

Pilot Assessment of the Repeatability of Indocyanine Green Fluorescence Imaging and Correlation with Traditional Foot Perfusion Assessments

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WHAT THIS PAPER ADDS

Critical limb ischemia (CLI) needs careful clinical examination, but also assessment of the arterial circulation by objective examinations. This prospective pilot study analyzed ankle brachial index (ABI), toe pressures (TP), transcutaneous oxygen pressure (TcPO₂), and indocyanine green fluorescence imaging (ICG-FI) in the assessment of CLI. This is the first study evaluating the repeatability of ICG-FI and its correlation with the traditional diagnostic tests, ABI, TP, and TcPO₂.

Background: Ankle brachial index (ABI), toe pressures (TP), and transcutaneous oxygen pressure (TcPO₂) are traditionally used in the assessment of critical limb ischemia (CLI). Indocyanine green (ICG) fluorescence imaging can be used to evaluate local circulation in the foot and to evaluate the severity of ischemia. This prospective study analyzed the suitability of a fluorescence imaging system (photodynamic eye [PDE]) in CLI.

Material and methods: Forty-one patients with CLI were included. Of the patients, 66% had diabetes and there was an ischemic tissue lesion in 70% of the limbs. ABI, toe pressures, TcPO₂ and ICG-fluorescence imaging (ICG-FI) were measured in each leg. To study the repeatability of the ICG-FI, each patient underwent the study twice. After the procedure, foot circulation was measured using a time-intensity curve, where T1/2 (the time needed to achieve half of the maximum fluorescence intensity) and PDE10 (increase of the intensity during the first 10 s) were determined. A time-intensity curve was plotted using the same areas as for the TcPO₂ probes ($n=123$).

Results: The mean ABI was 0.43, TP 21 mmHg, TcPO₂ 23 mmHg, T1/2 38 s, and PDE10 19 AU. Time-intensity curves were repeatable. In a Bland-Altman scatter plot, the 95% limits of agreement of PDE10 was 9.9 AU and the corresponding value of T1/2 was 14 s. Correlation between ABI and TP was significant ($R=.73$, $p<.001$), and it was weaker in diabetic patients ($R=.47$, $p=.048$) compared with non-diabetic patients ($R=.89$, $p=.002$). Correlations between ABI and TcPO₂ and TP and TcPO₂ were weak ($R=.37$, $p=.05$ and $R=.43$, $p=.037$, respectively). Correlation between TcPO₂ and PDE10 was strong in diabetic patients ($R=.70$, $p=.003$).

Conclusions: According to this pilot study, ICG-FI with PDE can be used in the assessment of blood supply in the ischemic foot.

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Article history: Received 18 January 2016, Accepted 28 June 2016, Available online 30 July 2016

Keywords: Critical limb ischemia, ABI, Toe pressure, TcPO₂, Indocyanine-green fluorescence imaging, Diagnostics, Repeatability

INTRODUCTION

Critical limb ischemia (CLI) is the most severe form of peripheral arterial disease. Evaluation of the circulation in the ischemic foot is crucial when methods of revascularization are planned as well as in the assessment of the results of the revascularization. In addition to careful clinical examination, evaluation of the blood supply of the foot is most important.

Conventional methods are systolic pressure measurements from ankle and toe level, ankle brachial index (ABI), and transcutaneous oxygen pressure (TcPO₂).¹ However, these methods have limitations. The repeatability of TcPO₂ and toe pressure (TP) is not good,^{2,3} and in many cases where there is loss of toes TP cannot be measured. ABI can be falsely high in many cases because of mediasclerosis. In diabetic patients, assessment of circulation is more complex than in non-diabetic patients because of the concomitant neuropathy and microvascular dysfunction.

Indocyanine green fluorescent imaging (ICG-FI) is a relatively recent method of imaging foot perfusion, and is still developing in many ways.⁴ The principle of ICG-FI is to illuminate the tissue of interest with light at the excitation wavelength (about 750–800 nm) while observing it at

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<http://dx.doi.org/10.1016/j.ejvs.2016.06.018>

longer emission wavelengths (over 800 nm). After an intravenous injection of ICG, perfusion of the foot can be visualized using an infrared camera and recorded for further analysis. The intensity of the fluorescence as a function of time can be analyzed from the recorded images allowing a time-intensity curve to be drawn. To get comparable numerical values, T1/2 and PDE10 can be determined from the time-intensity curve.⁵ T1/2 is the time needed to achieve half of the maximum fluorescence intensity. PDE10 is the intensity of the fluorescence 10 s after the starting point of uprising of the time intensity curve.⁵

The current prospective study tested ICG-FI in the assessment of critically ischemic feet. The aim was to evaluate the repeatability of the method and also to compare how it relates with the conventional methods, ABI, TP, and TcPO₂. Furthermore, any correlation between these methods was studied in assessment of the circulation in critical ischemia.

MATERIAL AND METHODS

Forty-one patients with critical limb ischemia treated at Helsinki University Hospital, Department of Vascular Surgery, were included in the study. Of the 41 limbs, 70% were Fontaine stage IV, chronic limb ischemia with tissue lesion. Of the patients, 66% had diabetes. The study was accepted by the ethical committee of Helsinki University Central Hospital. All patients were informed of the details of the study and patients signed informed consent to accept participation in the study. Exclusion criteria were allergy to iodine, history of liver disease, and heart failure of New York Heart Association (NYHA) classes III and IV.

ABI, toe pressures, and TcPO₂ were measured in each patient at three different points in the foot. In addition, all patients underwent ICG-fluorescence imaging twice, with at least 30 min between the measurements. All measurements were done by the same investigator (H.T.). A fluorescence imaging system (photodynamic eye [PDE], Hamamatsu Photonics K.K., Hamamatsu, Japan) was used. The system includes a 760 nm light emitting diode (LED) as an excitation light source and a charge coupled device (CCD) camera covered with a lens that filters out light with wavelength below 820 nm. The method of fluorescence imaging has been described previously.³ All images were electronically stored for later analysis. A certain range was fixed on the recorded images, which were displayed with 256 luminance

intensity, and signal intensity values were plotted against time as a time-intensity curve using regions of interest (ROIs) analysis program (Hamamatsu Photonics K.K., Hamamatsu, Japan).

ICG-FI was performed in a room with no windows or daylight and at room temperature between 20 and 25 °C. Before the examination, patients had at least 15 min of rest. Patients were investigated in the supine position and the PDE was set 20 cm above the dorsum of the foot. A 5 min video recording of the foot with PDE was started after injecting ICG (0.1 mg/kg) intravenously into the cephalic vein.

After the procedure, the recorded images were analyzed according to a previously described protocol.⁵ The fluorescence intensity of a standard sample in the dorsum of the foot was measured and plotted against time, and a time-intensity curve was drawn. From this curve, the time required to achieve half of the maximum fluorescence intensity was determined (T1/2). A time-intensity curve was also plotted from small areas of the foot using the same areas as for the TcPO₂ probes. The fluorescence intensity increase was measured at 10 s (PDE10) and the PDE10 value was compared with the TcPO₂ value from the same area.

Statistical analysis

Data are expressed as mean, standard deviation, and range. Independent variables Student *t* test and chi-square test were used to test significance between diabetic and non-diabetic patients. A Bland-Altman scatter plot was used in repeatability analysis. The correlation coefficient was used to describe correlation among ABI, TP, TcPO₂, and PDE10. A receiver operation characteristic (ROC) curve was used to test PDE10 to detect severe limb ischemia at the threshold of TcPO₂ 40 mmHg. In the analysis of correlation, ABI values >1.4 were excluded as they were considered to be unreliable. Statistical analysis was performed using software IBM SPSS Statistical Software version 22.0 (IMB, Armonk, NY, USA).

RESULTS

ICG-FI measurements were successful in 41 patients. The mean age of the patients was 73 years and one third were female (Table 1). No allergic reactions or other side effects were caused by ICG. The mean ABI was 0.43 (SD 0.43, range 0–1.6), TP 21 mmHg (SD15, range 0–56), TcPO₂ 23 mmHg

Table 1. Basic demography, Fontaine classification, and circulation measure of the study patients.

	All (n=41)	Diabetics (n=19)	Non-diabetics (n=22)	<i>p</i> (dm vs. non-dm)
Age, mean, SD (range)	73, 10.1 (58–98)	70, 10.1 (58–98)	77, 9.0 (58–93)	0.04
Sex, female, %	31.7	21.1	31.7	0.32
Fontaine IV, %	65.9	63.1	68.2	0.46
ABI ^a , mean, SD (range)	0.43, 0.4 (0–1.6)	0.50, 0.35 (0.18–1.6)	0.34, 0.39 (0–1.6)	0.17
Toe pressure, mean, SD (range)	21, 15 (0–56)	25, 14 (0–45)	17, 15 (0–56)	0.07
TcpO ₂ , mean, SD (range)	23, 14 (3–48)	24, 12 (5–48)	21, 16 (3–46)	0.6
T1/2, mean, SD (range)	38, 24 (10–110)	33, 23 (10–91)	42, 26 (13–110)	0.24
PDE10, mean, SD (range)	19, 17 (1–73)	21, 19 (3–73)	17, 16 (1–60)	0.24

^a ABI could not be measured in seven patients (one diabetic and six non-diabetics) because of incompressible calf arteries.

(SD 14, range 3–48), T1/2 38 s (SD 24, range 10–110), and PDE10 19 AU (SD 19, range 1–73). ABI was >1.5 in nine patients (20.0%). TP could not be measured in two patients (4.4%). TcPO₂ and ICG-FI were successful in all cases. Altogether 123 TcPO₂ values were measured from 41 patients and PDE10 was analyzed for all 123 TcPO₂ values using the same measurement point (Fig. 1).

PDE showed patchy ischemia indicating an uneven distribution of the circulation at foot level (Fig. 1). The time-intensity curve and thereafter T1/2 and PDE10 were repeatable at various levels of blood supply (Fig. 2). In the Bland-Altman scatter plot, 95% limits of agreement of PDE10 was 9.9 AU (Fig. 2) and the corresponding value of T1/2 was 14 s (Fig. 3).

Correlation between ABI and TP was significant ($R=.73$, $p<.001$). It was weaker in diabetic patients ($R=.47$, $p=.048$) compared with non-diabetic patients ($R=.89$, $p=.002$). Some correlation existed between ABI and TcPO₂ and TP and TcPO₂ ($R=.37$, $p=.05$ and $R=.43$, $p=.037$, respectively) (Fig. 4), and when patients were divided according to diabetes status the correlation remained in non-diabetic patients ($R=.63$, $p=.02$ and $R=.54$, $p=.04$, respectively), when in turn no correlation was seen in diabetic patients between ABI and TcPO₂ and TP and TcPO₂ ($R=.005$, $p=.8$ and $R=.20$, $p=.11$, respectively).

Correlation between ABI and PDE10 existed ($R=.42$, $p=.04$) and was more clear in non-diabetic patients ($R=.58$, $p=.01$) than in diabetic patients ($R=.30$, $p=.055$). TP and

PDE10 had stronger correlation than ABI and PDE10 ($R=.54$, $p=.02$; non-diabetic $R=.59$, $p=.04$, diabetic $R=.46$, $p=.1$). Overall correlation between TcPO₂ and PDE10 was moderate ($R=.47$, $p=.48$), being strong in diabetic patients ($R=.70$, $p=.003$) (Fig. 5) and weak in non-diabetic patients ($R=.31$, $p=.058$).

In the ROC curve using TcPO₂ level 40 mmHg, the cut off value of PDE10 was 21 AU (AUC 0.73; sensitivity 67%, specificity 72%; in diabetic patients AUC 0.83, sensitivity 88%, specificity 68%, in non-diabetic patients AUC 0.69; sensitivity 60%, specificity 65%) (Fig. 6).

DISCUSSION

Assessment of the critically ischemic foot is challenging, especially in diabetic patients. Traditional ABI and toe pressure are not feasible in all patients because of medial sclerosis and in many cases missing toes. Furthermore, the repeatability of TcPO₂ and TP has been shown to be weak or moderate.^{2,3} Although ICG-FI has been used for decades, for example in ophthalmology, it has not been widely used in vascular surgery. Yet, it gives interesting information on the circulation of the whole foot area and gives both visual and numeric information, which can be extracted afterwards from recorded images of the region of interest. However, it is important to assess the reliability and repeatability of new examinations before implementation to clinical practice. Repeatability of the ICG-FI has not been reported

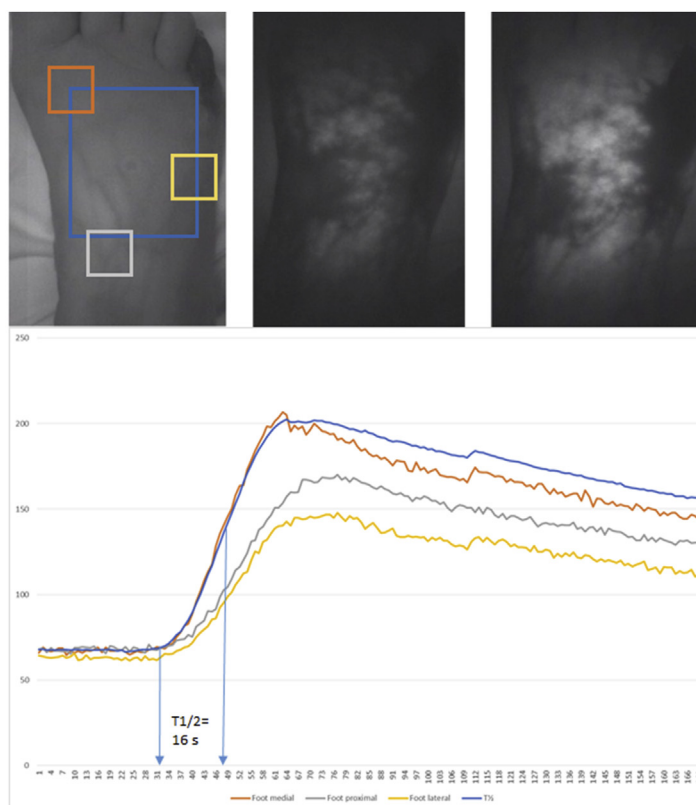
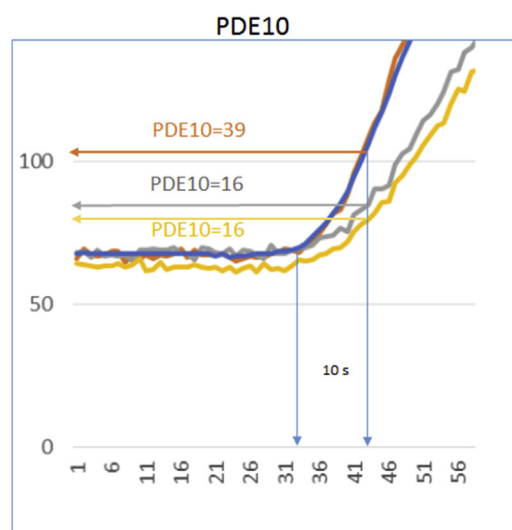


Figure 1. Fluorescence imaging of a foot with gangrene of the fifth toe showing the four ROIs where the time-intensity curves have been drawn after ICG-FI with PDE. From these curves, PDE10 was determined from three spots (left). T1/2 was derived from the time-intensity curve of a larger region (blue ROI) in the dorsal side of the foot.

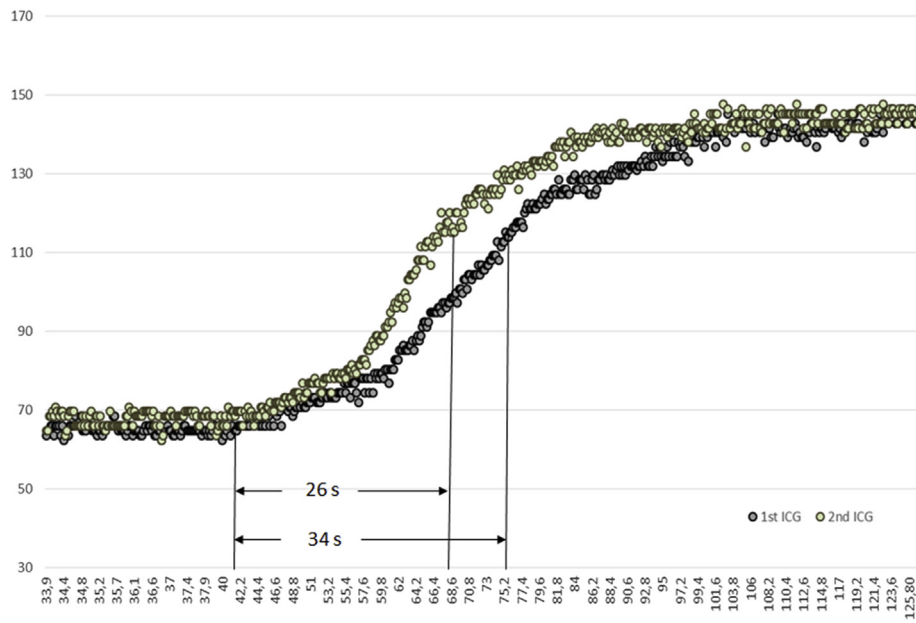


Figure 2. Two time-intensity curves taken from the dorsum of the same foot with 30 min of each other. T_{1/2} has been drawn to the curve. In the second measurement the time-intensity curve is quicker, perhaps because of some residual ICG in the body.

previously. This study reports a first experience of use of ICG-FI in the assessment of foot level perfusion, as well as the repeatability of the examination. The method was well tolerated, quick to perform and feasible for all patients. PDE showed ischemic patches on the foot, indicating variability in the circulation at this level. Direct correlation with traditional measures, ABI, TP, or TcPO₂, varied among the methods, which can be attributed to their different perspectives. The repeatability of the time-intensity curve was good and comparable with that of other methods used to assess foot circulation.

Indocyanine green imaging is a relatively recent imaging method, although it has been used in some clinical applications for almost 60 years.^{6,7} ICG has been used for decades in ophthalmology to image retinal blood vessels. It

has several clinically excellent properties, which have been carefully verified during its long clinical use.⁸ It is non-toxic and non-ionizing. It is ideal for angiography because it binds efficiently to blood lipoproteins and thus does not leak from circulation. Its lifetime is short, making repeated measures possible. Also, the signal to noise ratio is good, meaning there is not much near-infrared auto-fluorescence in tissue giving low background noise.⁹ Currently, there are several devices available in the market, the median cost of the devices being the same as in a well equipped laser Doppler tower including tools for ABI and toe pressure measurements. The cost of the actual examination is low as ICG is not expensive. The cost of the equipment starts from 60 000 euros and the cost of ICG in one examination is less than 10 euros.

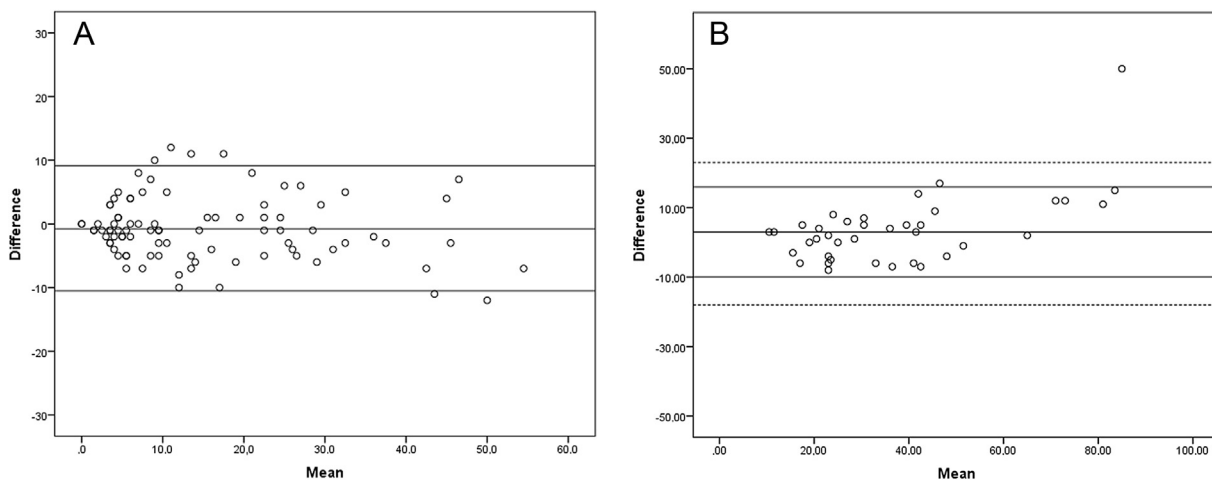


Figure 3. (A) Bland-Altman scatterplot showing the repeatability of PDE10 measurement. Horizontal lines show 95% limits of agreement. (B) Bland-Altman scatterplot showing the repeatability of T_{1/2} measurement. Horizontal lines show 95% limits of agreement (solid line=95% CI without one outlier, dashed line=95% agreement with all values).

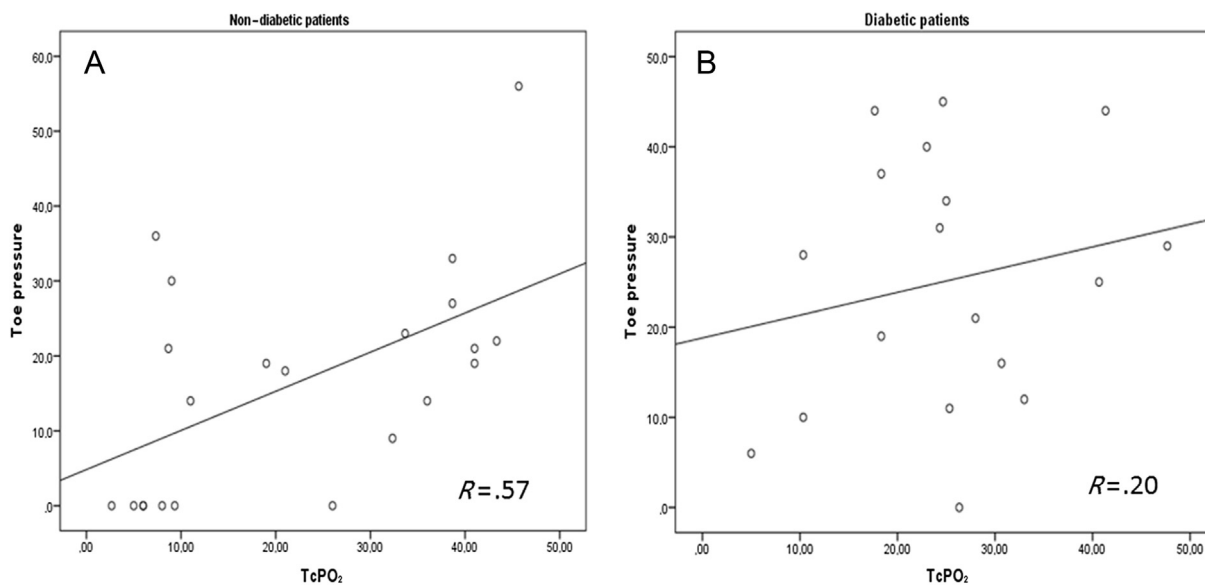


Figure 4. Correlation between toe pressure and TcPO₂ was moderate in non-diabetic patients and did not exist in diabetic patients.

In vascular surgery, ICG-FI was first used intra-operatively to ensure quality control. In a preliminary report published in 2007,¹⁰ nine patients were recruited for intra-operative angiography using PDE. At the end of the procedure before wound closure, ICG was injected in a central intravenous line reaching the leg artery about 30 s later. In eight out of nine cases, ICG angiography showed good fluorescent signals as the ICG passed through the graft. In one case, no fluorescence was detected and this graft was found to be thrombosed. Kang et al. proposed a perfusion rate model based on ICG dynamics, which they later applied to human patients to diagnose peripheral arterial disease with a commercially available system.^{11,12} The perfusion rates in normal legs were significantly higher compared with those

of PAOD legs and the method was also found to be sensitive for mild cases of PAOD.¹¹ In the current study, use of PDE was tested in the assessment of PAOD. Unlike Kang et al., the focus was on critical limb ischemia. The advantage of this method is the use of a time-intensity curve both to get a universal measure of blood supply to the foot area (T1/2), but also to assess circulation at different sites using PDE10. PDE10 can be used, for example, at areas near wounds and even at wound areas or the plantar side of the foot, which is not possible with TcPO₂. The ICG-FI does not show which vessel supplies an ischemic area. However, the possibility of assessing the circulation in several regions of interest afterwards gives interesting possibilities to study, for example, changes in perfusion after revascularization, and thus the success of the revascularization.

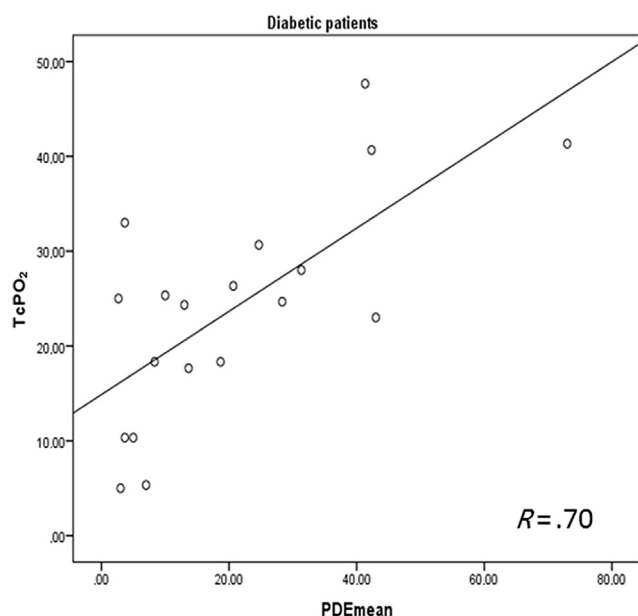


Figure 5. Correlation between TcPO₂ and PDE10 was strong in diabetic patients.

Correlation of the conventional methods, ABI, TP, and TcPO₂, with each other as well as with ICG-FI related variables, varied between strong correlation and no correlation. This can be understood because all these methods measure circulation at different sites. TcPO₂ reflects local skin blood supply. ICG-FI also describes the circulation at skin level, and 3–5 mm below the skin. Ankle pressure and ABI gives information on the blood pressure at ankle level, but is affected by mediasclerosis. TP in turn gives blood pressure very distally indicating the level of ischemia there, which may differ from tissue blood supply in the dorsum of the foot. This can be confusing however, as they may give different answers on the severity of the ischemia. All these measures are used in the assessment of circulation in the critically ischemic foot and each has strengths and weaknesses. ICG-FI with PDE is an additional tool in this assessment. It can be used in all patients, despite for example, the lack of toes or a wound in the foot. An additional advantage over the other methods is the recorded visual image of the foot area that gives information on variation of fluorescence intensity, that is blood supply at different sites. As analysis is done after image recording

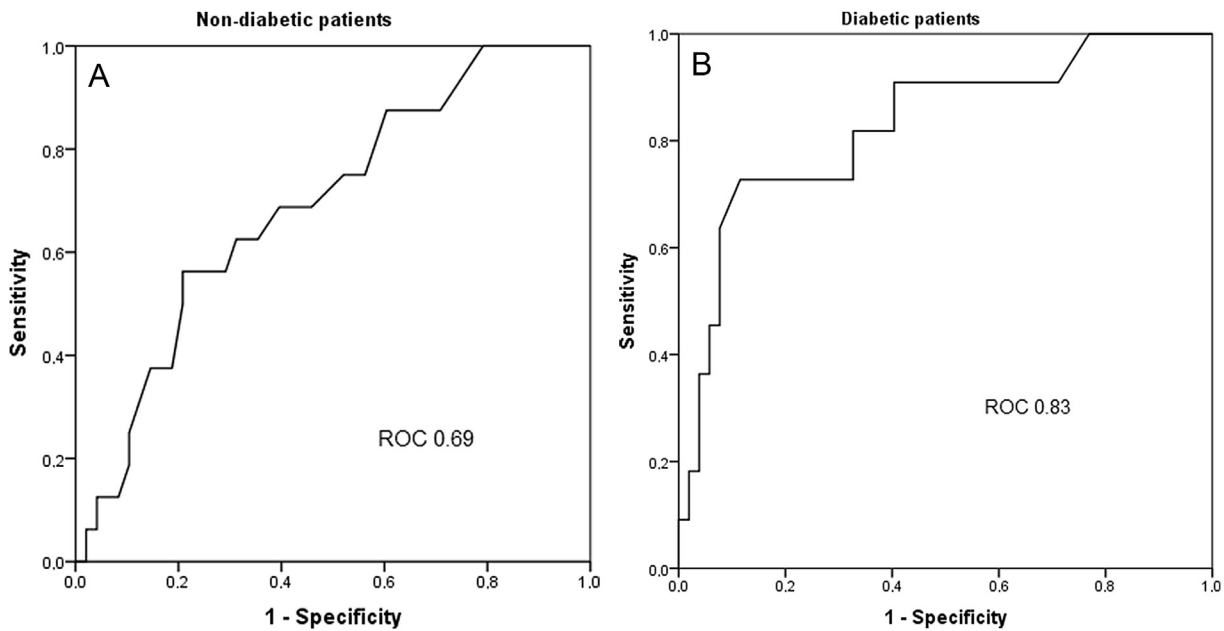


Figure 6. Receiver operation characteristic (ROC) curves for PDE10 to detect severe limb ischemia at the threshold of TcPO₂ 40 mmHg; sensitivity 60% and specificity 65% for non-diabetic patients (A); sensitivity 88% and specificity 68% for the diabetic patients (B).

using software which creates a time-intensity curve, several regions of interest can be measured, and later new regions of interests can be added. The study is also independent of the operator, so no inter-observer variability exists. In diabetic patients media sclerosis does not have an impact on the results.

Repeatability of a measurement is important when used in clinical decision making, considering therapeutic options, and evaluating the influence of therapy, as well as when assessing the progress of the disease. All the methods, ABI, TP, and TcPO₂, have limitations regarding reproducibility.^{2,3,13,14} In a study by de Graaff et al. evaluating intra-observer repeatability, the 95% limits of agreement for ABI was 0.09, toe pressure 10 mmHg, and TcPO₂ 14 mmHg.¹⁴ In the authors' previous study on the repeatability of TP, the 95% limit of agreement was 38 mmHg. The 95% limit of agreement for PDE10 was 9.8 AU and for T1/2 14 s. These are comparable or better than the values of the other methods. T1/2 tended to be systematically shorter in the second measurement (Fig. 1), probably because of some residual ICG in the body from the first imaging. Similarly, PDE10 values tended to be higher in the second round, which could be explained by the same hypothesis.

Although ICG-FI is easy to use and seems to give reliable information, it has certain pitfalls and limitations. For example, inflammation caused by infection in the foot increases microcirculation and this leads to increased fluorescence intensity and T1/2 and PDE10 values that are too high. Furthermore, the influence of low cardiac output is unknown and long T1/2 may be partially related to slow circulation rather than stenosis or occlusions in the lower extremity arteries. PDE is not completely non-invasive, because intravenous access is needed to get the images. Furthermore, the device used in this study was difficult to

set up for the imaging and it took time to draw the time-intensity curve and to extract the T1/2 and PDE10 values. In the current study, as well as in earlier reports, ICG has been extremely well tolerated, but although very rare, allergic reaction is possible.¹⁵

This study has several limitations. The number of patients is small and thus this can only be seen as a pilot assessment. This was because the availability of the assessment and study period was limited. The study does not have a control group with normal circulation, which would allow analysis of the threshold values for CLI. Further research is needed to evaluate the value of ICG-FI in predicting wound healing, determining the optimal amputation level, and in the assessment of the success of revascularization. So far, ICG-FI is not widely used. One possible reason is that vascular surgeons are not familiar with the method and its interpretation. The concept is old, but the first generation devices do not include reporting software, which is available in the newer devices. Newer ICG-FI equipment has quick reporting programs which create immediate time-intensity curves, allowing performance of the whole assessment process from the beginning of the examination until the report is ready, in 15 min. The most interesting future research area is quality control of the revascularization using ICG-FI.

In conclusion, according to this pilot study, ICG-FI with PDE can be used in the assessment of blood supply in the ischemic foot. It is quick to perform, well tolerated, and feasible. In addition to the quantitative evaluation, it gives a visual image of the variation in circulation at foot level. The small number of patients and lack of control group mean that the power of the study is limited and further research is needed to evaluate the threshold values for CLI and the accuracy of the examination.

CONFLICT OF INTEREST

None.

FUNDING

None.

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