Endocrine
https://doi.org/10.1007/s12020-023-03506-4

ORIGINAL ARTICLE

Incoherent optical fluctuation flowmetry for detecting limbs with hemodynamically significant stenoses in patients with type 2 diabetes

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Received: 22 May 2023 / Accepted: 22 August 2023

Abstract

Introduction The development of new highly accurate, inexpensive and accessible methods for the detection of lower-extremity peripheral artery disease (LE-PAD) in diabetic patients is required. The aim of this study was to evaluate the accuracy of a new incoherent optical fluctuation flowmetry (IOFF) method in detecting legs with hemodynamically significant stenoses compared to ankle brachial index (ABI) and transcutaneous oximetry (TcPO2) in patients with diabetes mellitus (DM).

Materials and methods Patients were recruited into 2 groups. Group 1 included patients with DM without LE-PAD and/or diabetic foot syndrome; Group 2 included patients with DM and LE-PAD. All patients underwent the following measurements: ultrasound (reference method), ABI, TcPO2, and the new IOFF method.

Results The new IOFF method showed a sensitivity of 79.5% and a specificity of 89.8% in detecting limbs with hemodynamically significant stenosis (AUC 0.890, CI 0.822–0.957). TcpO2 allows the diagnosis of LE-PAD with 69.2% sensitivity and 86.2% specificity (AUC 0.817, CI 0.723–0.911). Using a standard ABI cut-off of less than 0.9, the sensitivity and specificity for this parameter were 34.5% and 89.7%, respectively. Increasing the diagnostic cut-off of the ABI on the study group to 0.99 improved sensitivity to 84.6% and specificity to 78% (AUC,0.824 CI 0.732–0.915).

Conclusions The new IOFF technique has demonstrated high sensitivity and specificity in the detection of LE-PAD in patients with DM. The high accuracy, rapid measurement, and potential availability suggest that the new IOFF method has a high potential for clinical application in the detection of PAD.

Keywords Ankle brachial index · Peripheral artery disease · Incoherent optical fluctuation flowmetry · Transcutaneous oximetry

Introduction

Lower extremity peripheral artery disease (LE-PAD) is caused by atherosclerosis of the arteries, resulting in arterial narrowing or occlusion [1, 2]. PAD is associated with a significant risk of limb loss and cardiovascular mortality. Diabetes is known to increase both the incidence of LE-PAD and the severity and progression of the disease [3]. In addition, LE-PAD is frequently asymptomatic in patients with diabetes [2].

Digital subtraction angiography is the gold standard for the diagnosis of LE-PAD. However, it is an expensive and

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Published online: 23 September 2023

invasive procedure with a risk of complications, so non-invasive vascular imaging methods such as duplex ultrasonography are more frequently performed to assess vessel patency and the degree of stenosis [4, 5]. This method is highly accurate in detecting stenosis and is a first-line method [5, 6].

The most widely used routine screening test for PAD is the ankle-brachial index (ABI) [6, 7]. However, it is known that the accuracy of ABI is limited in patients with diabetes mellitus (DM) due to medial arterial calcification and neuropathy [8, 9]. Thus, in a large systematic review with the meta-analysis by V. H. Chuter et al. (2021), ABI was shown to have a sensitivity of 60% (95% CI 0.48 to 0.71; $P = 0.097$) and specificity of 87% (95% CI 0.78 to 0.92; $P < 0.001$) for the detection of LE-PAD. According to various studies included in the meta-analysis, the sensitivity ranged from 17% to 100% and the specificity from 59% to 99% [10]. The authors conclude that ABI has limited efficacy for the early detection of LE-PAD in patients with DM [10]. In addition, ABI measurement is an operator-dependent procedure and can be painful for patients with lower limb ischemia [6].

Therefore, the assessment of ABI in patients with diabetes has limited accuracy. So, a new, inexpensive, convenient, and accurate method for LE-PAD screening tests is required. A number of optical methods have been proposed as promising techniques for the detection and management of patients with LE-PAD (pulse oximetry, Laser-Doppler flowmetry, Laser speckle contrast imaging, near-infrared spectroscopy, fluorescence imaging etc.) [5, 7, 11–14]. However, due to a number of limitations, none of these methods has found widespread clinical applications.

Incoherent optical fluctuation flowmetry (IOFF) is a new optical method for assessing perfusion developed at the Laboratory of Medical and Physics Research, Moscow Regional Research and Clinical Institute ("MONIKI"). Previous studies have shown a high correlation of IOFF parameters with TcPO2 and the high sensitivity and specificity in identifying limbs with critical ischemia [15]. However, to date, no studies have evaluated the potential of the IOFF to detect limbs with hemodynamically significant stenosis.

The aim of this study was to evaluate the accuracy of the new IOFF method in identifying legs with hemodynamically significant stenosis compared to ankle-brachial index (ABI) and transcutaneous oximetry (TcPO2) in patients with diabetes mellitus (DM).

Materials and methods

Study design, population, and data sources

This observational, two-center study was conducted on patients with type 2 DM undergoing inpatient treatment. Patients were recruited into 2 groups:

- group 1 included patients without hemodynamically significant stenosis/occlusion of lower limb arteries and/or diabetic foot syndrome;
- group 2 included patients with hemodynamically significant stenosis/occlusion of lower limb arteries.

Arterial duplex ultrasonography was used as the reference standard for the determination of the presence of hemodynamically significant LE-PAD. Hemodynamically significant LE-PAD was defined as the presence of stenosis of more than 50 % of the lumen according to results of ultrasound duplex scanning [5, 16].

Exclusion criteria (common to both groups): pregnancy; skin diseases that prevent the study; diagnosed systemic autoimmune diseases; severe heart rhythm disorders (atrial fibrillation, frequent extrasystoles); blood diseases thrombocytopenia, anemia (hemoglobin less than 90 g/L); fever of any origin; exacerbation of concomitant chronic diseases; stage 5 chronic kidney disease; use of hormone replacement therapy, oral contraceptives; regular use of steroids, nonsteroidal anti-inflammatory drugs (therapy with antiaggregants was not an exclusion criterion).

The study was conducted at 2 centers: (1) Moscow Regional Research and Clinical Institute ("MONIKI"); (2) Federal State Budgetary Institution "V.A. Almazov National Medical Research Center" of the Ministry of Health of the Russian Federation.

Assessment of hemodynamics

All patients were assessed for lower limb hemodynamic parameters using the following methods: ultrasound (as reference method), ABI, transcutaneous oximetry (TcPO2), IOFF. During all measurements, patients were placed in the supine position on an examination bed, and all measurements were performed sequentially on two limbs. All patients were asked to refrain from smoking for 3 h prior to testing.

Duplex ultrasound

Duplex ultrasound of lower limb arteries was performed using the Vivid 7 Dimension, GE Healthcare, USA, and Philips Affinity 50, Philips Ultrasound, USA. The ultrasound protocol included an assessment of the presence of hemodynamically significant stenoses in 6 major arteries of each lower limb (common femoral, deep femoral, superficial femoral, popliteal, anterior tibial, posterior tibial). Hemodynamically significant stenosis was defined as a diameter reduction of 51–99%, including occlusions. [5, 15, 16].

Ankle-brachial index

The ABI measurement was carried out according to the recommendations of the American Heart Association [17]. After a 10-minute rest, the systolic blood pressure was measured at the posterior tibial, dorsal pedis, and brachial arteries. To record blood pressure, a Doppler probe was placed over the pulsing artery at an angle of 45° to 60° to the surface of the skin.

The ABI of each leg was calculated by dividing the highest ankle pressure (in the posterior tibial or dorsal pedis arteries) by the highest arm pressure.

Transcutaneous oxygen pressure measurement (TcPO2)

TcPO2 was measured using the TCM4, Radiometer, Copenhagen, Denmark. The patient was in the supine position for 10 min prior to the measurement. TcPO2 was measured by placing a probe against the skin on the dorsal surface of the foot at the first intermetatarsal space. If ulcers were present in this area, the sensor was moved to the nearest ulcer-free area. The probe was heated up to 44 °C. Registration of TcPO2 was carried out after 15–20 min of local heating.

Perfusion measurement using the IOFF method

Tissue perfusion was assessed using the IOFF method. The IOFF method is based on the spectral analysis of low-frequency fluctuations (0–10 Hz) of the optical signal back-scattered from the tissue with subsequent calculation of perfusion [18]. Herewith, the calculated perfusion is proportional to blood volume changes in a tissue per unit time. In contrast to all known laser-based flowmetry techniques, IOFF does not require the use of optical fibers and lasers and makes it possible to obtain a signal from a larger volume of tissue (tens of mm^3) [19, 20].

Prototypes of a new diagnostic device made by joint-stock company “Elatma Instrument-Making Enterprise” (Ryazan, Russia) were used to measure foot tissue perfusion. The optical sensor of the device uses three LED sources operating in the wavelength range of 560–580 nm and one silicon photodiode. The heating metallic plate is incorporated into the sensor to perform functional tests with skin heating, as well as to ensure conditions for thermal stabilization of the measurement area. Herewith, the temperature of the plate can vary in the range from 30 to 45° C. An operator can set a desired heating temperature with a given heating rate (0.1–1.5° C/s).

Prior to measurement, all patients rested for 10 min in the supine position in a room at a comfortable temperature. The sensor was applied to the dorsal surface of the foot (same site as the TcPO2 electrode). During the first 60 s, the temperature of sensors was kept thermoneutral at 32 ± 0.5 °C and baseline perfusion was assessed. After that a thermal test was performed, the sensor was heated at a rate of 1.5 °C/s to a temperature of 42 ± 0.5 °C. This sensor temperature was maintained until the end of the measurement. Perfusion was measured for 6 min. The following parameters were evaluated: Baseline perfusion was calculated as the median baseline perfusion level for the first 60 s of measurement; Local thermal hyperemia 1–5 min (LTH 1–5 min) was calculated as the median perfusion for each minute of heating.

An example of a perfusion curve obtained during the local heating test is shown in Fig. 1.

Statistical analysis

Statistical analysis was performed in RStudio 2022.02.1 Build 461 using R language version 4.2.1. Gtsummary 1.6.2 package [21]. was used to calculate descriptive statistics and compare groups. Medians and quartiles were calculated for quantitative variables and absolute (n) and relative (%) frequencies for qualitative variables. Quantitative variables in the two groups were compared using the Mann-Whitney test. Qualitative characteristics were analyzed using Chi-square or Fisher’s exact test. Diagnostic accuracy of quantitative measures was assessed using ROC-analysis (pROC 1.18.0 package [22]). The type I error rate (α) was set at 0.05 and null hypotheses were rejected at $p < 0.05$.

Power analysis of the study was performed using the pROC package (power.roc.test function). To detect features with an AUC ≥ 0.7 with $\alpha = 0.05$, a power level of 93.7% was achieved in the current study with 39 limbs with stenosis (cases) and 59 limbs without stenosis (controls).

Results and discussion

This study included 49 patients (98 lower limbs). The characteristics of the groups are shown in Table 1. There were 26 patients in group 1 and 23 patients in group 2.

Table 2 summarizes results of the lower extremity blood flow assessment in patients from two groups.

It was shown that all the studied parameters differed significantly in the two groups.

The table shows that some patients from group 1 (people without hemodynamically significant stenoses) had a pathological ABI value (more than 1.4 or less than 0.9) and a decrease of TcpO2 less than 30 mm Hg. This may be due to both the limited accuracy of these methods and the initial perfusion disorders that can develop in patients without major vessel involvement.

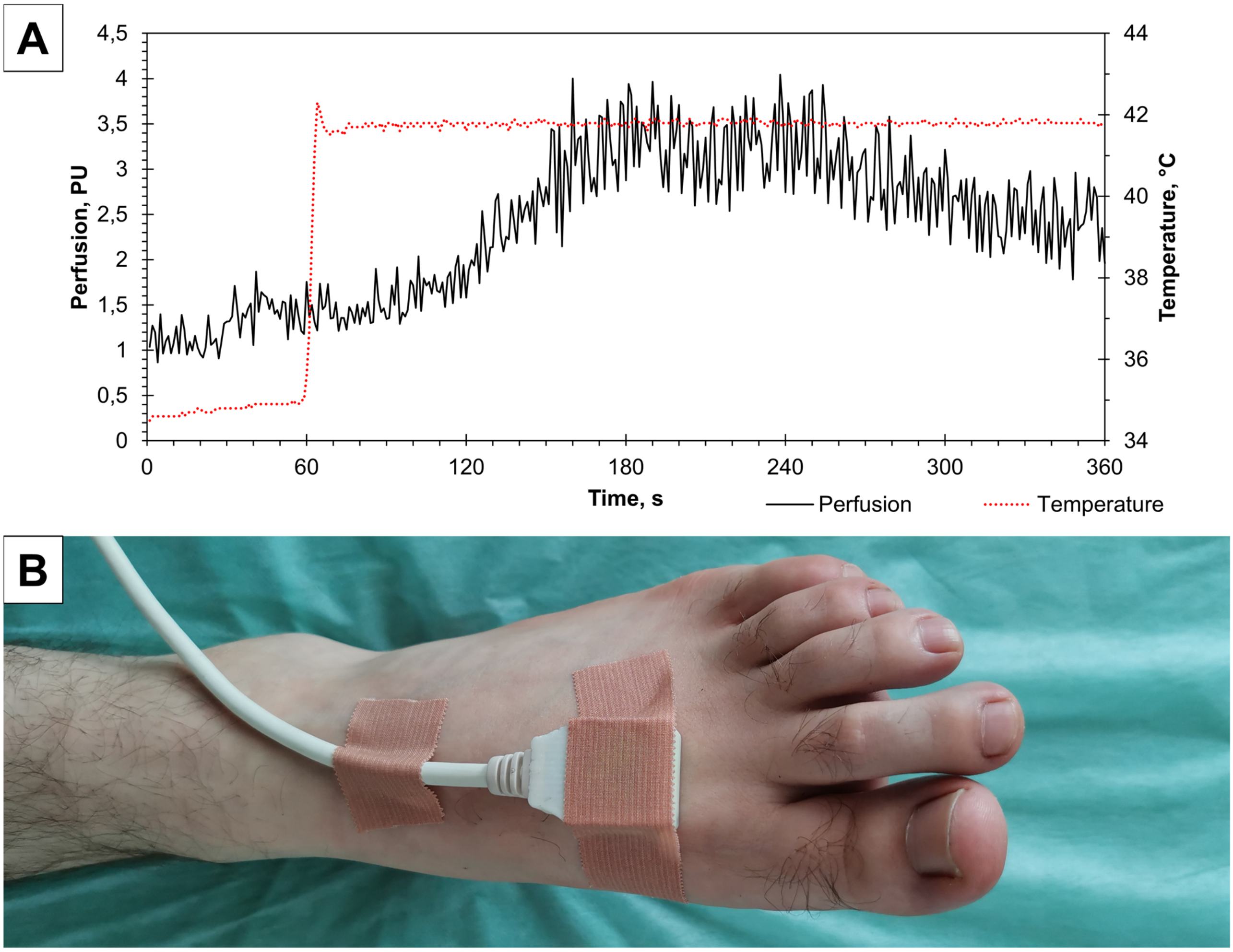

Fig. 1 **A** An example of a perfusion curve obtained by IOFF. **B** Measurement procedure

Additionally, limbs with and without ulcers were compared in group 2. No statistically significant differences were found (Appendix 1, Table 5).

Asymmetric lesions of the lower limb vessels were noted in a number of patients in groups. It is clinically important to identify a specific limb with hemodynamically significant stenoses. Therefore, further analysis was performed on 98 limbs (39 limbs with stenoses, 59 limbs without stenoses).

Evaluation of the sensitivity and specificity of methods in detection of extremities with hemodynamically significant stenoses

The diagnostic accuracy of different methods used and the optimal cut-off values were evaluated using receiver operating characteristic (ROC) curve analysis (Table 3).

As shown in Table 3, the area under the ROC curve was higher for all IOFF parameters than for TcPO2 and ABI. The parameter "LTH, 3 min" had the maximum area under the ROC curve and the maximum diagnostic accuracy (AUC 0.890; 0.822–0.957) (AUC_{IOFF}). Therefore, further analysis of the informativity of the IOFF method was carried out using this parameter. Figure 2A shows the ROC curves for the detection of limbs with hemodynamically significant stenoses.

Although the AUC values for IOFF, were higher than the AUC values for ABI and TcPO2, this advantage was not sufficient to achieve statistical significance. The difference $AUC_{IOFF} - AUC_{ABI}$ (with 95% confidence interval) was 0.066 (−0.034, 0.166), and the difference $AUC_{IOFF} - AUC_{TcPO2}$ was 0.073 (−0.012, 0.156) (Fig. 2B). These difference values do not allow us to claim that the difference between $AUC_{IOFF} - AUC_{ABI}$ or $AUC_{IOFF} - AUC_{TcPO2}$ are significantly greater than 0 and to accept the superiority hypothesis. However, if we consider the non-inferiority margin of AUC difference as 0.05, we can accept the non-inferiority hypothesis, and conclude, that the effectiveness

Table 1 Clinical characteristics of the groups

Part 1. Characteristics of patients	Group 1 ($N = 26$ patients)	Group 2 ($N = 23$ patients)	*p*
Age, years, Me (IQR)	58 (56, 64)	64 (58, 69)	0.050
Body mass index, kg/m^2, Me (IQR)	32.4 (26.6, 36)	28.4 (26.9, 33.9)	0.4
Sex: male/female, *n* (%)	22 (84.6%)/ 4 (15.4%)	15 (65.2%)/ 8 (34.8%)	<0.001
MNSI (Part A), Me (IQR)	6 (5, 7)	9.00 (8.00, 9.50)	<0.001
MNSI (Part B), Me (IQR),	4.25 (3.25, 6)	7.00 (5.50, 8.00)	0.001
HbA1c, %, Me (IQR)	8.8 (7.73, 9.87)	8.10 (7.12, 9.34)	0.3
eGFR using CKD-EPI (ml/min/1.73 m^2), Me (IQR)	90 (80, 98)	79 (66, 85)	0.044

Part 2. Characteristics of lower limbs	Group 1 ($N = 52$ limbs)	Group 2 ($N = 46$ limbs)	*p*
Feet with lower-extremity ulcers, *n*			
Neuropathic foot ulcers	0	4 (8.7%)	
Ischemic foot ulcers	0	2 (4.3%)	
Neuro-ischemic foot ulcers	0	5 (10.9%)	
Lower limbs with hemodynamically significant artery stenoses by duplex ultrasound			<0.001
Absence	52 (100.0%)	7 (15.2%)	
Presence	0 (0.0%)	39 (84.8%)	
Lower limbs with different numbers of stenoses			
Stenosis of 1 artery	0	11 (23.9%)	
Stenosis of 2 artery	0	13 (28.3%)	
Stenosis of 3 artery	0	6 (13.0%)	
Stenosis of 4 artery	0	9 (19.6%)	

eGFR using CKD-EPI estimated glomerular filtration rate according to chronic kidney disease epidemiology collaboration, *HbA1* glycated hemoglobin, *LQ* lower quartile, *Me* median, *UQ* upper quartile, *MNSI* The Michigan Neuropathy Screening Instrument

of IOFF in the detection of limbs with stenosis is not worse than of ABI or TcPO2.

The sensitivity and specificity of these three analyzed methods in detecting limbs with stenosis were calculated. The results of the analysis are presented in Table 4.

It was shown that the IOFF method allows the identification of limbs with hemodynamically significant stenosis (optimal cut-off LTH, 3 min ≤1.96 PU) with a sensitivity of 79.5% and a specificity of 89.8%. Both the sensitivity and specificity of the IOFF method were higher than that of TcpO2 and higher than that of ABI (although these advantages were statistically significant only for the sensitivity of standard thresholds of ABI, Table 4).

Our results showed sensitivity of 69.2% and specificity of 86.2% for TcpO2 ≤ 38 mmHg in detecting >50% stenosis. The use of transcutaneous oximetry as a method for identifying extremities with stenosis has been discussed in a number of publications [23, 24]. However, in clinical practice, TcpO2 is usually applied to assess the prognosis of the ischemic limb and to choose optimal management tactics, rather than to diagnose or screen for LE-PAD. Despite its relatively high sensitivity and specificity, this method is poorly suited as a screening method due to the long duration of measurements and the high cost of consumables.

The ABI is the most widely used and recommended screening test for PAD. The test of ABI ≤ 0.90 is used for the diagnosis of PAD. Our study showed that the sensitivity and specificity of ABI ≤ 0.90 in detecting hemodynamically significant stenoses were 34.5% and 89.7%, respectively. However, it is known that in patients with diabetes the ABI is often falsely overestimated due to medial arterial calcification. An increase in the ABI above 1.4 is also considered as a poor prognostic sign and an indication for further investigation [8, 25]. In addition, in our study, ABI could not be assessed in 11 limbs due to lack of pulsation in the foot arteries or due to severe pain, which is a contraindication for measurement. Since in clinical practice such test results would be considered abnormal and would require further investigation for PAD, we performed an additional analysis in which we considered ABI less than 0.9, greater than 1.4, and inability to assess ABI due to the absence of pulsation in the foot arteries or presence of pain syndrome as abnormal results of the ABI test. In this analysis, the sensitivity and specificity of the ABI were 53.8% and 84.7%, respectively.

Receiver Operator Characteristics (ROC) analysis was performed to assess the optimal threshold ABI value ("closest to the top left corner" threshold) that predict LE-PAD. The 0.99 was the optimal ABI cut-off associated with 84.6% sensitivity and 78% specificity for detecting hemodynamically significant stenosis (lesions >50%). For this ROC analysis, limbs in which it was impossible to assess the ABI due to the absence of pulsation in the foot arteries or severe pain were assigned an ABI value of 0. In the study group, an ABI cut-off of 0.99 was optimal instead of the conventional 0.9. This result is in line with previous studies. It is known that highly frequent arterial medial calcifications in diabetes may increase ABI. And a number of researchers are actively discussing the increase in the thresholds of ABI for patients with DM [8, 26]. Thus, the review by A. Abouhamda (2019) suggested increasing the diagnostic limit of ABI for patients with DM to 1–1.1 [27]. A higher incidence of intermittent claudication has been reported when ABI values were within the lower normal range (0.90–0.99) than in the upper normal one (1.0–1.39) [28]. According to Clairotte et al., in patients with diabetes mellitus, the cut-off values that provide the highest sensitivity and specificity for

Table 2 Results of hemodynamic assessment by different methods

	Group 1 (without stenoses), $N = 52$ lower limbs	Group 2 (with stenoses), $N = 46$ lower limbs	*p*-value
ABI, Me (IQR)	1.07 (1.00, 1.13)	0.94 (0.88, 0.98)	<0.001
ABI,			
>1.4, *n* (%)	2 (3.8%)	1 (2.2%)	<0.001
1–1.4, *n* (%)	36 (69.2%)	7 (15.2%)	
0.91–0.99, *n* (%)	11 (21.2%)	14 (30.4%)	
0.4–0.9, *n* (%)	3 (5.8%)	12 (26.1%)	
0.4, *n* (%)	0 (0.0%)	1 (2.2%)	
not defined, *n* (%)	0 (0.0%)	11 (23.9%)	
TcpO2 mm Hg. Me (IQR),	49 (42, 55)	32 (10, 44)	<0.001
TcpO2 mm Hg			
<30 mmHg, *n* (%);	2 (3.8%)	20 (43.5%)	<0.001
30–39 mmHg, *n* (%);	6 (11.5%)	9 (19.6%)	
40–59 mmHg, *n* (%);	38 (73.1%)	15 (32.6%)	
≥60 mmHg, *n* (%);	6 (11.5%)	2 (4.3%)	
The new IOFF method			
Baseline perfusion, PU, Me (IQR)	1.10 (0.87, 1.47)	0.55 (0.40, 0.87)	<0.001
LTH, 1 min, PU, Me (IQR)	1.82 (1.56, 2.07)	1.13 (0.87, 1.56)	<0.001
LTH, 2 min, PU, Me (IQR)	3.03 (2.59, 3.84)	1.15 (0.84, 1.88)	<0.001
LTH, 3 min, PU, Me (IQR)	3.62 (2.87, 4.50)	1.24 (0.83, 1.97)	<0.001
LTH, 4 min, PU, Me (IQR)	3.34 (2.70, 4.14)	1.18 (0.87, 1.81)	<0.001
LTH, 5 min, PU, Me (IQR)	2.91 (2.36, 3.82)	1.18 (0.85, 1.81)	<0.001

ABI ankle-brachial index, *IQR* interquartile range, *LTH 1–5 min* local thermal hyperemia for each minute of heating, *p* statistical significance

Table 3 Area under the ROC curve showing the diagnostic potential of IOFF, TcPO2, and ABI for identifying legs with hemodynamically significant stenosis

Parameter	AUC	LCL	UCL
ABI	0.824	0.732	0.915
TcpO2	0.817	0.723	0.911
The new IOFF method			
Baseline perfusion	0.831	0.745	0.916
LTH, 1 min	0.830	0.738	0.921
LTH, 2 min	0.877	0.802	0.952
LTH, 3 min	0.890	0.822	0.957
LTH, 4 min	0.865	0.783	0.948
LTH, 5 min	0.852	0.765	0.939

ABI ankle-brachial index, *AUC* area under the ROC curve, *LCL* lower 95% confidence limit, *UCL* upper 95% confidence limit, *LTH 1–5 min* local thermal hyperemia for each minute of heating

PAD screening are between 1.0 and 1.1 [29]. With standard diagnostic thresholds, the ABI method showed significantly lower sensitivity than the IOFF method, while with an increase in the diagnostic threshold of ABI to 0.99, the sensitivity and specificity of the methods are comparable.

Sometimes the presence of single hemodynamically significant stenoses and even occlusions can be compensated by well-developed collateral blood flow. In this regard, an additional analysis was performed to assess the correlation of blood flow parameters with a number of hemodynamically significant stenoses. TcpO2 and all perfusion parameters assessed by the IOFF method negatively correlated with the number of stenoses (Rs from −0.44 to −0.54). This additional data is presented in more detail in Appendix 2 (Table 6).

The IOFF method has a higher sensitivity and specificity than ABI and TcPO2 in detecting PAD (statistical significance for these differences is achieved only for sensitivity of standard thresholds of ABI, Table 4). The use of the IOFF technique allows a quick and painless examination for the patients. For example, the average time to measure unilateral ABI on one side is 14 min [30], TcPO2 is 15–20 min [31], the perfusion measurement time by IOFF in this study was 6 min. The most informative parameter was LTH, 3 min, which allows the

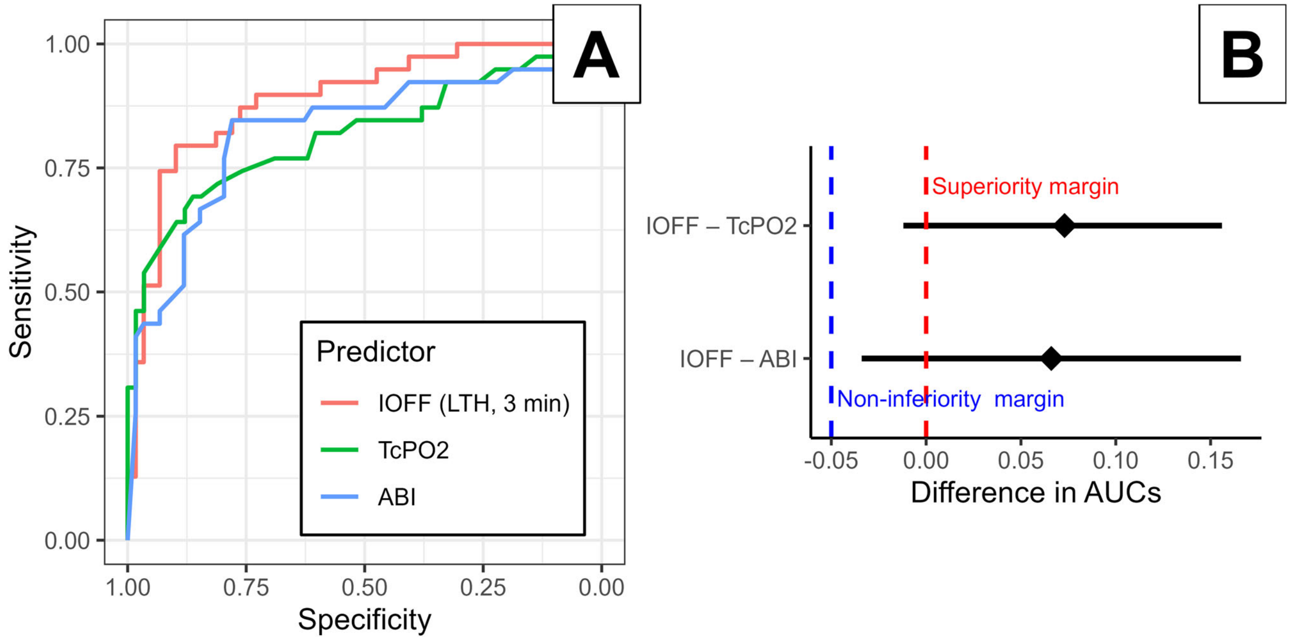

Fig. 2 **A** Receiver operating characteristics (ROC) curves of IOFF (LTH, 3 min), TcPO2, and ABI. ROC curves for detecting >50% stenosis. **B** Differences between the areas under the ROC-curves ($AUC_{IOFF} – AUC_{ABI}$ and $AUC_{IOFF} – AUC_{TcPO2}$) with two-sided 95% confidence intervals

Table 4 Sensitivity and specificity of IOFF, TcPO2, and ABI for detecting hemodynamically significant stenoses of lower limb arteries

Parameter	Diagnostic threshold	Sensitivity	Sensitivity, difference to IOFF (95% CI)	Specificity	Specificity, difference to IOFF (95% CI)
IOFF (LTH, 3 min), PU	≤1.96	79.5%	–	89.8%	–
TcpO2, mmHg.	≤38	69.2%	−10.3% (−28.8%, 9.3%)	86.2%	−3.6% (−15.6%, 8.5%)
ABI	<0.9	34.5%	−45% (−63.6%, −21.5%)[a]	89.7%	−0.1% (−11.6%, 11.2%)
ABI[b]	<0.9/ > 1.4/ unable to reliably assess[a]	53.8%	−25.6% (−44.2%, −4.6%)[a]	84.7%	−5.1% (−17.2%, 7.3%)
ABI[c]	<=0.99	84.6%	5.1% (−12.2%, 22%)	78%	−11.9% (−24.7%, 1.8%)

[a]95% confidence interval does not include “0” value, which means, that difference to IOFF is statistically significant

[b]Abnormal ABI was defined as less than 0.9, more than 1.4 and the impossibility of assessing the ABI due to lack of pulsation in the arteries of the foot or severe pain

[c]The optimal cut-off value was evaluated using receiver operating characteristic (ROC) curve analysis

sample time to be reduced to 4 min without compromising its informative value. In addition, the ABI measurement procedure is sometimes painful for patients with ischemia, whereas perfusion assessment with the IOFF method is painless. Importantly, the IOFF method does not require expensive consumables, and the use of LEDs makes the technology easy to implement and potentially inexpensive.

The study contains several limitations. Operators measuring ABI, perfusion by IOFF and TcPO2 were not blinded to the occurrence of hemodynamically significant LE-PAD (lack of blinding may be a potential cause of bias in the result). In this study, group 2 included patients with already diagnosed PAD and many patients had advanced symptomatic atherosclerosis. A large cross-sectional study including asymptomatic at-risk patients with an evaluation of the reproducibility of the method is required to clearly evaluate the IOFF method as a screening tool for lower extremity peripheral artery disease.

Statistically significantly higher sensitivity of IOFF compared to standard ABI thresholds was demonstrated. For the ROC-analysis the current sample size and the difference between the methods allow us to confirm only the hypothesis that the IOFF method is not worse to ABI and TcPO2 in stenosis detection (non-inferiority hypothesis), but not superior.

Good sensitivity and specificity in the detection of extremities with stenoses and high-speed measurement make the method potentially promising both in the diagnosis and screening tool for LE-PAD. The ability of the IOFF method to detect hemodynamically stenoses not worse than ABI and TcPO2 on the studied contrast groups allows us to suggest a clinical potential of the new IOFF method. Thus, our study showed that the IOFF method is a promising tool for detecting lower extremity peripheral artery disease in adults with type 2 DM.

Conclusion

Thus, the IOFF method has been shown to detect limbs with hemodynamically significant stenoses in patients with DM with high sensitivity and specificity. In the study group of patients with diabetes mellitus, an ABI cut-off of 0.99 was optimal instead of the conventional 0.9.

Data availability

Data are available from the corresponding author (P.G.) upon reasonable request.

Acknowledgements We sincerely thank the reviewer who worked with our article. The deep and substantiated recommendations of the reviewer allowed us not only to refine the article, but also influenced the further research plans of our scientific group.

Author contributions Conceptualization: P.G., D.R., A.G.; Methodology: P.G., D.R., A.G., D.K., D.L., A.B.; Software: D.L.; Formal analysis: A.G.; Investigation: P.G., A.G., S.Z., R.L., A.B.,Yu Kon, Yu Kov, E.K., N.M., T.B.; Resources D.R., R.L., A.B., T.B., D.K.; Writing – original draft preparation: P.G.; Writing – review and editing: all authors; Visualization: P.G., A.G.; Supervision: D.R., D.K.; Project administration: D.R., D.K., P.G., A.G. All authors have read, and agreed to the published version of the manuscript.

Funding Patient recruitment and examination were funded by JSC "Elatma Instrument-Making Enterprise" (Ryazan, Russia). Data analysis and interpretation were performed by researchers without funding from JSC "Elatma Instrument-Making Enterprise" as part of the research project "New Approaches to the Comprehensive Assessment of Peripheral Hemodynamic Parameters in the Management of Patients with Diseases of Various Etiologies", funded by the State Budget of the Moscow Region. The funding source had no role in this manuscript.

Compliance with ethical standards

Conflict of interest The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval and consent to participate Written informed consent was obtained from each patient. The study protocol was approved by the research ethics committees of the participating institutions: Moscow Regional Research and Clinical Institute Independent Ethics Committee (Protocol No. 13, dated 7 November 2019); Almazov National Medical Research Centre Ethics Committee (No. 27112019, meeting No. 11–19, dated 11 November 2019). The principles of the Declaration of Helsinki were followed.

Appendix

Tables 5, 6

Table 5 Comparison of hemodynamic parameters in limbs with and without ulcers in group 2

	Group 2 with ulcers, $N = 11$ lower limbs	Group 2 without ulcers, $N = 35$ lower limbs	*p*-value
ABI, Me (IQR)	0.96 (0.92, 0.97)	0.94 (0.85, 0.99)	0.5
TcpO2 mm Hg. Me (IQR)	21 (4, 54)	34 (20, 42)	0.3
The new IOFF method			
Baseline perfusion, PU, Me (IQR)	0.55 (0.28, 1.28)	0.54 (0.42, 0.84)	0.9
LTH, 1 min, PU, Me (IQR)	1.00 (0.73, 1.68)	1.14 (0.89, 1.38)	0.8
LTH, 2 min, PU, Me (IQR)	1.20 (0.71, 2.73)	1.13 (0.86, 1.63)	>0.9
LTH, 3 min, PU, Me (IQR)	1.62 (0.70, 3.07)	1.23 (0.89, 1.70)	0.8
LTH, 4 min, PU, Me (IQR)	1.40 (0.67, 2.99)	1.16 (0.89, 1.58)	0.9
LTH, 5 min, PU, Me (IQR)	0.94 (0.70, 2.69)	1.18 (0.88, 1.66)	0.8

We performed an additional analysis and did not find statistically significant differences in perfusion, ABI, and TcPO2 measurements between Group 2 patients with and without ulcers. This lack of significance may be attributed to the fact that the ulcers primarily had a mixed etiology (neuro-ischemic ulcers), where the development of ulcerative defects was influenced not only by perfusion impairment but also by the presence of neuropathy

Table 6 Correlation coefficient between blood flow parameters and a number of stenoses

Parameter	Rs	*p*
ABI	−0.23	0.223
TcpO2	−0.53	<0.001
The new IOFF method		
Baseline perfusion	−0.47	0.003
LTH, 1 min	−0.54	<0.001
LTH, 2 min	−0.54	<0.001
LTH, 3 min	−0.45	0.004
LTH, 4 min	−0.49	0.001
LTH, 5 min	−0.44	0.005

ABI did not significantly correlate with the number of stenoses ($p = 0.223$). TcpO2 and all perfusion parameters assessed by the IOFF method negatively correlated with the number of stenoses (Rs from −0.44 to −0.54). The presence of negative correlations suggests an association between the number of stenoses and perfusion parameters assessed by IOFF method and TcpO2. The lack of correlation between the ABI parameter and the number of stenoses may indeed be due to a smaller sample taken for analysis (in 11 legs, it was not possible to assess the ABI due to lack of pulsation in the foot arteries or due to severe pain). A follow-up larger study using ROC analysis is needed to further investigate this phenomenon and calculate the ability to predict the number of stenoses by instrumental assessment (IOFF, TcpO2)

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