RESEARCH ARTICLE

Microcirculatory Dysfunction in Patients With Diabetes Mellitus Detected by a Distributed System of Wearable Laser Doppler Flowmetry Analysers

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ABSTRACT

The paper is devoted to the study of perfusion and amplitude-frequency spectra of laser Doppler flowmetry (LDF) signals in patients with diabetes mellitus (DM) in different skin areas of the upper and lower extremities using a distributed system of wearable LDF analysers. LDF measurements were performed in the areas of the fingers, toes, wrists and shins. The mean perfusion values, the amplitudes of blood flow oscillations in endothelial, neurogenic, myogenic, respiratory and cardiac frequency ranges, and the values of nutritive blood flow were analysed. The results revealed a decrease in tissue perfusion and nutritive blood flow in the lower extremities and an increase in these parameters in the upper extremities in patients with DM. A decrease in the amplitudes of endothelial and neurogenic oscillations was observed. The obtained results confirm the possibility of using wearable LDF analysers to detect differences in the blood flow regulation in normal and pathological conditions.

1 | Introduction

The blood microcirculatory system has been the subject of extensive research for many years due to its direct involvement in the processes of vital activity of cells and tissues of living organisms. Many diseases, such as diabetes mellitus (DM) or arterial hypertension, in the early stages of their development are characterised by the onset of microcirculatory dysfunction [1–3].

Diabetes in particular is a major challenge for healthcare system [4], not only because of its high prevalence (more than 537 million people worldwide according to the IDF Diabetes Atlas 2021 [5]), but also because of the frequency of its disabling complications. Impaired microcirculation and the development of diabetic polyneuropathy in patients with DM lead to the development of diabetic foot ulcers, which are difficult to heal, lower extremities amputation, end-stage renal disease and other adverse outcomes [6]. DM is considered to be the leading cause of non-traumatic lower limb amputation in developed countries. Early diagnosis and appropriate treatment of microcirculatory disorders can help reduce the risk and the prevalence of diabetic complications.

Laser Doppler flowmetry (LDF) is one of the most widely used non-invasive methods to study the functional state of microcirculation in human skin [7]. This method is based on probing tissue with a laser beam and analysing the light scattered by the tissues. In LDF light scattered by erythrocytes moving in the biological tissue under investigation undergoes a Doppler frequency shift, while the frequency of light scattered by stationary objects remains unchanged. In general, the result of LDF measurements is the absolute value of perfusion measured at each time point (index of microcirculation, I_m), measured in perfusion (arbitrary) units. In addition to general local perfusion of biological tissues, LDF also allows to assess the function of the blood flow regulation mechanisms by various factors including

heartbeat, respiration, intrinsic myogenic activity of vascular smooth muscles, sympathetic nerve activity and vascular endothelial activity [8, 9].

Numerous papers by different teams of authors have already demonstrated the potential of LDF in the diagnosis of microcirculatory disorders associated with DM. Using LDF, changes in skin perfusion in the upper and lower extremities of patients with DM at rest and during application of local heating [10–13], impaired vasomotion [14, 15], decreased level of maximal vasodilation achieved under the influence of acetylcholine and sodium nitroprusside [16], inadequate response of microcirculation to application of occlusion test [16, 17] were shown.

A large number of studies have investigated the altered response of the cutaneous microcirculation in patients with DM to local heating, cooling, local pressure application or injection of vasodilators [18]. A large number of experimental protocols have been proposed using long periods of LDF signal recording and complex sequences of functional tests.

Studies of basal skin perfusion in patients with DM without the use of functional tests have so far produced conflicting results. Some papers report basal perfusion values in patients does not differ from the control group (CG) values [18–21]. Other authors report increased microcirculation levels in patients [13, 22, 23]. The third group of papers shows a decrease in absolute values of local perfusion [24]. This discrepancy in results may be explained by differences in study methodology: the choice of different anatomical skin sites for the study, the inclusion of patients with or without diabetic complications (such as diabetic polyneuropathy, retinopathy or nephropathy), the influence of comorbidities, and so on.

Some studies using two- and four-channel LDF monitors have presented the results of comparisons of microcirculatory parameters in patients with DM and healthy controls in different anatomical areas, including upper and lower extremities [21, 25–27]. The article by Urbancic-Rovan et al. [25] presents the results of a comparison of absolute values of perfusion and amplitudes of microcirculatory blood flow oscillations in groups of patients with diabetes with and without diabetic neuropathy and a CG in four measurement sites—right and left hand and right and left foot, demonstrating the importance of studying local features of microcirculation in different anatomical areas. Glazkov et al. in their work [26] demonstrated simultaneous measurement of perfusion in the finger pad and toe in patients with DM with and without diabetic foot syndrome and in healthy controls. The results reveiled higher absolute values of perfusion in the upper extremities in both groups of patients, but lower perfusion in the lower extremities in patients with diabetic foot syndrome compared to patients without this complication. Tikhonova et al. in their work studied skin perfusion and spectral characteristics of LDF and heart rate variability signals in the upper and lower extremities of diabetic patients at rest and during local heating [21] or under postural exposure [27].

The development and commercialisation of a wearable version of LDF devices ('LAZMA PF' by LAZMA Ltd., Russia; in EU/UK this device is made by Aston Medical Technology Ltd., UK as 'FED-1b') [28, 29] have provided researchers with new opportunities to study microcirculatory disorders, including in diabetes. These devices are gradually finding their application in medical practise, especially in the diagnosis of complications associated with DM [30, 31] and hypertension [32], have been tested in the assessment of smoking status [33], effectiveness of drug therapy for microcirculatory disorders [34], in assessing the influence of the body position on microcirculation parameters [35], assessment of blood microcirculation changes after COVID-19 [36] and under the microgravity conditions during the space flight [29, 37].

Hu et al. [38] in their recent work utilized wearable LDF analysers to evaluate foot microcirculation in several measurement places in patients with DM at rest. The authors were able to demonstrate both differences in perfusion parameters and mechanisms of blood flow regulation between patients and healthy controls, as well as regional differences in the regulation of microcirculation in different regions of the foot.

The aim of the present work was to investigate differences in perfusion levels and detect changes in the amplitude-frequency spectra of LDF signals in patients with DM in different anatomical skin areas of upper and lower extremities using a distributed system of wearable LDF analysers.

2 | Materials and Methods

2.1 | Experimental Studies

Twenty-six patients from the Endocrinology Department of the Orel Regional Clinical Hospital (Orel, Russia) diagnosed with Type 2 DM were selected to participate in the study. At the time of selection for the study, the patients were undergoing annual preventive treatment at the hospital. A CG of 31 conditionally healthy volunteers of similar age was recruited for comparison with the data obtained from the patient group. The subjects were informed about the study before it began, and each signed an informed consent form to participate in the experiment. The volunteers underwent a questionnaire, including questions about limb injuries and their innate features, as well as about the presence of a family DM history and cardiovascular diseases. The study was approved by the local ethics committee of the Orel State University named after I.S. Turgenev (protocol No. 15, dates of meeting 21.02.2019). The main characteristics of the groups studied are presented in Table 1.

Patients with comorbidities of the bronchopulmonary and neuroendocrine systems, coronary, peripheral or cerebral artery disease, cardiac or renal insufficiency, as well as with gastrointestinal, hepatic, renal, blood and other severe chronic diseases that could affect the diagnostic results were excluded from this study. Volunteers with a history of alcoholism, drug and substance abuse were also excluded from the study. Patients with diagnosed diabetic foot syndrome or active foot ulcers were not included in the study. The CG included conditionally healthy volunteers without a history of chronic diseases.

A diagram of the study protocol is shown in Figure 1. Before the beginning of the study, the subjects were asked to take a horizontal position on the couch. The sensors were fixed on the subject's body, and the subject adapted to the experimental conditions

and room temperature regime for at least 15 min. The wearable microcirculatory blood flow analysers 'LAZMA PF' (LAZMA Ltd., Russia; in EU/UK this device is made by Aston Medical Technology Ltd., UK as 'FED-1b') [28], implementing the LDF method with the probing radiation wavelength of 850 nm, allowing diagnostics of skin vessels at a depth of up to 1.2 mm [39], were used during the study. The devices were fixed in the area of the pads of 3rd fingers and 1st toes, in the dorsum of the wrist in the place of wrist watch fastening, as well as on the inner side of the upper third of the shins. The study lasted 10 min, during which the patient was in a relaxed state.

2.2 | Data Processing

For each volunteer, in each study area the mean perfusion values over a 10-min measurement period were calculated (index of microcirculation—I_m, measured in arbitrary (perfusion) units, p.u.). Each measured LDF signal was subjected to wavelet analysis using a continuous Morlet wavelet [40] in the following form:

$$W_x(f_{osc},\tau) = \sqrt{f_{osc}}\int_{\infty}^{\infty} I_m(t)\psi^*\left[f_{osc}(t-\tau)\right]dt, \quad (1)$$

where t is the time, τ is the time shift of the wavelet, f_{osc} is an estimate of the oscillation frequency ($\sim 1/a$, where a is the time-scale of the wavelet), and the symbol $*$ indicates the operator of complex conjugation. A Morlet wavelet is defined as follows:

$$\psi(t) = e^{2\pi i t}e^{-t^2/2\sigma^2}. \quad (2)$$

The decay parameter $\sigma = 1$. This wavelet allows one to ensure sufficient time-frequency resolution and is well localized in the time domain.

Using this function, it is possible to study amplitude and phase properties of oscillations of different frequencies in the analysed signal. The integrated wavelet spectrum is calculated by integrating Equation (2) over the period T of the recording time series as follows:

$$M_{osc}(f_{osc}) = \frac{1}{T}\int_0^T \left[W_x(f_{osc},\tau)\right]^2 dt. \quad (3)$$

In our study, based on the results of wavelet analysis, the amplitudes of microcirculatory blood flow oscillations were calculated in frequency ranges corresponding to endothelial regulation of microcirculation — endothelial range (0.0095–0.021 Hz), neurogenic parasympathetic regulation — neurogenic range (0.021–0.052 Hz), myogenic regulation — myogenic range (0.052–0.145 Hz), the influence of venous pressure dynamics due to mechanical activity of the chest — respiratory range (0.145–0.6 Hz) and changes in erythrocyte velocity with systolic and diastolic phases of cardiac activity — cardiac range (0.6–2 Hz) [8, 41].

The assignment of specific frequency bands in the wavelet analysis of LDF signals strongly depends on the length of the signal registration, as well as the logarithmic frequency resolution provided by the continuous wavelet transform. It is traditionally used to distinguish 5 frequency bands in wavelet analysis of LDF signals [8]. The use of longer recording times (from 30 min) allowed the identification of an additional frequency range (0.005–0.0095 Hz), the physiological significance of which is attributed to endothelial regulation independent of NO [42].

Based on the calculated amplitudes of blood flow fluctuations, the value of nutritive blood flow, a parameter reflecting the

TABLE 1 | Main characteristics of the studied groups.

Parameter	Control group (CG)	Patients with DM (DM)
Mean age, years	51 ± 10	56 ± 13
Sex, m/f	16/15	12/14
BMI, kg/m^2	27 ± 4	32 ± 7
SBP, mm.Hg.	123 ± 11	133 ± 14
DBP, mm.Hg.	76 ± 8	79 ± 8
Diabetes duration, years	—	13 ± 8
Fasting glucose, mmol/l	—	6.03 ± 1.96
HbA1c, %	—	8.5 ± 2.1

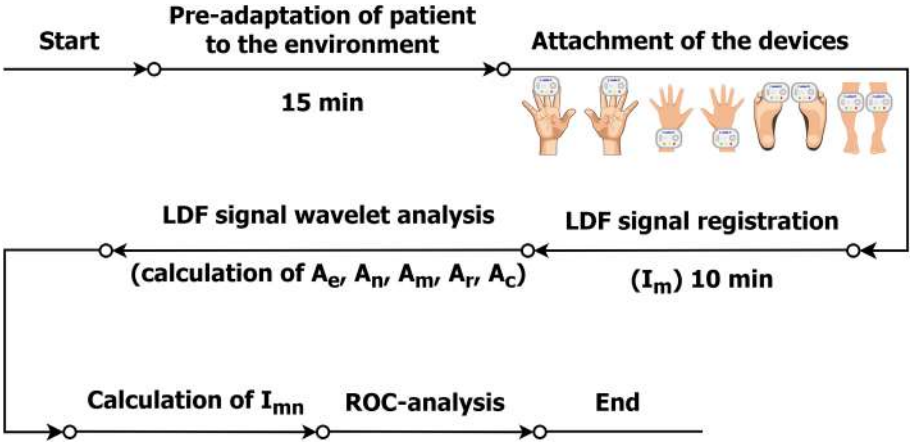

FIGURE 1 | A diagram of the experimental design.

efficiency of capillary perfusion, was calculated for each volunteer in accordance with the previously stated principle [28, 43], using the formula:

$$I_{mn} = I_m \frac{A_m}{A_n + A_c}, \quad (4)$$

where I_m is the index of blood microcirculation in the corresponding area of the study, A_m—is the amplitude of the oscillation in the myogenic range, A_n—is the amplitude of the oscillation in the neurogenic range, A_c—is the amplitude of the oscillation in the cardiac range.

To estimate the dominant factor in microvascular tone regulation, we also applied an approach to LDF signal processing in which the contribution of each rhythmic component of the LDF signal spectrum was estimated by the power of its spectral range as a percentage of the total power of the whole spectrum. The total power of the spectrum was determined as the sum of the squares of the amplitudes of the oscillations in each frequency range:

$$P = A_e^2 + A_n^2 + A_m^2 + A_r^2 + A_c^2, \quad (5)$$

where A_e and A_r are the amplitudes of the oscillation in the endothelial and respiratory ranges, respectively. And the contribution of individual rhythmic components to the spectra was calculated using the formula:

$$\frac{A_i^2}{P} 100\%, \quad (6)$$

where A_i is the amplitude of oscillations in the estimated frequency range.

To build a classification model and identify features that best separate subjects according to the presence and absence of peripheral blood flow disorders in patients, an approach based on linear discriminant analysis with the construction of a receiver operating characteristic (ROC) curve was applied in the present work.

2.3 | Statistical Methods

Origin Pro 2021 software (OriginLab Corporation, USA) was used to analyse the data. Data from different groups were compared using the nonparametric Mann–Whitney *U* test. The differences were considered statistically significant at $p < 0.05$.

In the Results section, unless otherwise stated, data are presented as whisker boxes for different groups and different measurement areas. The data for the patient group (DM) are presented in red and for the CG in green. The top and bottom lines of the boxes represent the 75th and 25th percentiles, respectively, while the lines within the boxes represent the medians. The top and bottom lines outside the boxes represent the highest and lowest values, and the squares inside the boxes are the average values.

3 | Results

The results of the comparison of perfusion parameters between the main and CGs are shown in Table 2. Statistically significant differences between groups are highlighted in bold. Figure 2 represents box charts for the values of index of blood microcirculation (Figure 2a) and nutritive blood flow (Figure 2b) for all study areas of patients with DM and CG.

TABLE 2 | Comparison of the parameters of microcirculation in a group of patients with DM and conditionally healthy volunteers (CG).

Parameters		Fingers	*p*	Toes	*p*	Hands	*p*	Legs	*p*
I_m, p. u.	DM	20.60 ± 1.70	0.126	13.97 ± 3.68*	**0.009**	9.23 ± 1.24*	**0.001**	7.52 ± 2.33	0.790
	CG	20.38 ± 5.14		18.04 ± 6.24		6.18 ± 2.08		7.17 ± 1.59	
I_{mn}, p. u.	DM	11.76 ± 3.67*	**0.019**	5.25 ± 2.45*	**0.004**	4.61 ± 2.06*	**<0.001**	3.04 ± 1.86	0.176
	CG	9.30 ± 3.43		8.07 ± 4.00		3.68 ± 1.35		3.22 ± 1.15	
A_e, p. u.	DM	0.70 ± 0.40	0.387	0.54 ± 0.35	0.059	0.14 ± 0.07*	**0.048**	0.15 ± 0.10*	**0.001**
	CG	0.70 ± 0.59		0.69 ± 0.44		0.18 ± 0.07		0.22 ± 0.12	
A_n, p. u.	DM	0.80 ± 0.51	0.414	0.61 ± 0.39	0.476	0.20 ± 0.13	0.404	0.18 ± 0.08*	**0.017**
	CG	0.77 ± 0.61		0.68 ± 0.50		0.20 ± 0.07		0.24 ± 0.11	
A_m, p. u.	DM	0.77 ± 0.33	0.120	0.54 ± 0.26	0.181	0.28 ± 0.18	0.186	0.21 ± 0.07*	**0.029**
	CG	0.68 ± 0.34		0.62 ± 0.29		0.22 ± 0.06		0.25 ± 0.07	
A_r, p. u.	DM	0.54 ± 0.05*	**<0.001**	0.38 ± 0.09	0.102	0.23 ± 0.05	0.202	0.26 ± 0.06*	**0.004**
	CG	0.48 ± 0.11		0.47 ± 0.22		0.26 ± 0.07		0.23 ± 0.06	
A_c, p. u.	DM	0.71 ± 0.33*	**0.048**	0.86 ± 0.31	0.378	0.42 ± 0.22	0.480	0.38 ± 0.06*	**0.001**
	CG	0.84 ± 0.33		0.76 ± 0.21		0.39 ± 0.13		0.33 ± 0.07	

Note: Statistically significant differences between groups are highlighted in bold (e.g. $p < 0.05$).
*The significance of the difference between the values of control group and patients was confirmed with $p < 0.05$ according to the Mann–Whitney U test.

3.1 | Mean Perfusion and Nutritive Blood Flow

Patients with DM were characterised by increased perfusion values in the wrist area and decreased values of this parameter in the toe area compared to the CG. No statistically significant differences were found in the perfusion parameters measured in the fingers and shins, although a slight increase in perfusion was found in the fingers. The nutritive blood flow parameter also showed increased values in the wrist and finger regions and decreased values in the toe region in patients with DM.

3.2 | Blood Flow Oscillations

Figure 3 demonstrates calculated amplitude-frequency spectra of the LDF signals of patients with DM and healthy controls, measured in the areas of 3rd fingers (Figure 3a), 1st toes (Figure 3b), dorsum of the wrists (Figure 3c) and inner part of the upper third of the shins (Figure 3d).

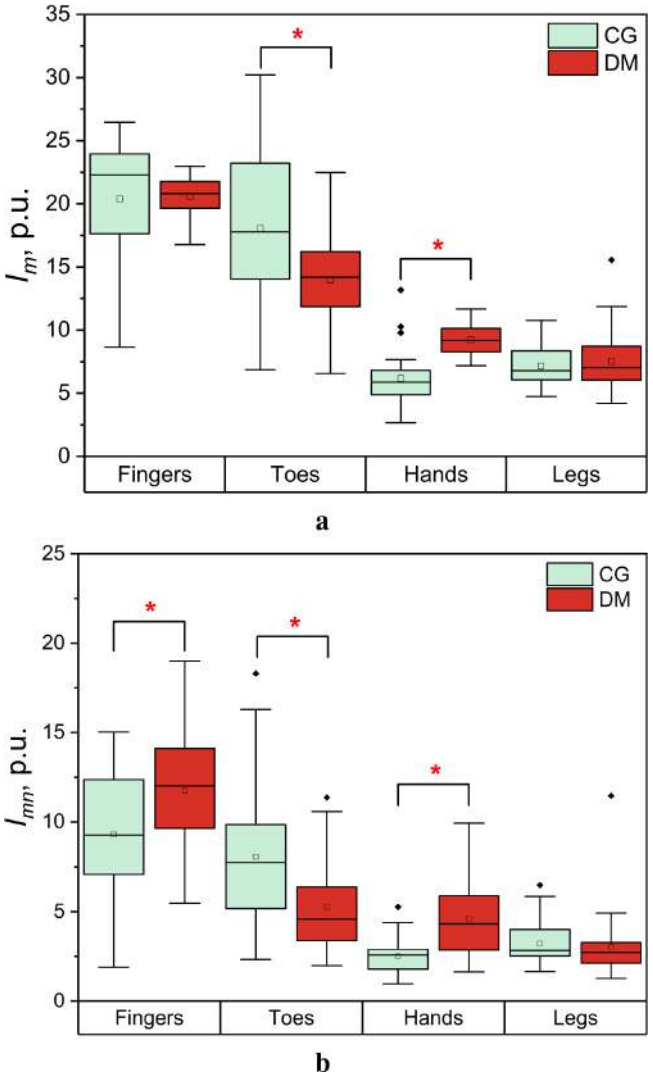

FIGURE 2 | Box plots of the index of blood microcirculation (a) and the nutritive blood flow (b). *—The significance of the difference between the values was confirmed with $p < 0.05$ according to the Mann–Whitney U test.

Table 2 and Figure 3 show that even at rest, patients with diabetes are characterised by reduced amplitudes of oscillations corresponding to active factors of blood flow regulation (endothelial and neurogenic) in all areas of the study. Statistically significant differences were found in the amplitudes of endothelial oscillations in the areas of the wrists and shins.

In the areas of the 3rd finger and shin, an increase in the amplitudes of respiratory oscillations was observed in patients compared to the CG.

The dynamics of cardiac oscillations are of interest. In almost all areas of the study, this parameter is higher in patients with DM compared to the CG. Statistically significant differences are found in the lower leg region. The exception is the area of the 3rd fingers, where this parameter is statistically significantly reduced in the group of patients with DM.

When calculating the power spectrum values of the skin blood flow oscillations in all investigated areas, no differences were found between the CG and the patients with DM. The contribution of each frequency band to the total power of the spectrum was also calculated (Figure 4). A reduced contribution of the endothelial band to the total power of the wavelet spectrum of the LDF signals was observed in the regions of the toes, shins and wrists in the patient group compared to the CG. At the same time, in the upper extremities, the contribution of myogenic oscillations to the total power of the spectrum was higher in the patients than in the CG in both the finger and the wrist regions. In the finger region, the contribution of cardiac oscillations was also lower in patients with DM. In the shin region, a decrease in the contribution of all active oscillations to the total power of the spectrum was observed in patients with DM, with a simultaneous increase in the contribution of passive oscillations (respiratory and cardiac).

3.2.1 | ROC-Analysis

To build a classification model to classify the studied population into the class of presence or absence of peripheral circulatory disorders associated with DM, different combinations of parameters were tested. The parameters I_m and I_{mn} in the areas of the toe pads and dorsal surface of the wrists were chosen as discriminant variables. The discriminant function is synthesised in such a way as to provide high sensitivity while providing excellent specificity. The use of these research areas is also the most optimal for the diagnosis of peripheral blood flow disorders in DM, as it allows the microcirculation to be analysed in the most vulnerable area (toes), where the disorders first manifest themselves, but also allows the state of blood flow in both the upper and lower extremities to be systematically assessed. Table 3 summarises the sensitivity and specificity for a different combination of measured parameters. The sensitivity and specificity parameters for the selected classification rule are shown in bold.

The discriminant function obtained was as follows:

$$f = -0.47 \times I_{mn}^{\text{toes}} + 1.30 \times I_{m}^{\text{hands}} - 7.09. \quad (7)$$

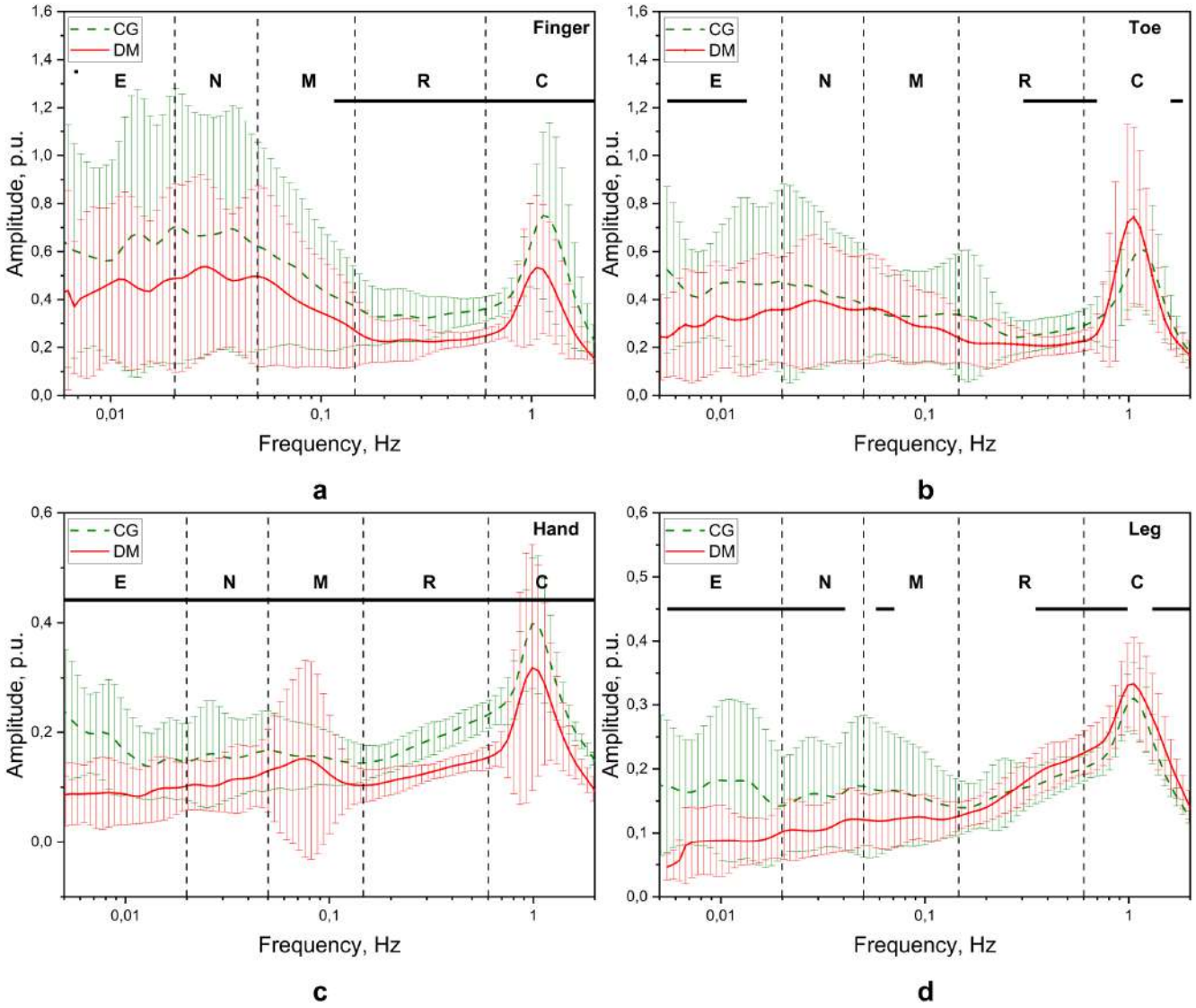

FIGURE 3 | Amplitude-frequency spectra of the LDF signals of the groups under the study measured in the region of the 3rd finger (a), 1st toe (b), dorsum of the wrist (c) and inner surface of the upper third of the shin (d). E, N, M, R and C correspond to the names of the frequency ranges of the endothelial, neurogenic, myogenic, respiratory and cardiac oscillations. The thick coloured line in the middle of the region corresponds to the mean value, error bars indicate a mean standard deviations from the sample. The thick black line at the top marks the frequency ranges in which a statistically significant difference between the parameters of the patient group and the CG was observed ($p < 0.05$ according to the Mann–Whitney U test).

For the obtained classification model, the sensitivity SE is 0.88 and specificity SP = 0.90, the area under the ROC-curve AUC = 0.94.

Figure 5 a shows the results of the linear discriminant analysis in the form of a scatter plot of the experimental data (I_m and I_{mn}), as well as the obtained discriminant function, which divides the experimental points into 2 groups (healthy volunteers and patients with peripheral blood flow disorders). Figure 5b shows the ROC-curve calculated for the obtained discriminant function.

Thus, if the obtained experimental point lies on the plot below the discriminant line, it is concluded that there are no peripheral blood flow disorders, and if the experimental point is above the discriminant line, it is concluded that there are peripheral blood flow disorders.

4 | Discussion

In the present work, using a distributed system of laser Doppler flowmetry analysers, a comprehensive study of the parameters of the blood microcirculation system in patients with DM at different points of the upper and lower extremities was carried out and the obtained data were compared with those of the CG. Previously, other teams of authors have already addressed the problem of studying microcirculation disorders as one of the factors in the development of diabetes complications, but the data obtained were scattered and did not allow making unambiguous conclusions about the direction of blood flow changes in diabetes.

There is no consensus in the scientific literature on how the value of skin perfusion at rest changes in patients with DM.

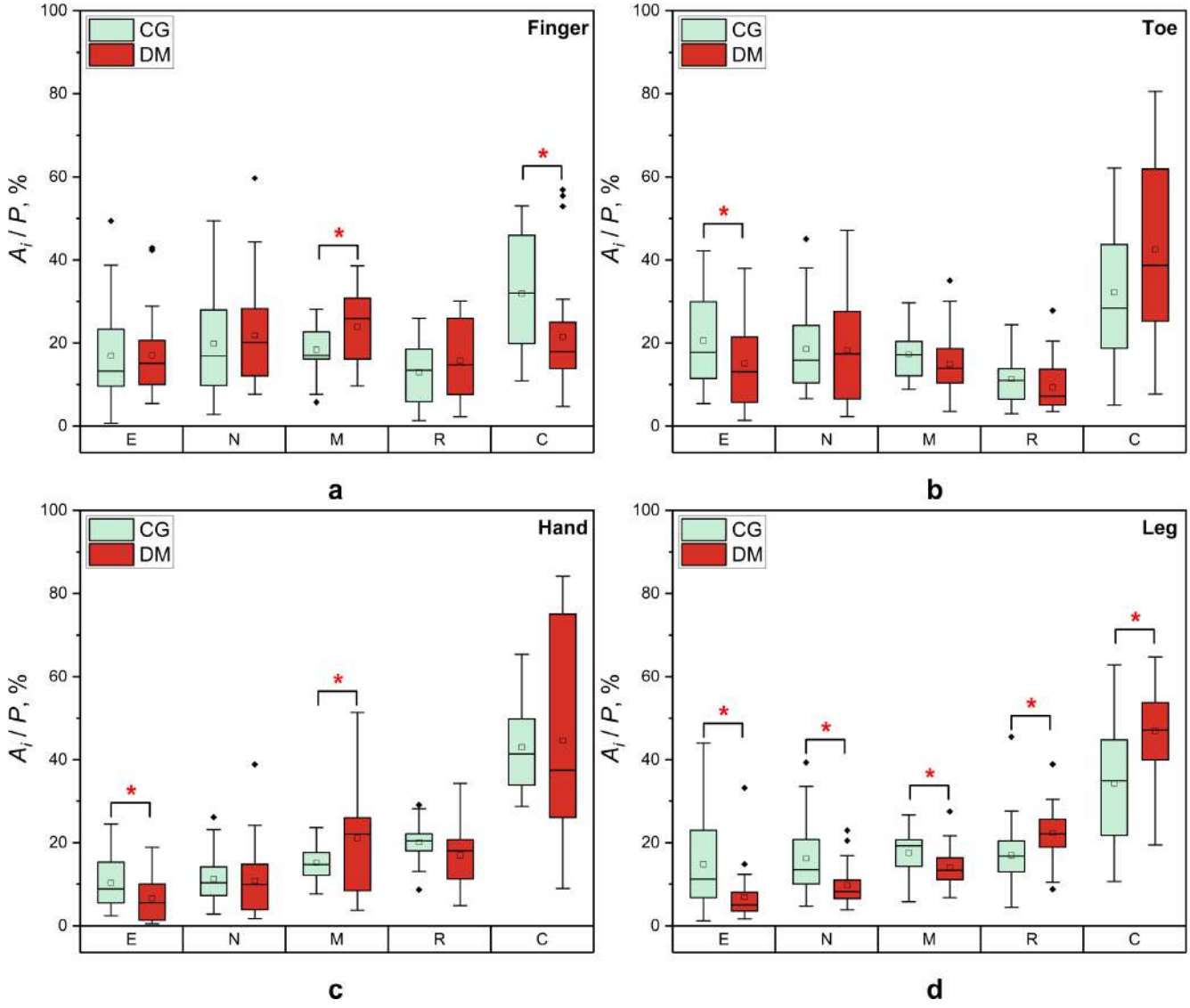

FIGURE 4 | The contribution of individual rhythmic components of blood microcirculatory oscillations to the total power of the wavelet spectrum of the LDF signal in the areas of the fingers (a), toes (b), wrists (c) and shins (d). *—The significance of the difference between the values was confirmed with $p < 0.05$ according to the Mann–Whitney U test.

TABLE 3 | Parameters characterising the reproducibility of classification rules: sensitivity (SE), specificity (SP), area under the ROC-curve (AUC), false positive (FP) and false negative (FN) errors.

Parameters	I_m^{toes} I_{mn}^{wrists}	I_{mn}^{toes} I_{mn}^{wrists}	I_{mn}^{toes} I_m^{wrists}	I_m^{toes} I_m^{wrists}
SE	0.69	0.65	**0.88**	0.85
SP	0.87	0.90	**0.90**	0.90
AUC	0.92	0.93	**0.94**	0.94
FP	0.13	0.10	**0.10**	0.10
FN	0.31	0.35	**0.12**	0.15

Note: Statistically significant differences between groups are highlighted in bold (e.g. $p < 0.05$).

While most of the works claim a decrease in perfusion in such patients [24], other authors show an increase in this parameter [23] or no significant changes [44]. One of the factors that may play a key role in the occurrence of such discrepancies is the different methodology of the studies, including differences in the localisation of LDF sensors on the patient's body (upper or lower extremities, glabrous or nonglabrous skin cites, etc.), and the possible influence of comorbidities and complications of DM, such as diabetic polyneuropathy. Among authors, noting increased perfusion in patients with DM there is an opinion about the influence of reduced venoarterial reflex due to sympathetic denervation associated with DM [22, 45]. Reduced perfusion is explained by the stealing of the microcirculatory bed due to arteriovenular shunting of blood [46, 47].

In this regard, the results obtained in the present study are of particular interest, showing multidirectional changes in the level of basal skin perfusion in patients with DM in the upper and lower extremities. While a decrease in the index of microcirculation was observed in the lower extremities, this parameter was increased in the upper extremities of the patients. Similar results can be found in the works of other authors [26].

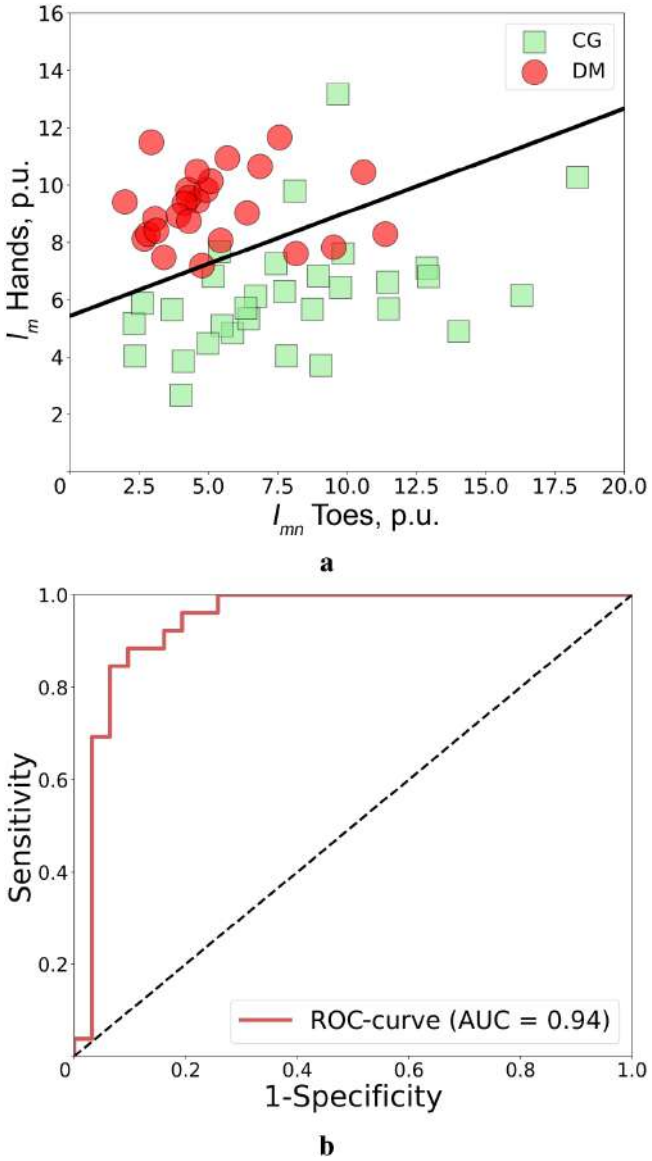

FIGURE 5 | Scatter plot of I_m^{hands} and I_{mn}^{toes} values for control group and patients with DM with applied discriminant function (a), and the ROC-curve calculated for the obtained discriminant function (b).

The decrease in the absolute values of perfusion and nutritive blood flow in the toe region of patients may be related to the negative effect of hyperglycaemia on the distal part of the microcirculatory bed of the lower extremities, leading to impaired blood flow in this region. It can be assumed that the increase in perfusion in the upper extremities in patients with DM may indicate an attempt of the organism to compensate for the emerging disorders of peripheral blood flow. This assumption is supported by the observation of an increased contribution of myogenic oscillation amplitudes to the total spectrum power in the fingers and wrists of patients with DM.

Another presumptive explanation for such changes may be the development of congestion in the microvessels of the upper extremities of patients with slowing of blood outflow from the microcirculatory channel. Some works suggest that the effect of diabetic neuropathy may lead to a decrease in vasoconstriction of arterioles and an increase in blood filling of arteriolar-venular anastomoses, which may explain the obtained result [23, 48]. Previously, we have shown an increase in mean perfusion in patients with DM in the wrist area with a decrease in the amplitudes of blood microcirculation oscillations in the low-frequency range [31].

The results obtained in the lower extremities of patients, with a decrease in absolute values of perfusion and amplitudes of all active blood flow oscillations, correspond to the findings of other authors' teams and indicate the development of spastic form of microcirculation disorders in patients, characterised by spasm of arterioles, decrease in values of blood microcirculation index, amplitudes of LDF signal oscillations in the low-frequency range and amplification of its high-frequency component.

Endothelial dysfunction is considered one of the factors in the development of complications in DM [49]. Numerous author teams using LDF for diagnostics of peripheral circulatory disorders have investigated endothelial function in patients with DM both simply by spectral analysis of LDF signals and with the use of various provocative effects in the form of local heating [10], application of local pressure or injections of vasodilators [50]. According to the results of spectral analysis of LDF signals in our study, it should be noted that patients with DM were characterised by a decrease in the amplitudes of endothelial blood flow oscillations with the exception of the finger area. There was also a significant decrease in the contribution of endothelial oscillations to the total power of the LDF signal spectrum in patients compared with controls. The results obtained may indicate the development of endothelial dysfunction and demonstrate the possibility of obtaining diagnostically significant information using wavelet analysis of LDF signals even at rest, without the use of functional tests.

The linear discriminant analysis of the recorded data demonstrated that using a distributed system of wearable LDF analysers it is possible to create approaches to the study that provide a high level of sensitivity and specificity in separating patients with the presence and absence of peripheral blood flow disorders. Based on the obtained discriminant model, a diagnostic method was proposed.

The proposed method of diagnosing peripheral blood flow disorders in Type 2 DM presents the following sequence of actions:

1. Preliminary preparation of the patient is carried out, consisting in his/her adaptation to the temperature regime of the room for 15 min, disinfection of the body areas to be examined, as well as the surfaces of the devices in contact with the patient's body.
2. The patient lies on the couch in a supine position with arms extended along the torso.
3. Fastening of 4 wearable LDF devices on the patient's body is carried out: 2 devices on the dorsal surface of the wrists on the midline at a point 2 cm above the styloid process, and 2 devices on the plantar surface of the 1st toes. The data measured at the toes and wrists have been shown to

be the most informative in terms of discrimination modelling. Therefore, it is recommended to use at least 4 devices in these areas in the proposed diagnostic method. It is proposed to fix the wearable devices using medical self-fixing bandages, so as not to create pressure on the measurement area.

4. Using the LDF method, the I_m values are recorded for 10 min without any effect on the blood microcirculation system (i.e., at rest, without performing any functional stress tests).
5. The recorded LDF-grams are subjected to the spectral analysis using wavelet transform to extract the amplitudes of peripheral blood flow oscillations in endothelial, neurogenic, myogenic, respiratory and cardiac frequency bands.
6. According to the recorded I_m values and calculated amplitudes of peripheral blood flow oscillations in the neurogenic, myogenic and cardiac ranges, the nutritive blood flow parameter I_{mn} is calculated for measurements in the lower extremities.
7. The obtained parameter values are substituted into the decisive rule (classification model), on the basis of which a conclusion is made about the presence or absence of peripheral blood flow disorders in the patient.

In the case of identifying a newly measured individual into the group of patients with the presence of peripheral blood flow disorders, it is suggested that additional parameters, such as the contribution of individual rhythmic components to the total spectrum power, should also be calculated to identify associated disorders, such as endothelial dysfunction, or reduced myogenic tone in the upper extremities.

5 | Conclusions

In the present work, a comprehensive study of blood microcirculation parameters in patients with DM and healthy controls was performed in the upper and lower extremities using a distributed system of wearable laser Doppler flowmetry analysers. The developed diagnostic approach with registration of parameters in different anatomical skin areas, analysis of amplitude-frequency spectra of LDF signals and calculation of nutritive blood flow values showed the possibility to detect the presence of microcirculatory dysfunction in patients with high sensitivity and specificity. The obtained results confirm the possibility of using wavelet analysis of LDF signals to detect differences in the regulation of the blood microcirculatory system in healthy and pathological conditions, even without the use of functional tests.

Author Contributions

Conceptualization, discussion and writing – original draft preparation, review and editing: E.Z. Investigation, formal analysis and writing – original draft preparation: Y.L. Investigation, supervision, conceptualization, and methodology: A.D. All authors edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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