Received: 25 December 2017 | Accepted: 26 March 2018

DOI: 10.1111/micc.12454

ORIGINAL ARTICLE

WILEY Microcirculation

Increase in perfused boundary region of endothelial glycocalyx is associated with higher prevalence of ischemic heart disease and lesions of microcirculation and vascular wall

Alexander Y. Gorshkov[1] | Marina V. Klimushina[1] | Sergei A. Boytsov[1] | Alexander Y. Kots[2] | Nadezhda G. Gumanova[1]

[1]National Research Center for Preventive Medicine, Ministry of Healthcare of Russian Federation, Moscow, Russia

[2]School of Medicine and Health Sciences, The George Washington University, Washington, District of Columbia

Correspondence
Nadezhda G. Gumanova, National Research Center for Preventive Medicine, Ministry of Healthcare of Russian Federation, Moscow, Russia.
Email: gumanova@mail.ru

Funding information
Russian Foundation for Basic Research, Grant/Award Number: 17-04-00157\17

Abstract

Objective: PBR characterizes penetration of red blood cells inside glycocalyx and its thickness can have profound impact on microcirculation and other vascular parameters. The goal of our study was to reliably quantify PBR and assess its potential use as a new marker of cardiovascular pathology.

Methods: The study included 208 patients (123 men and 85 women from 40 to 65 years of age) with various grades of cardiovascular SCORE risk index and IHD. PBR was quantified by sidestream dark field capillaroscopy with green light excitation. Cutaneous microcirculation was evaluated with laser Doppler fluorometry.

Results: Elevated PBR values over 2 mm were associated with morphological and functional lesions of arterial wall and microcirculation and lowered levels of ApoA1 lipoprotein. Moreover, elevated PBR values were associated with 2.07-fold increase in prevalence of cerebral atherosclerosis (P = .015) and 2.42-fold increase in prevalence of IHD (P = .024). Increase in PBR was associated with elevated systolic blood pressure.

Conclusions: Thus, PBR can be considered a new highly reproducible and promising marker candidate for non-invasive diagnostics of IHD and cerebral atherosclerosis suggesting important role of microcirculation in development and progression of cardiovascular diseases.

KEYWORDS

cardiovascular disease, endothelial glycocalyx, microcirculation, perfused boundary region, vascular endothelium

1 | INTRODUCTION

Endothelial monolayer is critical for normal vascular function. Lesions of endothelium manifested as endothelial dysfunction are linked to various cardiovascular diseases through pathological changes in microcirculation,[1] atherosclerosis of major arteries,[2] and left heart remodeling.[3] Endothelial glycocalyx covers the monolayer and plays important role in maintenance of endothelial integrity.[4] Endothelial glycocalyx regulates endothelial barrier,[5] shear stress,[6] and microenvironment of endothelial monolayer as well as its interaction with various blood cells.[7,8]

Recently, a group of scientists introduced a novel technology for assessment of sublingual microcirculation based on PBR parameter that characterizes penetration of erythrocytes inside endothelial glycocalyx.[9,10] PBR represents deviation of erythrocytes from

Abbreviations: ABI, ankle-brachial index; CAVI, cardio-ankle vascular index; CV, coefficient of variation; IHD, ischemic heart disease; IMT, intima-media thickness; LDL, low density lipoprotein; NOx, nitrite and nitrate; PBR, perfused boundary region; PWV, pulse wave velocity.

normal blood flow. Deeper penetration of erythrocytes is associated with lesions of microvascular perfusion.[9] Normal glycocalyx is quite dense and erythrocytes cannot penetrate deep inside it whereas less dense glycocalyx decreases microvascular perfusion.[9]

The goal of this study was to estimate possible correlation of PBR values with cutaneous microcirculation, condition of major blood vessels, and endothelial functional markers in patients with cardiovascular diseases. We have discovered that increase in PBR is strongly associated with elevated prevalence of IHD and SCORE risk index.

2 | MATERIALS AND METHODS

2.1 | Patients

The study included 208 subjects (123 men and 85 women) aged from 40 to 65 years examined for preventive counseling in the National Medical Research Center for Preventive Medicine, Russian Ministry of Health Care, Moscow, Russia. Cardiovascular risk was estimated based on SCORE index during outpatient examination in 177 subjects and other 31 patients were admitted to the hospital and diagnosed with IHD. IHD was verified by anamnesis, routine clinical evaluation, and various tests according to the local guidelines for patients with stable angina pectoris.[11]

The following exclusion criteria were applied: any acute inflammation including oral or dental inflammation; haematological diseases; left ventricular ejection fraction below 40%; diabetes mellitus; chronic kidney or liver failure; oncological diseases; mental illness; autoimmune diseases; any types of blood sugar lowering therapy; any types of cholesterol-lowering therapy except patients diagnosed with IHD who were prescribed statins; pregnancy; lactation. Patients with IHD who had an acute cardiovascular event <6 months before the study were also excluded.

Protocol of the study was approved by a local ethics committee according to the guidelines of the Helsinki Declaration and WHO. All participants gave their written consent to participate in the study and to grant access to their personal data.

2.2 | Perfusion of sublingual endothelial glycocalyx

Subjects were asked to rest quietly for 10 minutes prior to acquisition of sublingual sample. PBR was measured by a sidestream dark capillaroscope equipped with a green LED at 525 nm wavelength (KK Research Technology Ltd, UK). GlycoCheck software was used to quantify the images by an automatic protocol according to manufacturer instructions and our previous report.[12] Briefly, a series of 40 frames of digital images (752 by 480 pixels at 25 frames per second) were automatically recorded at 325-fold magnification to ensure optimal image quality, suitable background to noise ratio of the signal, focus of the image, hematocrit over 50%, and sufficient number of segments of microcapillaries of appropriate diameter from 5 to 25 μm. Median width of red blood cell column (P50) was 8.9 ± 0.7 μm. Approximately 3000 segments were identified and marked by the software at 0.5 μm steps for a total of 840 fragments per video frame. Then, distribution of distance of red blood cells from median of the capillary column was plotted and checked to fit appropriate statistical distribution. PBR was calculated as difference between total perfused diameter and median column width (25-75 percentile) divided by two. An average value for each patient was used for subsequent analysis. Specific set of samples was taken from few selected subjects on several consecutive days to estimate general variability of the assay, which was always below 10% CV. Measurements were not influenced by time of the day as long as the procedure was performed at least 2 hours after a meal. Sublingual areas either on the left or right side of the frenulum gave identical results in repeated estimations.[12] The applications of the method to various clinical studies have been extensively validated in a number of recent publications.[13-16]

2.3 | Cutaneous perfusion

Cutaneous microvascular perfusion within terminal vascular regions was performed by laser Doppler fluorometry[17,18] using a standard single-channel laser apparatus LAKK-02 (Lazma, Russia) in the visible red spectrum at 780 nm. A specific LAKK-TEST block was used to accurately measure skin temperature and timing in the case of brachial arterial occlusion at 1.0-1.2 mm depth. All patients were allowed to rest on a bed in a horizontal position in a room with controlled temperature (23 ± 1°C) and humidity (40%-60%). Baseline perfusion was measured for 6 minutes at the median brachial line close to the wrist joint. The sphygmomanometer cuff at the shoulder was then inflated for 5 minutes and rapidly decompressed. Restoration of blood flow was registered for additional 6 minutes after decompression. Times from removal of occlusion to maximal reperfusion (T_{max}, seconds) and to half-maximal reperfusion ($T½$, seconds) were calculated using the recordings.

2.4 | Vascular wall morphology and function

IMT in carotid artery was estimated by duplex scanning (Philips iU22 ultrasound system equipped with linear 9-11 MHz sensor, USA). Average IMT and maximal IMT were calculated by the software as well as maximal stenosis of carotid arteries expressed in percentage.

Rigidity of vascular wall was measured by PWV within carotid-femoral segment (VaSera 1500 ECG apparatus, Fukuda Denshi, Japan). CAVI was calculated based on rigidity parameters according to modified Bramwell-Hill equation as described.[19,20]

Ratio of systolic blood pressure measured at the shoulder and ankle, the ABI, was used to estimate presence of peripheral artery dysfunction.[21]

Increase in brachial artery diameter was assessed by flow-dependent vasodilation (Philips iU22 apparatus, USA) as described by Celermajer et al.[22]

Systolic and diastolic blood pressure was measured on the right arm after 5-10 minutes quiet rest twice and average values were

used to calculate pulse pressure Δ*P*. Normal Δ*P* values were considered to be 30-50 mm Hg.

2.5 | Biochemical markers

Blood was sampled from the ulnar vein after 12-14 hours of fasting. The patients were specifically asked to limit smoking and consumption of high nitrate-containing dinner prior to their visit. Serum was aliquoted and stored at −26°C.

Concentration of NOx was assayed in the deproteinized serum filtered through Spin-X^{R}UF500 5k molecular weight cutoff concentrators (Corning, UK). Nitrate was reduced to nitrite with vanadium (III) chloride (Sigma) and NOx levels were measured by the Griess reaction as described by Miranda et al[23] with modifications[24,25] using a microplate reader (Multiscan MCC/340; Labsystems, Finland).

Endothelin was measured using an ELISA kit from Affymetrix Bioscience, USA with a microplate reader. Total and LDL cholesterol, apolipoproteins A1 and B, creatinine, and C-reactive protein were assayed using an automatic analyzer Architect C 8000 (Abbott, USA).

2.6 | Statistical evaluation

Data were analyzed using Statistica software package version 8.0 (StatSoft Inc, USA). Some clusters of data did not pass the Kolmogorov-Smirnov test for normality of distribution and therefore, we used non-parametric tests throughout the calculations. Significance of differences between the groups was estimated by the Mann-Whitney test. Median values between multiple groups were compared using multiple comparison *P* values Kruskal-Wallis test (2-tailed) with subsequent comparison between specific groups. In the case of categorical data, significance of odds ratio and corresponding differences were confirmed using the chi-squared Wald test. *P* values of <.05 were considered significant. All values shown are mean ± SD unless stated otherwise in the text.

3 | RESULTS

The study comprised 208 subjects aged 55 ± 7.4 years including men and women (N = 177) with various degrees of cardiovascular risks by SCORE or without any degree of cardiovascular risks and 31 patients with IHD. Demographics and general characteristics of the group are shown in Table 1. It should be noted that 14 of 31 patients (45.2%) were treated with statins for at least 1 month prior to blood sampling. Participants that did not have IHD were not treated with statins. Hypertension was diagnosed in 57.7% of the patients while only 39% of these patients were regularly using medications for control of their blood pressure. However, average blood pressure in the whole group was within normal range; systolic, diastolic, and pulse pressure were 134 ± 15 mm Hg, 83 ± 9 mm Hg, and 51 ± 10 mm Hg, respectively (mean ± SD).

In general, lipid profile of the group indicates presence of certain atherogenic tendencies with median levels of total cholesterol and LDL cholesterol (6.0 mmol/L and 4.3 mmol/L, respectively) exceeding normal values (5.1 mmol/L and 3 mmol/L, respectively). Slightly elevated serum concentrations of C-reactive protein, an inflammatory marker, at 2.9 mg/L suggest minor chronic inflammatory process without significant clinical manifestation. Average rate of glomerular filtration according to creatinine clearance was estimated to be 106 mL/min and none of the patients had abnormally high or low values of this parameter.

The PBR values were ranged from 1.35 to 2.5 μm. Comparison of PBR values in men vs women indicates that there were no sex-related differences and therefore, during subsequent analysis, statistical evaluations were not adjusted for sex of the subjects (Table 1). To assess potential associations of parameters characterizing endothelial glycocalyx with functional conditions of the subjects, we split the whole cohort into terciles according to individual PBR values as shown in Table 2. Borderline of terciles were 1.8 μm and 2.0 μm and terciles were compared to each other using Kruskal-Wallis 2-tailed test. Results shown in Table 2 indicate that subjects of tercile III had considerable differences in a number of important cardiovascular parameters vs patients of terciles I or II suggesting patients with higher PBR have higher severity of cardiovascular lesions. For example, median systolic and pulse pressure values were significantly higher in tercile III vs tercile II (Table 2). Moreover, subjects of tercile III had higher IMT (average and maximum), CAVI on the left and right sides, and substantially altered response in cutaneous arterial occlusion test (both T_{max} and $T½$) vs subjects of terciles I and II. This suggests higher degree of atherosclerotic remodeling of carotid artery and higher stiffness of arterial walls. The data were in agreement with biochemical test of Apo A1 lipoprotein as its concentration was also lower in tercile III vs terciles I and II (Table 2) suggesting potential association between lesions of cholesterol metabolism and PBR. It should be noted that other parameters listed in Table 1 had no significant differences between the terciles and are not shown in Table 2.

Some differences between male and female participants in various cardiovascular and biochemical parameters (T_{max} and $T½$ and Apo A1) and the same parameters with PBR values as listed in Table 1 may suggest possible association between PBR values and gender. However, we have not detected significant correlation between PBR value and gender (Spearman rank order correlation $r = .0039$, $P > .05$). Moreover, ratio between male and female subjects was similar between the terciles thus ruling out these associations (Table 2).

Thus, elevated PBR values in tercile III may be linked to certain pathological processes and to clinical manifestations of atherosclerosis. To test this hypothesis, we have split subjects into 2 subgroups according to their PBR values by combining terciles I and II and comparing prevalence of IHD vs subjects of tercile III. Corresponding odds ratios indicate that prevalence of IHD is 2.42-fold higher ($P = .015$) in patients with PBR exceeding 2.0 μm vs patients with PBR <2.0 μm (Table 3). Similar to this, prevalence of validated cerebral atherosclerosis was higher by 2.07-fold ($P = .024$) in patients with higher PBR.

TABLE 1 Demographics, general characteristics, and biochemical markers in the cohort

	Total cohort; N = 208			Men; N = 123			Women; N = 85		
Parameters	**Median**	**25%**	**75%**	**Median**	**25%**	**75%**	**Median**	**25%**	**75%**
General characteristics									
Age, years	55	48	60	53	47	58	57*	49	62
Height, m	1.72	1.65	1.77	1.76	1.72	1.82	1.64*	1.60	1.67
Weight, kg	80.0	70.0	92.0	86.5	77.0	95.0	70.0*	64.0	80.0
BMI (kg/m^2)	27.0	24.5	29.7	27.7	25.5	21.6	25.8*	23.5	29.2
Heart rate, bpm	63	58	71	63	58	72	62*	58	68
Systolic blood pressure, mm Hg	133	122	144	136	124	144	126*	120	142
Diastolic blood pressure, mm Hg	82	80	90	82	80	90	80*	72	82
ΔP, mm Hg	50	42	58	50	44	58	50	40	60
Morphology and functional parameters of vascular wall and microcirculation									
Maximal IMT, mm	0.9	0.8	1.1	1.0	0.8	1.1	0.9	0.7	1.1
Average IMT, mm	0.85	0.7	1.0	0.9	0.7	1.0	0.8	0.7	1.0
Cardio-ankle parameters									
L-ABI	1.09	1.03	1.15	1.11	1.04	1.17	1.06*	1.01	1.11
R-ABI	1.07	1.00	1.12	1.08	1.01	1.13	1.06	0.99	1.10
L-CAVI	7.70	7.00	8.30	7.70	7.00	8.40	7.80	7.20	8.30
R-CAVI	7.75	7.00	8.40	7.80	7.00	8.50	7.70	7.10	8.40
Pulsewavevelocity, m/s	7.95	7.30	8.90	8.30	7.50	9.00	7.50*	6.90	8.40
Test of Celermajer (%)	9.05	5.60	12.50	8.20	4.50	11.70	11.60*	7.50	14.00
Parameters of glycocalyx and cutaneous microvascular perfusion									
PBR, µm	1.90	1.75	2.04	1.90	1.74	2.04	1.89	1.76	2.04
Cutaneous occlusion T_{max}, s	28.60	21.10	40.00	33.00	22.10	45.50	26.60*	20.60	31.60
Cutaneous occlusion $T½$, s	61.50	42.60	79.40	66.60	50.00	89.00	54.10*	37.30	68.70
Biochemical markers									
NOx, µmol/L	26.3	17.6	39.8	26.1	18.4	41.4	26.5	17.1	37.6
Endothelin, fmol/mL	1.11	0.52	2.25	1.04	0.48	2.68	1.27	0.71	2.17
Total cholesterol, mmol/L	6.0	5.3	6.9	5.8	5.2	6.9	6.2	5.5	7.0
Triglycerides, mmol/L	1.20	0.89	1.69	1.35	0.96	1.89	1.03*	0.78	1.43
LDL cholesterol, mmol/L	4.32	3.63	5.10	4.15	3.50	4.95	4.39	3.76	5.14
HDL cholesterol,mmol/L	0.99	0.79	1.23	0.85	0.70	1.06	1.12	0.95	1.42
Apo AI, mg/dL	150	135	170	143	128	161	156*	146	178
Apo B, mg/dL	98	80	115	101	83	116	94	78	109
Lp(a), mg/dL	14.8	7.3	48.8	130.0	79.0	173.0	15.4	8.2	31.4
C-reactive protein, mg/L	2.9	1.8	4.5	3.2	5.3	1.9	2.6	1.5	3.7
Main diagnosis and tobacco smoking status, N (%)									
Hypertension	120 (57.0)			76 (61.7)			43 (50.5)		
IHD	31 (14.9)			26 (21.1)			5 (6.02)		
Smoking	68 (32.7)			87 (70.3)			23 (27.0)		
Current medication status, N (%)									
Main condition treated	77 (37)			47(38)			30 (36)		
Beta blockers	30 (14.4)								
ACE inhibitors	24 (11.5)								

(Continues)

TABLE 1 (Continued)

Parameters	Total cohort; N = 208			Men; N = 123			Women; N = 85		
	Median	25%	75%	Median	25%	75%	Median	25%	75%
Angiotensin receptor II inhibitors	15 (7.2)								
Calcium antagonists	11 (5.3)								
Diuretics	8 (3.8)								
Statins (IHD)	13 (6.3)			11 (7.2)			2 (2.4)		

*$P < .05$, Mann-Whitney Test.

Moreover, general cardiovascular risk SCORE index had a tendency to be somewhat more prevalent by 1.65-fold ($P = .09$).

It is possible that PBR values are influenced by current pharmacotherapy administered to the patients. To evaluate this, we compared PBR values in groups treated with various drugs as shown in Table 4. Our results indicate that use of calcium antagonists and diuretics was associated with higher PBR values. In patients with IHD, use of statins was associated with lower PBR values (Table 4). It should be noted that statins were prescribed only to IHD patients while other subjects have not used statins. Other medications administered to the patients had no apparent associations with PBR, which might be due in part to small number of patients that were treated with the drugs. Thus, certain types of drug therapy can have considerable influence on PBR.

While considering possible use of PBR as a potential biomarker, it is important to compare it to other functional endothelial markers. We have measured levels of stable nitric oxide metabolites, NOx, as they were shown to be linked to regulation of blood pressure in a similar group of patients on a short-term nitrate-poor diet.[25] Results presented in Table 5 indicate that serum NOx concentrations exhibit significant negative correlation with systolic, diastolic, and pulse pressure values (Table 5). However, PBR values did not correlate with systolic and diastolic blood pressure while having relatively weak correlation with pulse pressure. It appears that in this particular group of patients, nitric oxide is more relevant for direct regulation of blood pressure while PBR is more likely to represent perfusion and elasticity of microvessels.

Thus, penetration of erythrocytes to deep layers of glycocalyx at a borderline of 2 μm in patients participating in the study can be linked to profound lesions of arterial walls as well as microcirculatory abnormalities manifested as higher prevalence of IHD and atherosclerosis of carotid artery. These effects appear to be independent from other functional endothelial markers.

TABLE 2 Characteristics of terciles split based on PBR values, PBR.Data are median (25-75 percentile range)

Parameter	Tercile I, N = 69 Men N = 42 Women N = 27	Tercile II, N = 69 Men N = 37 Women N = 32	Tercile III, N = 70 Men N = 44 Women N = 26	Multiple Comparisons Kruskal-Wallis test, *P*
PBR	≤1.81 μm	>1.81 μm; ≤2.00 μm	>2.00 μm	
Age, years	53 (48-58)	55 (45-60)	56 (51-62)	NS
Systolic blood pressure, mm Hg	130 (122-140)	128 (120-142)[a]	140 (125-146)	.01
Δ*P*, mm Hg	49 (42-56)[b]	50 (42-54)[a]	52 (46-62)	.001
Apo AI, mg/dL	157 (143-176)[b]	152 (137-174)[a]	143 (130-154)	.004
C-reactive protein, mg/L	2.7 (1.42-3.9)	3.1 (1.8-4.6)	3.2 (2.0-5.2)	NS
Maximal IMT, mm	0.8 (0.7-1.0)[b]	0.9 (0.7-1.1)[a]	1.0 (0.8-1.2)	.001
Average IMT, mm	0.8 (0.7-1.0)[b]	0.8 (0.7-1.0)[a]	1.0 (0.8-1.1)	.006
Maximal stenosis of carotid artery, %[c]	26.8 ± 6.3[b]	32.7 ± 15.6	32.0 ± 10.3	.003
CAVI left	7.5 (6.9-8.2)[b]	7.6 (7-8.1)[a]	8.1 (7.3-8.8)	.001
CAVI right	7.6 (6.9-8.1)[b]	7.7 (7.0-8.3)[a]	8.1 (7.4-8.9)	.002
Cutaneous occlusion T_{max}, s	28 (20-34)[b]	27 (21-37)[a]	34 (26-46)	.004
Cutaneous occlusion *T*½, s	56 (37-71)[b]	62 (38-85)[a]	66 (51-85)	.04

NS, not significant.
[a]$P < .05$, Kruskal-Wallis test between tercile II and tercile III.
[b]$P < .05$, Kruskal-Wallis test between tercile I and tercile III.
[c]Data are mean ± SD.

4 | DISCUSSION

Degradation and molecular modification of endothelial glycocalyx are believed to be one of the early stages of pathogenesis of vascular disorders.[26] Dysfunction of endothelial glycocalyx can contribute to arterial thrombosis.[27] Damaged glycocalyx permits deeper migration of erythrocytes toward endothelial monolayer inducing increase in PBR and release of certain components of glycocalyx into the bloodstream.[9,10] While mechanistic processes underlying disruption of glycocalyx are described in the literature, specific functional role of PBR in vascular pathology is poorly understood.

PBR is an indirect functional method and relies on monitoring flow of red blood cells through sublingual microcapillaries to estimate glycocalyx permeability. Recent studies in animals[9] and patients[15] showed high correlation between actual thickness of glycocalyx and PBR. However, in addition to glycocalyx, a number of physiological and instrumental parameters might influence PBR including local hematocrit, viscosity, and thickness of cell-free plasma layer as well as general image acquisition quality.[15] In our study, only those capillary segments that had about 50% hematocrit were used for calculations (section 2). Moreover, none of the subjects or patients had any haematological and blood-clotting diseases or acute inflammatory processes that may potentially influence local viscosity. Since the method has been introduced into clinical studies only a few years ago, additional validation of direct associations between glycocalyx thickness and PBR in patients and healthy subjects is certainly warranted.

TABLE 3 Odds ratio of occurrence of cardiovascular diseases and high SCORE index (over 5%) in patients with PBR <2.0 μm vs PBR over 2.0 μm

Condition	N, low PBR/total vs high PBR/total	Odds ratio (95% CI)	*P*
Cerebral atherosclerosis	43/69 vs 60/137	2.07 (1.15-3.72)	**.015**
IHD	16/71 vs 15/137	2.42 (1.12-5.27)	**.024**
High SCORE index, >5%	30/55 vs 43/122	1.65 (0.91-3.00)	.09

Marked values of $P < .05$ (in bold).

TABLE 5 Spearman rank order correlations (*r*) between PBR or NOx levels and blood pressure parameters

Parameter	Systolic blood pressure	Diastolic blood pressure	Pulse pressure, Δ*P*
PBR	.11	.03	**.16***
NOx	**−.25***	**−.15***	**−.24***

*$P < .05$ (in bold).

Our study is the first comprehensive report describing associations between PBR and various vascular parameters in patients. Our data indicate that higher levels of PBR may be linked to multiple components of pathological remodeling and include functional lesions of cutaneous microcirculation as well as major characteristics of carotid artery remodeling in atherosclerosis (Table 2). The list of parameters includes systolic blood pressure, pulse pressure, IMT (maximal and average), time of restoration of perfusion after occlusion (T_{max} and $T½$), and even serum concentrations of Apo A1. Moreover, we were able to determine a threshold level of PBR (2 μm) that shows strong association with prevalence of documented clinical IHD (2.42-fold) and stenosis of carotid artery (2.07-fold) according to Table 3.

Interestingly, PBR effects appear to be independent from a number of known atherosclerotic markers including serum lipids, ApoB levels, stable metabolites of nitric oxide, and endothelin. Also, PBR does not correlate with inflammatory processes as there was no association with levels of an inflammatory marker, C-reactive protein.

It should be noted that PBR was substantially affected by drug therapy. Specifically, in patients treated with calcium antagonists and diuretics, PBR was increased (Table 4). This is in disagreement with known antiatherogenic effects of calcium antagonists and may be one of the factors contributing to lowered risk of complications and lower mortality in patients treated with these drugs.[28,29] It is quite possible that increased arterial stiffness in remodeled vessels adversely impacts microcirculation and condition of endothelial glycocalyx.[30] In some patients, calcium antagonists and diuretics were prescribed for treatment of hypertension and since PBR over 2.0 μm is

TABLE 4 PBR values in patients medicated with various drugs

Medication	PBR, μm; mean ± SD; (N)		*P*
	Treated	Not treated	
Beta blockers	1.91 ± 0.24 (30)	1.91 ± 0.20 (178)	.96
Calcium antagonists	2.03 ± 0.18 (11)	1.91 ± 0.20 (197)	**.04**
ACE inhibitors	1.94 ± 0.21 (24)	1.91 ± 0.20 (184)	.44
Angiotensin II receptor antagonists	2.00 ± 0.25 (15)	1.91 ± 0.20 (193)	.30
Diuretics	2.05 ± 0.16 (8)	1.91 ± 0.20 (200)	**.04**
Statins (in the IHD group)	1.86 ± 0.20 (14)	2.05 ± 0.22 (17)	**.02**

Marked values of $P < .05$ (in bold).

associated with elevated blood pressure (Table 2), the link may be circumstantial and unrelated to pharmacological effects of the drugs. However, use of statins had an opposite effect and appears to decrease PBR. Obviously, the data warrant further investigation due to rather low number of patients treated with calcium antagonists and diuretics. As statins were only administered to patients with current IHD, sampling number is quite low in this case as well. Unfortunately, this is one of the main limitations of current study.

It is unlikely that PBR may be a general marker of endothelial function because it was not linked to circulating levels of stable nitric oxide metabolites, NOx, or endothelin (Table 5). It is however reasonable to suggest that high values of PBR exceeding 2 μm result in pathological microcirculation that is generally associated with prevalence of IHD and carotid atherosclerosis. Thus, PBR can be potentially used as a highly reproducible (CV <10%) non-invasive marker for assessment of cardiovascular risks in complex with other known biomarkers.

5 | PERSPECTIVE

Elevated PBR values over 2 micrometers were associated with morphological and functional lesions of arterial wall and microcirculation and lowered levels of ApoA1 lipoprotein. Elevated PBR values were associated with 2.07-fold increase in prevalence of cerebral atherosclerosis ($p = 0.015$) and 2.42-fold increase in prevalence of ischemic heart disease ($p = 0.024$). Thus, PBR can be considered a new highly reproducible and promising marker candidate for non-invasive clinical diagnostics of ischemic heart disease and cerebral atherosclerosis suggesting important role of microcirculation in development and progression of cardiovascular diseases.

ACKNOWLEDGMENTS

The study was funded by Russian Foundation for Basic Research, Grant number 17-04-00157\17.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

ORCID

Nadezhda G. Gumanova http://orcid.org/0000-0002-6108-3538

REFERENCES

1. Jacob M, Chappell D, Becker BF. Regulation of blood flow and volume exchange across the microcirculation. *Crit Care*. 2016;20:319.
2. Davignon J, Ganz P. Role of endothelial dysfunction in atherosclerosis. *Circulation*. 2004;109:27-32.
3. Paulus WJ, Tschöpe C. A novel paradigm for heart failure with preserved ejection fraction: comorbidities drive myocardial dysfunction and remodeling through coronary microvascular endothelial inflammation. *J Am Coll Cardiol*. 2013;62:263-271.
4. Broekhuizen LN, Mooij HL, Kastelein JJ, Stroes ES, Vink H, Nieuwdorp M. Endothelial glycocalyx as potential diagnostic and therapeutic target in cardiovascular disease. *Curr Opin Lipidol*. 2009;20:57-62.
5. Vink H, Duling BR. Capillary endothelial surface layer selectively reduces plasma solute distribution volume. *Am J Physiol Heart Circ Physiol*. 2000;278:H285-H289.
6. Jacob M, Rehm M, Loetsch M, et al. The endothelial glycocalyx prefers albumin for evoking shear stress-induced, nitric oxide-mediated coronary dilatation. *J Vasc Res*. 2007;44:435-443.
7. Vink H, Duling BR. Identification of distinct luminal domains for macromolecules, erythrocytes, and leukocytes within mammalian capillaries. *Circ Res*. 1996;79:581-589.
8. Ushiyama A, Kataoka H, Iijima T. Glycocalyx and its involvement in clinical pathophysiologies. *J Intensive Care*. 2016;4:59.
9. Lee DH, Dane MJ, van den Berg BM, et al. Deeper penetration of erythrocytes into the endothelial glycocalyx is associated with impaired microvascular perfusion. *PLoS ONE*. 2014;9:e96477.
10. Vlahu CA, Lemkes BA, Struijk DG, Koopman MG, Krediet RT, Vink H. Damage of the endothelial glycocalyx in dialysis patients. *J Am Soc Nephrol*. 2012;23:1900-1908.
11. National recommendations about diagnostics and treatment of stable angina. *Cardiovasc Ther Prev*. 2008;7(Suppl 4):1-37.
12. Gorshkov AY, Gumanova NG, Boytsov SA. Dark field microscopy for the endothelial glycocalyx study: first Russian experience; the reproducibility of the method. *Reg Haemodynamics Microcirc*. 2016;15/1:33-41.
13. Jaarsma C, Vink H, van Haare J, et al. Non-invasive assessment of microvascular dysfunction in patients with microvascular angina. *Int J Cardiol*. 2017;248:433-439.
14. Wadowski PP, Hülsmann M, Schörgenhofer C, et al. Sublingual functional capillary rarefaction in chronic heart failure. *Eur J Clin Invest*. 2018;48:e12869.
15. Machin DR, Gates PE, Vink H, Frech TM, Donato AJ. Automated measurement of microvascular function reveals dysfunction in systemic sclerosis: a cross-sectional Study. *J Rheumatol*. 2017;44:1603-1611.
16. Ikonomidis I, Pavlidis G, Lambadiari V, et al. Early detection of left ventricular dysfunction in first-degree relatives of diabetic patients by myocardial deformation imaging: the role of endothelial glycocalyx damage. *Int J Cardiol*. 2017;233:105-112.
17. Roustit M, Cracowski JL. Non-invasive assessment of skin microvascular function in humans: an insight into methods. *Microcirculation*. 2012;19:47-64.
18. Stefanovska A, Sheppard LW, Stankovski T, McClintock PV. Reproducibility of LDF blood flow measurements: dynamical characterization versus averaging. *Microvasc Res*. 2011;82:274-276.
19. Shirai K, Utino J, Otsuka K, Takata M. A novel blood pressure-independent arterial wall stiffness parameter; cardio-ankle vascular index (CAVI). *J Atheroscler Tromb*. 2006;13:101-107.
20. Sun CK. Cardio-ankle vascular index (CAVI) as an indicator of arterial stiffness. *Integr Blood Press Control*. 2013;6:27-38.
21. Geisel MH, Bauer M, Hennig F, et al. Comparison of coronary artery calcification, carotid intima-media thickness and ankle-brachial index for predicting 10-year incident cardiovascular events in the general population. *Eur Heart J*. 2017;38:1815-1822.
22. Celermajer DS, Sorensen KE, Gooch VM. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet*. 1992;340:1111-1115.
23. Miranda KM, Espey MG, Wink DA. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide*. 2001;5:62-71.

24. Metelskaya VA, Gumanova NG. Screening as a method for determining the serum level of nitric oxide metabolites. *Klin Lab Diagn*. 2005;6:15-18.
25. Gumanova NG, Teplova NV, Ryabchenko AU, Denisov EN. Serum nitrate and nitrite levels in patients with hypertension and ischemic stroke depend on diet: a multicenter study. *Clin Biochem*. 2015;48:29-32.
26. Dane MJC, van den Berg BM, Avramut MC, et al. Glomerular endothelial surface layer acts as a barrier against albumin filtration. *Am J Pathol*. 2013;182:1532-1540.
27. Noble MI, Drake-Holland AJ, Vink H. Hypothesis: arterial glycocalyx dysfunction is the first step in the atherothrombotic process. *QJM*. 2008;101:513-518.
28. Lichtlen PR, Lippolt P, Rafilenbeui W. INTACT Group Investigators. Retardation of angiographic progression of coronary artery disease by nifedipine: the 6-year follow-up study of INTACT (International Nifedipine Trial on Antiatherosclerotic Therapy). *J Cardiovasc Pharmacol*. 1996;28:S10-S21.
29. Brown MJ, Palmer CR, Castaigne A, et al. Morbidity and mortality in patients randomized to double-blind treatment with a long-acting calcium-channel blocker or diuretic in the International Nifedipine GETS study: intervention as a goal in hypertension treatment (INSIGHT). *Lancet*. 2000;356:366-372.
30. Hansson L, Zanchetti A, Carruthers SG, et al. Effects of intensive blood-pressure lowering and low-dose aspirin in patients with hypertension: principal results of the Hypertension Optimal Treatment (HOT) randomised trial. HOT Study Group. *Lancet*. 1998;351:1755-1762.

How to cite this article: Gorshkov AY, Klimushina MV, Boytsov SA, Kots AY, Gumanova NG. Increase in perfused boundary region of endothelial glycocalyx is associated with higher prevalence of ischemic heart disease and lesions of microcirculation and vascular wall. *Microcirculation*. 2018;25:e12454. https://doi.org/10.1111/micc.12454