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Evaluation of Laser-Doppler-Fluxmetry for the diagnosis of microcirculatory disorders

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Abstract

Background: The laser Doppler fluxmetry (LDF) is a non-invasive method to assess skin blood perfusion, measuring the flow of blood cells inside a tissue volume without harming the tissue. In the diagnosis of skin circulation disorders, the results of the LDF measurement are generally used in such a way that "normal" (or non-ill) or "pathological" values are achieved by comparison with a reference sample, for example of apparently healthy subjects.

Material and Methods: In this study, the values of LDF for the diagnosis of microcirculatory disorders in patients with coronary artery disease (n=20) or in patients with microcirculatory disorders, already diagnosed by capillary microscopy (n=46), were examined.

Results: The mean values of LD amplitudes in the four frequency windows for patients with coronary artery disease were in the reference range. However, some of the patients showed reduced LD values: in eleven of the twenty patients, one or more mean LD amplitudes were below the reference range. Four of the eleven patients had pathologically decreased capillary erythrocyte velocities of

$\mathbf{v}_{ery}$=0.09-0.21 [mm/s], while the other seven patients had normal blood circulation at rest.
For all patients with a proven cutaneous microcirculatory disorder, the mean LD amplitude in at least one of the frequency windows FF2 to FF4 was pathologically reduced.
Conclusion: The Laser-Doppler fluxmetry method used in the study allows the reliable diagnosis of cutaneous microcirculatory disorders.

1. Introduction

Laser Doppler measurements are frequently used in research and clinical routine. The laser Doppler fluxmetry (LDF) is a noninvasive method of skin blood perfusion (blood flow, volume, and velocity) measuring the flow of blood cells inside a tissue volume without harming the tissue [1]. Blood cells moving within the tissue volume illuminated by the laser beam will cause a frequency shift of the reflected laser light useful for measuring the cutaneous blood flow in humans [2, 3] or animals [4, 5]. This approach was first used in the 1980s [6, 7, 8] and has since been applied for many tissues.
However, the diagnostic reliability or effectiveness of the LDF has not yet been sufficiently tested. It is indispensable in the context of medical diagnostics, but also for monitoring progress and therapy [9]. In the diagnostic process, the results of the LDF measurements are generally used in such a way that by comparison with a reference collective of apparently healthy subjects (for definition see e.g. [10, 11]), a decision is made which values are "normal" (or non-ill) and which are "pathological".
Therefore, a clear interpretation of LDF values of individual patients requires the knowledge of the reference range. Reference ranges are statistically determined from test results of healthy persons. The reference range is made up of 95% of all values measured in healthy individuals. The highest and lowest 2.5 percent are excluded. The additional measurements of patient collectives then allow the assessment of the diagnostic effectiveness of the measurement method.
In this study, the values of the LDF for the diagnosis of microcirculatory disorders in patients with coronary artery disease or in patients with microcirculatory disorders, already diagnosed by capillary microscopy, were examined.

2. Material and Methods

Three clinical studies were performed: 62 apparently healthy subjects, 20 patients with coronary artery disease diagnosed by coronary angiography and 46 patients with cutaneous microangiopathy suffering from arterial occlusive disease, diabetes mellitus, hypertension or lipid metabolism disorders diagnosed by periungual video-capillary microscopy were included.

LD System

The LDF-DOP-system (Laser Medicine Center Berlin) used is based on an IBM-compatible computer equipped with two additional expansion boards and further provided with two LD gauge heads [12]. Each gauge head contains a focused Laser-Diode with a wave length 670mm and an emission power of 5 mW. The reflected light was received by two photodiodes, electronically pre-amplified in the gauge head and conducted via cable to the control- and converter board. The software-controlled gauge heads, the tunable amplification and the real-time frequency analysis (FFT) of the signals with tunable filters enabled the immediate display and evaluation of the signals. All measurements were performed at room temperature without additional heating of the gauge head.

In a previous study, it could be shown that the LD-amplitudes were in good agreement with erythrocyte velocities in small channels *in vitro* (CV = 0.9) [13].

Capillary Video-Microscopy

The microscopic examinations were performed under standardized conditions, for details see previous publication [14]. Erythrocytes in nailfold capillaries were visualized with a stereomicroscope (Zeiss AG, Germany), which was connected to a video system. Due to the absorption of green light by hemoglobin, epi-illuminating light with a wavelength of 480 nm allowed erythrocyte detection. So-called plasma gaps are formed at sites without erythrocytes, and can be observed clearly. The mean capillary erythrocyte velocity $\mathbf{v}_{ery}$ was quantified by frame-to-frame analysis of the video pictures following the motion of plasma gaps using an image analysis system 'Cap-Image' (Zeintl Engineering Office, Heidelberg, Germany [15]). Details of the assessment and error analysis are described elsewhere [16].

The reference range of the mean erythrocyte velocity $\mathbf{v}_{ery}$ in the arteriolar leg of cutaneous capillaries under standardized resting conditions ranges between 0.38 mm/s and 0.94 with a mean value of 0.66±0.28 mm/s [17]. The reference range of the duration of the post-ischemic hyperemia (after 3 minutes of stasis) in cutaneous capillaries is 76-340 s with a mean value of 208±66 s [17].

Statistical Analysis

The performance of a method as a diagnostic criterion can be described by its sensitivity and specificity [9]. The specificity (also called the true negative rate) of a measurement procedure is defined as the rate with which the procedure detects non-cases (true-negative, TN) divided through the total number of real negative cases in the data (N):

Specificity [%] = (TN / N) ∗ 100

The sensitivity (also called the true positive rate) of a measurement method indicates the rate of true positive results (TP) divided by the number of real positive cases in the data (P) which is the sum of true-positive plus false negative test results:

Sensitivity [%] = TP / P

3. Results

Patients with coronary artery disease

20 patients with angiographically proven coronary artery disease were examined. The mean capillary erythrocyte velocity $\mathbf{v}_{ery}$ in nail fold capillaries was 0.54 ± 0.29 mm/s on average and was therefore not different from an age-matched control group of apparently healthy subjects. The individual values were Gaussian distributed (Kolmogoroff-Smirnow test; $p > 0.05$). However, the coefficient of variation of 45% was significantly higher than in healthy individuals.

Figure 1 shows the corresponding LD amplitudes (mean ± 2 sd) and the respective coefficients of variation VC for FF1 to FF4. The individual values of the LD amplitudes were Gaussian distributed in all frequency windows ($p > 0.05$).

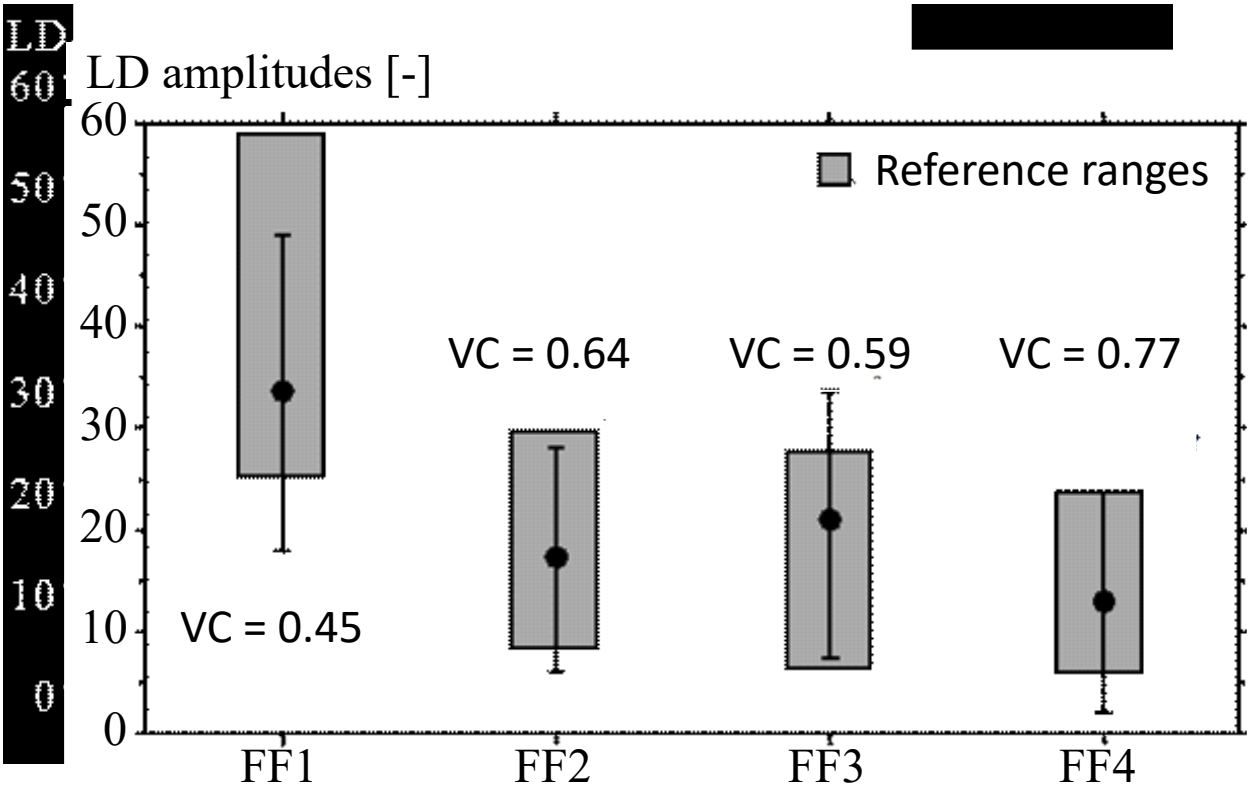


Figure 1: LD amplitudes (mean ± 2 sd) and coefficient of variation (VC) in patients with coronary artery disease in the four frequency windows

Mean values of LD amplitudes for patients with coronary artery disease were also within the reference ranges. However, some of the patients had lower values: 11 of the 20 patients had one or more mean LD amplitudes outside the reference range, see Table 1. Four of the 11 patients had a pathologically lowered erythrocyte velocity between 0.09 and 0.21 mm/s. (Values below the reference range and thus smaller than 0.38 mm/s were considered pathological.) The other seven patients had normal capillary erythrocyte velocities at rest.
In three of the patients with normal erythrocyte velocity, the LD amplitude in FF3 was increased.

Table 1: Mean LD amplitudes and $\mathbf{v}_{ery}$ in patients with coronary artery disease (-/↓/↑ = within/below/above the reference range).

Patient	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Σ
Very	–	↓	–	–	–	–	↓	–	–	–	–	–	–	↓	–	–	↓	–	–	–	4
FF1	–	↓	–	–	↓	–	↓	–	–	↓	↓	–	–	↓	–	–	↓	–	↓	–	8
FF2	–	↓	–	↓	–	–	↓	–	–	–	↓	–	↓	↓	–	–	↓	–	–	–	7
FF3	–	–	↑	–	–	–	–	↑	–	–	–	–	–	–	–	–	–	–	–	↑	3
FF4	–	–	–	–	–	–	↓	–	–	↓	–	–	–	–	–	↓	–	–	–	–	3

Table 2 shows the diagnostic quality criteria for the detection of a cutaneous microcirculatory disorder in patients with coronary artery disease for the four frequency windows of LD amplitudes.

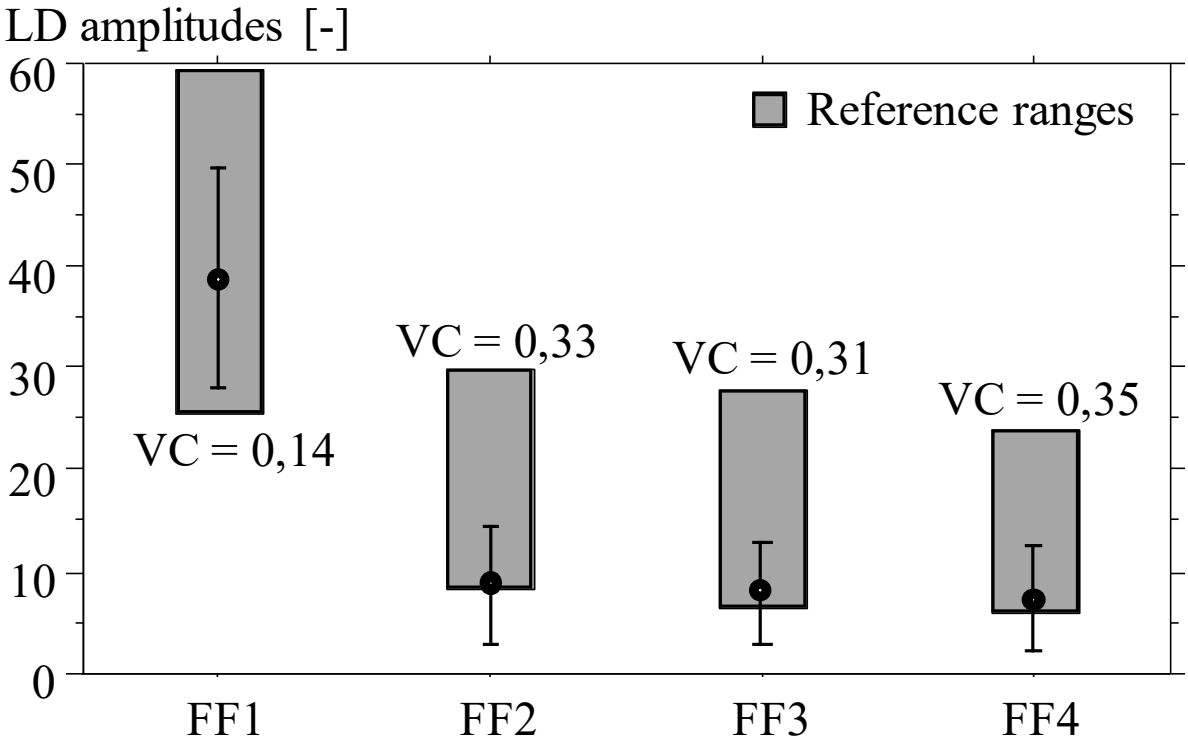
Table 2: Sensitivity, specificity and positive and negative predictive values for the LD-values in the four frequency windows for patients with peripheral arterial occlusive disease

[%]	FF1	FF2	FF3	FF4
Sensitivity	100.0	100.0	0.0	25.0
Specificity	75.0	81.3	81.3	87.5
Positive predictive value	50.0	57.1	0.0	33.3
Negative predictive value	100.0	100.0	76.5	82.4

Patients with cutaneous microcirculatory disorders

In 46 of 71 patients with arterial occlusive disease a cutaneous microcirculatory disorder was detected ($\mathbf{v}_{ery}$ less than 0.38 mm/s or duration of reactive hyperemia less than 76 s). In this patient group, the mean value of $\mathbf{v}_{ery} = 0.12 \pm 0.06$ mm/s was significantly below the reference range. The erythrocyte velocities in these patients differed significantly from those of apparently healthy subjects ($p = 0.03$). The VC of $\mathbf{v}_{ery}$ was 0.47 and was thus significantly higher than in the reference sample of healthy subjects of the same age. Figure 2 shows the corresponding LD amplitudes (mean ± 2 sd) and the VC for FF1 to FF4. The individual values of the LD amplitudes were Gaussian distributed in all frequency windows ($p > 0.05$).

Figure 2: LD amplitudes (mean ± 2 sd) and coefficients of variation (VC) in patients with cutaneous microcirculatory disorder (n=46)



The mean LD amplitudes of patients with microcirculation disorders were still within the reference range in all frequency windows - but at the lowest edge in FF2 to FF4. For all patients included here, the mean LD amplitude in at least one of the frequency windows FF2 to FF4 was pathologically reduced (see Table 3). In 23 patients (50 %) the LD amplitude in two of the three frequency windows was pathologically reduced. In four cases (9 %) the LD amplitude in FF2, FF3 and FF4 was pathologically reduced. The fact that in this group of patients the LD signal in FF1 was in the reference range in over 80% of cases and the amplitude in FF2, FF3 or FF4 was simultaneously reduced indicates a disproportionately high number of slowly flowing blood cells. At the same time, the proportion of fast-flowing cells was significantly lower than in healthy people or patients with coronary heart disease.

Table 3: Mean LD amplitudes and $\mathbf{v}_{ery}$ in patients with microcirculatory disorder (-/↓/↑= within / below / above the reference range).

Pat.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	Σ
Very	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	46
FF1	–	–	–	–	↓	–	–	–	–	–	–	↓	↓	–	–	–	–	–	–	–	↓	–	–	↓	–	–	–	–	–	–	–	–	↓	–	–	–	–	↓	–	–	↓	–	–	–	–	8
FF2	↓	–	–	↓	↓	↓	↓	–	↓	↓	–	↓	↓	↓	–	↓	–	↓	↓	–	↓	↓	–	↓	–	↓	↓	↓	↓	↓	–	–	–	↓	–	↓	↓	–	–	–	↓	↓	↓	–	↓	–	–	27
FF3	↓	–	↓	–	–	↓	–	↓	–	↓	↓	–	↓	↓	↓	–	↓	–	–	↓	↓	–	↓	↓	↓	–	–	↓	–	–	–	↓	↓	↓	–	↓	–	↓	–	↓	↓	–	–	–	↓	–	24
FF4	–	↓	–	–	–	–	↓	–	–	–	–	–	↓	–	–	–	↓	–	–	–	↓	–	↓	–	↓	↓	–	–	↓	↓	↓	–	↓	–	–	–	↓	–	–	↓	–	–	↓	↓	–	↓	17

Table 4 shows the diagnostic quality criteria for the recognition of a cutaneous microcirculatory disorder in patients with cutaneous microcirculatory disorders and concomitant atherosclerotic diseases for the four frequency windows of LD values.

Table 4: Sensitivity, specificity and positive and negative predictive values for the four LD-values for patients with cutaneous microcirculatory disorder

[%]	FF1	FF2	FF3	FF4
Sensitivity	17.4	58.7	52.2	36.9
Specificity	-	-	-	-
positive predictive value	100.0	100.0	100.0	100.0
negative predictive value	-	-	-	-

Since only patients with microcirculatory disorders were included, specificity and negative predictive values cannot be calculated (true negative cases are missing as only patients with microcirculatory disorders were included).

4. Discussion

Former studies have shown that endothelial dysfunction occurs in atherosclerotic patients at an early stage of the disease [18, 19], which can lead to reduced capillary blood flow and microcirculatory disorders [20, 21]. Such early changes detected by capillary microscopy were referred to as surrogate markers for the detection of subclinical atherosclerosis [19]. However, capillary microscopic tests are time-consuming and are therefore seldomly used in clinical routine.
Therefore, the present study examined whether such microcirculatory disorders described in the skin of patients with atherosclerotic diseases can be detected by

means of laser Doppler flux measurements, which can be carried out much faster and are clinically widespread. For this purpose, the patients - who already had a cutaneous microcirculatory disorder under rest conditions - were identified by capillary microscopic examination [14, 22].
The laser Doppler measurement system (DOP2) used in the study allows a differentiation of up to four speed ranges [12, 13] by numerical real-time frequency analysis of the signal in four adjustable frequency windows. The measurements were performed directly in nail fold capillaries in which the capillary erythrocyte velocities were measured and averaged in several capillaries under rest conditions for three minutes.

Conclusion
The LDF-DOP method allows the reliable diagnosis of cutaneous microcirculatory disorders in patients with atherosclerotic disease and microcirculatory disorder.

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