Improving the clinical applicability of laser Doppler perfusion monitoring
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Chapter 3

How to assess post-occlusive reactive hyperaemia by means of laser Doppler perfusion monitoring: application of a standardised protocol to patients with peripheral arterial obstructive disease

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Summary

The standardisation of manoeuvres to perform clinically discriminative microvascular flow-reserve tests is still poorly developed, as well as the response analysis. The aim of this study was to establish a reproducible analysis method for the post-occlusive reactive hyperaemia (PORH) test measured using laser Doppler perfusion monitoring (LDPM). LDPM data were measured from the PORH response of 24 Fontaine class II-III peripheral atherosclerotic/arterial obstructive disease (PAOD) patients and 30 healthy subjects. The PORH response was recorded from the dorsum of the foot after 3 min of arterial occlusion at the thigh. The resulting tracings were analysed by describing their morphology through five defined parameters: resting flux (RF), time to RF level (t_{RF}), maximum flux (MF) during reactive hyperaemia, time to maximum flux (t_{MF}), and time to half recovery (t_{HR}). While the time parameters were discriminative between patients and controls, flux parameters were not. The time to resting flux (t_{RF}) led to the most discriminative model that correctly predicted 88.5% of the cases. Hence, we concluded that obtaining t_{RF} with the presented procedures provides an optimal model to quantify the patient’s microvascular condition from the PORH response.

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3.1 Introduction

Laser Doppler perfusion monitoring (LDPM) is a well-known technique for assessing tissue microcirculation in a continuous and non-invasive way [16]. A typical LDPM monitor makes use of two optical fibres [7]: one to bring the laser light to the tissue (i.e. illuminating or source fibre), and another that collects the back-scattered light from the tissue (i.e. detecting or collecting fibre). The resulting LDPM flux signal is related to the blood perfusion of the illuminated volume of tissue [13].

In the past, a typical LDPM monitor used a 633 nm He-Ne laser, but nowadays diode laser light of 780 nm is widely used. The advantage is that measurements at this wavelength are less influenced by the oxygenation of blood [11,19]. Another factor that affects LDPM measurements is the fibre separation: the wider the separation is, the more light is detected that has travelled through deeper skin [10,12]. These technical aspects of LDPM were studied in a joint effort by several research and technical European groups (SMT project) aiming to standardise the LDPM technique [3,15].

In clinical practice, LDPM is not generally applied for daily use. One of the limitations of the LDPM technique is that it does not allow measurements of the microcirculation in absolute units, i.e. ml s\(^{-1}\) (100 g\(^{-1}\))\(^{-1}\), but in arbitrary units called perfusion units (PU) [11]. Furthermore, several studies have reported the low clinical value of resting blood flux values [8]. However, using provocation tests, e.g. post-occlusive reactive hyperaemia (PORH), differences between healthy subjects and patients with peripheral arterial obstructive disease (PAOD) have been reported [6,8,17]. These studies agree on the usefulness of quantitative parameters that consider the response times (e.g. time to maximum flux). Particularly interesting was del Guercio’s study in which nine different parameters of the PORH response were investigated and compared between healthy controls and PAOD patients. These differences were more evident performing the occlusion at the thigh than at the ankle.

This chapter, within the framework of a European project for standardisation of the LDPM technique, studied PORH on both POAD patients and healthy subjects at several clinical centres in Europe, using standardised equipment and methods. This study aims to propose a standardised method to assess post-occlusive reactive hyperaemia response on PAOD patients by means of LDPM.
3.2 Methods

Three different European research groups participated in this study, they are referred to as partners A, B and C. All partners agreed to perform post-occlusive reactive hyperaemia (PORH) measurements in peripheral arterial obstructive disease (PAOD) subjects and control subjects according to a common protocol (Table 3.1).

3.2.1 Patients

The inclusion criteria for the PAOD patients are shown in Table 3.2. The respective local ethical committees approved the studies. A total number of 54 subjects were measured: 24 patients were type II-III Fontaine class (mean age: 65.5 years; range: 45-80 years), with an ankle-brachial index (ABI) less than 0.9, without critical limb ischemia (according to the second European consensus document on chronic critical leg ischemia (1991)) and no signs of diabetes mellitus. The control group consisted of 30 subjects without clinical symptoms and signs of vascular disease (mean age: 53.8 years; range: 22-74 years).

Table 3.1. Protocol for the PORH measurements.

1. LDPM monitor:
   - Laser wavelength of 780 nm.
   - Probe with a fibre separation of 0.25 mm.
   - Laser warming up for 30 minutes.
   - Time constant set to minimum.
   - Bandwidth set to maximum.
   - Calibration according to manufacturer’s instructions.
   - No local heating.

2. Subject preparation and manoeuvres:
   - Room temperature of 25 ±1 °C.
   - Subjects in the supine position.
   - The dorsum of the feet 10 cm above heart level.
   - A blanket covers the legs up to the ankle.
   - 30 minutes for acclimatisation.
   - 18 cm-wide pneumatic cuff installed around the thigh of the leg on which the measurements are performed.
   - No clothing allowed between the cuff and the skin.
   - The probe installed preferably on the dorsum of the foot between the second and third metacarpal radius.
   - The arterial pressure is measured on the arm.
   - The maximum pressure of the cuff is set to 30 mm Hg above the systolic arm pressure.
   - 3 min occlusion time; 1 min in case of strong pain.
3.2.2 LDPM monitors

Each research partner used similar equipment and its own patient and control groups. Partners A and B used a Pf4001 (Perimed AB, Järfälla, Sweden) and partner C used a Pf5001 (Perimed). These laser Doppler perfusion monitors (LDPM) generate low power laser light at a wavelength of 780 nm. All partners used similar multi-purpose LDPM probes for skin measurements, having a core diameter of 0.125 mm and a fibre separation centre-to-centre of 0.25 mm. The LDPM monitors were set to the minimum time constant (0.2 seconds), and the maximal cut-off frequency was set at 12 kHz. The instruments were calibrated using the manufacturer’s aqueous suspension of polystyrene microspheres and instructions. Partners A and C connected the analogue output channels of the monitor to an analogue-to-digital acquisition card sampling at a rate of 40Hz. Recording software was developed using Labview v5.1 (National Instruments Co., Austin, USA). Partner B used the digital output of the LDPM monitor in combination with the manufacturer’s software (Perisoft, Perimed).

3.2.3 Protocol

The common protocol (Table 3.1) requires that the subjects comfortably rest on a bed in supine position for 30 min prior to the test. The recording session lasted 33 min without stops, and had three stages: baseline, occlusion, and reactive hyperaemia. The first stage always lasted 15 min for recording enough reference flux signal. The occlusion lasted 3 minutes at the most; in case of strong leg pain 1 minute was the minimum time. After the occlusion, the signal was recorded for another 15 minutes to obtain the PORH response.

The probe site, according to the protocol, was at the dorsum of the foot because the underlying skin is less influenced by the thermoregulatory blood flow due to the absence of arteriovenous anastomoses [5].

The occlusion place was the thigh, in line with del Guercio, and was performed using an 18 cm wide pneumatic cuff attached around the measured leg. Partner A used a rapid cuff inflator system (E-20 rapid cuff inflator and AG-101 air source, D.E. Hokanson, Bellevue, USA) to quickly inflate/deflate the cuff. The other two partners used a handheld sphygmomanometer pump to inflate the cuff.

3.2.4 Tracings analysis

Before calculating the parameters given in Table 3.3, the measurements were converted to perfusion units (10 mV = 1 PU, following the manufacturer’s instructions), and averaged over one second. Next, the data was
plotted for visual inspection. The resting flux (RF) was calculated from the longest excerpt free of artefacts and representative of the pre-occlusion period. The Biological Zero (BZ) [18] was calculated from the most representative excerpt free of large signal fluctuations during occlusion. The time at which the flux signal reappeared after occlusion was considered as \( t = 0 \). The BZ value was then subtracted from all data points. Once the recording was corrected for BZ (fig. 3.1), the value of the time to RF level (\( t_{RF} \)) was determined as the first flux data-point higher or equal to the RF value. Then, the signal was smoothed even more using a 21-seconds moving average. This 21-seconds average produced an acceptable smoothed signal without loosing much of its characteristics. From the resulting averaged tracing, we determined the maximum flux (MF) and the time to MF level (\( t_{MF} \)) by inspection of the individual data-points of the averaged curve. In case of several flux peaks we used the highest one. Before obtaining the time to half recovery (\( t_{HR} \)) we calculated the half recovered flux (HRF) as described in Table 3.3. Then \( t_{HR} \) was obtained from the first flux data-point lower or equal to HRF.

3.2.5 Statistics

The two-tailed Mann-Whitney u-test was used to compare each of the parameters between groups, and \( p < 0.05 \) was considered significant. Furthermore, a binomial logistic regression analysis [1] was performed to find a relation among the studied parameters (Table 3) that could optimally separate PAOD patients from healthy subjects. A value \( p_{PAOD} = 0 \) was considered to be a control, and \( p_{PAOD} = 1 \) a PAOD subject. The cut-off value was \( p_{PAOD} = 0.5 \). All the data analysis was performed on a personal computer with MSExcel v9 and SPSS v10 programs.

3.3 Results

In three patients, the occlusion lasted one minute due to strong leg pain. Apart from that, in two patients it was not possible to derive reliable results for \( t_{RF} \) and \( t_{HR} \), the times to reach resting flux and half-recovered flux respectively, due to the presence of movement artefacts caused by tremors that disturbed the flux signal. However, it should be noted that none of these subjects were excluded from the study.

Figure 3.1 shows the method of analysis of the post occlusive reactive hyperaemia (PORH) response for which results are summarised in Table 3.4. The resting flux values (RF) measured at the dorsum of the foot before the occlusion were generally low. These were similar between groups, hence RF did not discriminate patients from controls. The maximum flux values
### Table 3.2. Inclusion criteria for the PAOD patients.

- Type II-III Fontaine class PAOD: claudication or rest pain (not fulfilling other criteria for critical limb ischemia).
- Ankle-Brachial Index < 0.9.
- Beta-blockers, calcium antagonists and other medication that influences the vasomotor tone are excluded. Buflomedil or Naftidrofuryl were withdrawn at least one week before the study. The following drugs were permitted:
  - Statins.
  - Aspirin or other thrombocyte aggregation inhibitors (no dipyridamol allowed).
  - ACE-inhibitors, provided the patient is stably instituted.
  - No (history of) congestive heart failure.
  - No diabetes mellitus (has to be excluded by recent, < 3 months, measurements of glucose or HbA1c levels according to ADA criteria).

### Table 3.3. Definitions of the parameters used in this study.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>BZ</td>
<td>Biological Zero: the remaining flux signal during occlusion.</td>
</tr>
<tr>
<td>RF</td>
<td>Resting Flux: the mean flux signal level during the pre-occlusion period in standardised perfusion units (PU).</td>
</tr>
<tr>
<td>t₀</td>
<td>Time of reference: the moment when the flux signal starts rising after the end of occlusion and becomes higher than BZ.</td>
</tr>
<tr>
<td>t₉RF</td>
<td>Time to resting flux: the first time after t₀ that the flux is higher than RF.</td>
</tr>
<tr>
<td>MF</td>
<td>Maximum Flux: the highest flux value observed during the PORH response in standardised perfusion units (PU).</td>
</tr>
<tr>
<td>t₉MF</td>
<td>Time to maximum flux: the time from t₀ when MF occurs.</td>
</tr>
<tr>
<td>t₉HR</td>
<td>Time to half recovery of PORH: the time from t₀ when the decreasing flux reaches half way between RF and MF. The flux value is calculated from: ( HRF = \frac{(MF+RF)}{2} )</td>
</tr>
<tr>
<td>MF/RF</td>
<td>Ratio of the maximum flux and resting flux.</td>
</tr>
</tbody>
</table>

(MF) for both groups during reactive hyperaemia were not discriminative as well. However, the ratio between MF and RF was significantly different between both groups \( (p = 0.002) \). The time to reach RF level \( (t₉RF) \) had the best discriminative properties of the studied parameters, as shown in Figure 3.2. In the control group, the 95% confidence interval for the mean of \( t₉RF \) was between 1.9 and 3.6 seconds, whereas for the patients it was between 17.3 and 35.1 seconds. The time to maximum flux \( (t₉MF) \) and the
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time to half-recovery \((t_{\text{RF}})\) were, albeit less strong, other discriminative parameters between patients and controls (fig. 3.2). Again, longer times were observed for the patients.

The modelled equations to calculate the probability of a subject to have peripheral arterial obstructive disease (PAOD), \(\logit(p_{\text{PAOD}})\), obtained from the logistic regression analysis are shown in Table 3.5. Only the four parameters \(t_{\text{RF}}, t_{\text{MF}}, t_{\text{HR}}\) and MF/RF resulted in significant models. Other parameters, RF, MF, or other combinations of all mentioned parameters did not. Ankle-Brachial Index of the PAOD subjects only correlated weakly \((R^2 = 0.21)\) with the calculated value of \(\logit(p_{\text{PAOD}})\) using the coefficients for \(t_{\text{RF}}\) in Table 3.5.

Finally, we tested whether \(t_{\text{RF}}\) with the model of Table 3.5 also correctly classified the controls and PAOD subjects for each partner, by using the criterion \(t_{\text{RF}} = -Z/X = 6.86\) s. From the Groningen, Pisa, and Toulouse control subjects, 8/9, 15/15, and 6/6 were classified correctly, respectively, using \(t_{\text{RF}} < 7\) s. For the PAOD patients these numbers were 3/3, 12/15, and 2/4, respectively, using \(t_{\text{RF}} \geq 7\) s.

### 3.4 Discussion and Conclusions

This study provides a method for recording and analysing of post occlusive reactive hyperaemia (PORH) by means of laser Doppler perfusion monitoring (LDPM), using a standardised protocol that has been used successfully to differentiate between patients with peripheral arterial obstructive disease (PAOD) and healthy controls. Equipment and procedures are used that might be available in most vascular research laboratories. The new aspect of this study is that a consortium of several research groups and manufacturers agreed about this common protocol, and that common results of measurements according to this protocol have been presented. The proposed analysis method showed that the time to resting flux \((t_{\text{RF}})\) was the parameter with the best discriminative power that could correctly classify most of the subjects (Table 3.5).

The differences between the time parameters of the control group and the patient group were highly significant, which confirms findings of other studies \([4,6,9,14,17]\). It should be noted that the measuring set-ups among the studies differed by occlusion area and measuring site, and that the mentioned studies used the end of occlusion as time reference \((t_0)\). However, we decided to use the time when flux signal rises again as a reference time, because this can easily be obtained from the flux registration. Thus, even investigators that did not measure the time of latency might compare their results with those given in this study.
An advantage of the time-analysis is its lower dependence on the flux signal value, certainly up to some extent. Several factors (e.g. probe geometry and its calibration, noise) influence the value of the resulting flux signal, complicating the comparison of LDPM flux data from different vascular laboratories. Nevertheless, this study showed that the time analysis of LDPM data obtained by different teams, using similar equipment and procedures, produce comparable results.

We chose the thigh as occlusion site because ankle occlusions in PAOD patients with mediasclerosis, diabetes mellitus, or renal insufficiency are not reliable, require very high pressures, and are difficult to perform. Furthermore, del Guercio et al. showed that thigh occlusion leads to better differences in the hyperaemic response between PAOD patients and healthy subjects [6].

We compared our results with data available in the literature that were obtained using older generations of LDPM monitors. Del Guercio et al., occluding on the thigh and measuring on the pulp of the hallux, found significant differences between healthy volunteers and PAOD patients for the “time of recovery” ($t_r$). The average of his mean $t_r$ values minus the mean “time of latency” is similar to our mean time to reach resting flux values ($t_{RF}$): for controls 7.2 and 2.7 seconds, respectively; and for PAOD 33 and 26 seconds, respectively. Furthermore, both Kvernebo and del Guercio also studied the time to peak flux with similar set-up [6,8]. Therefore, we could calculate mean $t_{MF}$ values from their results as well: 17 s, 35 s and 23 s for controls, and 57 s, 93 s and 75 s for PAOD subjects, respectively for Kvernebo, del Guercio and ours. It is concluded that these older studies confirm our results by showing similar trends of the times to reach resting flux and maximum flux ($t_{RF}$ and $t_{MF}$).

In this study, the resting flux and maximum flux (RF and MF) were not significantly different between the controls and PAOD patients. Similar conclusions regarding RF and MF were found previously, using various measuring set-ups and earlier generations of LDPM monitors [2,6,14]. Although in this study only one brand of LDPM monitor was used, other modern devices with similar specifications may be used. Some differences among LDPM brands exist, which may cause flux values not being directly comparable. However, since the flux values (RF and MF) in this study were found not significantly different between the controls and PAOD patients, the differences among brands are probably less important for the case of the PORH response.

One might consider the exclusion of patients with diabetes mellitus as a limitation of our method, as diabetes mellitus patients represent a majority
of patients with peripheral arterial obstructive disease. However, microvascular disease is also one of the hallmarks of diabetes mellitus. Diabetic microvascular disease presents clinically with a loss of microvascular flow reserve and a resting hyperaemia in the feet. These features affect the laser Doppler flow response in a manner that may be distinct from that in

Table 3.4. Statistical results of PORH in PAOD (n=24) and controls (n=30).

<table>
<thead>
<tr>
<th>Group</th>
<th>Median</th>
<th>95% interval for mean Lower</th>
<th>Upper</th>
<th>U-test p (Z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RF*</td>
<td>Control</td>
<td>4.60</td>
<td>4.2</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td>PAOD</td>
<td>5.37</td>
<td>4.64</td>
<td>7.92</td>
</tr>
<tr>
<td>MF*</td>
<td>Control</td>
<td>16.69</td>
<td>15.86</td>
<td>25.41</td>
</tr>
<tr>
<td></td>
<td>PAOD</td>
<td>9.41</td>
<td>9.91</td>
<td>19.15</td>
</tr>
<tr>
<td>tRF†</td>
<td>Control</td>
<td>2.00</td>
<td>1.88</td>
<td>3.59</td>
</tr>
<tr>
<td></td>
<td>PAOD</td>
<td>31.50</td>
<td>17.25</td>
<td>35.11</td>
</tr>
<tr>
<td>tMF†</td>
<td>Control</td>
<td>19.00</td>
<td>18.85</td>
<td>27.75</td>
</tr>
<tr>
<td></td>
<td>PAOD</td>
<td>56.50</td>
<td>50.92</td>
<td>87.74</td>
</tr>
<tr>
<td>tHR†</td>
<td>Control</td>
<td>68.00</td>
<td>54.57</td>
<td>76.76</td>
</tr>
<tr>
<td></td>
<td>PAOD</td>
<td>117.00</td>
<td>97.03</td>
<td>147.24</td>
</tr>
<tr>
<td>MF/RF</td>
<td>Control</td>
<td>3.16</td>
<td>0.25</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>PAOD</td>
<td>2.10</td>
<td>0.39</td>
<td>0.62</td>
</tr>
</tbody>
</table>

* Flux in perfusion units (PU), † time in seconds. RF: resting flux; MF: maximum flux; tRF: time to RF; tMF: time to MF; tHR: time to half-recovery. N.S. is p > 0.05

Table 3.5. Significant logistic regression coefficients applied to the discrimination of PAOD patients from control subjects, and the percentages of correctly classified measurements. Z is the constant value of the model, X is the resulting coefficient for the tested parameter: \[ \log(p_{PAOD}) = 10\log \left( \frac{p_{PAOD}}{(1 - p_{PAOD})} \right) = Z + X \text{ Parameter} \].

<table>
<thead>
<tr>
<th>Parameter tested</th>
<th>Regression coefficients</th>
<th>Significance (p)</th>
<th>Controls (%)</th>
<th>PAOD (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>tRF</td>
<td>X = 0.492</td>
<td>0.005</td>
<td>96.7</td>
<td>77.3</td>
<td>88.5</td>
</tr>
<tr>
<td></td>
<td>Z = -3.373</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tMF</td>
<td>X = 0.092</td>
<td>&lt; 0.001</td>
<td>90.0</td>
<td>75.0</td>
<td>83.3</td>
</tr>
<tr>
<td></td>
<td>Z = -3.954</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tHR</td>
<td>X = 0.037</td>
<td>0.001</td>
<td>83.3</td>
<td>54.5</td>
<td>71.2</td>
</tr>
<tr>
<td></td>
<td>Z = -3.639</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MF/RF</td>
<td>X = -0.812</td>
<td>0.007</td>
<td>66.7</td>
<td>70.8</td>
<td>68.5</td>
</tr>
<tr>
<td></td>
<td>Z = 2.178</td>
<td>0.010</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
atherosclerotic PAOD. Although we agree that our analysis might also be of value in patients with diabetes mellitus and PAOD, we have limited for the current purpose our analysis to patients with PAOD without diabetes mellitus.

Another limitation is that our study design does not allow to assess the diagnostic power of our method, because our method was tested in measurements in clinically very distinct groups. A next step should be to assess the diagnostic validity and power of the $t_{RF}$ in a less well-defined population of patients presenting with possible PAOD, which should then be compared to conventional diagnostic tools like (exercise) pressure measurements or duplex scanning.

In summary, this study proposes standardisation of procedures and equipment to assess the characteristics of the PORH response by means of LDPM. The time parameters used in this study ($t_{RF}$, $t_{MF}$, and $t_{HR}$) better discriminated patients from controls than the flux signals (RF and MF). When applied to control subjects and patients with mild to moderate PAOD, the time to resting flux ($t_{RF}$) alone was able to classify subjects with good accuracy. Furthermore, the logistic regression analysis showed that addition of other parameters to $t_{RF}$ did not improve the model. Therefore, we conclude from this multi-centre study that the use of $t_{RF}$ provides an optimal model.

![Figure 3.1. Detail of a PORH response of a control subject. The black line is the signal averaged over one-second. The grey line is the 21-seconds moving average of the black line data. This smoother line was used to obtain $t_{MF}$, $MF$ and $hr$.](image_url)
Figure 3.2. Box-plots of the studied parameters for the control (open boxes) and PAOD (close boxes) groups. The boxes represent (top to bottom): highest value, 3rd quartile, median, 1st quartile and lowest value. The outliers (O) are 1.5-3 box lengths, and the extreme values (*) are beyond 3 box lengths from the median.
to quantify the patient’s microvascular condition from the standardised PORH response with respect to PAOD. A first next step will consist of the comparison of $t_{RF}$ measured with different LDPM systems. Other future studies should focus on the value of $t_{RF}$ in comparison with conventional diagnostic tools, as well as on the use of it in patients with both PAOD and diabetic mellitus.

3.5 References


