Summary of Ph.D. thesis

Assessment of endothelial function and microvascular reactivity with laser Doppler flowmetry

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I. Introduction

Scientific results of the past 25 years demonstrated that endothelium is not only a barrier, but it is an organ controlling the coagulation and fibrinolytic system, platelet and leukocyte adhesion and regulating the vascular tone. Dysfunction of endothelial cells plays an important role in the development of different pathological processes, like atherosclerosis, diabetic angiopathy and hypertension. In the last decades the research on the physiology and pathophysiology of microcirculation developed substantially. Laser Doppler flowmetry (LD) is a noninvasive, objective and reproducible method to estimate skin microcirculation in different vascular disorders. LD gives the possibility for the objective assessment of different treatments affecting the microcirculation. Iontophoresis coupled with LD makes it possible to assess the real-time changes of the skin blood flow after the administration of different vasoactive substances without systemic effects.

II. Objectives and thesis

The main objective of the study was the assessment of endothelial function and microvascular reactivity in different high risk states (hypertension, end stage renal disease, dyslipidemia, peripheral artery disease, diabetes mellitus) and the measurement of different (drug and non-drug-induced) therapeutic effects. For this purpose we have chosen LD, a method, that has not been used previously in the clinical praxis in Hungary.

We wanted to clarify whether our simple protocol of LD combined with iontophoresis is suitable for the demonstration of endothelial dysfunction in different cardiovascular risk states. Our goal was the determination of normal and pathological values of endothelium-dependent vasodilatation in the skin microcirculation. We wanted to evaluate whether the effect of different therapies, affecting the microcirculation, could be measured with our modified LD method.

1. We assumed that the endothelium-dependent vasodilatation to acetylcholine (ACh) is reduced in the skin microcirculation of essential hypertensive patients (*hypothesis 1*).
2. Our LD method is suitable to measure the improvement of endothelial function after a drug therapy (BRX-235) affecting the endothelium (hypothesis 2).

3. Cardiovascular disease is the major cause of mortality in hypertensive patients under chronic hemodialysis. We supposed that there is a difference in the endothelial function of essential hypertensive and hemodialysed hypertensive patients (hypothesis 3).

4. Hypercholesterolemia has a negative effect on endothelial function. We assumed that in the skin microcirculation of hypercholesterolemic patients the endothelium-dependent vasodilatation to acetylcholine (ACh) is reduced (hypothesis 4).

5. Our LD method is suitable to measure the improvement of endothelial function after statin therapy (hypothesis 5).

6. Homozygous familial hypercholesterolemia is associated with early and high cardiovascular morbidity and mortality. We supposed that the acute effect of LDL-apheresis (H.E.L.P. treatment) could be measured in the skin microcirculation by LD (hypothesis 6).

7. Peripheral artery disease (PAD) is one manifestation of atherothrombosis. We assumed that in these patients endothelial dysfunction can be demonstrated not only in the affected limb, but as a consequence of the generalized process also in the forearm skin (hypothesis 7).

8. Inhibition of platelet aggregation has been proved to decrease cardiovascular risk. We supposed that clopidogrel treatment can have a beneficial effect on vascular reactivity through decreased platelet derived growth factor (PDGF) release (hypothesis 8).

9. We supposed that in Fontaine II stage PAD patients the effect of pentoxifyllin therapy, which improves microcirculation through many different mechanism, could be measured by LD (hypothesis 9).

10. We presumed that LD is suitable for the objective assessment of the effect of lumbar sympathectomy in Fontaine III stage PAD patients (hypothesis 10).

11. We wanted to observe the effect of lumbar sympathectomy on different LD provocation tests mediated partly by the sympathetic nerve system (hypothesis 11).

12. The prognosis of diabetic patients is determined by vascular complications. We supposed that diabetic microangiopathy can be diagnosed earlier by LD comparing to other noninvasive methods, used in the clinical praxis (hypothesis 12).
III. Methods

Laser Doppler flowmetry

We performed our LD measurements with a one-channel Periflux 4001 equipment between 1993 and 1997, and with a two-channel Periflux 5001 and with the Perilont (PF480-1) micropharmacological system (Perimed, Sweden) since 1998. LD measurements were carried out in a temperature-controlled room (24±1°C), between 11 and 13 ours with the subjects in supine position, after 20 minutes acclimatisation period.

*Postocclusive reactive hyperaemia test (PORH)*

To assess endothelial function, we performed the PORH test on the forearm. During the PORH test after the registration (60 sec) of the baseline flow (RF) arterial occlusion was performed with a suprasystolic pressure (220 mmHg) by the help of a pneumatic cuff of a sphygmomanometer for 3 minutes, then after the release of the pressure we observed the skin hyperaemia (PF) on the volar surface of the left forearm 10 cms below the elbow with a standard LD probe. An other standard probe was put on the skin of the right forearm as a control.

In PAD patients we performed the PORH test on both lower limbs, over the inner malleolus or on the dorsum of the foot, under the I.toe.

*Venoarterial response (VAR)*

The LD probe was attached to the skin over the inner malleolus or on the dorsum of the foot, depending on the protocol. After registering the baseline flow in lying position we detected skin blood flow changes in sitting position for at least 2 minutes. We performed the test on both limbs. We evaluated both resting flow (RF) and sitting flow (SF).

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\text{VAR(\%)} = \frac{\text{RF-SF}}{\text{RF}} \times 100
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*Effect of local heating*

We applied the local heating test for the assessment of microvascular reactivity in PAD patients. Skin microcirculation was assessed on the dorsum of the foot with a special LD probe for heating. We registered the skin perfusion for 1 minute at unheated temperature and during local heating, when we used 44°C for 1 minute. After the heating we observed the perfusion till the end of the hyperemic response. We evaluated the resting flow (RF) and the peak flow (PF).
Assessment of the effect of iontophoretically delivered vasoactive substances with LD

Effect of acetylcholine (Ach):
The drug delivery electrode of the PeriIont system was filled with 140 µl Acetilcholine 1% (Clinalfa AG, Switzerland) and was attached with the special, heated laser probe to the volar surface of the right forearm. The dispersive electrode was attached to the volar aspect of the wrist to complete the circuit. We placed a control standard probe 4 cm laterally from the drug delivery electrode. The probe temperature was standardized to 32°C during drug tests. After registration of the baseline flow (60 sec) two doses of ACh was delivered using an anodal current (0.1 mA for 30 s and 0.16 mA for 30 s) with a 120 sec interval.

Effect of sodium nitroprusside (SNP):
The test was performed after the Ach test, the probe was attached min. 4 cm distance from the field of Ach iontophoresis. Using a new delivery electrode two doses of sodium nitroprusside 1% (Nitropress, ABBOTT, USA) was delivered using a catodal current (0.1 mA for 20 s and 0.1 mA for 30 s) with a 120 sec interval. The electric current strength chosen for our study were well tolerated by the subjects.

Measurement of endothelial markers

Plasma von Willebrand factor (vWF) level and activity
Plasma vWF level was determined by immune-turbidimetric method (STA-LIATEST® vWF), ristocetin cofactor activity by agglutination method (Von Willebrand Reagent, Dade Behring).

Plasma endothelin-1 (ET-1) and big-endothelin (big-ET) level
Plasma ET-1 and big-ET levels were determined by quantitative enzyme immune assay (Biomedica).

IV. Statistical evaluation
All results are presented as average ± SD. If the examined variables showed normal distribution and the sample size made it possible we used Student’s t-test comparing two samples or ANOVA and Scheffé’s post hoc analysis comparing more samples. To verify relation between different variables we applied Pearson’s linear correlation. When these conditions weren’t met nonparametric procedures were performed.
Results were compared in two different groups by Mann-Whitney’s U test, in the self control studies by Wilcoxon’s test. We used Friedman’s ANOVA for the analysis of the different LD tests. Differences were considered as statistically significant when P<0.05. Statistical analysis was performed by Statistica for Windows software.

V. Studies and results

1. Demonstration of endothelial dysfunction in essential hypertension. Effect of drug therapy on endothelial function.

1.1. Demonstration of endothelial dysfunction in essential hypertension.
The aim of the study was to investigate the endothelium-dependent vasodilatation in the forearm skin using two noninvasive laser Doppler applications in patients with essential hypertension and in normotensive control subjects. The study population included 44 patients with essential hypertension (EHT group) and 26 normotensive control subjects (CONT group). The vasodilatation to the two doses of ACh was significantly smaller in the EHT group than in the CONT group (199 ± 166, 561 ± 358; 444 ± 376, 804 ± 444 %, respectively; p<0.05). The vasodilatation to SNP was smaller but not significantly different in the EHT group from that in the CONT group (322 ± 264, 667 ± 411; 386 ± 267, 779 ± 366 %, respectively, ns). The PF and TP was significantly smaller during the PORH test in the EHT group than in the CONT group (PF: 300 ± 171; 410 ± 174 %, p<0.05). The vWF level was significantly higher within the normal range in the EHT group (p<0.05), and the ristocetin cofactor activity tended to be higher (not significant) as compared with the CONT group. The ET-1 and big-ET levels were in the normal range in the CONT group and elevated in the EHT group.

1.2. Effect of BRX-235 therapy on the endothelial function in essential hypertensive patients

BRX-235 acts on at least three different pathways involved in the pathogenesis of atherosclerosis, namely it is a heat shock protein (HSP) co-inducer, it has an antioxidant effect and it also decreases circulating
level of triglycerides (experimental results). The aim of our study was to evaluate the effect of a 12 week BRX-235 treatment on endothelial dysfunction in essential hypertensive patients with impaired (<5%) flow mediated dilatation (FMD) of the brachial artery. 21 non-smoker men with stable, treated hypertension were selected in the LD study, 19 completed the study. Patients must have had stable blood pressure (<160/95 mm Hg) and their antihypertensive medication had not been changed during the preceding 6 months. In these patients the average vasodilatation induced by the two doses of ACh was smaller compared to the vasodilatation of healthy subjects, observed in our previous study. Parameters of endothelium-dependent vasodilatation tended to be improved after 12 weeks BRX-235 therapy. 12 patients had decreased microvascular endothelium-dependent vasodilatation (<500%), demonstrated by LD at baseline. In these patients however there was a significant increase in the vasodilatation after the higher dose of ACh after 12 week BRX-235 therapy (136 ± 124, 328 ± 95; 264 ± 322, 597 ± 530 %, respectively; p<0.05).

2. Demonstration of endothelial dysfunction in patients with uremia and hypertension.

The aim of the study was to compare the endothelium-dependent and independent vasodilatation in the forearm skin microcirculation in patients with essential hypertension, in hypertensive hemodialysed patients and in normotensive control subjects. The study population included 34 patients with essential hypertension (EHT), 21 hemodialysed hypertensive patients (DHT) and 22 normotensive control subjects (NT). LD examination was performed between 11h and 13h in hypertensive and control patients, and 2 hours before dialysis treatment in hemodialysed hypertensives. The vasodilator response for the two doses of ACh was significantly smaller in the EHT group (192 ± 26% and 531 ± 47 %) than in the NT group (474 ± 83 % and 836 ± 97 %, p<0,05). In the DHT group (160 ± 26 % and 360 ± 67 %), the vasodilatation to the first dose of ACh was significantly smaller compared to the NT group (p<0,05), and the effect of the second dose of ACh was significantly smaller compared to both EHT and NT groups (p<0,05; p<0,01, respectively). The vasodilatation to SNP was smaller but not significantly different in the EHT group (335 ± 47 % and 660 ± 72 %) compared to the NT group.
(381 ± 60 % and 782 ± 81 %). The vasodilatation to SNP in the DHT group (186 ± 42 % and 379 ± 63 %) was significantly smaller compared to the NT and EHT groups (p<0.05). The percent changes of the peak flow were significantly smaller in the EHT (284 ± 26 %) and DHT (234 ± 48 %) groups than in the NT group (434 ± 36 %, p<0.05). The vWF level (174 ± 25 %) and the ristocetin cofactor activity (103 ± 10 %) were significantly higher in the DHT group than in the NT and the EHT group (p<0.01). There was no significant correlation between vWF levels and values for LD parameters, blood pressure, age and duration of hypertension. The ET-1 and big-ET levels were in the normal range in the NT group (0.46 ±0.55; 0.75 ±0.48 fmol/l). The ET-1 level was above the normal range both in the EHT (0.80 ±2.04 fmol/l) and DHT (1.4 ±2.87 fmol/l) groups. The big-ET level was above the normal range in the EHT group (1.29 ±1.2 fmol/l). The elevated big-ET level (2.67 ±1.61 fmol/l) in the DHT group was significantly higher compared to the NT (p<0.001) and EHT (p<0.01) groups, as well.

3. Assessment of microcirculation and endothelial function in different type hypercholesterolemias.

3.1. Effect of atorvastatin treatment on endothelial function in hypercholesterolemia.

In a multicenter, open study we investigated the effect of atorvastatin treatment on microvascular reactivity in 29 patients with high cardiovascular risk. Besides hypercholesterolemia (5.2-8.0 mmol/l) co-existing vascular disease or stable type 2 diabetes mellitus or impaired glucose tolerance were the inclusion criteria. After a 4-8 week screening period (diet only) the patients were treated with 10 mg atorvastatin for 8 weeks, thereafter, depending on the achieved cholesterol level, they continued the study with 10 mg atorvastatin for 4 weeks or 20 mg for 8 weeks period. Patients, who had achieved the target cholesterol level (<5.0 mmol/l) with the 20 mg treatment, continued with open label period for consecutive 4 weeks. The LD investigations were performed before atorvastatin treatment and also after 12 weeks treatment, if the target lipid level was achieved (20 patients). The average vasodilatation to the two doses of ACh was reduced as compared to the vasodilatation of healthy subjects, observed in our previous study (p<0.05). There was no significant change in the
endothelium-dependent vasodilatation after the 12 weeks atorvastatin treatment. The impaired endothelium-independent vasodilatation to SNP was not changed after the treatment. From the 29 patients, only 20 had impaired (<800%) vasodilatation to ACh at the beginning, and only 10 of them completed the study. In this smaller group of patients the vasodilatation tended to increase with both substances after the 12 week treatment, but this change wasn’t statistically significant.

3.2. Effect of H.E.L.P. treatment on the microcirculation in homozygous familial hypercholesterinaemia.

In the treatment of drug resistant familial hypercholesterolemic (FH) patients the therapeutic approaches are the extracorporal LDL-aphereses, one of them is the Heparin-induced Extracorporal LDL-cholesterol Precipitation (H.E.L.P.) system.

In Hungary the first homozygous FH child was treated with H.E.L.P. therapy in the 1st Dialysis Center of the Eurocare Nephrological Network. The boy aged 15 has in his medical history coronary endarterectomy and aortic valve implantation. Before the H.E.L.P. treatment the average serum cholesterol (Ch) level was 20.1 mM/l, the LDL-Ch level was 18.20 mM/l.

We’ve been treating the patient for three years (95 treatments with 1-3 weeks intervals) without any adverse events. Removing LDL-Ch, Lp (a) and fibrinogen from plasma, the H.E.L.P. treatment can result in significant improvements in hemorheology and also in endothelial function. Therefore we investigated the changes in peripheral microcirculation during the treatment. First we measured the skin blood flow at the first finger tip by laser Doppler during 20 H.E.L.P. treatments. After that we measured the effect of local heating on the skin blood flow on the dorsum of the foot before and after the treatment.

The average Ch and the LDL-Ch levels decreased significantly to 8.71±2.40 mM/l, and to 7.49±2.46 mM/l, respectively during the last two years. The levels of HDL-Ch and apo-A1 did not change but the apo-B level decreased significantly. The skin blood flow increased twofold during the apheresis (44.1 ± 32.2, 85.36 ±48.92 PU, p<0.01), and the improvements of these parameters of the microcirculation had significant negative correlations (p<0.05) with the serum Ch and fibrinogen levels.

The hyperaemic response after local heating was significantly higher
(65.69±20.43; 89.17±26.88 PU; p<0.05) and faster (slope of the curve: -0.29±0.21; -0.62±0.44; p<0.05) after the treatment.

4. Microcirculatory changes in peripheral artery disease

4.1. Effect of clopidogrel on the vascular dysfunction in peripheral artery disease

The aim of the study was to evaluate the effect of clopidogrel treatment on the endothelium-dependent and independent vasodilation in Fontaine II stage peripheral artery disease (PAD) patients. We investigated the correlation between microvascular reactivity and some markers of inflammation (hsCRP and sCD40L) as well.

39 patients (27 men, 12 women) were selected into the open labeled, prospective, self-controlled, phase IV, study. All included patients needed antiplatelet therapy as secondary prevention but because of inefficacy of aspirin, previous gastrointestinal ulcer, known asthma or allergy to aspirin their antiplatelet therapy should be changed to or started with clopidogrel.

LD performed before clopidogrel-treatment showed significantly reduced endothelium-dependent and independent vasodilatation. After the 12-week clopidogrel-treatment there was no significant change in the endothelium-dependent vasodilatation, meanwhile the endothelium-independent vasodilatation increased significantly (p=0.004). At the second measurement there was a significant difference (p=0.029) in the endothelium-dependent vasodilatation of patients with (CVPAD) and without (PAD) other previous cardiovascular events (AMI, TIA, stroke).

According to the high-risk study population the levels of hsCRP and sCD40L were elevated and did not changed significantly after the 12-weeks clopidogrel treatment. We did not found correlations between the changes in LD and the levels of sCD40L or hsCRP.

4.2. Effect of pentoxifyllin treatment on the microvascular reactivity in Fontaine II stage peripheral artery disease

We investigated the effect of pentoxifyllin 100 and 400 mg per day administered by intravenous infusion for 14 days. 40-40 patients suffering from PAD (Fontaine-II. stage) were randomly selected for the double-blind treatment in the two groups. Walking distance and
ankle/brachial index were measured on every third day, whereas skin blood flow at the dorsum of the foot in resting state and during postocclusive reactive hyperemia (PORH) was measured at baseline and on the 14th day of the therapy. The walking distance significantly increased in both groups. The ankle/brachial index didn’t change significantly. No significant differences have been found related to the walking distance and to the ankle/brachial index between the two groups. In the higher dose group, during the PORH-test the time to reach peak flow (TP) decreased significantly (p<0.05), demonstrating the improvement of microvascular reactivity.

4.3. Effect of lumbar sympathectomy in patients with Fontaine III stage peripheral artery disease

Lumbar sympathectomy (LS) is often the only alternative treatment which is capable to improve the distal circulation. We investigated whether LD is a useful method to measure the effect of the LS on the foot microcirculation. We evaluated the effect of LS on different LD provocation tests as well.

20 consecutive patients with severe ischemia of lower limbs (vascular reconstruction was not possible) were investigated with LD on both lower limb (on the dorsum of the foot) before and average 5 days (4-14 days) after the LS. After 20 minute resting two tests were performed: the hyperaemic response after local heating and postural venoarterial response (VAR).

On the operated side the average RF changed from 10,26±4,69 PU to 14,29±7,69 PU (t-test: p<0,01), the PF from 31,89±19,78 PU to 31,3±18,23 PU (t-test: non sign.), the PF% from 238±198 % to 156±147 % (t-test: p<0,05), the VAR from 16,7±45,3 % to 21,2±51 % (t-test:non sign.) and the T from 29,42±2,06 °C to 30,34±1,96 °C (t-test: p<0,05).

On the non-operated side there were no significant changes observed in any of these parameters.

5. Diagnosis of diabetic microangiopathy with Laser Doppler flowmetry

In diabetic patients impaired skin microcirculation is often present. Methods used for the investigation of the microcirculation so far are either expensive (e.g. fluorescens capillaroscopy) or have low sensitivity
(e.g., photoplethysmography). LD with different provocation tests can be suitable for the early diagnosis of diabetic microcirculatory changes.

We investigated the circulation of the lower limbs with continuous wave Doppler (CWD), photoplethysmography (PPG) and also with LD in type 1 (N=22) and type 2 (N=20) diabetic patients and also in non-diabetic control (N=20) patients.

No significant differences have been found between the three groups based on the macro- or microangiopathy confirmed with CWD (ankle/brachial index) and PPG (arterial curve) investigations. The venoarterial response (VAR) related to posture measured by LD was significantly higher in the control group compared to diabetic groups. The LD investigations showed a significant difference between the diabetic and control patients while other angiological investigating methods (CWD, PPG) did not show any difference.

VI. Summary of the results

With our simple method of laser Doppler flowmetry, which was introduced in our day-to-day clinical practice, similarly to other invasive and noninvasive procedures, we could demonstrate endothelial dysfunction in the forearm microcirculation of patients with hypertension, uremia and hypertension, hypercholesterolemia and peripheral artery disease. Like other authors we found the maximal endothelium-dependent and independent vasodilatation in healthy subjects approx. 700-800%. Based on measurements in 200 patients and 40 healthy control subjects we consider a maximal vasodilatation below 500% to Ach or SNP iontophoresis as pathological. Postocclusive reactive hyperaemia is partly mediated by the endothelium (induced by the shear stress) in skeletal muscle, but their mechanisms have not been specially studied in dermal microvessels. We found a 400% average peak flow in healthy subjects, and based on the investigation of hypertensive patients we consider a peak flow below 250% as pathological.

We demonstrated that in the skin microcirculation of essential hypertensive patients the endothelium-dependent vasodilatation to acetylcholine (ACh) and during PORH is reduced (hypothesis 1).
Our LD method was suitable to measure the improvement of endothelial function after BRX-235 therapy. BRX-235 had no effect on blood pressure, lipid and glucose metabolism; accordingly the improvement of endothelium-dependent vasodilatation could be the consequence of its heat shock protein (HSP) co-inducer and/or antioxidant effect (hypothesis 2). We demonstrated the decrease of endothelium-dependent and independent vasodilatation and increased serum level of biomarkers of endothelial damage in hemodialysed hypertensive patients. Our results confirmed a significant difference in the endothelial function of essential hypertensive and hemodialysed hypertensive patients (hypothesis 3).

We demonstrated that in the skin microcirculation of patients with hypercholesterolemia the endothelium-dependent vasodilatation to acetylcholine (ACh) is reduced (hypothesis 4). The coexisting impairment of endothelium-independent vasodilatation could be the consequence of disturbed glucose metabolism, as was confirmed with different invasive and noninvasive methods by other authors. The impaired SNP effect in our non-diabetic patients with vascular disease refers to the presence of vascular dysfunction. We couldn’t demonstrate the improvement of microvascular reactivity after statin therapy (hypothesis 5). The lack of the supposed increase of endothelium-dependent vasodilatation can be explained by the low dose of atorvastatin used or by the co-existing diabetes mellitus. Differences in vascular beds and mechanism involved in the hyperemic response can also play a role (macro vs. microcirculation, muscle vs. skin) in our results. In the CARATS study, similar to our findings, the vasodilatation to ACh didn’t improved in the coronary microcirculation after 6 month simvastatin therapy.

We demonstrated that in homozygous familial hypercholesterolemia the acute effect of LDL-apheresis (H.E.L.P. treatment) could be measured in the skin microcirculation by LD (hypothesis 6). The H.E.L.P. system decreased significantly the serum LDL-cholesterol, fibrinogen and Lp (a) levels and the changes of these parameters significantly correlated with the improvement of capillary perfusion. The significant increase of maximal vasodilatation to local heating supports the theory, that LDL-apheresis directly improves the endothelial function.
We confirmed that in peripheral artery disease (PAD) patients endothelial dysfunction can be demonstrated not only in the affected limb, but as a consequence of the generalized process also in the forearm skin. (hypothesis 7). The significantly decreased endothelium-dependent vasodilatation of PAD patients with a cardiovascular event supports the prognostic significance of endothelium-dependent vasodilatation. The elevated levels of hsCRP and sCD40L in our patients also refer to the high cardiovascular risk.

In our study after 12 week clopidogrel treatment the endothelium-dependent vasodilatation did not change but the endothelium-independent vasodilatation significantly increased (hypothesis 8). The average level of sCD40L did not change after the treatment, but the increased vasodilatation to SNP refers to the changed behavior of vascular smooth muscle cells, i.e. restoration of NO sensibility. In the later process a modification of CD40/CD40L interaction or the decreased platelet derived growth factor (PDGF) release also may have a role.

We demonstrated first time in the literature with LD the beneficial effect of pentoxifyllin therapy in Fontaine II stage PAD. (hypothesis 9).

We confirmed that LD is suitable for the objective assessment of the effect of lumbar sympathectomy in Fontaine III stage PAD patients (hypothesis 10). The degree of the maximal vasodilatation after local heating didn’t change after LS supporting the importance of non neurogen, NO mediated mechanism in this test. We demonstrated first time in the literature that in PAD patients the magnitude of the impaired VAR didn’t decreased after LS. This observation supports the greater importance of local neurogen and myogen mechanism compared to central sympatic innervation in the venoarterial response. (hypothesis 11).

We demonstrated that diabetic microangiopathy can be diagnosed in an earlier, asymptomatic stage with LD, then with other noninvasive methods, used in the clinical praxis (hypothesis 12). Our results confirmed that the most important factor in the development of the VAR is the position of the leg below 50 cm of the heart level, the sitting or standing position had no importance. We demonstrated the impaired VAR in type 2 diabetes mellitus, which was described only in type 1 diabetes by other authors.
The LD method, used and introduced by our group in Hungary, is noninvasive and technically easy to acquire. The computerized recording and evaluation of the perfusion curves guarantees the detached, investigator independent results. Using standard operation procedures the good reproducibility of the LD method was confirmed by many authors. The noninvasive assessment of endothelial dysfunction and impaired microvascular reactivity can support the selection of more efficient drug therapy in the primary and secondary prevention to decrease cardiovascular morbidity and mortality.
Publications in the topic


III. Farkas Katalin: Lézer Doppler áramlásmérés. LAM. 1998, 8, 4-12.


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