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A Toe Flexion NIRS assisted Test for Rapid Assessment of Foot Perfusion in Peripheral Arterial Disease: Feasibility, Validity, and Diagnostic Accuracy

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WHAT THIS PAPER ADDS

The present study offers a new add on tool for non-invasive ambulatory screening and monitoring of peripheral arterial disease. The novelty of the test, assisted by near infrared spectroscopy, is its capacity to provide a rapid assessment of haemodynamics in tissues under natural conditions and to obtain a dynamic assessment of the foot, which makes possible exploration of its macro- and microvascular reserve. This discriminative capacity makes the present test of particular interest in diabetics, and in other pathological conditions when the ABI is not measurable or reliable.

Objectives: Feasibility, validity, and diagnostic accuracy of a non-invasive dynamic ambulatory test were assessed with near infrared spectroscopy (NIRS) evaluating foot perfusion in peripheral arterial disease (PAD).

Methods: This was a prospective observational study. Eighty PAD patients (63 males, 71 ± 9 years), including 41 patients with coexisting diabetes, participated. Thirteen healthy subjects (8 males, 26 ± 8 years) were also studied by echo colour Doppler providing 160 diseased and 26 non-diseased limbs. Under identical clinostatic conditions, participants performed a 10–repetition toe flexion tests with NIRS probes on the dorsum of each foot; the area under the curve of the oxygenated haemoglobin trace ("toflex area") was calculated and the ankle–brachial index (ABI) was measured. Time of execution, rate of wrong tests, and adverse reactions were recorded. Within session reliability was assessed by administering the test twice, with a 5 minute interval between tests. The validity was assessed determining whether the toflex area was (a) dependent on the oxygen delivery from the lower limb arteries simulating PAD conditions by a progressive blood flow restriction (40–120% of systolic pressure) in healthy subjects; (b) consistent with the degree of PAD ranked by ABI and correlated with ABI and ankle pressure values in PAD patients. The diagnostic accuracy in detecting PAD was compared with examination using echo colour Doppler ultrasound.

Results: All tests were rapidly, satisfactorily (<1% mistakes), and safely performed. Toflex area values, superimposable in the two sessions (intra-class correlation coefficient 0.92), were comparable to PAD values following blood flow restriction, consistent with PAD severity, correlated with dorsal pedis artery pressure ($r = .21$; $p = .007$) and ABI ($r = .65$; $p < .001$) in PAD, but not in the presence of diabetes. Toflex area was similar to echo colour Doppler for detecting PAD following receiver operating characteristic curve analysis (area = 0.987, $p < .001$; toflex area values ≤ -28 arbitrary units, sensitivity/specificity 95.6/100).

Conclusion: The toe flexion test enables ambulatory assessment of foot perfusion and PAD detection, even in the presence of non-measurable ABI or diseases affecting the microcirculation.

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Keywords: Exercise test, Peripheral arterial disease, Near infrared spectroscopy, Diabetic foot, Sensitivity and specificity

INTRODUCTION

Non-invasive haemodynamic assessment of the lower limbs enables screening and monitoring of peripheral arterial disease (PAD), activities that are of particular importance in the presence of diabetes.[1] Validated tools are available, including the ankle–brachial index (ABI), toe brachial index, and echo colour Doppler, as well as techniques to assess tissue perfusion in the lower limbs such as the

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transcutaneous partial pressure of oxygen ($TcPO_2$).[2] However, these tools have limitations. They are static measures or selectively investigate the macrovascular circulation, or require post-exercise measurements; in addition, they may potentially be limited by calcification of blood vessels, or may represent a measure of skin perfusion.[1–9] A rapid non-invasive ambulatory assessment of the haemodynamic reserve of the foot under natural and dynamic conditions may provide an adjunctive method for the testing and monitoring of PAD patients, especially in the presence of medical conditions affecting both the larger vessels and the microcirculation. Near infrared spectroscopy (NIRS) non-invasively explores tissue microvascular haemodynamics,[4,5,10–12] monitoring the local balance between oxygen delivery and consumption. As it is suitable for dynamic and bedside measurements,[10,12] NIRS has been used for PAD assessment[4,13–15] to study perfusion of the foot,[16,17] or monitoring foot oxygenation in patients undergoing endovascular revascularisation[18] or intermittent pneumatic compression treatment.[19] On the basis of previous experience,[13,19–21] it was hypothesized that the oxygenation changes of the foot, when studied by the NIRS technique during a standardised task of toe flexion, might represent a measure of foot perfusion that is (a) both feasible and valid, (b) informative regarding the main arterial blood flow, and inclusive of the assessment of the peripheral microvascular blood flow when PAD is complicated by diabetes (c) with diagnostic capacity in detecting PAD.

MATERIALS AND METHODS

Study design and setting

This was a prospective observational study involving PAD patients and healthy subjects carried out at the Department of Rehabilitation Medicine, University Hospital of Ferrara. The local ethics committee approved this study (number: 04/2015).

Participants

From October 2015 to December 2016, eligible consecutive patients affected by PAD and referred to the program of Vascular Rehabilitation of the Department of Rehabilitation Medicine were invited to participate. Patients at Fontaine's stages II—III, without severe limitation of oxygen transport (severe anemia) and amputations or severe impairment of range of movement of the metatarsophalangeal joints were included in the study. Three skilled operators blinded to the new test at the Department of Vascular Surgery performed clinical examination and echo colour Doppler ultrasound. The abdominal aorta and iliac arteries, common, superficial, and deep femoral arteries, popliteal arteries, and both tibial axes were evaluated. High resolution imaging integrated with pulsed wave Doppler analysis and peak-flow velocity were used according to published standards.[22]

In addition, university students attending the laboratory and hospital personnel were approached to take part in the study as healthy volunteers. Written informed consent was obtained from all the participants. All healthy subjects aged $\geq$18 years underwent a clinical examination to exclude the presence of any chronic pathological condition.

Testing procedures

All the measurements necessary for the different phases of the study (Table 1) were carried out sequentially in a temperature controlled environment between 8:30 and 12:30 a.m. by the same expert operator within 15 days of the echo colour Doppler examination.

Ankle brachial index measurement. All participants underwent ABI measurement according to the standard[23] using a Doppler ultrasound device (Stereodop 448.S, Ultrasomed, Lavello, Italy) with a 9.3 MHz probe and a standard blood pressure cuff. PAD legs were ranked according to disease severity on the basis of the following ABI

Table 1. Phases of the study.

Aim	Action	Population (legs)	Method
Feasibility	Implementation and safety: time of completion including data analysis, rate of incorrect executions and/or adverse events	PAD, healthy (n = 186)	Descriptive statistics
Validity	Toe flexion test repeated two times on same day (consistency)	PAD, healthy (n = 186)	Intra-class correlation coefficient analysis
	Progressive external blood flow restriction (40—80—120% of systolic blood pressure) to simulate PAD	Healthy (n = 26)	One way analysis of variance
	Toflex area values ranked according to the ABI value only when measurable	PAD, healthy (n = 152)	One way analysis of variance
	Toflex- area correlation with the ABI value of: whole PAD population; D_{free}-PAD only; D-PAD only (discriminant)	PAD (n = 160)	Spearman's rho rank correlation
	Toflex area correlation with *posterior tibial* and *dorsal pedis* arteries pressure values	PAD (n = 160)	Spearman's rho rank correlation
Accuracy	Toflex area compared with Echo Colour Doppler for PAD detection	PAD, healthy (n = 186)	ROC curve analysis

Abbreviations: ABI, ankle brachial index; D_{free}-PAD, peripheral arterial disease patients without diabetes; D-PAD, peripheral arterial disease patients with diabetes; PAD, peripheral arterial disease; ROC, Receiver operating characteristic.

values: normal—borderline, 0.91—1.30; moderate, 0.90—0.70; severe, ≤0.70; incompressible, >1.31.

For further analyses, the legs of PAD patients also affected by diabetes were labeled as diabetes PAD (D-PAD); all the other PAD legs were labeled as diabetes free PAD (D_{free}-PAD).

Toe flexion test

Five minutes after ABI measurement, patients and healthy subjects performed the toe flexion test.

NIRS system. A continuous wave system (Oxymon MKIII, Artinis Medical Systems, Elst, The Netherlands) comprising two channels (2 equivalent pulsed light sources and 2 avalanche photodiode detectors) was used. The system uses intensity modulated light at 1 Hz frequency and laser diodes operating at three wavelengths corresponding to the absorption wavelengths of oxyhemoglobin (O_2Hb) and deoxyhemoglobin (HHb). The light generated and conducted to the tissue is partly absorbed and scattered by the tissues and re-collected by the detector, providing a direct measurement of O_2Hb and HHb concentrations.

Protocol. With the subject lying in a supine position, NIRS optodes, connected to the abovementioned instrument, were placed and secured with tape on the dorsum of each foot, approximately 2 cm proximal to the metatarsophalangeal joints between the first and the fourth intermetatarsal space. The interoptode distance was set at 30 mm, allowing a maximum light penetration depth of approximately 15—20 mm.

After a brief explanation, subjects were asked to perform a toe flexion movement for 10 consecutive times, following the rhythm imposed by a metronome set at 40 beats per minute. The pattern allowed a complete toe flexion—extension movement lasting 3 s, with the cumulative test duration of 30 s. The semi-quantitative data obtained were extracted by the software provided by the NIRS system manufacturer (Oxysoft 2.0.47, Artinis Medical Systems) and were transferred to a spreadsheet (Microsoft Excel 2013). After normalisation to zero (the baseline value collected at the beginning of the test was set to 0), the data for the O_2Hb trace were analysed to determine the area under the curve recorded during the test ("toflex area") (Fig. 1).

Blood flow restriction in healthy subjects. Young healthy volunteers repeated the toe flexion test with progressive blood flow restriction. A blood pressure cuff was placed around the thigh and connected to a manometer that was inflated at 40%, 80%, and 120% of the systolic arterial pressure previously measured in the supine position. The procedure was interrupted if the subject requested termination of the procedure because of intolerable discomfort. Between each grade of compression, a resting period of 5 minutes was scheduled. At the end of the test, subjects were asked to grade their discomfort on a 0—10 scale.

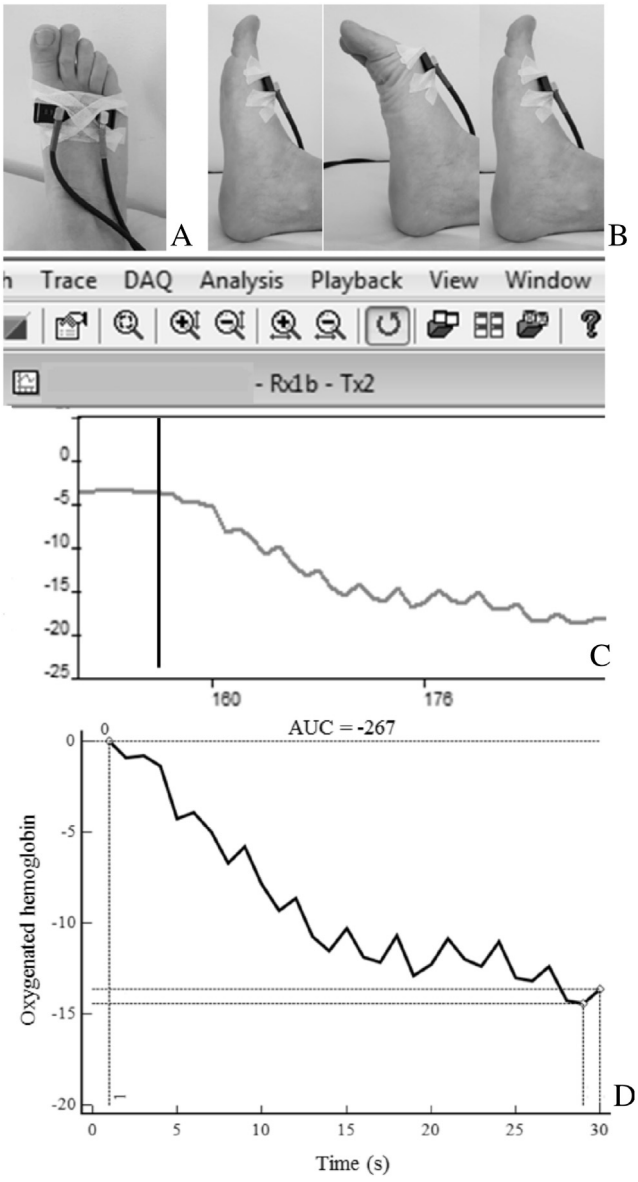

Figure 1. The phases of the toe flexion test. (A) Optode positioning; (B) task execution (to be repeated 10 times); (C) oxygenation monitoring by software; (D) toflex area calculation.

Diagnostic accuracy

Sample size calculation. The formula proposed by Hajian—Tilaki was employed.[24] Setting a desired sensitivity of 0.90 and a disease prevalence of 0.75 considering both healthy and PAD legs, with a maximum marginal error of estimate of 0.05, the total sample size required was $n = 184$ legs.

Positivity cutoffs. For the toe flexion test an arbitrary positivity cutoff value for toflex area <−20 arbitrary units (a.u.) was set, considering that in healthy legs a negligible area of oxygen debt is foreseeable whereas in PAD legs a significant imbalance between oxygen delivery and oxygen request during toe flexion occurs.

For echo colour Doppler, the positivity cutoff was set for both limbs when at least one haemodynamically significant arterial lesion was present in at least one leg of that subject.

Endpoints

The endpoints of the study are shown in Table 1.

Statistical analysis

Continuous variables are expressed as mean ± standard deviation or median (interquartile range) according to there being a normal or non-normal distribution, and categorical variables as a percentage. The normal distribution of data was verified by the Kolmogorov–Smirnov test. Test–retest reliability was assessed using the intra-class correlation coefficient, selecting the absolute agreement method of analysis. The comparisons of toflex area values in healthy individuals following blood flow restriction and in PAD subjects according to the leg categories were carried out by a one way analysis of variance. A Spearman rank correlation analysis was conducted to evaluate the relationship between the toflex area and other parameters. Receiver operating characteristic (ROC) curves were calculated to identify potential toflex area cutoff values for separation of healthy and PAD legs according to echo colour Doppler positivity. A p value $\leq .05$ was considered to be statistically significant. Data were analysed using MedCalc Statistical Software version 16.8.4 (MedCalc Software bvba, Ostend, Belgium).

RESULTS

Eighty PAD patients (63 males, age 71 ± 9 years) and 13 healthy subjects (8 males, age 26 ± 8 years) were enrolled into the study. The characteristics of the participants are shown in Table 2.

In PAD patients, following ABI measurement, 36 legs were labeled as normal–borderline, 33 as moderate, 57 as severe, and 34 as incompressible. Moreover, 78 were D_{free}-PAD legs, and the remaining 82 were D-PAD legs, belonging to the 41 PAD subjects with coexisting diabetes.

Feasibility

All patients completed the toe flexion test twice without reporting any local or general adverse effects during NIRS measurement. Two patients repeated the test an additional time because of a misunderstanding of the rules ($n = 1$) and/or to difficulties to follow the rhythm imposed by the metronome ($n = 1$). The test execution lasted about 5 min, including explanation, positioning, and setting of NIRS probes, and data analysis. All the NIRS traces collected were analysed for the toflex area calculation.

Validity

The repetition of the test, performed in the same session (consistency) for all the tested legs, showed values of toflex area of −125 ± 123 a.u. for the first attempt and −131 ± 124 a.u. for the second, with an intra-class correlation coefficient equal to 0.92 (95% CI 0.89–0.94).

Blood flow restriction in healthy subjects. All healthy volunteers completed the toe flexion test with progressively increasing degrees of blood flow restriction. No adverse events occurred during the procedure. The subjects rated their degree of discomfort with a median value of three out of 10 (interquartile range 2–4).

The total of 26 healthy legs showed mean values of toflex area of 35 ± 57 a.u. at baseline without blood flow restriction, of −22 ± 82 a.u. with a 40% flow restriction, of −40 ± 90 a.u. with 80% of flow restriction and of −163 ± 105 a.u. with 120% of flow restriction. The one way analysis of variance resulted in a significant model ($p < .001$) with significant differences between toflex area

Table 2. Characteristics of study participants.

	PAD patients (n = 80)	Healthy subjects (n = 13)
Age (years)	71 ± 9	26 ± 8
Male sex, n (%)	63 (79)	8 (62)
Leriche-Fontaine's stage		
II A	21 (26)	0 (0)
II B	58 (73)	0 (0)
III	1 (1)	0 (0)
Lesion location and classification[a], n (%)		
Aorto-iliac	14 (18)	0 (0)
A–B–C–D	3–6–4–1	
Femoral-popliteal	58 (73)	0 (0)
A–B–C–D	6–22–26–4	
Infra-popliteal	49 (61)	0 (0)
Risk factors, n (%)		
Diabetes	41 (51)	0 (0)
Hypertension	71 (89)	0 (0)
Hyperlipidaemia	58 (73)	0 (0)
Smoking	65 (81)	0 (0)
Current smoking	11 (14)	0 (0)
Familiarity	10 (13)	0 (0)
Comorbidities, n (%)		
Myocardial infarction	23 (29)	0 (0)
Coronary heart disease	34 (43)	0 (0)
Cerebrovascular disease	9 (11)	0 (0)
Lung disease	12 (15)	0 (0)
Lower limb revascularisation	33 (41)	0 (0)
Endovascular–Surgical–Combined	9–14–10	0 (0)
Charlson Comorbidity Index	5.8 ± 1.8	0.1 ± 0.5
Drug therapy, n (%)		
Anticoagulants	14 (18)	0 (0)
Antiaggregants	69 (86)	0 (0)
Cilostazol and/or pentoxifylline	0 (0)	0 (0)
Statins	58 (73)	0 (0)
Antihypertensive	71 (89)	0 (0)
Hypoglycemic agents and/or insulin	41 (51)	0 (0)

Abbreviations: PAD, peripheral arterial disease; SD, standard deviation.

Values are mean ± SD for continuous variables and number (percentage) for categorical variables.

[a] Lesion classification according to the "Inter-Society Consensus for the Management of Peripheral Arterial Disease".

without restriction and toflex area at all degrees of flow restriction, and between the 120% compression and both the 40% and 80% flow restriction (Fig. 2).

Ranking of toflex area and haemodynamics in PAD subjects. Considering all legs with a reliable ABI, significant differences ($p < .001$) were observed between toflex area of healthy legs (mean value 35 ± 57 a.u.) and all the other categories. In addition, both normal—borderline (−103 ± 108 a.u.) and moderate (−110 ± 59 a.u.) legs differed from severe (−178 ± 115 a.u.) legs ($p < .001$).

Considering the correlation between ABI and toflex area, in the whole PAD population no significant correlation was observed ($r = .03$; $p = .752$). The same analysis performed on D_{free}-PAD legs, resulted in a moderate correlation ($r = .65$; $p < .001$), whereas no significant correlation was observed for ABI and D-PAD legs ($r = -.10$; $p = .248$) (Fig. 3).

Considering the correlation between ankle pressure and toflex area among all PAD legs a weak but significant correlation with the pressure measured in the dorsal pedis artery ($r = .21$; $p = .007$) was observed, but not with the pressure in the posterior tibial artery ($r = .08$; $p = .305$).

Diagnostic accuracy

The flow diagram of participants, in relation to the arbitrarily set positivity cutoffs previously described and according to STARD,[25] is reported in Fig. 4.

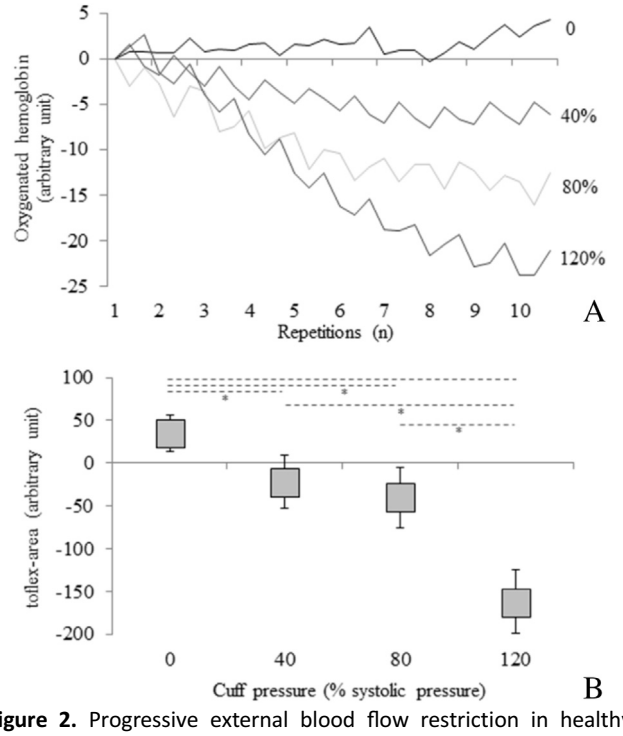

Figure 2. Progressive external blood flow restriction in healthy subjects performed at 40%, 80%, and 120% of systolic blood pressure. (A) Oxygenated haemoglobin traces at different degrees of compression; (B) one way analysis of variance between different categories. *Significant difference.

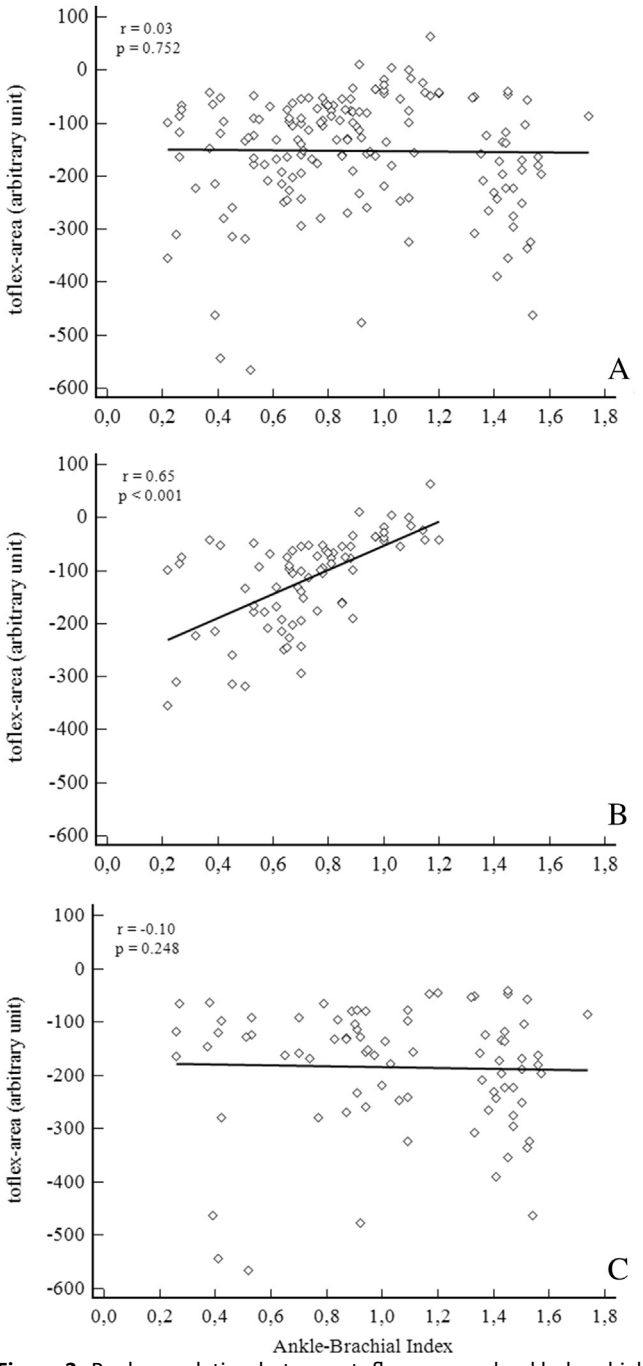

Figure 3. Rank correlation between toflex area and ankle brachial index in PAD patients. (A) Whole population; (B) diabetes free PAD; (C) diabetes PAD.

Compared with the total 26 healthy non-diseased legs, the ROC analysis of toflex area showed an area under the ROC curve of 0.987 ($p < .001$; 95% CI 0.959—0.998) with sensitivity/specificity of 95.6/100.0 for values ≤−28 a.u. (negative likelihood ratio of 0.04) (Fig. 5).

DISCUSSION

The study illustrated the feasibility, validity, and diagnostic accuracy of a toe flexion test with non-invasive continuous recording of tissue oxygenation provided by NIRS to study and monitor the dynamic foot perfusion in PAD in an

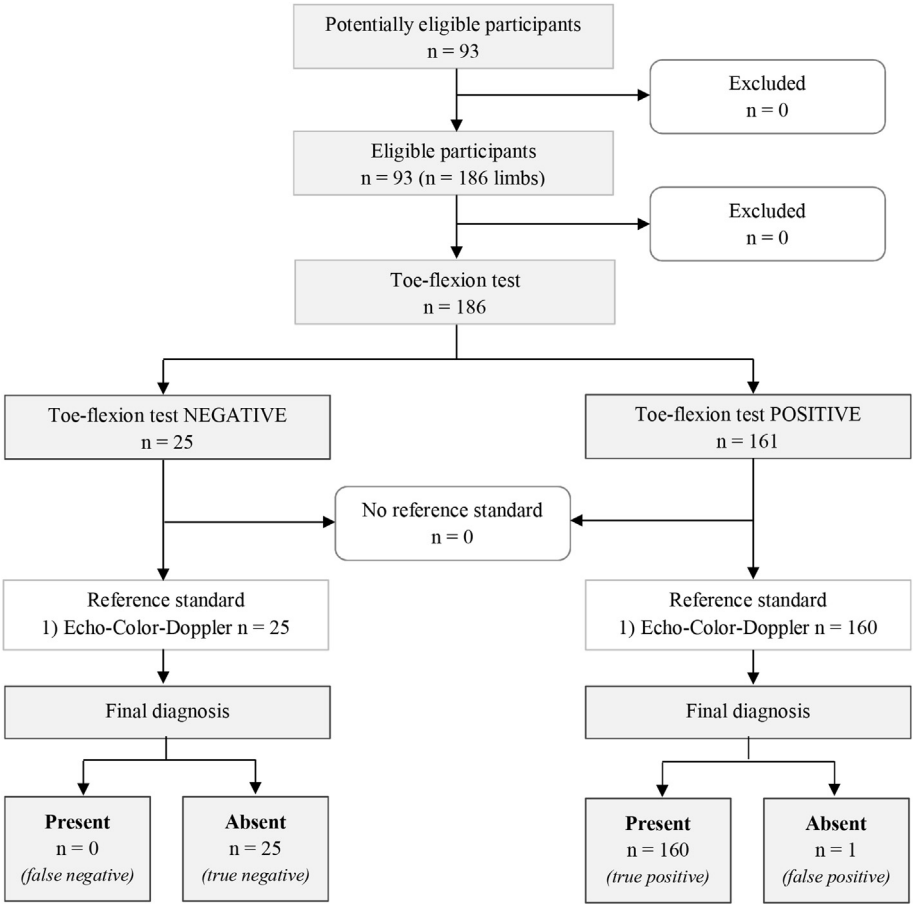

Figure 4. Flow of participants through the study in relation to the accuracy phase.

outpatient setting. Reproducible, non-invasive, and objective measures of tissue perfusion in the lower limbs are considered important tools for managing patients with PAD as well as for monitoring patients with diabetes.[1,2] The present study aimed to offer an option in this direction by the development of a new test. In the absence of an accepted gold standard to measure foot perfusion by a dynamic test, multiple test results were combined to construct a reference standard outcome. The relationship between the toflex area and oxygen delivery deriving from the larger vasculature has been well demonstrated, with values of the new parameter determined in healthy subjects turning into values consistent with the presence of severe PAD. A relationship between toflex area and validated haemodynamic parameters was also observed: a correlation with the dorsalis pedis artery pressure selectively perfusing the dorsum of the foot was found, but not with the posterior tibial artery pressure. The correlation with ABI was moderate, as expected when comparing a dynamic with a static measurement. The presence of a relationship between ABI and different NIRS measurements at rest is controversial[5,26–29]; however, in this study the correlation was present in the D_{free}-PAD patients and absent in the presence of a disease evoking microcirculatory impairment. This fact represents a discriminant validity factor for the toe flexion test, which is not a simple measure of oxygen delivery from the main arterial blood flow but a comprehensive measure including the peripheral microvascular circulation.

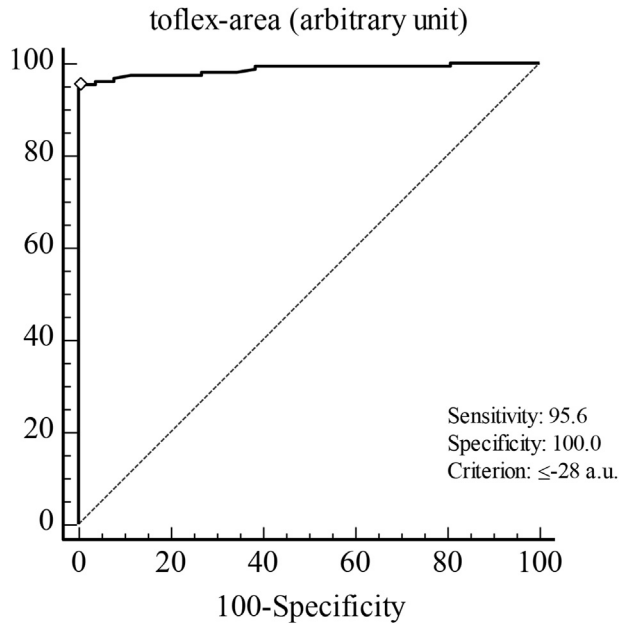

Figure 5. Receiver operating characteristic curve of the toflex area compared with the echo colour Doppler positivity for the presence of peripheral arterial disease.

This microvasculature efficiency, which affects the distribution of peripheral tissue perfusion in the ischaemic

foot,[30] can be uncovered by the dynamic phase: the requested toe flexion movement increases metabolic activity and local oxygen consumption, inducing thermal and chemical changes with the production of metabolites and vasoactive factors. These factors, normally influencing blood redistribution according to haemodynamic compensatory strategies, are abnormally exploited in the diabetic foot in the presence of ischaemia and neuropathy.[31] These altered strategies may be responsible for the observed toflex area/ABI decoupling in the presence of microcirculatory disturbance. In terms of diagnostic accuracy, the test proposed can distinguish between PAD and healthy legs when compared with the echo colour Doppler examination, with high values of sensitivity and specificity. Therefore, the toe flexion test might be considered for routine clinical use: it would enable stage monitoring, and screening of PAD patients in an outpatient setting, representing a possible add-on test in the presence of altered microcirculation, or a replacement test when the ABI is not measurable.

In this study the NIRS technique, which stands in between the haemodynamics and tissue perfusion measures,[1] offers semi-quantitative measurements of the oxygen balance. Applied to a dynamic protocol, this technique enables the quantification of the oxygen debt caused by the activation of the muscles of the dorsum of the foot in the presence of PAD. The measurement differs from other validated static measurements such as ABI or the toe brachial index, but also of skin perfusion from TcPO_2. The NIRS technique, previously employed in the study of foot ischaemia,[19] allows investigation of the metabolism of the tissues to be investigated and not only of foot skin oxygenation.[10,11,32] Moreover, it works under normal physiological conditions, not requiring a heating process of the zone under examination.[33–35] In addition, the NIRS technique is suitable for outpatient examination, and does not require calibration or warm-up procedures, or disposables and supplies, or specifically trained personnel.

The toe flexion test proved to be feasible for use in an outpatient facility, being both rapid (lasting around 5 minutes for the whole procedure) and safe. Moreover, being based on a simple protocol, the test was easy for the patient and repeatable. Finally the test provides objective results, not being related to symptoms, which are sometimes confounding for coexisting comorbidities; it allows the assessment of PAD patients with a not-computable ABI who are unable to walk and therefore unable to undergo the evaluation of post-exercise ABI, or in presence of altered microcirculation. Abnormal toflex areas, similar to those observed in the presence of diabetes, were also noted in patients with PAD and coexisting arteritis/vasculitis or rheumatic diseases (personal observation). However, it is accepted that the test could be of difficult to perform in patients with severe foot deformities and in patients with partial amputations.

The study presents several limitations related to the relatively small sample of patients and inherent to the NIRS technique. A difficulty to transfer NIRS measurements into clinical practice was reported, despite their good reproducibility for tissue oxygen saturation assessment.[5] As for other validated techniques of perfusion assessment (laser Doppler flowmetry or TcPO_2),[2] NIRS analyses a localised and limited region of muscle under the probes, although oxygen saturation might vary, within and between specific muscles. Factors such as individual skinfold thickness, oedema, and especially atrophy of the foot muscles may have theoretically affected the results. However, these measurement issues are minimised as toflex area is not an absolute value but an area of oxygen debt contracted during the task: the measure is obtained with respect to the individual baseline level determined under the same analytical conditions. Repeatability was assessed in terms of consistency, on the same day without removing the NIRS probes, but not of stability, which needs to be confirmed. Moreover the sensors might have been positioned in slightly different regions among patients, although the same skilled operator performed the procedure. In addition, despite the analytical measurements that were based on objective analysis, the NIRS operator was not blinded to the group assignment of each patient. The toe flexion test also presents internal limitations derived from the different flexion movement possible for each patient, but the limited articular range of motion of the exercise (about 40°), could partially smooth these discrepancies. Finally the toflex area parameter was not correlated with a local measure of TcPO_2 for the discussed difference between the two techniques; however, in future this comparison might be performed to add to the specific knowledge related to this issue.

In conclusion, the present tool assisted by NIRS, in absence of specific dynamic tests, could represent a quick outpatient test to objectively define PAD presence or severity and foot perfusion. Further studies are necessary to support its suitability in the vascular field to evaluate the effects of pharmacological, surgical or rehabilitative interventions as well the risk of developing wounds.

CONFLICT OF INTEREST

None.

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