., 64 years old, was admitted to the hospital with complaints of constant aching pain in the wrist joints as well as the metacarpophalangeal, proximal interphalangeal, and metatarsophalangeal shoulder joints. The pain was experienced with the greatest severity in the second half of the night and in the morning, accompanied by morning stiffness for 5 hours. The anamnesis showed that at the age of 40, the patient first started to notice recurrent aching pains and swelling of the ankle joints, for which she did not consult a doctor. She independently took nonsteroidal anti-inflammatory drugs with a positive effect. Fifteen years later, she experienced pain in the small joints of the hands, accompanied by morning stiffness, for which she was hospitalized. During hospitalization, the diagnostic presentation was formulated as seropositive cyclic citrullinated peptide antibodies (CCPA)-positive erosive RA. The anamnestic assessment of the articular syndrome was 7 points according to the 2010 EULAR/ACR criteria. Sulfasalazine was prescribed at a dose of 2000 mg per day, along with prednisolone at a dose of 20 mg per day, followed by a dose reduction to 5 mg per day. During her intake, the patient noted an improvement in well-being in the form of decreased joint pain. However, with a decrease in the dose of prednisolone to 5 mg per day, pain in the wrist and small joints of the hands resumed; therefore, instead of sulfasalazine, methotrexate was prescribed at a dose of 10 mg per week. Despite the improvement in health, during therapy with methotrexate, a twofold excess of the upper limit of the norm of liver enzymes was recorded. Then, leflunomide was prescribed as a baseline therapy at a dose of 20 mg per day. The patient tolerated this therapy satisfactorily; she did not notice pain, swelling, or stiffness in the joints. In 2019, the patient developed leukopenia, so leflunomide was canceled. After achieving a normal number of blood leukocytes, methotrexate was prescribed again as a baseline therapy at a dose of 15 mg per week.

At the beginning of 2020, due to the deterioration in her condition, she was hospitalized at the Research Institute of Rheumatology and

Оценка эндотелиального гликокаликса с помощью темнопольной микроскопии
Evaluation of an endothelial glycocalyx using dark-field microscopy

Date	Density valid	Red blood cell filling, %	Perfused boundary region (PBR)	PBR at high flow	Microvascular health score TM
14.01.2020	406	75.4	1.99	1.66	2.54
22.01.2020	430	77.2	1.82	1.42	3.73

N o t e. Results of the dark-field microscopy of the sublingual mucosa vessels before (line 2) and after (line 3) the complex therapy are presented. Date: date of the study; Density valid: the number of vessels containing more than 50% of erythrocytes; Red blood cell filling: the average count of erythrocytes in the vessels studied; Perfused boundary region: boundary region of perfusion; Microvascular health score™ (MVHS): assessment of the microvasculature condition.

Allergology of the First Pavlov Saint Petersburg State Medical University for the treatment adjustment. Upon admission, the articular syndrome (7 swollen joints, 10 painful joints, VAS 80 mm) was observed during the clinical presentation. The examination revealed ESR of 18 mm/h, positive rheumatoid factor, and positive CCPA. An X-ray of the hands revealed multiple erosions with the development of ankylosis in the wrist joints. There was also a complication of the underlying disease in the form of systemic osteoporosis with a predominant loss of bone mass of the vertebral bodies (T-score L_1–L_4 "–2.8 SD") without fractures, for which bisphosphonates were prescribed.

No data were received for lesions of internal organs within the RA. The activity of the process upon admission corresponded to high (DAS28: 5.65). Therefore, the doctors decided to increase the dose of methotrexate to 20 mg per week. As part of the complex treatment, physiotherapy (magnetotherapy and phonophoresis of hydrocortisone on the joint area) and therapeutic exercises were also performed. A good clinical and laboratory response was obtained during therapy (DAS28: 5.65 → 3.88; SDAI: 100 → 38.3; CDAI: 97 → 38; parameters of articular syndrome upon discharge included 6 swollen joints, 2 painful joints, VAS 30 mm, and ESR 17 mm/h).

During hospitalization, before and after the start of complex treatment, the eGC was assessed using a dark-field video microscope with LED illumination in the green region of the spectrum

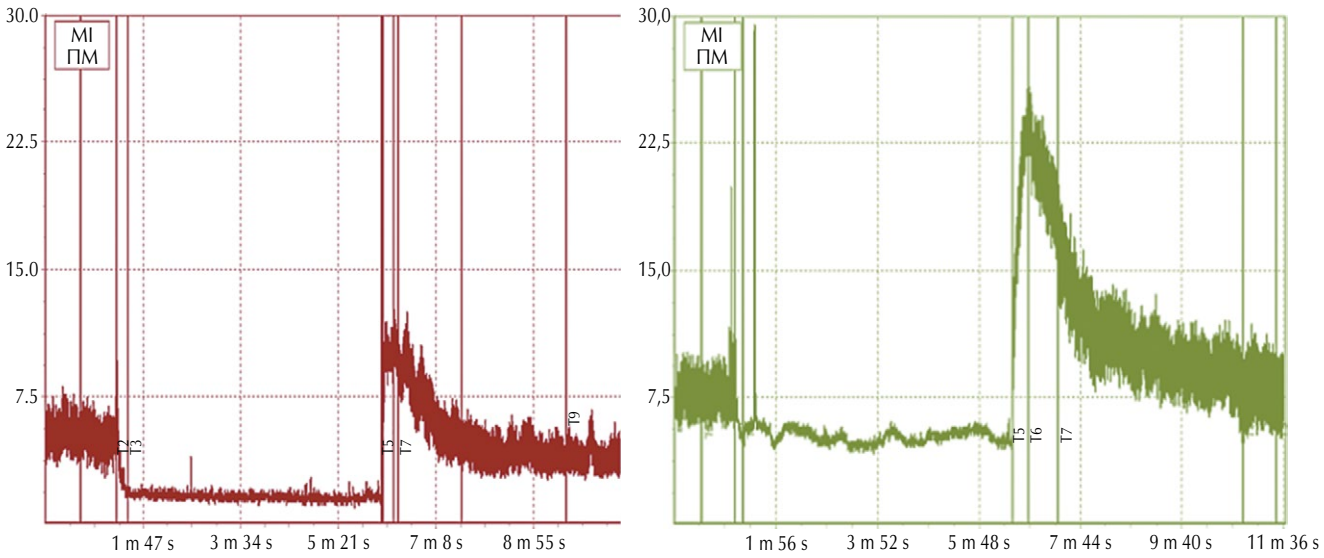

Fig. 1. An arterial occlusion test with laser Doppler flowmetry. LDF-gram before (on the left) and after (on the right) sampling with short-term arterial occlusion. The area of study is the locus of the anterior surface of the distal third of the forearm skin. The abscissa shows the values of the microcirculation index (MI), measured in perfusion units, the ordinate — time scale

Рис. 1. Проба с артериальной окклюзией при лазерной допплеровской флоуметрии. ЛДФ-грамма до (слева) и после (справа) пробы с кратковременной артериальной окклюзией. Область исследования — локус передней поверхности дистальной трети кожи предплечья. По оси абсцисс расположены значения показателя микроциркуляции (ПМ), измеренные в перфузионных единицах, по оси ординат — временная шкала

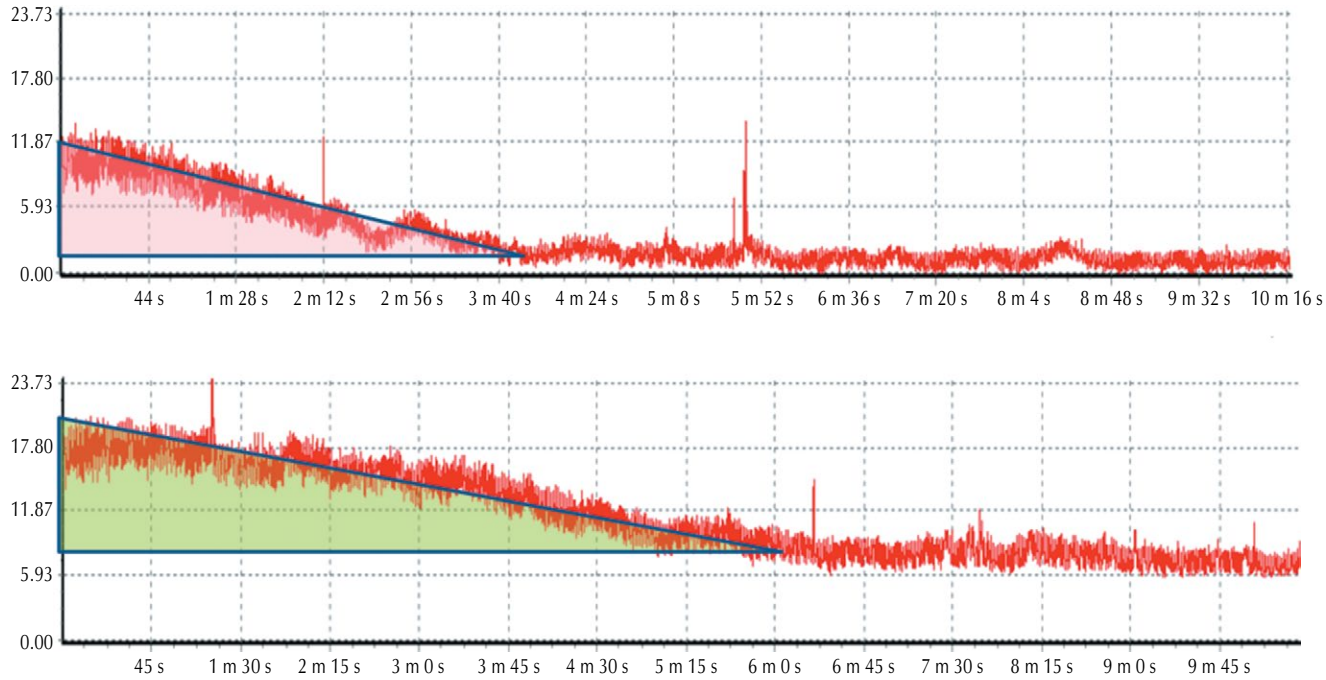

Fig. 2. A test with iontophoresis of 0.1% acetylcholine solution with laser Doppler flowmetry. LDF-gram before (above) and after (below) the application of 0.1% acetylcholine solution. The area of study is the locus of the anterior surface of the distal third of the forearm skin. The abscissa shows the values of the microcirculation index, measured in perfusion units, the ordinate — time scale

Рис. 2. Проба с ионофорезом 0,1 % раствора ацетилхолина при лазерной допплеровской флоуметрии. ЛДФ-грамма до (сверху) и после (снизу) аппликации 0,1 % раствора ацетилхолина. Область исследования — локус передней поверхности дистальной трети кожи предплечья. По оси абсцисс расположены значения показателя микроциркуляции, измеренные в перфузионных единицах, по оси ординат — временная шкала

(KK Research Technology Ltd., Great Britain) and GlycoCheckTM software (Glycocheck BV, the Netherlands). Moreover, the vasomotor function of the endothelium was studied using a laser diagnostic multifunctional complex LAKK-M (Research and Production Enterprise LAZMA, Russia). The interval between studies was 9 days. The research results are presented in Table 1 and in Figures 1 and 2.

According to the results of dark-field microscopy (Table), after the start of the complex treatment, the patient showed a decrease in the boundary region of perfusion and, consequently, an increase in the eGC thickness. The number of vessels containing over 50% of erythrocytes also increased along with the average count of erythrocytes in the vessels studied. These changes probably indicate an increase in vascular blood filling, the number of functioning capillaries and, quite possibly, the process of angiogenesis. We registered the same positive changes for the integral indicator and the assessment of the microvasculature condition. Thus, changes in the parameters of dark-field microscopy may indicate indirectly an increase in the regenerative potential of the microvasculature during treatment. In this case, the key change is the thickening of eGC.

When performing LDF, native peripheral blood flow was recorded with the frequency-response spectra of oscillations, after which functional tests were performed with arterial occlusion (Fig. 1) and iontophoresis of 0.1% acetylcholine solution (Fig. 2). The study results revealed an increase in the amplitude of the myogenic spectrum (vasomotion), indicating a tendency for the predominance of vasodilation over vasoconstriction after the start of treatment. Therefore, this reflects a shift in the central regulation of arteriole tone toward the trophotropic aspect. An important factor to be considered here is the increase in the proportion of nutritional perfusion relative to the shunt one due to a decrease in the bypassing rate. On the one hand, this may be due to an increase in the number of functioning capillaries; on the other hand, this can be attributed

to a decrease in blood flow through arterial and venular anastomoses.

The occlusion test (Fig. 1) results revealed an extension of the half-recovery period of blood flow to the basal level, which was largely caused by an increase in the amplitude of the microcirculation index after the start of treatment. An increase in the severity of such a reaction during treatment may indicate a tendency to restore the vasomotor function of the microvasculature, because the test with arterial occlusion assesses the incretion of nitric oxide(I), which is the main vasodilator, and the endothelium's ability to synthesize.

The area under the curve of the microcirculation index was calculated (triangle in Fig. 2) according to the results of the test, which used acetylcholine for a quantitative assessment of the reaction severity. According to the literature, the latent period should normally be absent, the duration of the reaction should be 6–7 minutes, and the increase should be about 45%. As shown in Figure 2, after the start of treatment, these indicators were closer to the above-described norm than before the treatment. Furthermore, the area under the curve was more pronounced after the start of treatment, which in general, may also indicate a tendency toward the restoration of the vasomotor function of the endothelium during treatment.

Thus, along with a decrease in the clinical and laboratory activity of RA, the thickening of the eGC and an improvement in the vasomotor function of the microvasculature vessels were revealed during treatment. Therefore, in assessing the state of the endothelium in RA patients, it may be promising to study the thickness of eGC and the characteristics of peripheral microcirculation, particularly the mechanisms of its regulation. Doing so facilitates the process of searching for new, unconventional risk factors for an unfavorable course of RA and cardiovascular risks in these patients and the designing of personalized treatment for each of them.

Conflict of interest. The authors declare no conflict of interest.

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