

Micro-lightguide spectrophotometry for tissue perfusion in ischemic limbs

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Objective: To validate micro-lightguide spectrophotometry (O2C) in patients with lower limb ischemia and to compare results with those obtained from toe blood pressure.

Methods: We prospectively examined 59 patients, 24 of whom complained of claudication, 31 had critical ischemia, and four were asymptomatic. Diabetes was present in 19 (32%) patients. Saturation (SO₂) and flow measured with O2C were determined with the limb in the horizontal position followed by a 55-cm elevation. Toe pressures were determined in the horizontal position only. In addition, 13 patients were examined before and, on average, 3 days after revascularization. **Results:** Median SO₂ was 62% (25%-75% percentile: 37%-75%) with the limb in the horizontal position and 16% (3%-41%) with the limb elevated. Comparing the individual toe pressures with SO₂ values measured in the horizontal position and elevated position revealed a significant correlation ($r_s = 0.40$; $P < .01$ and $r_s = 0.56$; $P < .01$, respectively). A low SO₂ (ie, <40% in the horizontal position and <20% in the elevated position) was highly predictive of a toe pressure of 40 mm Hg or less. In the horizontal position, the positive predictive value was 100%, whereas the negative predictive value was 47%. The similar figures in the elevated position were a positive predictive value of 97% and a negative predictive value of 68%. Postoperatively, SO₂ increased significantly from 27% (P25%-75%: 11%-75%) to 79% (68%-87%) in the horizontal position ($P = .008$) and from 14% (P25%-75%: 2%-39%) to 55% (30%-73%) in the elevated position ($P = .011$), respectively. Looking at the individual 13 cases in which revascularization was performed, three patients had a partial reconstruction (ie, superficial femoral artery occlusion distal to a central reconstruction or reconstruction to a popliteal blind segment). These patients had significantly lower postoperative SO₂ as well as toe pressure compared with the 10 patients with unobstructed flow to the foot.

Conclusions: O2C was easy to use, fast, and painless. The most useful finding was the high predictive value of a low saturation and the rise in O2C values after successful revascularization. (*J Vasc Surg* 2012;56:746-52.)

Ankle or toe pressures are generally accepted for diagnostic purposes as well as for an indirect measure of severity of peripheral arterial disease (PAD).¹ However, this is not the case in all patients (ie, some exhibit only mild symptoms at low pressures, whereas others have pronounced symptoms in spite of relatively high pressures). Also for the prediction of wound healing the pressure measurements are of limited value.^{1,2} In addition, incompressible leg arteries make ankle pressures unreliable in some patients, mainly diabetics. Even so, pressure measurements are the dominating objective parameter for assessing lower limb perfusion in vascular surgery.^{1,3}

A new technique has been developed for noninvasive assessment of tissue oxygenation and microcirculation: the micro-lightguide spectrophotometer (“Oxygen-to-see”

[ie, O2C]). This method allows determination of oxygen saturation (SO₂) and relative blood flow and hemoglobin amount (rHb) in tissue by use of a small probe. The probe is attached directly to the skin under observation.⁴ Investigations have been made using micro-lightguide spectrophotometer measurements in various organs.⁵⁻¹³ The method’s ability to predict ulcer healing in patients with diabetes has been studied,^{14,15} as well as the method’s ability to predict healing after amputation.¹⁶ Consecutive measurements in healthy volunteers have provided reproducible data.¹⁴ These results suggest that O2C can assess the microcirculation. Combined with its simplicity and speed, the technique may prove useful in a busy vascular clinic.

However, O2C has neither been validated systematically in patients with lower extremity arterial insufficiency, nor has it been compared face-to-face with distal pressure measurements. Accordingly, we set up this study using the O2C technique to examine a group of patients representing different levels of arterial insufficiency and to compare the results with those obtained with peripheral blood pressures. Additionally, a subgroup was re-examined after revascularization.

METHODS

Subjects. This study was conducted at the department of Vascular Surgery, Rigshospitalet, Denmark. Subjects were ambulatory and hospitalized patients referred to the department on suspicion of PAD. Subjects were excluded from the study if they had acute limb ischemia. One person

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Table I. Characteristics of the patients

	Total	Non-PAD	Rutherford 1-3	Rutherford 4	Rutherford 5-6
Number of patients	59	4	24	11	20
Age median, years (range)	66 (45-88)	62 (49-68)	63 (45-83)	71 (54-88)	70 (54-88)
Men:women	36:23	2:2	12:12	5:6	17:3
Diabetes	19	0	6	2	11
Smoker: yes/former/never	27/30/2	1/3/0	15/8/1	5/5/1	6/14/0
Edema: none/moderate/heavy	44/9/6	4/0/0	22/2/0	10/1/0	8/6/6
BMI >30	11	0	7	0	4
Pulse oximetry (\leq SaO ₂ 95%)	20	2	9	3	6

BMI, Body mass index; PAD, peripheral arterial disease; SaO₂, systemic oxygen saturation attained using a pulse oximeter. Data are shown for the total study group and grouped according to Rutherford classification.

only (L.P.) handled all examinations during an 8-month research fellowship in 2009. Of about 250 patients referred for assessment, the number of patients to be included was thus restrained to one or two each day. The selection of patients included in the study was random, aiming at including patients with claudication and patients with critical limb ischemia by turns. In addition, logistical problems and competitive investigations limited the availability of patients. Informed consent was obtained from the included subjects. The local ethics committee approved the study (Protocol number HB-2008-147).

All participants had bilateral measurements; however, data were included only for the most symptomatic limb, as paired data would violate the independence assumption of the statistical analysis.¹⁷ Moreover, symptoms in the most symptomatic limb could mask symptoms from the least symptomatic limb.

During the last half of the study period, a subset of patients who underwent revascularization was re-examined postoperatively.

Clinical assessment included vascular history and clinical examination to categorize patients according to the Rutherford (R) classification.¹⁸ In addition, it assessed potential risk factors (eg, smoking, diabetes, body mass index [BMI], and presence of lower limb edema; Table I).

Procedures. All measurements were performed at room temperature after the patient had rested for 15 minutes and was positioned horizontally. The patients' systolic and diastolic arm blood pressures were determined on both arms. The highest values were used. The systemic oxygen saturation was attained using a pulse oximeter. The sensor was positioned on the index finger.

O2C. The O2C (LEA Medizintechnik, Giessen, Germany; Fig 1, *a*) sends continuous white light (20 W, 500-800 nm, 1-nm resolution) and laser light (830 nm and <30 mW) into the tissue where it is scattered. Light is emitted through a flat probe, measuring 2 mm in depth, fastened to the skin using double adhesive tape. The small probe collects the scattered light, splits it into its spectral components, and converts it into an electrical signal. The white light is used to determine SO₂ by means of the changing color of the reflected light caused by a wavelength-dependent absorption. The laser light is

used similarly for calculating the relative blood flow (flow expressed as arbitrary units [AU]). The technique behind using O2C for measuring blood flow and oxygenation has previously been described in detail.^{4,14}

In this trial, we fixed the O2C probe to the plantar surface of the first toe (Fig 1, *b*). First, SO₂ and flow were determined with the limb in horizontal position. All measurements were performed in double, and the average value calculated. Thereafter, measurements were repeated following a 55-cm elevation of the foot using a hard pillow (Fig 1, *c*). Patients who underwent revascularization were re-examined the same way postoperatively (circa 3 days later). Each measurement took about 2 minutes, and the whole sequence of four determinations—two in the horizontal position and two in the elevated position—took <10 minutes.

Peripheral blood pressures. Peripheral blood pressures were measured at the ankle and toe level using appropriately sized cuffs. Ankle blood pressures were determined using the Doppler technique, and toe blood pressures (TBP) were obtained with strain gauge technique¹⁹ (Medimatic, Copenhagen, Denmark) in the first or second toe. Pressures were measured twice; the mean value of the highest pressure was used.

Revascularization. During the last 2½ months of the study period, a group of patients were examined before and after revascularization. At the re-examination, all reconstructions were patent when assessed clinically. The reconstructions were confirmed by duplex ultrasound in case of doubt.

Statistics. Nonparametric statistics were used for the analysis, and results were expressed in median and percentiles (25 and 75 percentile) or range. Reliability based on duplicate measurements was also expressed in terms of the coefficient of variation, calculated from standard deviation. Paired observations were analyzed with Wilcoxon signed rank test; groups were compared with Mann-Whitney *U* test and Kruskal-Wallis test. Correlations were assessed with Spearman's rank correlation. *P* values less than .05 were considered significant. All calculations were performed with IBM SPSS statistics versions 18 and 20 (Armonk, NY).



Fig 1. a, The O2C apparatus consists of a computer using the Windows system and a small probe. On the screen, readings of SO_2 , rHb, and flow are shown as curves during the examination. This enables the examiner to register any fluctuations. Readings can be made as a continuous reading or over a certain time span and the apparatus calculates a mean value. b, The O2C probe attached to the plantar surface of the first toe using double adhesive tape. c, The limb was elevated 55 cm using a hard pillow. O2C, Micro-lightguide spectrophotometry; rHb, relative blood flow and hemoglobin amount; SO_2 , saturation.

RESULTS

In total, 75 patients were asked to participate. Four declined due to lack of time or because they felt the additional examinations would be too strenuous. Of the 71 patients included, four patients turned out not to have PAD but were included in the analysis as a non-PAD group. It was not possible to measure TBP in three limbs due to severe pain and/or ulcerations. Additionally, TBP was unavailable in nine limbs because of time limitations or logistical problems.

Because a group of the patients suffered from diabetes, we decided to use only patients in which toe pressure measurement had been performed (59 patients).

Potential risk factors (eg, smoking, diabetes, BMI, and presence of lower limb edema) are listed in Table I.

O2C saturation and flow. Table II summarizes the saturation and flow obtained with O2C categorized according to the clinical degree of limb ischemia (ie, the Rutherford classification). Overall, the median SO_2 was 62% (P25%-75%: 37%-75%) with the limb in the horizontal position. Replicate measurements revealed a median difference of 0% (-2%-6%), and in terms of coefficient of variance, 15%. With the limb elevated, SO_2 was in median 16% (3%-41%). Repeated measurements revealed a median difference of 0% (-5%-2%). Pairwise comparison of horizontal versus elevated readings revealed a decrease in median values of 26% (9%-49%) comparable to a relative decrease of 58% ($P < .001$).

When comparing SO_2 values between the three Rutherford categories R1-3, R4, and R5-6, no significant difference could be shown in the horizontal position. On the other hand, patients with claudication (R1-3) had significantly higher values in the elevated position compared with R4 ($P < .006$) but not when compared with R5-6 patients ($P = .24$). R4 patients also had significantly higher SO_2 in elevated position compared with R5-6 patients ($P < .04$). Non-PAD subjects turned out to have significantly higher SO_2 values compared with PAD patients in both positions (horizontal: $P < .05$; elevated: $P < .01$).

Fig 2 illustrates the individual SO_2 data measured with the limb in the horizontal position and the corresponding reading with the limb elevated 55 cm. Although the correlation was significant ($r_s = 0.57$; $P < .01$), the individual results varied substantially. The drop in SO_2 on elevation was of the same extent in the three Rutherford categories ($P = .65$). In addition, patients who had diabetes, marked in Fig 2, could not be distinguished from nondiabetics in regard to saturation. This was true for both the horizontal and the elevated positions ($P = .54$ and $.93$, respectively).

The O2C technique also provides a relative flow measure. As indicated in Table II, flow behaved similarly to SO_2 on elevation of the limb. When comparing flow in the three Rutherford categories, no significant difference could be shown in any of the positions. Non-PAD patients, however, had significantly higher values in the elevated position ($P < .02$). The flow in the horizontal and elevated positions was 30 (P25%-75%: 9-85) AU and 2 (0-7) AU, respectively.

Table II. Results of the three noninvasive methods

	Non peripheral arterial disease		Rutherford 1-3		Rutherford 4		Rutherford 5-6	
	n = 4 90 (35-120)		n = 24 55 (18-110)		n = 11 25 (8-80)		n = 20 25 (0-80) ^a	
TBP [mm Hg]	Horizontal	Elevated	Horizontal	Elevated	Horizontal	Elevated	Horizontal	Elevated
SO ₂ (%)	82 (69-85)	77 (59-84)	62 (6-93)	30 (0-83)	54 (0-80)	2 (0-32)	59 (4-83)	10 (0-74)
Flow (AU)	86 (74-141)	54 (46-58)	29 (2-200)	2 (0-113)	13 (0-210)	0 (0-13)	28 (0-176)	1 (0-78)

AU, Arbitrary units; n, number of limbs; O2C, micro-lightguide spectrophotometry; SO₂, saturation; TBP, toe blood pressure.

The unit of O2C-SO₂ is %, and the unit of flow is AU. These values were obtained in horizontal and in elevated limb positions. The unit of TBP is mm Hg. Ranges are shown in parenthesis. Values are shown in whole numbers. Data are grouped according to Rutherford classification.

^aA toe pressure of 0 mm Hg must be interpreted as a pressure below the detectable limb. Only one patient in Rutherford 5 to 6 had a toe pressure of 80 mm Hg.

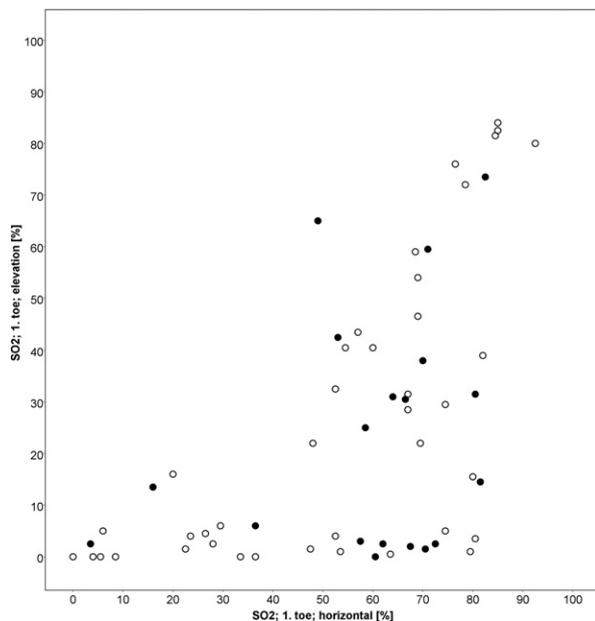


Fig 2. O2C SO₂ measured with the limb in horizontal position compared with the value obtained when the limb was elevated. The unit of SO₂ is %. Data are grouped according to the presence of diabetes. *White circles* indicate the patients without diabetes; *black circles* indicate the patients suffering from diabetes. O2C, Micro-lightguide spectrophotometry; SO₂, saturation. SO₂; 1. toe; elevation, SO₂ value measured with the limb elevated at the plantar surface of the first toe. SO₂; 1. toe; horizontal %, SO₂ value measured with the limb in horizontal position at the plantar surface of the first toe.

Peripheral blood pressures. The median TBP was 35 mm Hg (P25%-75%: 23-60 mm Hg). Table II shows the distribution according to the clinical classification. Pressures were significantly higher in claudicants (R1-3) compared with the group with critical ischemia (R4 and R5-6; $P < .001$). Non-PAD subjects also had significantly higher TBP than R4 and R5-6 patients ($P < .007$).

Comparing O2C with TBP. In Fig 3, we plotted the individual toe pressures against SO₂ values measured in the horizontal position (Fig 3, a) and on elevation (Fig 3, b). A

significant correlation was found in both ($r_s = 0.40$; $P < .01$ and $r_s = 0.56$; $P < .01$, respectively). More interesting than a correlation was the finding that low SO₂ (ie, <40% in the horizontal position and <20% in the elevated position) was predictive of a toe pressure of 40 mm Hg or less. In the horizontal position, the positive predictive value (PPV) was 100% (16/16), where the negative predictive value (NPV) was only 47% (20/43). The similar figures in the elevated position were a PPV of 97% (30/31) and an NPV of 68% (19/28).

We have not depicted flow data but, similar to SO₂, flow values were significantly correlated to TBP horizontally ($r_s = 0.30$; $P < .05$) as well as in the elevated position ($r_s = 0.50$; $P < .01$).

Patient subgroups. The distribution of diabetes was uneven within clinical classes (Table I). Consequently, we looked for systematic differences within the three classes in O2C, but found none. In addition, we checked for any influence of systemic SO₂ below 95%, from smoking, having limb edema, or being obese (BMI >30 kg/m²). There was no evident systemic effect on the O2C measurements.

Comparing pre- and postoperative measurements. Three (range, 2-5) days postoperatively, 13 patients were re-examined.

Central, aorto-iliac revascularization was performed on eight patients. Six of these patients had angiographically uninterrupted infrainguinal run-off. In addition, two had an untreated superficial femoral artery occlusion (Table III). The remaining five patients had peripheral, infrainguinal reconstructions. In four, the outflow to the foot was uninterrupted. The last patient had reconstruction to a popliteal blind segment (Table III).

In the 13 patients, SO₂ increased significantly from median 27% (P25%-75%: 11%-75%) to 79% (68%-87%) in the horizontal position ($P = .008$) and from 14% (2%-39%) to 55% (30%-73%) in the elevated position ($P = .011$). The same was true of TBP: 30 mm Hg (14-58 mm Hg) to 60 mm Hg (40-105 mm Hg; $P = .003$). Looking at the individual cases, three patients (Table III) had a partial reconstruction (ie, superficial femoral artery occlusion distal to a central reconstruction [cases 7 and 8] or reconstruction to a popliteal blind segment [case 13]). These patients

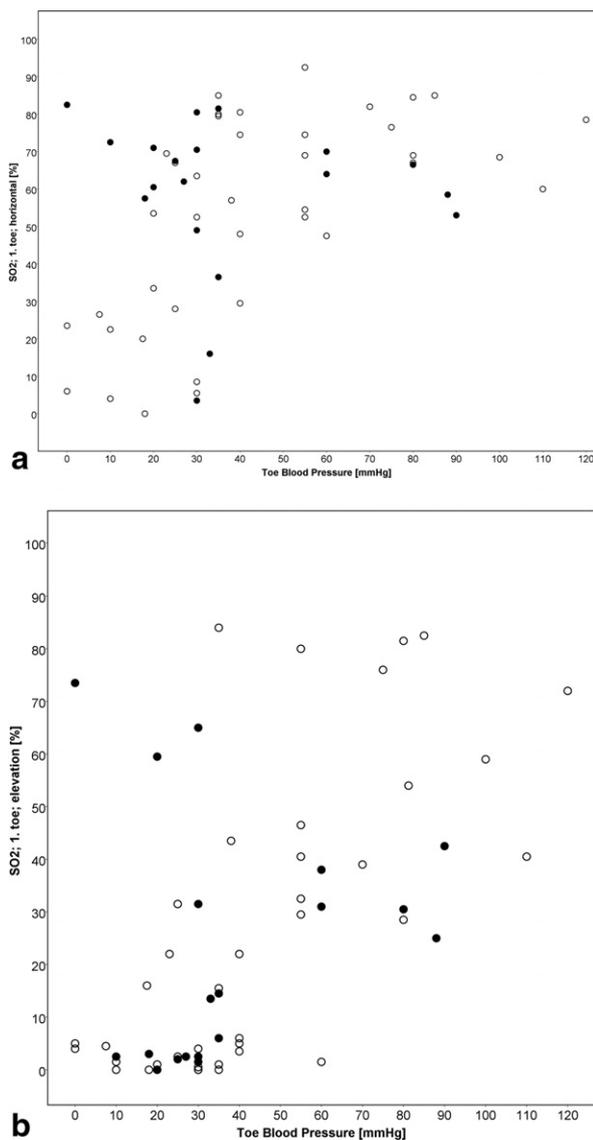


Fig 3. a, TBP compared with O2C SO_2 measured with the limb in horizontal position. Unit of TBP is mm Hg and the unit of SO_2 is %. Data are divided according to the presence of diabetes. SO_2 ; *I. toe; horizontal*, SO_2 value measured with the limb in horizontal position at the plantar surface of the first toe. b, TBP compared with SO_2 measured with the limb elevated. Unit of TBP is mm Hg and the unit of SO_2 is %. Data are grouped according to the presence of diabetes. *White circles* indicate the patients without diabetes; *black circles* indicate the patients suffering from diabetes. O2C, Micro-lightguide spectrophotometry; SO_2 , saturation; SO_2 ; *I. toe; elevation*, SO_2 value measured with the limb elevated at the plantar surface of the first toe. TBP, toe blood pressure.

had significantly lower postoperative saturation as well as toe pressures compared with the ten patients with unobstructed flow to the foot. This is illustrated by the following figures: 47% (21%-65%) versus 82% (71%-91%; $P < .005$), 18% (0%-25%) versus 62% (36%-89%; $P < .005$), and 25 mm Hg (25-40 mm Hg) versus 90 mm Hg (40-125 mm

Hg; $P < .007$) for SO_2 horizontal, SO_2 elevated, and toe pressures, respectively.

Fig 4 illustrates TBP and SO_2 in the horizontal position in the individual patients before and after revascularization. It clearly visualizes the effect of revascularization in both parameters. Looking at SO_2 patients who had low values preoperatively and who had low values postoperatively shows a noticeable increase in SO_2 . Patients having a high SO_2 preoperatively (65% or below) remain at a high level, and four out of five of these cases were claudicants. The three patients (7, 8, and 13) who had a partial reconstruction are marked and easily identifiable as having the lowest postoperative SO_2 values.

DISCUSSION

This is the first systematic validation of the use of O2C in monitoring skin perfusion in ischemic limbs.

Group-wise O2C could separate claudicants from patients with critical ischemia, but only when examined in the elevated position. These results were somewhat similar to the ability of distal pressures to separate groups. Group-wise results differ significantly, but with a considerable overlap. Disappointingly, O2C examinations in the horizontal position revealed only insignificant differences between groups.

In a clinical setting, individual predictions are crucial, and, in this case, results were difficult to interpret. Through our O2C measurements, made in the elevated position, we had hoped to simulate the clinical findings of a deathly pale foot achieved by elevating a critically ischemic limb. In fact, O2C proved valuable in detecting low toe pressures, as almost all patients with low SO_2 readings also had low toe pressures. Thus, the PPV of an SO_2 in predicting a TBP of 40 mm Hg or less was 100% in horizontal and 97% in the elevated position (Fig 3, a and b).

Previous investigations have been made using O2C measurements. Measurements were made in the following: heart,⁵ brain,⁶ skin flaps,^{7,8} the nerves of diabetic patients,⁹ the optic nerve,¹⁰ in models with venous hypertension,¹¹ in determination of mitochondrial metabolism,¹² and to assess the effect of hemodialysis on skin's microcirculation.¹³ Also as mentioned, the method's ability to predict ulcer healing in patients with diabetes has been studied,^{14,15} as well as the method's ability to predict healing after amputation.¹⁶ However, study populations in these publications have been small, and no direct comparisons with our results were possible. One study²⁰ investigated O2C reliability in diabetic and nondiabetic subjects and found a coefficient of variance of 18%. This was similar to our finding of 15% (SO_2 in horizontal position).

Assessing perfusion before and after revascularization may eliminate some inherent variability. In fact, we noted significantly higher postoperative values when revascularization reached the foot compared with patients who had only a partial revascularization and who had an increase neither in SO_2 nor in TBP.

Further, there was no overlap in SO_2 values between these two groups, making these results even more convinc-

Table III. Patients who underwent revascularization

No.	Rutherford classification	Preoperative diagnosis	Revascularization	Postoperatively
1	1-3	Aorta occlusion, open SFA	Aortobifemoral prosthesis	PPP
2	1-3	Aorta occlusion, open SFA	Aortobifemoral prosthesis	PPP
3	1-3	Aorta occlusion, open SFA	Aortoiliac stenting	Graft open, but no PPP
4	4	Aorta occlusion, open SFA	Aortobifemoral prosthesis	PPP
5	1-3	Iliac occlusion, open SFA	Femorofemoral cross-over graft	PPP
6	1-3	Iliac occlusion, open SFA	Femorofemoral cross-over graft	PPP
7	4	Iliac and SFA occlusion	Femorofemoral cross-over graft	Graft open, but no PPP
8	4	Iliac and SFA occlusion	Iliac-femoral prosthesis	Graft open, but no PPP
9	5-6	SFA occlusion	Femoropopliteal graft	PPP
10	1-3	SFA occlusion	Fem-pop UK in situ bypass	PPP
11	5-6	SFA occlusion	Fem-tib in situ bypass	Bypass open, with several arteriovenous fistulas
12	5-6	SFA occlusion	Fem-pop UK in situ bypass	PPP
13	5-6	SFA and all crural arteries occlusion	Fem-pop UK in situ bypass to popliteal blind-segment	Bypass open, but no PPP

No., Case number; *Fem-pop*, femoropopliteal; *Fem-tib*, femorotibial; *PPP*, palpable pedal pulse; *SFA*, superficial femoral artery. Case number, Rutherford classification, preoperative diagnosis, type of procedure, and postoperative findings are shown.

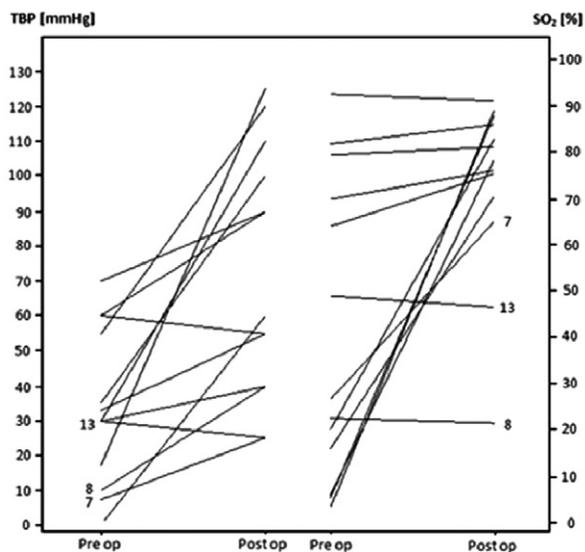


Fig 4. TBP and SO_2 measured in horizontal position in each case before and after revascularization. The measurements are plotted individually on the left and right of the figure, respectively. Unit of TBP is mm Hg and the unit of SO_2 is %. SO_2 , Saturation; *TBP*, toe blood pressure.

ing (Fig 4). These results indicate that O2C might be valuable in monitoring SO_2 and flow in the individual patient. This could be, for example, during an operation or, as we did, before and after revascularization. On the O2C computer screen, readings of SO_2 , rHb, and flow are shown as curves during the examination. This makes continuous

readings possible and makes O2C useful as a monitoring device.

PAD patients with diabetes often respond differently compared with nondiabetics. In this study, we were unable to demonstrate any difference, but this patient population was heterogeneous. Moreover, we did not have a large enough sample of patients to allow a thorough analysis of subgroups (diabetic vs nondiabetic, smokers vs nonsmokers, etc).

During the examinations, we noticed that large variations occurred when the patient inadvertently moved the toe. This may lead to artifacts or false values, which in turn suggests that perhaps O2C is too sensitive and therefore demands special attention during the examination. We did not repeat O2C measurements when the difference between consecutive results was large.

Depending on the type, O2C probe measurements can be made in 2- and/or 6-mm depth. Our O2C measurements were made in 2-mm depth. The question is whether we are making measurements relevant for diagnosing lower limb ischemia or merely assessing natural fluctuations in the microcirculation of the skin.²¹ The foot was heated before examination, but this does not guarantee that variations do not occur during the examination. Potentially, a better choice would be to make measurements of 6 mm in depth in patients suffering from PAD to prevent disturbance from these fluctuations. Further, patients suffering from critical ischemia often experience pain related to the toes and the skin in contrast to the claudicants where the symptoms are related to the muscles. This fact indicates that perhaps measurements (ie, SO_2 and flow) obtained at skin level give

additional and useful information about the patients with critical ischemia.

An O2C apparatus costs about USD \$35,000 (€26,500), all inclusive. Assuming depreciation over 3 years and about 200 measurements performed annually, the cost of one examination is circa USD \$5.

However, the ease and speed with which O2C examinations may be performed are important factors for the patient as well as the examiner. A full examination for the evaluation of limb ischemia takes about 10 minutes with the O2C technique and is painless for the patient. The probe is fixed to the patient, making readings possible with the limb lifted – as in our case – or with limb lowered from the bed or, as mentioned above, fixed to the toe during an operation.

CONCLUSIONS

In conclusion, our experience indicates that O2C was easy to use, fast, and painless, and might add valuable clinical information regarding patients with lower limb ischemia. Probably the most useful finding was the high predictive value of a low SO₂ and the convincing rise in O2C values after successful revascularization. This made the technique useful for postoperative surveillance. However, additional studies in specific populations are necessary to ensure and define the future clinical application in the vascular setting.

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AUTHOR CONTRIBUTIONS

Conception and design: LJ, TS
Analysis and interpretation: LJ, TS
Data collection: LJ
Writing the article: LJ, TS
Critical revision of the article: TS
Final approval of the article: LJ, TS
Statistical analysis: LJ, TS
Obtained funding: LJ, TS
Overall responsibility: LJ, TS

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