

## Editor's Choice

# Novel Assessment Tool Based on Laser Speckle Contrast Imaging to Diagnose Severe Ischemia in the Lower Limb for Patients With Peripheral Arterial Disease

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**Objective:** We propose a new assessment tool to diagnose severe ischemia of the lower limb in peripheral arterial disease, using laser speckle contrast imaging to evaluate heating-induced microcirculatory fluctuations in the proximal and distal sites of the dorsal foot.

**Study Design:** A cross-sectional study.

**Methods:** We recorded the slope describing the behavior of perfusion values (decrease or plateau) following the initial, heating-induced increase in perfusion in 63 feet of patients with clinical signs of peripheral arterial disease.

**Results:** The plateau and decrease groups were defined as having perfusion slopes of  $<0.20$  and  $\geq 0.20$  PU/min, respectively. Transcutaneous oxygen tension was significantly lower ( $P < 0.001$ ) in the plateau than in the decrease group (8 vs. 45 mmHg), indicating more severe ischemia. The laser speckle contrast imaging thermal load test discriminated transcutaneous oxygen tension  $<30$  mmHg with good sensitivity (78.7%) and specificity (96.2%), and an area under the curve of 0.908.

**Conclusions:** Both transcutaneous oxygen tension and the laser speckle contrast imaging thermal load test are useful in diagnosing severe ischemia in the foot. *Lasers Surg. Med.* 49:645–651, 2017.

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**Key words:** severe limb ischemia; laser speckle contrast imaging; thermal load test

## INTRODUCTION

It is recommended that symptomatic peripheral arterial disease (PAD) patients be diagnosed by objective examination [1]. The ankle brachial index (ABI) and transcutaneous oxygen tension ( $tcPO_2$ ) are the typical tools used for non-invasive assessment of the severity of limb ischemia in terms of blood pressure and tissue oxygenation state, respectively. Measurement of  $tcPO_2$  is one of the most popular methods for evaluating cases of severe ischemia. Meanwhile, laser speckle contrast imaging (LSCI), a non-invasive blood flow

imaging technique, involves irradiating tissues with a laser beam and monitoring the spatial and temporal fluctuations of the scattered light, yielding an equivalent to the tissue perfusion value (PV) [2–4]. LSCI has been used to study various conditions such as Raynaud's disease [5], sclerodermas [6], burns [7], and PAD with reactive hyperemia induced by arterial occlusion [8].

LSCI has recently been applied to clarify various mechanisms of microcirculation in normal perfusion induced by thermal load [2,4,9]. Several methods based on blood-flow imaging devices have demonstrated a reproducible, triphasic response to local cooling; this fluctuation, termed “hunting reaction” or “cold-induced vasodilation,” consists of an initial, rapid vasoconstriction, followed by transient vasodilation, and finally, prolonged vasoconstriction [2,4,10–13]. However, an LSCI-based protocol for assessing the severity of limb ischemia via thermal load has not yet been established.

We aimed to establish an LSCI-based protocol for assessing the severity of limb ischemia, which involves evaluation of blood perfusion following local heating to the foot, considering that the subsequent relative cooling process involves a transient increase in perfusion followed by prolonged decrease, similar to the “hunting reaction.” We hypothesized that higher ischemic severity would show a distinctive LSCI-detected

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pattern of perfusion fluctuation following thermal load, expressed in terms of the slope of the decrease in PV, which would correlate with the values of the conventional parameters used to assess ischemia (ABI and  $tcPO_2$ ).

## MATERIALS AND METHODS

### Patients and Study Protocol

Between January and June 2015, patients with clinical signs of PAD were referred to our vascular surgery unit. PAD was diagnosed based on the presence of >50% vessel stenosis due to lesions in the lower limbs. We predominantly assessed the vessel stenosis by computed tomography angiography. In cases with contraindications for the use of contrast media, such as allergies or chronic kidney disease, we evaluated the stenosis by duplex ultrasound sonography and/or magnetic resonance angiography. We excluded patients with acute leg ischemia due to emboli. Furthermore, we excluded patients with an axillary temperature over 37.0°C, inflammatory diseases such as collagen disease, malignancy, and infectious disease. All patients were evaluated using three methods: ABI,  $tcPO_2$ , and the LSCI thermal load (LTL) examination, as discussed below.

The medical records of the patients were reviewed to collect data regarding underlying diseases. Hypertension was diagnosed in patients with a systolic blood pressure of >140 mmHg or a diastolic blood pressure of >80 mmHg, as well as in patients treated for hypertension. Coronary artery disease was diagnosed as the presence of angina pectoris, myocardial infarction, or both, and was confirmed by coronary angiography or a history of vascular reconstruction of any coronary artery. Cerebrovascular disease was diagnosed in patients with a history of stroke, transient ischemic attacks, carotid artery revascularization, or cerebral hemorrhage. Chronic kidney disease was defined as having a daily hemodialysis treatment. Smoking status, either at the time of the examination or in the past, was recorded via survey.

All protocols, surveys, and consent forms were approved by the Institutional Review Board of Tokyo Medical and Dental University Hospital. Written informed consent was obtained from all patients.

### Measurements

A commercially available LSCI device (Omegazone™, Omegawave, Inc., Tokyo, Japan) was used to measure PV. The LSCI has a laser (diode) with a wavelength of 780 nm, and the output power is 40 mW. The laser light spreads and is irradiated on the skin, and the laser power on the skin was calculated to be about 0.3 mW/cm<sup>2</sup> when the LSCI was placed 40 cm from the skin. The evaluation took place in a dedicated room, with a room temperature of 23–25°C and constant ambient lighting. The patient remained at rest in the supine position for 15 minutes. The PV was measured based on the optical power contrast in each pixel at the domain of approximately 0.5 mm, which is produced by scattering light with a charge-coupled device camera. LSCI data can be expressed as arbitrary perfusion units (i.e., perfusion units

[PU]) as a relative value corresponding to “mL/min/100 g” [4,14]. The LSCI camera was placed above the dorsal foot, at a fixed distance of precisely 40 cm from the skin, which was ensured by focusing the two laser sources equipped on each side of the camera. The resolution of the perfusion color image was 639 × 480, and the frame rate was 60 frames per second. PV was calculated from 20 real sequenced images. The time interval of the LSCI camera was set to 1 second.

The dorsal foot PV at rest ( $PV_{rest}$ ) was first recorded for 1 minute as the baseline value just before measuring  $tcPO_2$ .

$tcPO_2$  was measured according to the instructions specified in the user manual of the monitoring device (TCM400™, Radiometer Medical, Inc., Copenhagen, Denmark). A  $tcPO_2$  probe was set at 44°C to measure  $tcPO_2$  at two sites, namely the proximal site (tarsal level) and the distal site (between the first and second metatarsal bone).  $tcPO_2$  was determined as the plateau value recorded at 20 minutes after local heating. After achieving sufficient heating and completing the  $tcPO_2$  measurement, the  $tcPO_2$  probe was removed, and the whole foot was monitored using the LSCI device; this was considered to be the start point of the 15-minute LTL examination (Fig. 1).

The ABI was measured using the Doppler method, with the patient in the supine position [1].

The reproducibility of all measurements was assessed by repeating the measurements on two different days and for same points on the foot in a subset of our sample of patients, who consented to these repeat measurements.

### LSCI Data Analysis

The LSCI data were analyzed after completion of the LTL test, using a dedicated analysis software program (LIA™, Omegawave, Inc.), and the mean PV in the regions of interest (ROIs) was calculated. The ROI was set as a 1-cm diameter circle, approximately the same size as the  $tcPO_2$  probe, at the same site of  $tcPO_2$  probe-induced heating. Therefore, the adopted ROI was compatible with both the  $tcPO_2$  and the LSCI measurements.  $PV_{rest}$  was calculated based on the average of the mode for 10 seconds before  $tcPO_2$  probe heating.

To evaluate the findings of the LTL examination, a new perfusion parameter was defined in terms of the slope of the PV decrease (PU/min) after the inflection point marking the end of the initial, transient increase induced by heating. The raw PV values were processed in terms of 30-second modes in order to minimize the signal noise related to body movement. The slope was obtained as a linear regression of the processed PV values, and described the PV fluctuation over the first 5 minutes after the inflection point (Fig. 2).

### Statistical Analysis

Data were presented in terms of median with 25th and 75th percentiles for continuous variables, and as percentages for categorical variables. To compare numerical data between two groups, the Mann–Whitney U test and the Wilcoxon signed-rank test were used for unpaired and paired groups,

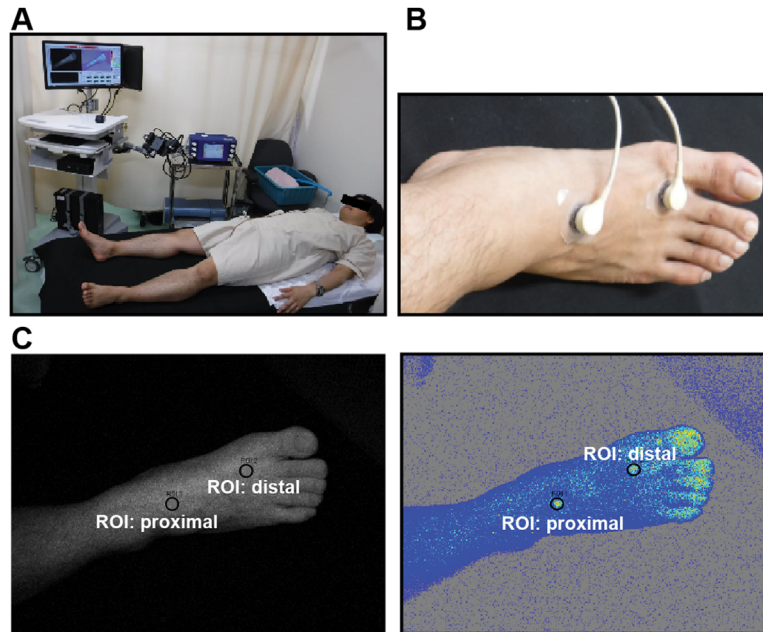


Fig. 1. Thermal load examination based on laser speckle contrast imaging (LSCI). (A) LSCI setup after heating and measurement using a transcutaneous oxygen tension (tcPO<sub>2</sub>) probe. (B) Two sites on the dorsal foot were tested for tcPO<sub>2</sub> and represented regions of interest (ROI1 and ROI2) during subsequent LSCI: the proximal site (tarsal level) and the distal site (between the first and second metatarsal bone). (C) Representative LSCI rendering including the two ROIs on the right dorsal foot.

respectively. Spearman's rank correlation analysis was applied to estimate the relationship between variables. Receiver operating characteristic (ROC) curves were plotted to evaluate the diagnostic value of the slope in detecting severe limb ischemia as an ABI <0.40 [15,16] and a tcPO<sub>2</sub> <30 mmHg [1,15]. The slope was calculated using single linear regression. Values of  $P < 0.05$  indicated statistical significance. All statistical analyses were performed using the SPSS software program (version 20.0, SPSS, Inc., Chicago, Illinois).

## RESULTS

### Patient Characteristics

A total of 36 patients were recruited in this study, and 63 limbs (126 sites) were evaluated via LSCI after excluding nine limbs either because of major amputations ( $n = 2$ ) or lack of consent from the patient to the evaluation of the non-affected limb ( $n = 7$ ). In terms of the ABI measurements, eight further limbs were excluded because ABI was  $>1.4$  [1].

The median age in our study sample was 74.0 years (range: 50–96 years), and 25 patients (69%) were men. The documented comorbidities were hypertension (72%), dyslipidemia (42%), diabetes mellitus (67%), coronary artery disease (33%), cerebral vascular disease (33%), chronic kidney disease requiring hemodialysis (22%), and smoking history (56%).

According to the Rutherford classification [17], 33 limbs were classified as PAD of categories 1–3 (grade I), although the treadmill walking test was not routinely performed. Of these, 14 were classified as stage IIb (severe claudication at

a distance <200 m) according to the Fontaine classification [18]. The remaining 30 limbs were classified into Rutherford categories 4–6 (grades II and III), with 6, 17, and 7 limbs classified as categories 4, 5, and 6, respectively.

### Group Classification According to the PV Slope

During the LTL examination, PV initially showed a transient increase, and then exhibited one of two distinct patterns, namely a sharp decrease (Fig. 2A) or a plateau with mild fluctuation (Fig. 2B). The slope was calculated to reflect the two distinct patterns of PV fluctuation after the initial, transient increase. A histogram analysis of the slope revealed two peaks of the frequency distribution, separated by a region with sparse frequency for PV ranging between 0.15 PU/min and 0.20 PU/min (Fig. 3A). Therefore, the threshold of the slope was set at 0.20 PU/min, and slope values <0.20 PU/min were assigned to the plateau group (group P,  $n = 40$  sites; Fig. 2B), while those  $\geq 0.20$  PU/min were assigned to the decrease group (group D,  $n = 86$  sites; Fig. 2A).

### Reproducibility

We evaluated the reproducibility of the measurements by performing measurements on two different occasions and at 16 different points on eight lower limbs belonging to either group P or group D (eight feet in total). In all eight cases, both measurements indicated the same behavior of the PV following the initial increase, and the slope was not significantly different between the two measurements ( $P = 0.78$ ) (Fig. 4A). The reproducibility of tcPO<sub>2</sub> is shown

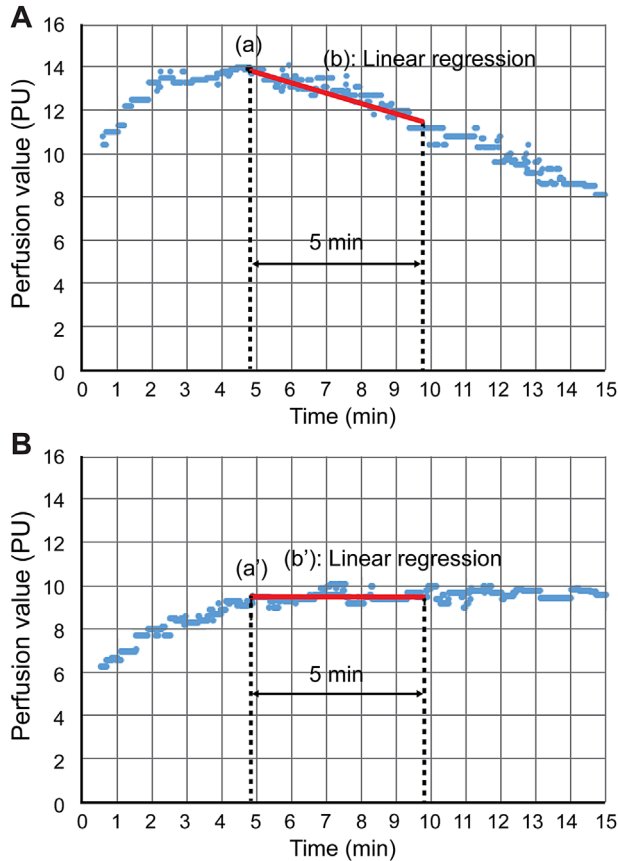


Fig. 2. Two typical patterns of perfusion value (PV) fluctuation described in terms of the slope of the PV distribution obtained using laser speckle contrast imaging (LSCI) during a thermal load test. (A) The PV decreased sharply after the inflection point marking the peak of the initial, transient PV increase following heating. (a) PV at the inflection point; (b) linear regression of the PV distribution in the first 5 minutes after the inflection point (slope). (B) The PV plateaued after the inflection point. (a') PV at the inflection point; (b') linear regression of the PV distribution in the first 5 minutes after the inflection point (slope).

in Figure 4B. Although one point of  $tcPO_2$  reached 30 mmHg in the second test after reaching 26 mmHg in the first test, the other points did not reach the 30 mmHg boundary value of  $tcPO_2$ .

#### Comparison Among ABI, $tcPO_2$ , and LTL Findings

Groups P and D were compared in terms of the PV slope,  $PV_{rest}$ ,  $tcPO_2$ , and ABI (Table 1). In group P, the median ABI was 0.35 (25–75% range: 0.11–0.61) and the median  $tcPO_2$  was 8.0 mmHg (25–75% range: 3–18), while these values were significantly higher in group D. In group P ( $n = 40$  sites), 21 sites (52.5%) showed ABI  $< 0.40$ , and 37 sites (92.5%) showed  $tcPO_2 < 30$  mmHg.

$PV_{rest}$  was not correlated with conventional ischemic parameters such as ABI and  $tcPO_2$ . In correlation analysis, Spearman's  $\rho$  value for  $PV_{rest}$  with ABI was 0.171, and  $tcPO_2$  was less than 0.001.

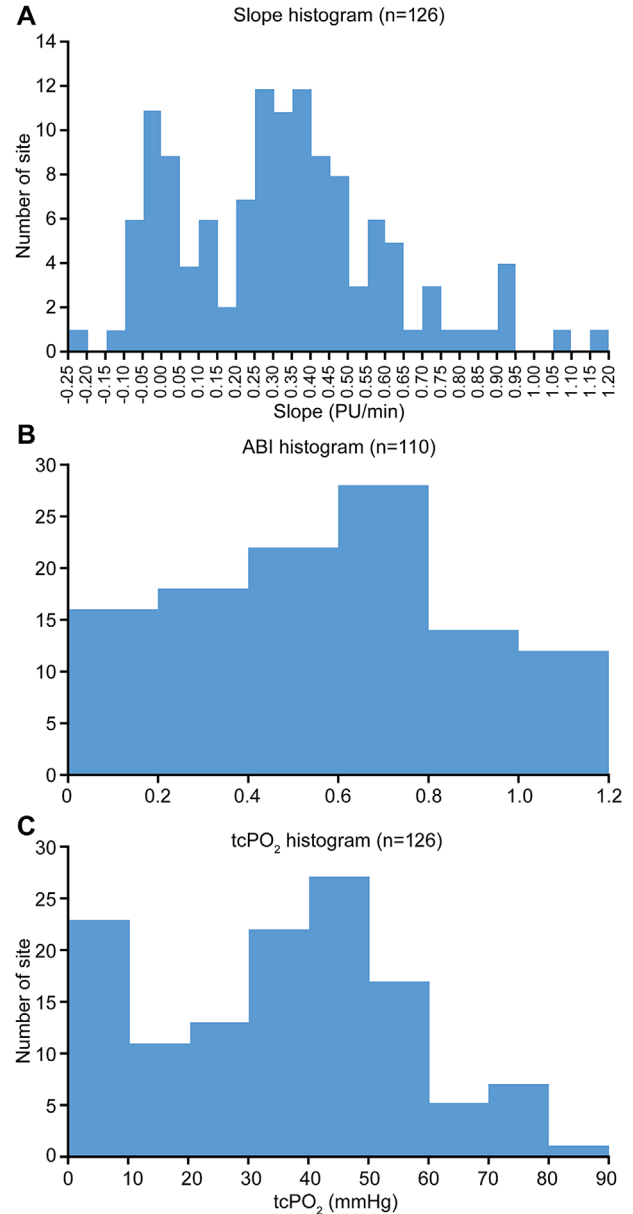


Fig. 3. Histogram analysis of the distribution of values of parameters indicating ischemia. (A) Slope of the perfusion value (PV) fluctuation (PU/min) measured by laser speckle contrast imaging at 126 sites. The histogram was built with a 0.05 PU/min interval, and shows two peaks of the frequency distribution, separated by an area of low frequency for PV between 0.15 PU/min and 0.20 PU/min. (B) The distribution of ankle brachial index (ABI) values ( $n = 110$  sites) does not show any distinctive feature. (C) The distribution of transcutaneous oxygen tension ( $tcPO_2$ ) values ( $n = 126$  sites) does not show any distinctive feature.

#### Predictive Ability of the PV Slope Regarding Ischemic Parameters

The ability of the PV slope to discriminate ABI  $< 0.4$  showed an area under the ROC curve of 0.723, with a sensitivity of 61.8% and a specificity of 75.0% at a cut-off value of 0.20 PU/min (Fig. 5A). The ability of the PV slope to discriminate  $tcPO_2 < 30$  mmHg showed an area under

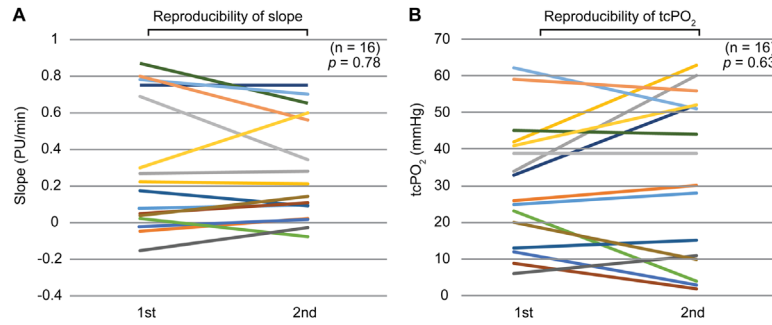


Fig. 4. Reproducibility of the slope of the perfusion value and  $tcPO_2$  measurements. The measurements were performed on two different occasions in same testing points, at 16 different points on eight feet. (A) The reproducibility of the slope. (B) The reproducibility of the  $tcPO_2$ .

the ROC curve of 0.908, with a sensitivity of 78.7% and a specificity of 96.2% at a cut-off value of 0.20 PU/min (Fig. 5B). A similar analysis regarding discrimination of  $tcPO_2 < 20$  mmHg showed an area under the ROC curve of 0.919, with a sensitivity of 88.2% and specificity of 88.2% (Table 2).

## DISCUSSION

The aim of our study was to establish a novel, non-invasive method for assessing the severity of limb ischemia. To this end, we examined the difference between conventional ischemic parameters (ABI and  $tcPO_2$ ) and the patterns of PV fluctuation recorded during the LTL test. To our knowledge, this is the first report to evaluate limb ischemia using LSCI via a thermal load test.

There is no definitive correlation between  $PV_{rest}$  and conventional ischemic parameters such as ABI and  $tcPO_2$ . We thought that the compensation mechanism contributed to the maintenance of  $PV_{rest}$ , regardless of the ischemic severity [19]. Therefore, a load test is needed to evaluate limb ischemia using the LSCI device. The slope seemed to be the most important parameter to describe the two distinct patterns of PV fluctuation. With regard to patient distribution, our study recruited patients with borderline ABI and  $tcPO_2$ , indicating severe limb ischemia. However, the histogram of the PV slope distribution showed two peaks, separated by an area with low frequency for PV

ranging from 0.15 PU/min to 0.20 PU/min. This represents one of the key results of our study, and suggests that there may be different PV behaviors associated with different stages of ischemia. Therefore, we stratified the evaluated sites into two groups according to a PV-slope cut-off of 0.20 PU/min, which served as a clear threshold distinguishing between the plateau pattern and the decrease pattern. These patterns showed reproducibility in all cases entering reproducibility test.

We further compared the two groups in terms of ABI and  $tcPO_2$ , which represent conventional indicators of ischemia previously shown to have sufficient predictive power for discriminating severe limb ischemia [20]. We found that ABI and especially  $tcPO_2$  were significantly lower in group P, implying that patients who show a plateauing pattern of PV fluctuation during the LTL test have a more severe ischemic condition than patients who show a decreasing pattern.

The Wound, Ischemia and foot Infection (WIFI) criteria [15] define ABI  $< 0.40$  as indicating severe ischemia. Our ROC curve analysis showed that a PV fluctuation slope of  $< 0.20$  PU/min has low sensitivity and specificity for discriminating ABI  $< 0.40$ . ABI has been generally used to screen for PAD and evaluate its severity. However, it is well known that the reliability of ABI may be limited in patients with medial arterial calcification [1,15]. A previous meta-analysis reported that the sensitivity and

**TABLE 1. Ischemic Parameters in Patients With Peripheral Arterial Disease, With Measurement Sites ( $n = 126$ ) Stratified Based on the Slope of the Perfusion Value Fluctuation**

	Plateau group			Decrease group			P-value
	Number	Median	25–75%	Number	Median	25–75%	
Slope	40	0.01	−0.04–0.07	86	0.41	0.31–0.56	–
$PV_{rest}$	40	7.85	4.82–9.91	86	7.50	5.65–9.08	0.95
ABI	40	0.35	0.11–0.61	70	0.66	0.52–0.84	$< 0.001$
$tcPO_2$	40	8	3–18	86	45	36–53	$< 0.001$

The slope (PU/min) indicates perfusion fluctuation after an initial, transient increase in perfusion value caused by local heating during a thermal load test, and was calculated based on data from laser speckle contrast imaging.  $PV_{rest}$  is the perfusion value at rest.  $tcPO_2$ , transcutaneous oxygen tension (mmHg); ABI, ankle-brachial pressure index.

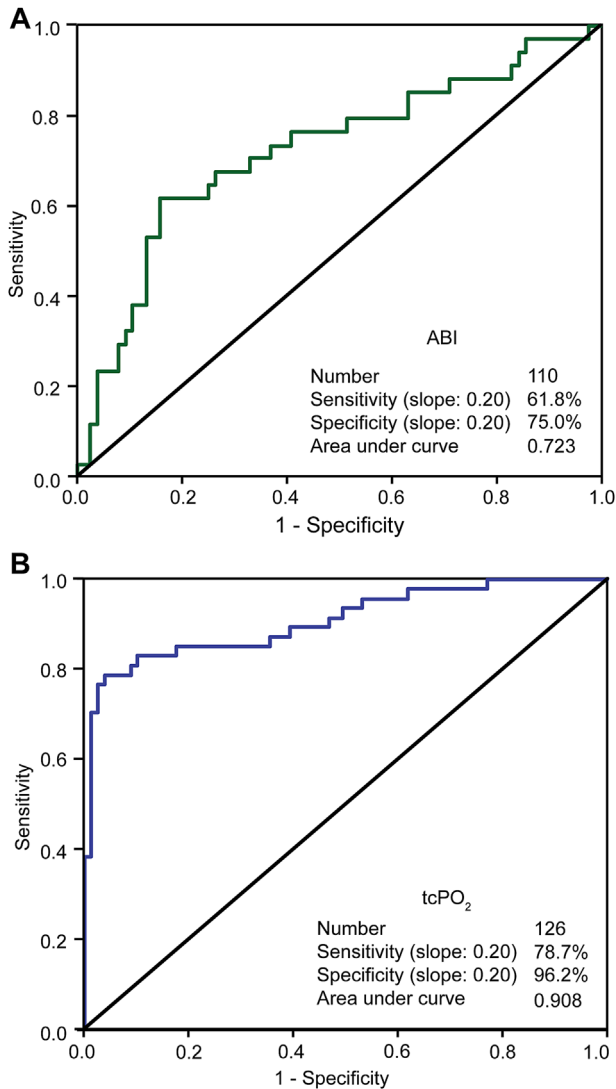


Fig. 5. Receiver operating characteristic (ROC) curve of the perfusion value (PV) fluctuation slope for discrimination of severe ischemia described in terms of ankle brachial index (ABI) and transcutaneous oxygen tension (tcPO<sub>2</sub>). (A) ROC curve of the PV slope for discriminating ABI <0.40. (B) ROC curve of the PV slope for discriminating tcPO<sub>2</sub> <30 mmHg.

specificity of ABI to predict limb amputation were 52% and 73%, respectively, and only 48% and 52%, respectively, to predict healing of the foot ulcer [20]. In our study population, 66% of patients had diabetes and 22% were on dialysis. As such patients are expected to have medial arterial calcification, it may explain why the sensitivity and specificity of the PV slope <0.20 PU/min were lower in terms of detecting ABI <0.40 than in terms of detecting tcPO<sub>2</sub> <30 mmHg. Therefore, the LTL test might be helpful for cases having ABI limitations due to medial arterial calcification.

Measurement of tcPO<sub>2</sub> is one of the most advantageous methods for diagnosing severe ischemia as well as diabetic foot, because it is not affected by arterial calcification [21].

**TABLE 2. The Discriminatory Ability of the Perfusion Value Fluctuation Slope, As Measured by ROC Curve Analysis With Several Cut-Off Values for tcPO<sub>2</sub>**

Cut-off value (mmHg)	Number of sites with tcPO <sub>2</sub> within the cut-off value	Sensitivity (%)	Specificity (%)	Area under the curve
10	23	100	83.5	0.950
20	34	88.2	88.2	0.919
30	40	78.7	96.2	0.908
40	69	56.5	98.2	0.845

The slope indicates perfusion fluctuation measured using laser speckle contrast imaging, after an initial, transient increase induced by local heating during a thermal load test. tcPO<sub>2</sub>, transcutaneous oxygen tension; ROC, receiver operating characteristic.

Some criteria including WIFI [15] and TASC II [1] define tcPO<sub>2</sub> <30 mmHg as indicating severe limb ischemia. The high AUC for discriminating tcPO<sub>2</sub> <30 mmHg suggests that foot PV with a slope of <0.20 PU/min, as in group P, might be equivalent to the conventional diagnostic method employed to evaluate the severity of limb ischemia.

Although there is no doubt regarding the predictive capability of tcPO<sub>2</sub> to diagnose severe limb ischemia, the optimal threshold value of tcPO<sub>2</sub> remains controversial [21–23]. Andersen [24] reported that tcPO<sub>2</sub> <20 mmHg indicates severe limb ischemia. In our study, a PV slope of <0.20 PU/min had good predictive capability for both tcPO<sub>2</sub> <20 mmHg and tcPO<sub>2</sub> <30 mmHg. Therefore, the LTL test may be able to clearly diagnose severe ischemia even in patients with a controversial level of tcPO<sub>2</sub> within the 20–30 mmHg range.

In the present study, the LSCI measurement was initiated just after discontinuing local probe-induced heating (44°C), and described the process of relative cooling-induced arteriolar vasodilation at room temperature (23–25°C). Unfortunately, the PV could not be evaluated prior to this moment because of the presence of the tcPO<sub>2</sub> probe used for heating and tcPO<sub>2</sub> measurements. Therefore, we described the prolonged vasoconstriction following the initial, transient vasodilation in terms of PV slope. Although the underlying mechanisms remain unknown, previous studies demonstrated that the skin vasoconstrictive response to local cooling represents the combined effect of an intact adrenergic nerve component as the vasoconstrictor, and nitric oxide synthase (NOS) as the predominant vasodilator [13,25]. One or more NOS isozymes are inhibited by local cooling [10]. However, chronic hypoxia amplifies neural NOS, which attenuates vascular smooth muscle contractility [26,27]. Therefore, we hypothesize that a similar phenomenon occurs in chronic severe hypoxia (as is the case for severe limb ischemia), resulting in a low tcPO<sub>2</sub>. Coats and Hillier reported that, in severe ischemic conditions, subcutaneous vessels were significantly impaired in terms of the sensitivity to noradrenaline [28]. As discussed above, we believe that arteriolar expandability by

neural NOS activation and decreased sensitivity to nor-adrenaline attenuated the prolonged vasoconstriction response to local skin cooling. However, further study is warranted to clarify the origin of this effect.

The present study has several limitations, the foremost being the small sample size. Nevertheless, our study demonstrated that LTL examination can provide a highly reliable and quantitative assessment of dorsal foot perfusion. Other limitations include the lack of an established procedural standard for evaluating the foot perfusion by LSCI during a thermal load test. Although these limitations should be addressed in future studies, the LTL test might be useful in the diagnosis of severe limb ischemia in terms of its non-invasiveness and accuracy, and it may be helpful in the diagnosis of cases difficult to diagnose by conventional tests, such as calcification on ABI or a  $tcPO_2$  in the controversial range of 20–30 mmHg.

## CONCLUSIONS

The LTL test can detect  $tcPO_2 < 30$  mmHg with high specificity. While  $tcPO_2$  measurements were the most accurate in diagnosing the severity of limb ischemia, the LTL test is also expected to serve as a useful non-invasive method for diagnosing severe limb ischemia.

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