Clinical Hemorheology and Microcirculation 78 (2021) 247–257
DOI 10.3233/CH-201016
IOS Press

Reactivity of skin microcirculation as a biomarker of cardiovascular events. Pilot study

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Abstract.
BACKGROUND: The role of microcirculatory disorders is progressively being accepted in the pathogenesis of cardiovascular diseases.
OBJECTIVE: The purpose of current study is to assess whether we can consider skin microcirculation disorders as a biomarker of cardiovascular events.
METHODS: Group 1 consisted of healthy volunteers ($n = 31$); group 2 ($n = 42$) consisted of patients with diseases that increase the risk of cardiovascular events; group 3 ($n = 39$) included patients with the history of cardiovascular events. Skin microcirculation measurement was performed using laser Doppler flowmetry during the heating test.
RESULTS: LDF parameters reflecting the rapid response of microcirculation to heating ("Slope 120 s" and "Slope 180 s") significantly differed in three groups ($p < 0.05$). A decrease in the "Slope 180 s" parameter less than 0.5 PU/s is associated with cardiovascular events (sensitivity 69.2%, specificity 66.7%; the area under the ROC curve, 0.667; 95% confidence interval [CI], 0.545–0.788, $p = 0.01$). Multivariable logistic regression analysis revealed that "Slope 180 s $\leq$ 0.5 PU/s" was significantly related to cardiovascular events (adjusted odds ratio = 3.9, $p = 0.019$, CI 95% 1.2–12).
CONCLUSIONS: Reduced reactivity of the skin microcirculation may be useful as a biomarker of severe damage to the cardiovascular system and is promising as a risk factor for cardiovascular events.

Keywords: Cardiovascular diseases, skin, microcirculation, laser-Doppler flowmetry, risk factors, diabetes mellitus, hypertension

1. Introduction

In recent years, the idea that microcirculation dysfunction plays an important role in the ethiopathogenesis of cardiovascular diseases is discussing [1, 2]. Due to its availability, skin microcirculation has been proposed as a model for the general function of microvessels [3]. There are many methods to assess skin microcirculation: video microscopy, thermography, laser speckle contrast imaging, laser Doppler flowmetry (LDF), etc. [4–7]. LDF is one of the most widely used optical methods for quantitative, non-invasive assessment of skin microcirculation. The method assesses index of skin perfusion

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ISSN 1386-0291/$35.00

in the studied area (the parameter is directly proportional to the velocity and concentration of erythrocytes) [8]. It should be mentioned that LDF measures not only the skin microcirculation, but LD signal might also reflect blood flux in tissues below the skin (especially if the skin is thin and atrophied) [9]. Still, the term "skin microcirculation" is usually used for simplicity.

There is not enough data to date to say unambiguously whether the skin microcirculation disorders are applicable in practice as prognostic or diagnostic biomarkers of cardiovascular diseases [10]. However, many studies showed the relationship between skin microcirculation disturbances and cardiovascular disease. It was demonstrated that arterial hypertension, diabetes mellitus, coronary artery disease, etc. are associated with disorders of skin microcirculation; moreover, microcirculation disorders are often considered as a pathogenetic factor for the development of these diseases [2, 11–14]. Thus, it is known that arterial hypertension is associated with changes in skin capillaries [14–16]. Moreover, it was shown that these changes can occur even in normotensive offspring of individuals with essential hypertension [17]. It is well known that diabetes mellitus causes disorders in skin microcirculation; several studies showed that, these disorders can be revealed not only in patients with diagnosed diabetes mellitus [11, 18] but also in patients with prediabetes [19, 20]. It was noticed that patients with coronary artery disease exhibit a lower reactivity of skin microcirculation compared with persons having no symptoms of ischemia [13, 21]. It has been reported that chronic heart failure is also associated with changes in skin microcirculation [22]. A common feature of these diseases is that they increase the risk of the cardiovascular events development [23].

The purpose of the current study is to assess whether we can consider skin microcirculation disorders as a biomarker of cardiovascular events.

2. Materials and methods

2.1. Participants

Our study included 112 participants who were divided into three groups. Group 1 consisted of healthy volunteers ($n = 31$; 15 men, 16 women). According to the survey and preliminary laboratory and instrumental examination, the participants had no arterial hypertension, cardiovascular diseases, and diabetes mellitus. Patients with no history of cardiovascular events, but who had diseases that increase the risk of cardiovascular events were enrolled in group 2 ($n = 42$; 15 men, 27 women). The diseases that increase the risk of cardiovascular events were defined as arterial hypertension, diabetes mellitus, chronic coronary artery disease, mild heart failure (NYHA functional class I-II). group 3 ($n = 39$; 19 men, 20 women) included patients with a history of nonfatal cardiovascular event/events. For our analyses, the definition of cardiovascular event included myocardial infarction, stroke, coronary revascularization (definition of cardiovascular events according to FIELD Study Investigators [24]).

The exclusion criteria for all groups were atrial fibrillation, diagnosed peripheral artery disease, dermatitis at the measurement sites. No exclusions in relation to current pharmacotherapy were applied.

2.2. Study protocol

All skin microcirculation tests were carried out by the LDF method on a LAKK-02 device (scientific productive enterprise (SPE) "LAZMA", Moscow, Russian Federation). To assess the reactivity of skin microcirculation, a thermal test was performed as the most convenient, simple and safe type of exposure, which may be further applied in clinical practice. Moreover, local heating is most often used in scientific studies [1]. Local heating was carried out on a LAKK-TEST device (SPE "LAZMA", Russia). An optic fiber sensor is mounted into the heating probe of the LAKK-TEST device, which makes it possible

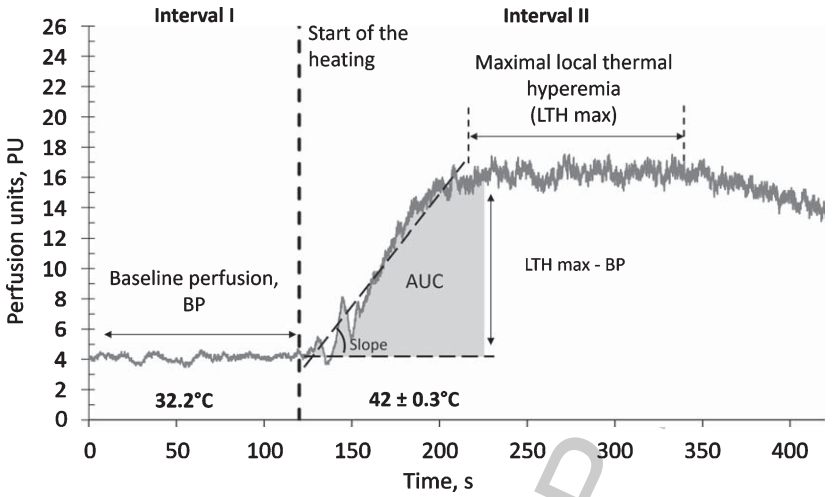

Fig. 1. The example of the microcirculation curve recorded during the heating test.

to heat and measure skin microcirculation simultaneously. Skin microcirculation was measured in perfusion units (PU). After 15 minutes of adaptation to the measurement conditions, a heating probe with the fiber optic sensor was fixed on the back of the right forearm (4 cm proximal to the radiocarpal articulation along the median line). Throughout the examination (7 min), the patient was in a sitting position, the arms lay on the table with the palms downwards, and the forearms were at the level of the heart. Within the first two minutes (interval I: 0–120 seconds) the baseline perfusion was recorded; during this period the temperature of the heating probe was 32.2°C. At 120 second, heating was turned on to a temperature of 42 ± 0.3°C (heating rate 2°C per second) (interval II, 120–420 seconds). After 5 min of heating, the registration of microcirculation was terminated. The scheme of examination with the example of the microcirculation curve is shown in Fig. 1.

For the microcirculation reactivity analysis, we have calculated the following parameters:

(1) the average baseline perfusion in interval I "BP", which reflects the level of skin microcirculation at rest;
(2) the maximum of heating vasodilatation parameter "LTH max", which is often evaluated in studies [25–28], was calculated as an average value of the representative region of the microcirculation curve of the duration no less than 30 seconds in interval II;
(3) the area under the hyperemia curve within the first 120 and 180 seconds of heating ("AUC 120 s" and "AUC 180 s"), which reflects the intensity of vasodilation and is used by researchers [27, 29];
(4) the relative increase in perfusion ("LTH max — BP"), which was estimated by calculating the difference between the maximum perfusion during heating ("LTH max") and the average baseline perfusion ("BP").
(5) the tangent of the angle between the regression line (for the microcirculation curve) and the time axis within the first 120 and 180 seconds of heating multiplied by 10 ("Slope 120 s" and "Slope 180 s").

Formula developed by us for calculating parameters "Slope 120 s" and "Slope 180 s":

$$Slope = \frac{\sum (t_i - \bar{t})\ (I_i * 10 - \bar{I} * 10)}{\sum (t_i - \bar{t})^2}, \text{ where}$$

I_i – microcirculation value (PU) at time t_i; $\tilde{I}$– arithmetic mean perfusion for the estimated interval; t_i – time from the beginning of the heating in seconds, $\bar{t}$ – mean time for the estimated interval.

2.3. Statistical analysis

The statistical analysis was performed in IBM SPSS Statistics v25 (IBM Corp., USA) and GraphPad Prism 8.0.1 (GraphPad Software, USA). The quantitative variables are represented by the median and interquartile range (Me [LQ; UQ]), nominal – by the absolute and relative frequencies (n, %). The quantitative variables in the three groups were compared using the Kruskal-Wallis test. The *post-hoc* pairwise comparisons were made using the Dunn test with Bonferroni correction. The nominal variables were compared with Fisher's exact test with Bonferroni correction. The cut-off values for the quantitative variables were selected using ROC-analysis. The biomarker assessment was performed with the logistic regression and the odds ratio (OR) estimation. The *p*-value less than 0.05 was considered to be statistically significant. Sample size calculation was performed in G*Power 3.1.9.4 (Universität Düsseldorf, Germany) for three groups comparison with one-way ANOVA [30]. It was shown that at least 111 patients should be included in the study to detect the difference between groups with 0.3 effect size at 0.05 significance level and 80% power. Effect size was estimated during the previous measurements.

2.4. Ethics

The informed consent was obtained from all the participants. The protocol of the study complies with the ethical principles of the Helsinki declaration (revision of 2013) and was approved by the Independent Ethics Committee at the Moscow Regional Research and Clinical Institute (Moscow, Russia) (Protocol No. 11 of 12 December, 2017).

3. Results

The general characteristics of the study participants are presented in Table 1. The patients from group 2 and group 3 were comparable by sex, age, body mass index (BMI), the presence of diabetes mellitus, and diabetic microangiopathies. There were significantly more patients with chronic coronary artery disease and chronic heart failure in group 3 than in group 2. The participants from group 1 did not have the diagnosed diseases of the cardiovascular system, obesity and were significantly younger than patients from groups 2 and 3. This group was included in the study as a reference group without microcirculatory disorders caused by diseases or age. Healthy volunteers in group 1 did not receive vasoactive drugs. Table 2 shows the cardiovascular drug therapy in groups 2 and 3. In groups 2 and 3, there were no significant differences in the frequency of drug therapy with angiotensin receptor blockers, calcium channel blockers, diuretics, centrally active agents, long-acting nitrates, alpha-receptor blockers, and anticoagulants. The frequency of therapy with beta-blockers, angiotensin-converting enzyme inhibitors, antiplatelet drugs, and lipid-lowering drugs was significantly higher in group 3 than in group 2 (Table 2).

Table 3 presents the comparison results of analyzed microcirculation parameters between groups. The average baseline perfusion "BP" didn't differ in all three groups. The parameters "LTH max", "AUC 120 s" "AUC 180 s", "LTH max BP" showed significant differences between the healthy volunteers and the patients with the diseases affecting the cardiovascular system (group 1 vs group 2; group 1 vs group 3); however, they did not differ inside the groups of patients with these diseases (group 2 vs group 3).

Table 1
Characteristics of the study participants

	Group 1 $n = 31$	Group 2 $n = 42$	Group 3 $n = 39$	All groups comparison, p-value	Pairwise post-hoc comparisons
Age, years, Me [LQ; UQ]	28 [25; 32]	59 [55; 67]	64 [57; 70]	$< 0.001^*$	$p_{1-2} < 0.001^*$ $p_{1-3} < 0.001^*$ $p_{2-3} = 0.635$
Sex (male/female), n (%)	15(48%)/ 16(52%)	15(36%)/ 27(64%)	19(49%)/ 20(51%)	0.419	–
Body mass index, kg/m2, Me [LQ; UQ]	21.9 [19.5; 24]	28.65 [26.1; 32]	29.8 [27.1; 33]	$< 0.001^*$	$p_{1-2} < 0.001^*$ $p_{1-3} < 0.001^*$ $p_{2-3} = 1$
Hypertension, n (%)	–	42 (100%)	39 (100%)	–	–
Diabetes mellitus, n (%)	–	21 (50%)	26 (67%)	–	$p_{2-3} = 0.177$
Diabetic retinopathy, n (%)	–	5 (24%) from 21	10 (38%) from 26	–	$p_{2-3} = 0.768$
Diabetic nephropathy, n (%)	–	14 (67%) from 21	16 (62%) from 26	–	$p_{2-3} = 0.355$
Chronic coronary artery disease, n (%)	–	13 (31%)	30 (77%)	–	$p_{2-3} < 0.001^*$
Heart failure, NYHA functional class, n (%)					
I	–	2 (5%)	4 (10%)		$p_{2-3} = 0.022^*$
II	–	8 (19%)	14 (36%)	–	
III	–	–	3 (8%)		
IV	–	–	–		
Cardiovascular events					
Previous myocardial infarction, n (%)	–	–	20 (51%)	–	–
Previous stroke, n (%)	–	–	13 (33%)	–	–
Previous coronary revascularization, n (%)	–	–	18 (46%)	–	–

LQ: lower quartile; Me: median; UQ: upper quartile; NYHA: New York Heart Association; *: statistically significant differences.

Table 2
Medication therapy

Medications	Group 2, $n = 42$	Group 3, $n = 39$	p-value
Beta-blockers, n (%)	15 (35.7%)	28 (71.8%)	0.002*
Angiotensin-converting enzyme inhibitors, n (%)	14 (33.3%)	23 (59.0%)	0.026*
Angiotensin receptor blockers, n (%)	19 (45.2%)	13 (33.3%)	0.364
Calcium channel blockers, n (%)	13 (31.0%)	17(43.6%)	0.259
Diuretics, n (%)	16 (38.1%)	21 (53.8%)	0.184
Centrally active agents, n (%)	6 (14.3%)	2 (5.1%)	0.267
Alpha-receptor blockers, n (%)	1 (2.4%)	0	1
Long-acting nitrates, n (%)	1 (2.4%)	6 (15.4%)	0.052
Antiplatelet drugs, n (%)	24 (57.1%)	33 (84.6%)	0.008*
Anticoagulants, n (%)	2 (4.9%)	4 (10.3%)	0.426
Lipid-lowering therapy, n (%)	18 (42.9%)	31 (79.5%)	0.001*

*statistically significant difference.

Table 3
Comparison of microcirculation parameters in three groups of patients

	Group 1 Me [LQ; UQ]	Group 2 Me [LQ; UQ]	Group 3 Me [LQ; UQ]	All groups comparison, *p*-value	Pairwise post-hoc comparisons
BP, PU	4.3 [3; 6.1]	4.5 [3; 7.5]	3.5 [2.5; 5.8]	0.31	–
LTH max, PU	22.8 [18.2; 26.9]	17.8 [13.9; 22]	15.1 [11.5; 19.4]	$<0.001^*$	$p_{1-2}<0.008^*$ $p_{1-3}<0.001^*$ $p_{2-3}=0.219$
Slope 120 s, PU/s	1.41 [1.12; 1.59]	1.1 [0.81; 1.31]	0.85 [0.6; 1.02]	$<0.001^*$	$p_{1-2}<0.001^*$ $p_{1-3}<0.005^*$ $p_{2-3}=0.02^*$
Slope 180 s, PU/s	0.79 [0.59; 0.93]	0.61 [0.45; 0.76]	[0.36; 0.57]	$<0.001^*$	$p_{1-2}<0.008^*$ $p_{1-3}<0.001^*$ $p_{2-3}=0.04^*$
AUC 120 s, PU*s	1368 [1227; 2131]	1037 [791; 1315]	810 [659; 1160]	$<0.001^*$	$p_{1-2}=0.001^*$ $p_{1-3}<0.001^*$ $p_{2-3}=0.133$
AUC 180 s, PU*s	2448 [2057; 3534]	1805 [1390; 2208]	1477 [1106; 1857]	$<0.001^*$	$p_{1-2}<0.001^*$ $p_{1-3}<0.001^*$ $p_{2-3}=0.119$
LTH max BP, %	410 [293.2; 600.5]	277 [180.7; 442.6]	282.9 [175.2; 464.4]	0.007^*	$p_{1-2}<0.012^*$ $p_{1-3}<0.023^*$ $p_{2-3}=1$

AUC 120 s and AUC 180 s: the area under the curve within the first 120 and 180 seconds of heating, respectively; BP: the average baseline perfusion; LTH max: the maximum of heating vasodilatation; Slope 120 s and Slope 180 s: the tangent of the angle between the regression line (for the microcirculation curve) and the time axis within the first 120 and 180 seconds of heating, respectively; LTH max – BP: a relative increase in perfusion after turning on the heat. LQ: lower quartile; Me: median; PU: perfusion units; UQ: upper quartile; *: statistically significant differences.

Parameters "Slope 120 s" and "Slope 180 s" significantly differed in all three groups (Table 3). The maximum value of "Slope 120 s" and "Slope 180 s" was recorded in healthy volunteers (group 1). In patients from group 2 these parameters were significantly lower compared with the healthy volunteers. In patients that had a history of cardiovascular events (group 3) "Slope 120 s" and "Slope 180 s" values were minimal and significantly differed from that for the healthy volunteers and the patients of group 2 (Fig. 2, Table 3).

Thus, "Slope 120 s" and "Slope 180 s" differed not only between the healthy volunteers and the patients with the diseases affecting the cardiovascular system, but also significantly differed between group 2 and group 3. Using the ROC analysis, the cut-off value was calculated, which allows to separate groups 2 and 3 according to the microcirculation parameter. For the "Slope 180 s" parameter, the cut-off value was 0.5 PU/s (the area under the ROC curve, 0.667; 95% confidence interval [CI], 0.545–0.788, $p=0.01$) (Fig. 3). The sensitivity of this criterion ("Slope 180 s $\leq$ 0.5 PU/s") in identifying patients with cardiovascular events was 69.2%, and the specificity was 66.7%. The possibility to apply this microcirculation parameter as a biomarker of cardiovascular events was estimated by constructing the logistic regression model and calculating the OR of cardiovascular events for patients with the diseases affecting the cardiovascular system (group 2 and group 3). The results of microcirculation reactivity assessment as a biomarker of cardiovascular events are shown in Table 4. The parameter "Slope 180 s $\leq$ 0.5 PU/s" was associated with cardiovascular events (OR, 4.5; 95% CI, 1.7–11.5; $p=0.002$).

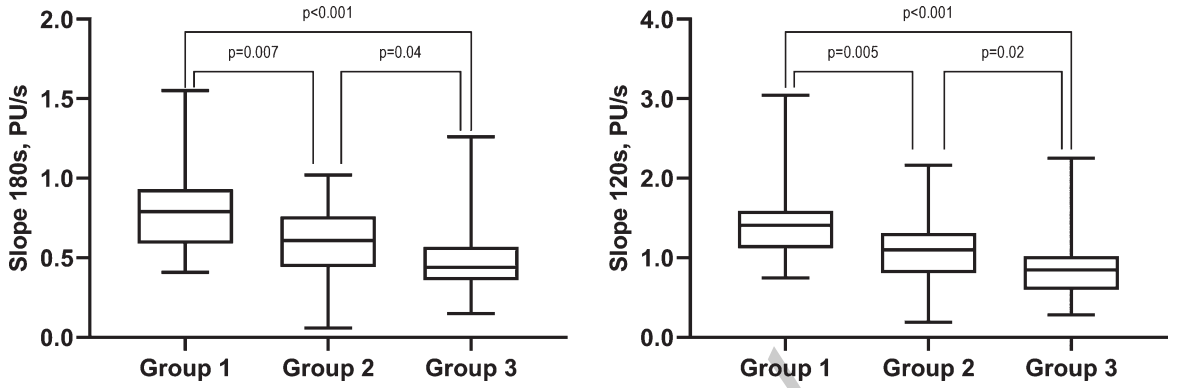

Fig. 2. Difference in the "Slope 120 s" and "Slope 180 s" parameters for three groups of patients. PU/ s— perfusion units per second.

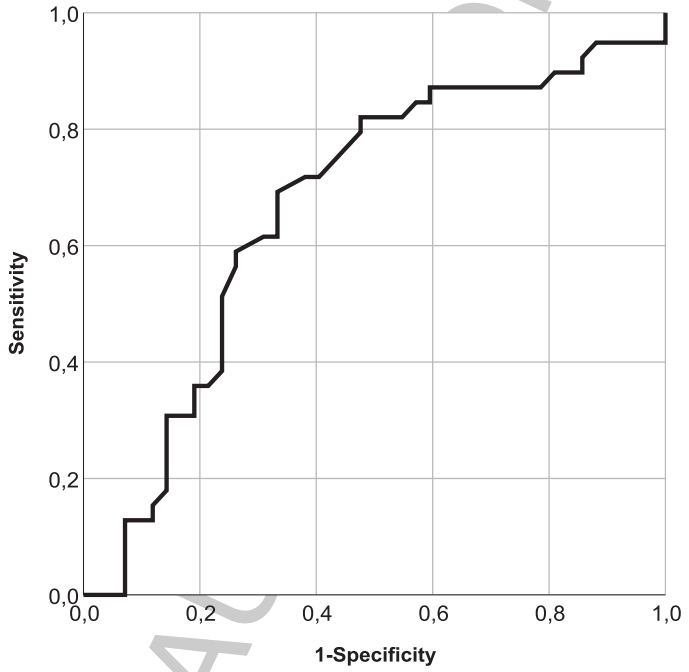

Fig. 3. ROC analysis for parameter "Slope 180 s" in identifying patients with cardiovascular events.

After correction for potential confounders (age, sex, body mass index, heart failure, diabetes mellitus, coronary artery disease with angina pectoris) with multivariable logistic regression model, OR for this parameter remained statistically significant (OR 3.9; 95% CI, 1.2–12, $p = 0.019$).

4. Discussion

In our pilot study, we consider the reactivity of skin microcirculation as a biomarker of cardiovascular events. There are some studies showing that LDF parameters of skin microcirculation are associated with cardiovascular risk, measured by the cardiovascular risk assessment scores [31, 32]. As for our study, we consciously refused to categorize patients according to the generally accepted stratification

Table 4
The results of microcirculation reactivity assessment as a risk factor for cardiovascular events

	Slope 180 s as a risk factor			
	>0.5 PU/s $n=40$	≤ 0.5 PU/s $n=41$	OR (95% CI)	Adjusted OR (95% CI)
No cardiovascular events (Group 2), $n=42$	28 (70%)	14 (33%)	4.5 (1.7 – 11.5), $p=0.002$	3.9 (1.2 – 12), $p=0.019$
A history of cardiovascular events (Group 3), $n=39$	12 (30%)	27 (67%)		

Adjusted OR is controlled for potential confounders: age, sex, body mass index, heart failure, coronary artery disease, diabetes mellitus. CI: confidence interval; OR: odds ratio; PU: perfusion units.

of the total cardiovascular risk (low, moderate, high and very high risk) or other cardiovascular risk assessment scores [23]. These scores are necessary for making a decision on treatment strategies and have some limitations. However, our task was not to compare microcirculatory disorders with risk factors already existing in the clinic, but rather to assess the possibility of their use as a biomarker of the severity of cardiovascular injury. So, we decided to include groups of patients with cardiovascular events and without them in the study.

As can be seen from Table 3, many microcirculation parameters significantly differed between the healthy volunteers (group 1) and the patients with the diseases affecting the cardiovascular system (groups 2 and 3). Group 1 was significantly different in the all analyzed clinical parameters from groups 2 and 3. This group was included in the study as a control group with a "reference" microcirculation; a younger age allowed to restrict age-related effects on microcirculation. These data are consistent with a number of scientific studies [11, 33]. However, we found it more relevant to practical medicine to assess microcirculation reactivity disorder as a marker of cardiovascular injury in patients with diseases affecting the cardiovascular system than in young healthy individuals.

"Slope 120 s" and "Slope 180 s" parameters significantly differed in all three groups. These parameters differed not only between healthy volunteers and patients with cardiovascular diseases, but also between groups 2 and 3. We showed that the "Slope 180 s" parameter less or equal to 0.5 PU/s can be considered as the biomarker of cardiovascular events.

As mentioned above, patients from groups 2 and 3 had diseases and conditions that could also affect skin microcirculation, such as sex, age, BMI, diabetes mellitus, stable angina, chronic heart failure. They are also considered well-known factors that increase the risk of cardiovascular events. Thus, the reduced microcirculation reactivity in patient with cardiovascular events may be explained by the influence of other factors correlated with microcirculation. Therefore, an adjusted odds ratio was calculated to prove that reduced reactivity of skin microcirculation is associated with increased odds of cardiovascular events independently of other factors. To exclude the influence of these factors multivariable logistic regression analysis was performed. And it was shown that the "Slope 180 s ≤ 0.5 PU/s" was significantly associated with cardiovascular events even after controlling for potential confounders. It was shown that the odds of cardiovascular events in patients with a decreased microcirculation reactivity ("Slope 180 s ≤ 0.5 PU/s") is 3.9 times higher than in patients with "Slope 180 s > 0.5".

The "Slope 180 s" parameter shows the dynamic changes of the perfusion curve in the first three minutes after the heating is turned on. The slope of the microcirculation curve quantitatively reflects the intensity of the swift response of the microcirculation bed to heating.

It is commonly supposed that primary vasodilatation upon heating is due to neuronal axon-reflex mechanisms, and further vasodilatation is associated with the release of NO from the vessel endothelium

[34, 35]. Consequently, in patients with cardiovascular diseases, neural dysfunction may significantly contribute to the decrease in the response of skin microcirculation to heating.

The algorithm of the heating test performed in current study involves heating to $42 \pm 0.3^{\circ}$C at the heating rate of 2°C/s [36]. In the majority of studies, a lower heating rate is used; therefore, the time required for attaining the maximum temperature and the total time of the test increases. Thus, common mean time of the heating test is 20–50 min [11]. The acquisition of informative results during a 7 min heating test makes this algorithm very convenient for the use in the clinical practice. Moreover, a 3-minute heating test is enough to evaluate the "Slope 180 s" parameter.

5. Conclusions

Thus, the assessment of the reactivity of skin microcirculation to local heating may be considered as a biomarker of damage to the cardiovascular system. LDF parameters of skin microcirculation are quantitative, objective, dynamically changing values that are convenient to apply in clinical practice.

The paradigm of the modern medicine is based on earlier detection of risk factors of pathologies and their elimination. At present, a number of diseases and symptoms have been recognized as cardiovascular risk factors, and this list is constantly being updated. Today it is considered that microcirculation disorders can be not only a manifestation, but also a pathogenetic link in several diseases leading to cardiovascular events. This study showed that skin microcirculatory disturbances can be used as a biomarker of severity of cardiovascular injury. We suppose that skin microcirculation reactivity may also be promising as a risk factor for cardiovascular events. This approach may be useful for a more accurate assessment of cardiovascular risk in patients and may provide physicians with additional information about the patient health status.

6. Limitations

The study design does not allow the effect of drug therapy on skin microcirculation to be assessed.

Acknowledgments

This work was supported by grant of the President of the Russian Federation (grant number MK-1786.2020.7, agreement No. 075-15-2020-354).

Conflicts of interest

The authors declare no conflict of interest.

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