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Повреждение эндотелиального гликокаликса у пациентов с ревматоидным артритом

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Обоснование. Важная роль в регуляции функционирования эндотелия принадлежит эндотелиальному гликокаликсу, который первым повреждается при развитии аутоиммунных заболеваний. Его истончение приводит к нарушению барьерной функции сосудов и развитию воспалительного процесса. В статье рассмотрено использование оценки эндотелиального гликокаликса для диагностики и мониторинга заболеваний.

Цель работы — установить взаимосвязь толщины эндотелиального гликокаликса с активностью, серологическим профилем и факторами риска неблагоприятного течения ревматоидного артрита.

Материалы и методы. В исследование включено 76 пациентов с ревматоидным артритом в возрасте от 18 до 65 лет обоих полов. В качестве основного показателя оценки состояния эндотелиального гликокаликса использована пограничная область его перфузии, определяемая с помощью темнопольного видеомикроскопа.

Результаты. Установлено относительное истончение эндотелиального гликокаликса у лиц женского пола и при наличии у пациента факторов риска неблагоприятного течения ревматоидного артрита. Взаимосвязь этого показателя с серологическим профилем заболевания не выявлена. Обратная корреляция между толщиной эндотелиального гликокаликса и показателями уровня синдекана-1 и скорости оседания эритроцитов подтверждает вовлечение эндотелиального гликокаликса в воспалительный процесс при ревматоидном артрите. Спрогнозировано назначение пациентам пульс-терапии метилпреднизолоном в стационаре. При повторном обследовании истончение эндотелиального гликокаликса отмечено при наличии факторов риска неблагоприятного течения ревматоидного артрита, негативности по циклическому цитрулиновому пептиду и отсутствии ответа на лечение.

Заключение. Темнопольная микроскопия дает возможность прижизненной и неинвазивной оценки толщины эндотелиального гликокаликса, предоставляя важную информацию как с фундаментальной точки зрения, углубляя знания о патологической физиологии заболевания, так и с клинической — позволяя рассматривать истончение эндотелиального гликокаликса как дополнительный маркер развития ревматоидного артрита.

Ключевые слова: эндотелиальная дисфункция; эндотелиальный гликокаликс; темнопольная микроскопия; ревматоидный артрит; эндотелий; синдекан-1.

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Disruption of endothelial glycocalyx in patients with rheumatoid arthritis

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BACKGROUND: Endothelial glycocalyx has an important role in the regulation of endothelial functioning. It is the first to be damaged during the development of autoimmune inflammatory diseases. Thinning of endothelial glycocalyx leads to a violation of the barrier function of blood vessels, the development and maintenance of the inflammatory process. In this context, using endothelial glycocalyx assessment for diagnostic and monitoring of diseases is considered of great interest.

AIM: To establish the relationship between endothelial glycocalyx thickness with activity, serological profile and risk factors for an unfavorable course of rheumatoid arthritis.

MATERIALS AND METHODS: The study included 76 patients from 18 to 65 years of both sexes with rheumatoid arthritis. The perfusion boundary region of endothelial glycocalyx determined using a dark-field microscope has been used as the main indicator for assessing the state of endothelial glycocalyx.

RESULTS: The obtained results have demonstrated a decrease in the initial endothelial glycocalyx thickness in females in the presence of risk factors for an unfavorable course of rheumatoid arthritis. At the same time, no relationship with the serological profile and endothelial glycocalyx thickness has been found. An inverse correlation between endothelial glycocalyx thickness and syndecan-1 level and erythrocyte sedimentation rate has been found. This confirms the fact that endothelial glycocalyxes are involved in the inflammatory process in rheumatoid arthritis. Endothelial glycocalyx assessment using dark-field microscopy made it possible to predict the subsequent appointment of pulse therapy with methylprednisolone in the hospital. On re-examination, endothelial glycocalyx thinning has been observed in the presence of unfavorable risk factors for the course of rheumatoid arthritis, in negative to anti-cyclic citrullinated peptide antibodies patients, or in the absence of response to treatment.

CONCLUSIONS: The use of dark-field microscopy is unique in its capabilities for intravital and non-invasive assessment of endothelial glycocalyx thickness. It provides important fundamental information deepening knowledge in the pathological physiology of disease. From a clinical point of view, it allows to consider a decrease in endothelial glycocalyx thickness as an additional prognostically unfavorable marker in rheumatoid arthritis.

Keywords: endothelial dysfunction; endothelial glycocalyx; dark-field microscopy; rheumatoid arthritis; endothelium; syndecan-1.

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BACKGROUND

Endothelial dysfunction is a key link in the pathogenesis of rheumatoid arthritis (RA) [7]. Endothelial glycocalyx (EGC), which is a thin dynamic layer of macromolecules (glycoproteins, proteoglycans, and glycosaminoglycans) on the vascular endothelium surface, plays an important role in the endothelium function regulation [6]. Acting as a biological barrier, it is the first to be damaged in the development of autoimmune diseases. The thinning of EGC impairs the barrier function of the vessels, which results in their increased permeability, increased platelet and leukocyte adhesion to the vascular wall, and changed microvasculature perfusion. All this leads to inflammatory process development [2, 4]. Despite its instability, EGC thickness measurement can be used to diagnose and monitor diseases [2]. Dark-field microscopy allows non-invasive and *in vivo* EGC assessment, with moderate reproducibility, which increases with repeated measurements, as well as insignificant daily variability [3, 5].

This study aimed to establish the relationship between EGC thickness and activity, serological profile, and risk factors for an unfavorable course of RA.

MATERIALS AND METHODS

The study included 76 patients aged 18 to 65 years (13 males and 63 females) with diagnosed RA according to the American College of Rheumatology and the European League Against Rheumatism criteria in 2010 [1]. The median age was 56 years (49–62.5 years). Rheuma-

toid factor positivity was established in 74.6% of patients and that for cyclic citrullinated peptide in 79.4% of cases. The study was conducted twice, in the first days after the patient's hospital admission and before discharge on average of after 9 days (8–11 days), to assess the effect of the therapy on the vascular endothelium. All patients received study-independent treatment according to current clinical guidelines and standards of specialized care.

The main indicator for assessing the EGC state was the size of its perfused boundary region (PBR) determined by the depth of erythrocyte immersion into the EGC thickness in microvessels with an internal diameter of 5–25 μm (PBR_{5–9}, PBR_{10–19}, PBR_{20–25}, and PBR_{5–25}, respectively). An increased PBR reflects a deeper immersion of erythrocytes into the EGC thickness, which is interpreted as to its thinning [3]. A dark-field video microscope with LED illumination in the green region of the spectrum (KK Research Technology Ltd, Great Britain) and GlycoCheck software (Glycocheck BV, the Netherlands) was used to measure the PBR. The video microscope was placed on the sublingual mucosa of the oral cavity. The analyzed indicators were automatically calculated using the software algorithm. The quantitative measurement of circulating syndecan-1 using the Human Syndecan 1 enzyme-linked immunoassay Kit (RayBiotech Inc., USA) was used as a laboratory marker of EGC damage.

Statistical data processing was performed using the International Business Machines Corporation Statistical Package for the Social Sciences version 26 program. The norm of distribution of indicators in the sample was determined using the Kolmogorov–Smirnov test with the Lilliefors

Table. Correlation between the size of the perfused boundary region of endothelial glycocalyx and the serological profile and risk factors for an unfavorable course of rheumatoid arthritis

Таблица. Взаимосвязь величины пограничной области перфузии эндотелиального гликокаликса с серологическим профилем и факторами риска неблагоприятного течения ревматоидного артрита

PBR, μm	Indicator analyzed		p level
	absent	present	
PBR _{5–9} *	1. Positivity for cyclic citrullinated peptide 1.16 $\pm$ 0.03	1.07 $\pm$ 0.02	p = 0.017
PBR _{5–25} *	2. Compromised history of rheumatic diseases 2.02 $\pm$ 0.06	2.20 $\pm$ 0.04	p = 0.014
PBR _{5–25}	3. Large joint involvement during rheumatoid arthritis in the first 2 years after the onset 2.07 $\pm$ 0.06	2.13 $\pm$ 0.03	p = 0.037
PBR _{5–9}	4. The need for genetically engineered biological therapy due to rheumatoid arthritis history 1.11 $\pm$ 0.01	1.21 $\pm$ 0.04	p = 0.035
PBR _{5–25} *	5. Osteopenia or bony rarefaction 2.05 $\pm$ 0.05	2.25 $\pm$ 0.06	p = 0.011
PBR _{5–25} *	6. Erosive rheumatoid arthritis 1.93 $\pm$ 0.05	2.11 $\pm$ 0.06	p = 0.027

Note: PBR — perfused boundary region; *PBR values after hospital treatment at the repeated examination.

correction, as well as the values of kurtosis and skewness, depending on which parametric or nonparametric methods were used.

The paired *t*-test or the Wilcoxon test was used to compare related populations and the Spearman coefficient for correlation analysis. Discriminant analysis was performed. Statistical significance was determined at $p < 0.05$. The clinical study was approved by the First Pavlov Saint Petersburg State Medical University (minutes No. 11/2019 dated December 28, 2019). All participants provided informed voluntary consent in advance.

RESULTS

The relationship between the PBR value and the serological profile and risk factors for an unfavorable course of RA was established (Table).

A statistically significantly direct correlation between the PBR₅₋₂₅ value and the level of syndecan-1 ($r_{xy} = 0.487$; $p = 0.012$) and erythrocyte sedimentation rate ($r_{xy} = 0.378$; $p = 0.002$) was established (Fig. 1, 2).

The dependence of PBR₅₋₂₅ on the treatment response, estimated by the DAS28 C-reactive protein index ($p = 0.019$), was revealed. The mean PBR₅₋₂₅ value was 2.27 ± 0.23 in the absence of treatment response, whereas 1.99 ± 0.32 μm in cases of a satisfactory response. Differences between groups with satisfactory and good treatment responses were not statistically significant ($p = 0.469$).

The relationship between the PBR value and the need to use methylprednisolone pulse therapy in a hospital was analyzed. The PBR₅₋₂₅ value in the group with subsequent pulse therapy was higher than those without it ($p = 0.001$) (Fig. 3).

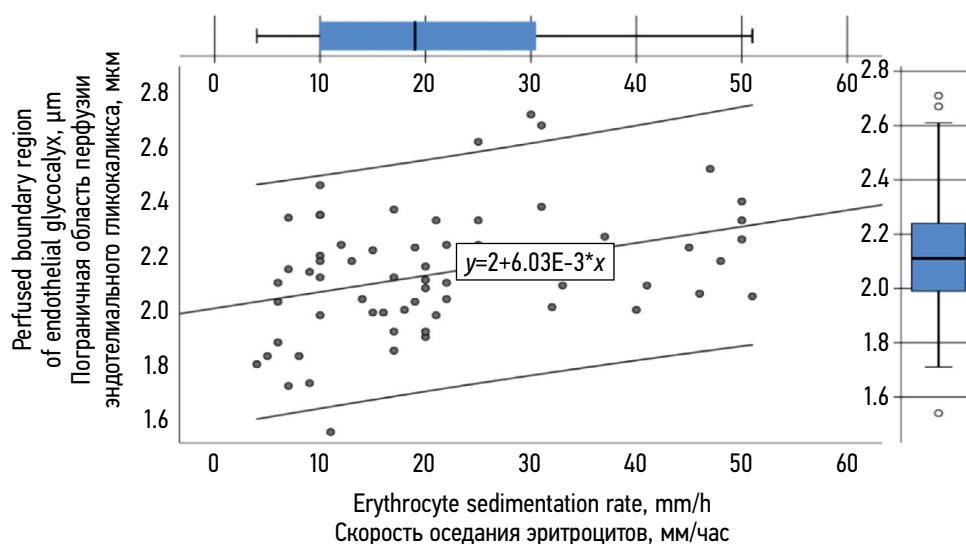


Fig. 1. Correlation between PBR₅₋₂₅ and erythrocyte sedimentation rate. PBR — perfused boundary region

Рис. 1. Корреляционная связь PBR₅₋₂₅ и скорости оседания эритроцитов. PBR — пограничная область перфузии

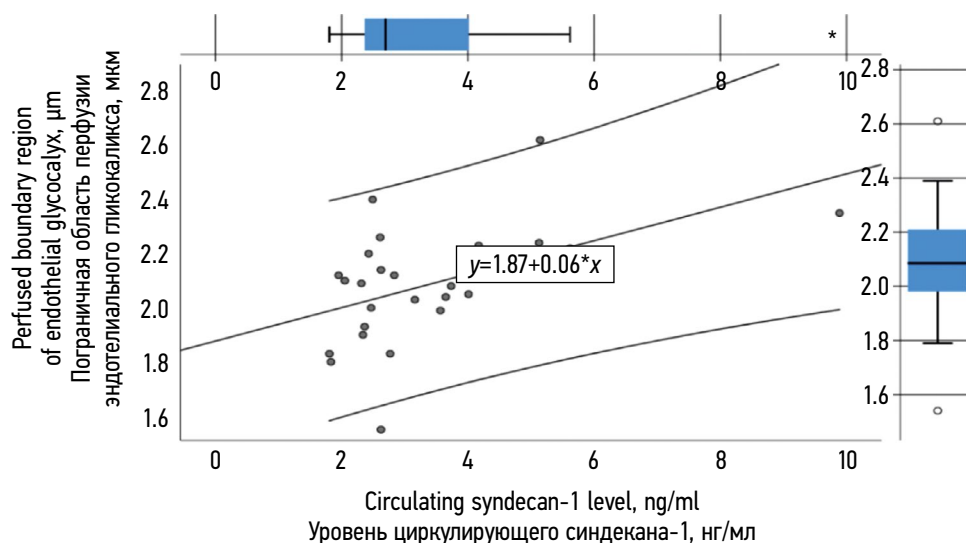


Fig. 2. Correlation between PBR₅₋₂₅ and syndecan-1. PBR — perfused boundary region

Рис. 2. Корреляционная связь PBR₅₋₂₅ и уровня синдекана-1. PBR — пограничная область перфузии

A discriminant analysis was performed to clarify the effect of baseline PBR on the probability of pulse therapy. The following model was obtained:

$$y = -9.107 + 3.831x,$$

wherein y is a discriminant function that characterizes the probability of pulse therapy and x is the PBR_{10-19} value. The discrimination constant was 0.156. Wilk's λ coefficient was used to establish statistically significant differences ($p = 0.020$). The model sensitivity was 68.2% and the specificity was 69%.

DISCUSSION OF RESULTS

As of September 2021, any original publications on the study of EGC in RA using dark-field microscopy were not found.

Our study results revealed the relative EGC thinning in females and patients with risk factors for an unfavorable course of RA. The relationship of this indicator with the serological profile of the disease was not revealed.

The inverse correlation between EGC thickness and syndecan-1 levels and erythrocyte sedimentation rate confirms the involvement of EGC in the inflammatory process in RA. The EGC evaluation using dark-field microscopy predicted the subsequent prescription of methylprednisolone pulse therapy to patients in the hospital.

Upon repeated examination, EGC thinning was noted in the presence of risk factors for an unfavorable course of RA, negativity for cyclic citrullinated peptide, or lack of treatment response.

CONCLUSION

Dark-field microscopy enables *in vivo* and non-invasive EGC thickness assessment, thus providing important

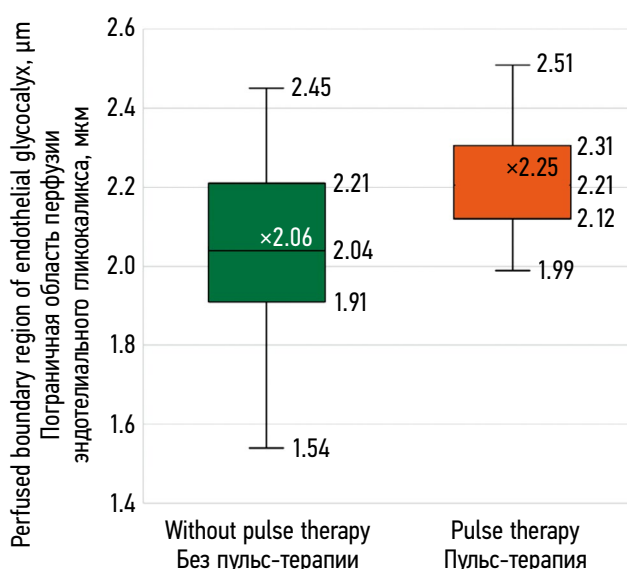


Fig. 3. Differences in PBR5-25 depending on subsequent methylprednisolone pulse therapy. PBR — perfused boundary region

Рис. 3. Различия PBR_{5-25} в зависимости от последующего проведения пульс-терапии метилпреднизолоном. PBR — пограничная область перфузии

information both from a fundamental point of view, advancing in knowledge about the pathological disease physiology, and from a clinical point of view, which enables the consideration of EGC thinning as a marker of an unfavorable course of RA.

ADDITIONAL INFORMATION

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

All authors made a significant contribution to the study and article preparation, read and approved the final version before its publication.

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