

ORIGINAL RESEARCH

Assessment of the Severity of Ischaemia and the Outcomes of Revascularisation in Peripheral Arterial Disease Patients Based on the Skin Microcirculatory Response to a Thermal Load Test

Yohei Yamamoto ^{a,*}, Yoshinori Inoue ^b, Kimihiro Igari ^a, Takahiro Toyofuku ^a, Toshifumi Kudo ^a, Hiroyuki Uetake ^a

^a Department of Surgical Specialties, Tokyo Medical and Dental University, Tokyo, Japan

^b Ambulatory Vascular Surgical Clinic Tokyo, Tokyo, Japan

Objective: This study investigated the skin microcirculatory response to a thermal load test using a laser Doppler flowmetry device to evaluate the severity of limb ischaemia and the outcomes of revascularisation in patients with peripheral arterial disease (PAD).

Methods: A total of 34 PAD patients (39 limbs) including 17 critical limb ischaemia (CLI) patients (21 limbs) who underwent revascularisation were enrolled. The skin microcirculation of the dorsal side of the affected foot was investigated for 15 minutes after local heating. The tests were performed both before and after revascularisation, and several parameters gleaned from the microcirculatory fluctuations were analysed and compared with the ankle brachial pressure index and the transcutaneous oxygen tension (tcPO₂) values.

Results: Among the parameters, significant differences were observed between the CLI patients and patients with claudication with regard to the increasing phase time (T_{inc}), the difference in the perfusion values at the onset and the peak of the transient increase in blood perfusion (PΔ), the slope of the transient increase in blood perfusion (S_{in}), and the slope of the decrease in blood perfusion after the peak (S_{de}). In CLI patients, the PΔ, S_{in}, and S_{de} values increased significantly after revascularisation. In the patients with claudication, the changes in the parameters after revascularisation were not statistically significant. The S_{de} showed the most statistically significant correlation with the tcPO₂ value ($\rho = .759, p < .001$).

Conclusions: Thermal load testing can be used to evaluate the severity of limb ischaemia in patients with PAD.

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OBJECTIVES

Appropriate assessment of the severity of limb ischaemia and the effectiveness of revascularisation is necessary for management of patients with peripheral arterial disease (PAD).¹

Several studies have shown that the pathophysiology of PAD, especially the subgroup of patients who develop critical limb ischaemia (CLI) affects multiple microvascular functions through a complex process.² Thus, a new method of assessing the microvascular function may help augment the information provided by standard tests. Previous studies have shown the usefulness of microcirculatory evaluations during the return to baseline temperature after

local heating in the diagnosis of severe limb ischaemia.^{3,4} To the present authors' knowledge, however, only one method based on laser speckle contrast imaging has been reported, and the correlation between blood flow parameters and the existing method has not been clarified.³

The present study investigated skin microcirculatory responses to a thermal load test using an experimental setup composed of a heating probe and laser Doppler flowmetry (LDF) before and after revascularisation in patients with various degrees of PAD, including patients with clinical signs of CLI. To evaluate the feasibility of the test, several parameters were compared that were gleaned from the microcirculatory fluctuations with transcutaneous oxygen tension (tcPO₂), a conventional clinical parameter.

MATERIALS AND METHODS

From November 2016 to April 2017, 58 PAD patients, including 27 with clinical signs of CLI required hospitalisation. They all underwent skin blood flow measurement of the dorsal foot after local heating [local heating test (LHT)]. All patients who were clinically diagnosed with CLI had at

* Corresponding author. Department of Surgical Specialties, Tokyo Medical and Dental University, 1–5-45 Yushima, Bunkyo-ku, Tokyo, 113–8519, Japan.

E-mail address: y-yamamoto.srg1@tmd.ac.jp (Yohei Yamamoto).

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least one $tcPO_2$ value (measured in the foot) of <30 mmHg and/or an absolute ankle pressure of <50 mmHg. Patients with collagen vascular disease, inflammatory disease, Raynaud's phenomenon, an axillary temperature of $>37.0^\circ\text{C}$ and patients undergoing conservative treatment were excluded. The LHT was performed before and after revascularisation in a total of 39 limbs in 34 PAD patients, including 21 limbs in 17 CLI patients (Fig. 1).

This study was approved by the ethics committee of Tokyo Medical and Dental University Hospital. Written informed consent was obtained from all patients.

The LHTs were performed in the morning (7:00–8:00 a.m.) in a 20 – 23°C room. Patients were not allowed to smoke or consume caffeine for at least 24 hours before each test. Prior to testing, patients rested in the supine position for 15 minutes. Local heating was performed using an electric moxibustion instrument (Shouki E09–04, Zen Iryoki Co., Ltd., Fukuoka, Japan). The skin heat probe was set to 48°C . After 15 minutes of local heating, the heating probe was removed, and the skin blood flow in the heated area was immediately measured by LDF (Omegaflo-lab, Omegawave Inc., Tokyo, Japan). The LDF values were recorded for 15 minutes (Fig. 2A). Pre-intervention measurements were performed on the morning of the day before revascularisation, and post-intervention measurements were performed within 14 days of revascularisation.

The ankle brachial pressure index (ABI) was measured before and after revascularisation using an ultrasound Doppler technique. In patients with CLI, the $tcPO_2$ values were also measured using a TINA TCM400 (Radiometer Medical, Copenhagen, Denmark) device according to the manufacturer's instructions. One of the $tcPO_2$

measurements on the dorsal side of the foot was taken at the same site as the LDF measurements.

LDF data analyses

The initial response during the return to baseline temperature after local heating was a decrease in blood perfusion. However, within the first 1–2 minutes, the perfusion values began to increase. After 2–5 minutes of increasing, the perfusion value reached the next inflection point that marked the peak, which was followed by another decrease (Fig. 2B). To assess the LHT, the following five parameters were analysed: increasing phase time (T_{inc}), perfusion value change ($P\Delta$), ratio of the perfusion value at the peak of the increasing phase (PV_p) to the perfusion value at the onset of the increasing phase (PV_o), increasing slope (S_{in}), and decreasing slope (S_{de}). The slope was obtained as the linear regression of the processed perfusion values. These parameters can be seen graphically in Fig. 2B.

Statistical analyses

Continuous variables are expressed as median and 25th and 75th percentiles. The Mann–Whitney U test was used to compare differences between the pre- and post-intervention data. Spearman's correlation was used to examine the relationship between the two values. All statistical analyses were performed using BellCurve for Excel (Social Survey Research Information Co., Ltd. Tokyo, Japan).

RESULTS

A total of 39 limbs in 34 PAD patients (male, $n = 29$; female, $n = 5$; median age, 72 years; range 66.5–79 years) were analysed. The patient characteristics are shown in Table 1.

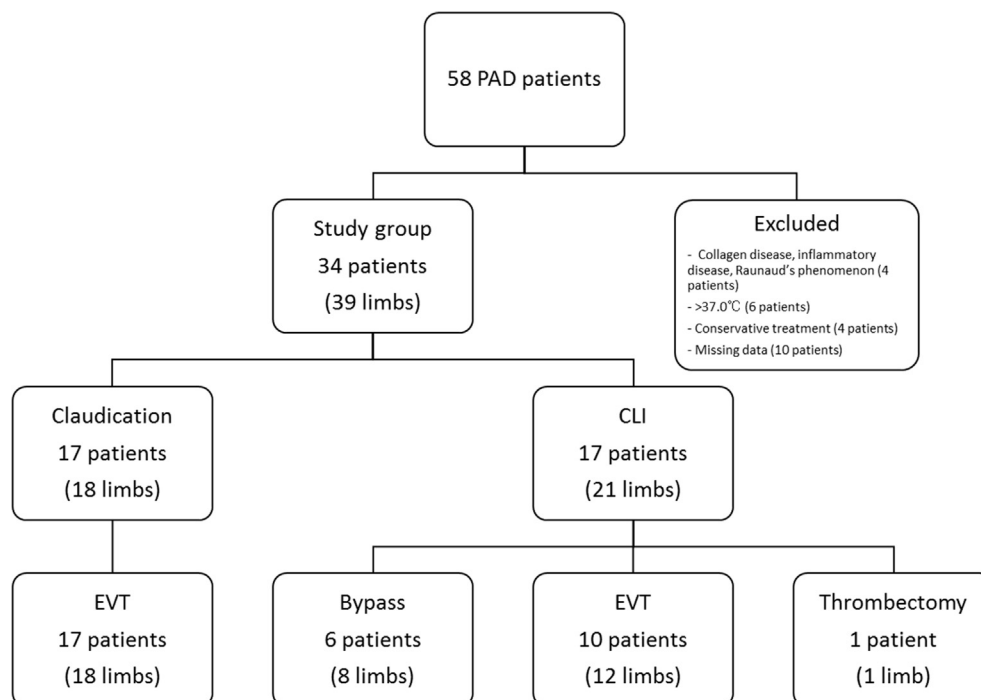


Figure 1. Flow chart of the study cohort. PAD = peripheral arterial disease; CLI = critical limb ischaemia; EVT = endovascular treatment.

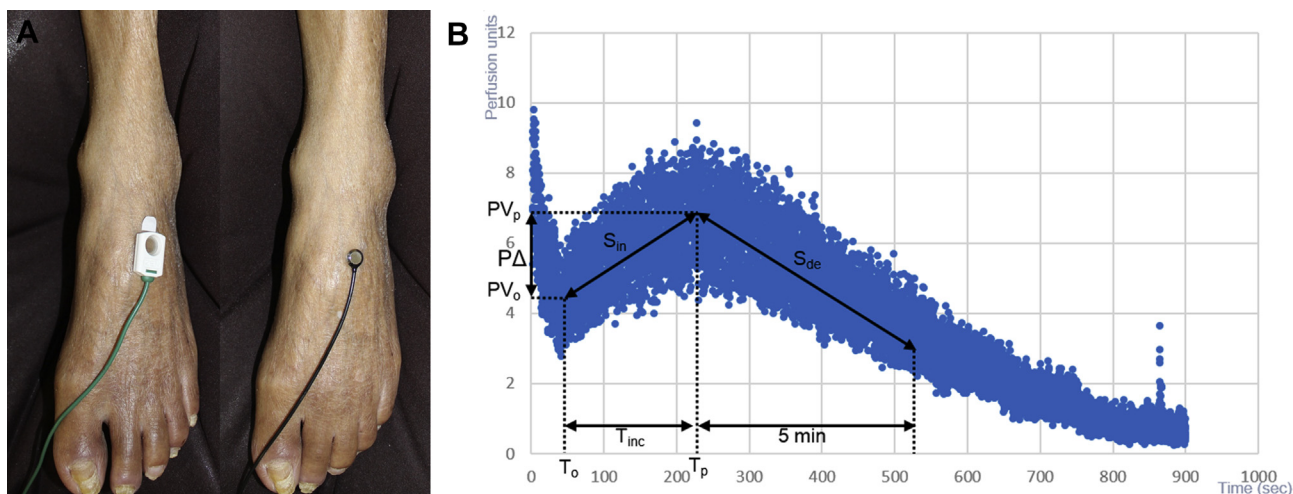


Figure 2. (A) Photographs of the local heating test (LHT). Local heating was achieved with an electric moxibustion instrument (left). After 15 min of local heating, the heating probe was removed, and the measurement of skin blood perfusion in the heated area was immediately initiated using a laser Doppler flowmetry (right). (B) Typical blood flow fluctuations during the LHT. The LHT parameters were defined graphically. T_{inc} = time from the onset of the increasing phase (T_o) to the inflection point that marked the peak perfusion value (T_p), also known as the increasing phase time. PV_o = perfusion value at the onset of the increasing phase. PV_p = perfusion value at the peak of the increasing phase. $P\Delta = PV_p - PV_o$. S_{inc} = the slope of the perfusion value distribution in the increasing phase. S_{de} = the slope of the perfusion value distribution in the first 5 min after the peak.

Distribution of disease severity in the limbs with PAD, stratified according to the Rutherford classification, was as follows: category 1 ($n = 2$), category 2 ($n = 13$), category 3 ($n = 3$), category 4 ($n = 9$), category 5 ($n = 11$), and category 6 ($n = 1$). Bypass operations were performed for six CLI patients (eight limbs). Six bypass operations were performed for CLI patients: two aortobifemoral bypass operations for long aorto-iliac occlusion (four limbs) and four bypasses to the arteries below the popliteal trifurcation. Twelve revascularisation procedures for CLI patients were endovascular. The target lesions of the endovascular procedures were femoropopliteal lesions in six limbs and infrapopliteal tibioperoneal lesions in six limbs. Femoropopliteal artery thrombectomy was performed in one case. All of the revascularisation procedures performed in the treatment of patients with claudication were endovascular procedures. The target lesions were iliac lesions in five limbs, femoropopliteal lesions in 12 limbs, and both iliac and femoropopliteal lesions in one limb.

The pre-operative ABI could not be measured in 12 of 21 limbs with CLI because of incompressible arteries in seven

Table 1. Patient characteristics and risk factors of the study population.

	Overall	Critical limb ischaemia (CLI)	Claudication
n (limbs)	34 (39)	17 (21)	17 (18)
Male (female)	29 (5)	13 (4)	16 (1)
Age	72 (66.5–79)	78 (69–81)	71 (65–75)
Hypertension (%)	29 (85%)	13 (74%)	16 (94%)
Diabetes (%)	18 (53%)	11 (64%)	7 (41%)
Haemodialysis (%)	10 (29%)	7 (41%)	3 (18%)
Current smoker (%)	8 (23%)	4 (23%)	4 (23%)

CLI = critical limb ischaemia.

limbs, an undetectable or weak Doppler signal in four limbs, and the presence of an ankle wound in one limb. The pre-interventional median ABI was 0.44 (0.32–0.48) in the nine patients with CLI in whom ABI could be determined and 0.71 (0.58–0.85) in patients with claudication. The pre-interventional LHT parameters of the patients with CLI and those with claudication are shown in Table 2. Significant differences were observed in the T_{inc} , $P\Delta$, S_{inc} , and S_{de} values of the two groups. The pre- and post-intervention ABI values, the number of patent runoff vessels, $tcPO_2$ values, and LHT parameters are shown in Tables 3 and 4. In the patients with CLI, the $P\Delta$, S_{inc} , and S_{de} significantly increased after revascularisation. In patients with claudication, the ABI significantly increased after revascularisation; however, the LHT parameters measured before and after revascularisation did not differ significantly.

The correlation between the $tcPO_2$ values and LHT parameters is shown in Table 5. S_{de} showed the most statistically significant correlation with the $tcPO_2$ value ($\rho = .759$, $p < .001$). Table 6 shows the number of patients that clinically benefited from each of the investigations for assessment of revascularisation.

DISCUSSION

This study investigated fluctuations in the skin blood flow of PAD patients during the return to baseline temperature after local heating using an LDF device. The fluctuations observed are presumed to be a form of cold induced vasodilation (CIVD)⁵ induced by relative cooling after local heating. CIVD is a paradoxical increase in skin blood flow following initial vasoconstriction during local cold exposure that has been suggested to have a cryoprotective function.

Preliminary results showed that the increase in skin blood flow was attenuated in patients with clinically diagnosed CLI

Table 2. Pre-interventional local heating test (LHT) parameters.

	Critical limb ischaemia (CLI)	Claudication	<i>p</i>
Increasing phase time (T_{inc}), s	181 (153–255)	155 (143–168)	.04
Perfusion value change ($P\Delta$), perfusion units, PU	1.5 (0.9–2.7)	3.40 (2.50–4.95)	.003
Perfusion value at the peak of the increasing phase (PV_p)/Perfusion value at the onset of the increasing phase (PV_o)	1.81 (1.26–1.93)	1.85 (1.57–2.46)	.06
Increasing slope (S_{in}), PU/min	0.35 (0.17–0.7)	1.07 (0.43–1.66)	.001
Decreasing slope (S_{de}), PU/min	0.22 (0.01–0.41)	0.43 (0.33–0.81)	.006

CLI = critical limb ischaemia; LHT = local heating test; $P\Delta$ = perfusion value change; PU = perfusion units; PV_o = perfusion value at the onset of the increasing phase; PV_p = perfusion value at the peak of the increasing phase; S_{de} = decreasing slope; S_{in} = increasing slope; T_{inc} = increasing phase time.

Table 3. Ankle brachial pressure index (ABI) values, number of patent runoff vessels, transcutaneous oxygen tension ($tcPO_2$) values, and local heating test (LHT) parameters pre- and post-intervention in critical limb ischaemia (CLI) patients.

	Pre-intervention	Post-intervention	<i>p</i>
ABI	0.44 (0.32–0.48)	0.93 (0.83–1)	<.001
Number of patent runoff vessels (0/1/2/3)	7/10/1/3	0/11/6/4	.010
$tcPO_2$, mmHg	13 (6–25)	37 (30–48)	<.001
Increasing phase time (T_{inc}), s	181 (153–255)	194 (160–232)	.950
Perfusion value change ($P\Delta$), perfusion units (PU)	1.5 (0.9–2.7)	3.8 (2.7–5.0)	<.001
Perfusion value at the peak of the increasing phase (PV_p)/Perfusion value at the onset of the increasing phase (PV_o)	1.81 (1.26–1.93)	1.48 (1.29–1.72)	.529
Increasing slope (S_{in}), PU/min	0.35 (0.17–0.7)	1.03 (0.4–1.57)	.014
Decreasing slope (S_{de}), PU/min	0.22 (0.01–0.41)	0.64 (0.31–0.91)	.001

ABI = ankle brachial pressure index; CLI = critical limb ischaemia; LHT = local heating test; $P\Delta$ = perfusion value change; PU = perfusion units; PV_o = perfusion value at the onset of the increasing phase; PV_p = perfusion value at the peak of the increasing phase; S_{de} = decreasing slope; S_{in} = increasing slope; $tcPO_2$ = transcutaneous oxygen tension; T_{inc} = increasing phase time.

Table 4. Ankle brachial pressure index (ABI) values, number of patent runoff vessels, and local heating test (LHT) parameters pre- and post-intervention in patients with claudication.

	Pre-intervention	Post-intervention	<i>p</i>
ABI	0.71 (0.58–0.85)	0.95 (0.87–1.03)	<.001
Number of patent runoff vessels (0/1/2/3)	0/4/8/6	0/4/8/6	1.000
Increasing phase time (T_{inc}), s	155 (143–168)	155 (141–179)	.874
Perfusion value change ($P\Delta$), perfusion units (PU)	3.40 (2.50–4.95)	3.45 (2.28–4.55)	.924
Perfusion value at the peak of the increasing phase (PV_p)/Perfusion value at the onset of the increasing phase (PV_o)	1.85 (1.57–2.46)	1.73 (1.51–2.69)	.924
Increasing slope (S_{in}), PU/min	1.07 (0.43–1.66)	1.21 (0.86–1.67)	.601
Decreasing slope (S_{de}), PU/min	0.43 (0.33–0.81)	0.58 (0.42–0.78)	.517

ABI = ankle brachial pressure index; LHT = local heating test; $P\Delta$ = perfusion value change; PU = perfusion units; PV_o = perfusion value at the onset of the increasing phase; PV_p = perfusion value at the peak of the increasing phase; S_{de} = decreasing slope; S_{in} = increasing slope; T_{inc} = increasing phase time.

compared with those with claudication and that the attenuated microcirculatory response in patients with CLI improved after revascularisation.

To know where to look and quantitatively assess the LHT, several parameters were defined. Analysis of the results showed that the severity of ischaemia was represented by parameters related to both a transient increase and the degree of decrease in blood flow after the peak. In the present study, S_{de} was found to have the most statistically significant correlation with $tcPO_2$, indicating that the secondary vasoconstriction observed several minutes after local heating is attenuated and thereby reflects the severity of limb ischaemia. Although the data were corrected using a separate device, the diagnostic value of this parameter in predicting severe ischaemia has been reported in previous

studies.^{3,4} These results may support the applicability of the thermal load test in assessing the severity of limb ischaemia and skin microcirculatory recovery in CLI patients.

At the same time, the skin microcirculatory reactivity to the thermal load test was mostly maintained and remained unchanged following revascularisation in the diseased limbs of patients with claudication. One possible explanation for this observation is that most patients with claudication in this study had mild to moderate and stable disease; however, the thermal load test does not appear to be suitable for quantitatively assessing PAD patients with mild ischaemia.

Several potential mechanisms may underlie the changes in the blood flow fluctuations observed in this study. For example, arterioles in CLI patients are reported to be almost

Table 5. Correlation between the parameters of the local heating test and the transcutaneous oxygen tension (tcPO₂) values in critical limb ischaemia (CLI) patients.

	ρ (Spearman)	p
Increasing phase time (T _{inc})	-.362	.019
Perfusion value change (P Δ)	.628	<.001
Perfusion value at the peak of the increasing phase (PV _p)/Perfusion value at the onset of the increasing phase (PV _o)	-.288	.065
Increasing slope (S _{in})	.561	<.001
Decreasing slope (S _{de})	.759	<.001

CLI = critical limb ischaemia; P Δ = perfusion value change; PU = perfusion units; PV_o = perfusion value at the onset of the increasing phase; PV_p = perfusion value at the peak of the increasing phase; S_{de} = decreasing slope; S_{in} = increasing slope; tcPO₂ = transcutaneous oxygen tension; T_{inc} = increasing phase time.

Table 6. Number of patients who benefited clinically from each of the investigations for assessment of revascularisation.

	Ankle brachial pressure index (ABI)	Transcutaneous oxygen tension (tcPO ₂)	Local heating test (LHT)
Claudication (n = 17)	15	—	7
Critical limb ischaemia (CLI) (n = 17)	7	15	15

ABI = ankle brachial pressure index; CLI = critical limb ischaemia; LHT = local heating test; tcPO₂ = transcutaneous oxygen tension.

maximally dilated and relatively insensitive to vasodilator stimuli.⁶ Impaired blood flow enhancement in patients with low tcPO₂ might mirror a low vasodilatory capacity secondary to a vasodilatory status.

In this study, the ABI could not be determined in 57% of the patients with CLI. Thus, thermal load testing may be valuable for assessing the severity of limb ischaemia in CLI patients with unreliable ABI results. Because of differences in the measuring principles used, the present method may be less markedly affected by tissue conditions, such as oedema, which leads to falsely low tcPO₂ values. In this series, the LHT appeared to be a more useful and effective modality than tcPO₂ in patients with CLI with post-operative limb oedema.

The present objective was not to exhaustively test the device; the aim was to assess the feasibility of thermal load testing. To establish the testing technique, it will be necessary to use a generally available heating technique. For example, an experimental setup with an infrared lamp

and a laser Doppler imager⁷ can be adopted as a non-contact method. The results of the present study do, however, provide a basis for further discussion.

CONCLUSIONS

The present study demonstrated the feasibility of an LDF based setup for assessing the skin blood flow via a thermal load test. Thermal load testing can be used to evaluate the severity of ischaemia in patients with PAD but needs further clinical research to confirm its value.

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CONFLICT OF INTEREST

None.

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