Ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: ready for the clinical arena?

Summary of PhD thesis

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I. Introduction

The vascular endothelium as a large paracrine organ senses and responds to a myriad of internal and external stimuli through complex cell membrane receptors and signal transduction mechanisms, leading to the synthesis and release of various vasoactive factors. The endothelium maintains normal vascular tone and blood fluidity, however, in the setting of traditional and recently discovered cardiovascular disease risk factors, such as smoking, aging, hypertension, hypercholesterolemia, diabetes and obesity, homocysteinemia, elevated C-reactive protein, chronic systemic infections, the endothelium loses its normal regulatory functions. Clinical syndromes such as stable and unstable angina, acute myocardial infarction, cardiac syndrome-X, claudication, and stroke also relate, in part, to a loss of endothelial control of vascular tone, thrombosis, and the composition of the vascular wall, thus, measurement of endothelial function in patients has emerged as a useful tool for vascular research.

Given this possible causal pathway from endothelial dysfunction to various vascular disease such as atherosclerosis, numerous methods have been employed to measure endothelial dysfunction in humans, each with its own advantages and disadvantages. There has been considerable interest in noninvasive examination of endothelium-dependent flow-mediated dilatation of the conduit brachial artery using vascular ultrasound. This response has been shown to depend in large part on NO synthesis, but also reflects release of other endothelium-derived vasodilators. The flow-mediated dilation of the brachial artery is induced by the shear stress during flow increase and the principal mediator is endothelium-derived nitrogen oxide. The absolute mean flow-mediated (FMD) increase in brachial artery diameter or the percent change in vessel diameter during reactive hyperaemia after release of a 4.5-minute occlusion may be the endpoint of measurements. Blood flow is measured as the mean Doppler flow velocity multiplied by the cross-sectional area.

Measuring FMD is attractive because it is noninvasive and allows repeated measurements.
A great interest exists in determining the clinical utility of brachial artery FMD. Investigators have hypothesized that endothelial function may serve as an integrating index of risk factor burden and genetic susceptibility, and that endothelial dysfunction will prove to be a preclinical marker of cardiovascular disease. Several studies suggest that the presence of endothelial dysfunction in the coronary circulation is an independent predictor of cardiovascular events. Numerous studies have demonstrated that brachial artery reactivity improves with risk factor modification and appropriate interventions targeted on the most likely underlying etiological factor. Therefore in the future, practitioners may use brachial artery FMD to assess response to drug therapy, as well.

II. Aims of the work

We hypothesized that the vascular ultrasound assessment of endothelium-dependent flow-mediated dilatation of the brachial artery is a useful tool for evaluation of effects of pharmacologic interventions in clinical setting. Therefore we aimed:

II.1. To compare methodologically the flow-mediated dilatation of the brachial artery with forearm microvascular reactivity in the assessment of endothelial dysfunction in essential hypertension

We investigated whether endothelium-dependent vasodilatation in the forearm microcirculation is related to endothelium-dependent flow-mediated dilation of the brachial artery in essential hypertensive patients and normotensive subjects.

II.2. To assess the effect of chronic statin therapy on exercise-induced ST segment depression and on systemic endothelial function in cardiac syndrome-X patients with mild hypercholesterolemia

Systemic endothelial dysfunction is associated with electrocardiographic positivity during stress testing even in the presence of normal coronary arteries. Statins can improve
endothelial functions due to its non-lipid lowering effects. Starting out from these points, we aimed in this part of the study, to determine whether statin therapy have any beneficial effect on systemic endothelial function and exercise induced ischaemia in cardiac syndrome-X patients with mild hypercholesterolemia.

II.3. To compare the effects of carvedilol and metoprolol on blood pressure, endothelial function and metabolic parameters in patients with hypertension and type-2 diabetes mellitus

The aim of this part of the study was to compare the effects of twelve weeks of antihypertensive therapy with carvedilol, a non-selective β-adrenoreceptor blocker with α1-blocking properties, with the selective β1-adrenergic receptor blocker metoprolol on the blood pressure, endothelial function and metabolic parameters of patients with hypertension and type 2 diabetes.

III. Patients and methods

III.1. For noninvasive assessment of endothelial dysfunction in essential hypertension; comparison of the flow-mediated dilatation of the brachial artery with forearm microvascular reactivity the patient population and methods used were as follows:

III.1.1 Patients

The study population included 22 patients with essential hypertension (mean age 50.5 ± 6.9 years, males, systolic blood pressure 141.2 ± 12.25 mmHg, diastolic blood pressure 85.8 ± 7.02 mmHg) and 11 normotensive control subjects (mean age 41± 9 years, males, systolic blood pressure 116.6 ± 10 mmHg, diastolic blood pressure 68.3 ± 5.59 mmHg). Patients older than 60 years, smokers or those with diabetes mellitus or with elevated (>6.5 mmol/l) total cholesterol plasma levels were excluded. No patient was on nitrate treatment, and the antihypertensive therapy had been unchanged for at least 6 months prior to inclusion. Essential hypertensive patients were screened for FMD of the brachial artery and only patients with FMD < 5 %, confirmed impaired endothelial function, were included.
First the laser Doppler flowmetry, thereafter the vascular ultrasound examination were done on the same day.

III.1.2 Laser Doppler flowmetry.

A laser Doppler instrument (Periflux 5001, wavelength 780 nm) and a micropharmacology system (Perilont) were used for noninvasive and continuous measurement of perfusion changes during vascular provocations in the skin (Perimed AB, Järfälla, Sweden). The drug delivery electrode was filled with 140 µl Acetylcholine 1% (Clinalfa AG, Swiss) and was attached with the laser probe to the volar surface of the left 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. After registration of the baseline flow (60 sec) two doses of acetylcholine was delivered using an anodal current (0.1 mA for 30 s and 0.16 mA for 30 s) with a 120 sec interval. With a new delivery electrode two doses of sodium nitroprusside 1% (Nitropress, ABBOTT, USA) were delivered using a cathodal current (0.1 mA for 20 s and 0.1 mA for 30 s) with a 120 sec interval. During the postocclusive reactive hyperemia test after the registration of the baseline flow (60 sec) arterial occlusion was performed with suprasystolic pressure by the help of a pneumatic cuff of a sphygmomanometer for 3 minutes (biological zero), after the release of the pressure we measured the skin hyperaemia on the volar surface of the left forearm 10 cms below the elbow with a standard laser Doppler probe. Another standard probe was put on the skin of the left forearm as a control. The laser Doppler output is semiquantitative and expressed in PU of output voltage (1 PU = 10 mV) in accordance with general consensus (European Laser Doppler Users Groups, London 1992). The laser Doppler outputs were recorded continuously by an interfaced computer with acquisition software (Perisoft). Calibration was performed by a device composed of colloidal latex particles, the Brownian motion of which provides the standard value. The magnitude of the changes in skin perfusion was calculated as the ratio between peak and mean baseline perfusions.
III.1.3. Evaluation of endothelial function by flow-mediated brachial artery dilatation

The evaluation of endothelial function was performed according to the method described by Celermayer et al and as stated in the report of the International Brachial Artery Reactivity Task Force. All patients were studied minimum 10 hours after the last meal. The patient lay supine in a quiet, temperature-controlled room (20-25°C) for 10 minutes before the study with the right arm gently immobilized in extension. The diameter of the brachial artery was measured from 2D ultrasound images using a commercially available system (Hewlett-Packard SONOS 2000, 7.5-MHz probe). Images were recorded on an S-VHS tape. In each study, scans were taken at rest, during reactive hyperemia, at rest again and after sublingual nitrates. The brachial artery on the dominant arm was scanned in longitudinal section, 2 to 15 cm above the antecubital fossa. The settings were adjusted to optimize the lumen/arterial wall interface and held constant during testing. Measurements were taken from the anterior to the posterior “m” line at end diastole, incident with the R-wave on the electrocardiogram, at a fix distance from an anatomic marker, such as a bifurcation. Three cardiac cycles were analyzed for each scan and measurements were averaged. Following the baseline measurements a forearm cuff occlusion was obtained for 4.5 minutes below the elbow by inflation of pneumatic tourniquet to a pressure of 270 mmHg. The artery diameters were performed within one minute after cuff deflation during reactive hyperemia. Following 10 minutes of recovery phase a resting scan was repeated. Sublingual nitrates (400 µg of glyceryl trinitrate) were then administered. The last set of scans was performed 3 minutes after nitrate intake, during nitrate-mediated dilatation (NMD). The maximum FMD and NMD diameter measurements were calculated as the average of the three consecutive maximum diameter measurements after hyperemia and sublingual nitrate. Then the FMD and NMD were calculated as the percent change in diameter compared with baseline resting diameters. The images were read independently by 2 separate observers blinded to the patient identity and the study phase.
III.2. For the assessment of the effect of chronic statin therapy on exercise-induced ST segment depression and on systemic endothelial function in cardiac syndrome-X patients with mild hypercholesterolemia the patient population, the study design and methods used were the following:

III.2.1. Patient population

Patient population consisted of 40 prospectively-enrolled cardiac syndrome-X patients with mild hypercholesterolemia (mean age 55.7 ± 1.05 years, 25 males). The diagnosis of cardiac syndrome-X was based on the presence of typical angina pectoris, transient > 1 mm ST segment depression during exercise stress test, transient perfusion defect during myocardial perfusion scintigraphy, angiographically normal coronary arteries in the absence of coronary artery spasm (excluded by hyperventilation), left ventricular hypertrophy and systemic hypertension. The patients underwent thallium scintigraphy and coronary angiography with hyperventilation test for exclusion of arteriospasm in 1-year period before enrolling to the study. Myocardial perfusion scintigrams with transient perfusion defects were considered as positive. Angiograms with visually no wall irregularities were accepted as normal. Patients were eligible if they met the following inclusion criteria: a normal coronary angiogram; positive exercise ECG test; positive myocardial perfusion scintigram; normal regional and global resting left ventricular function; mildly elevated total serum-cholesterol level (>5.2 mmol/L). The exclusion criteria were previous myocardial infarction, valvular heart disease including mitral valve prolapse, congestive heart failure, cardiomyopathy, sinus node dysfunction or conducting disturbances (including left bundle branch block), diabetes mellitus, impaired renal or liver functions, smoking. All patients received and were taught American Heart Association steps 2 diet and received antianginal treatment consisted of beta-blockers and/or calcium antagonists. None of them were on long-acting nitrate, NO-donor, trimetazidine and ACE-inhibitor therapy. Only sublingual nitrates were allowed for the relief of chest pain during
the study. Medications were held constant through the entire study. Postmenopausal women were not on hormone replacement therapy.

**III.2.2. Study design**

At baseline and at the end of the study laboratory measurements, exercise ECG, and FMD studies were done. After baseline measurements the patients were randomized to placebo (n=20) or simvastatin 20 mg daily at bedtime (n=20) for a duration of 12 weeks.

**III.2.3. Laboratory measurements**

Blood samples were analyzed for total cholesterol, high-density lipoprotein cholesterol and triglycerides. Low-density lipoprotein cholesterol levels were determined by using the formula of Friedewald. The measurements were expressed in mmol/L.

**III.2.4. Evaluation of endothelial function by flow-mediated brachial artery dilatation**

The evaluation of endothelial function was performed according to the method as described above under III.1.3.

**III.2.5. Exercise stress test**

All patients performed a multistage treadmill test according to modified Bruce protocol. Patients were not allowed to take beta-blockers and sublingual nitrate 24 h before the exercise stress tests. Blood pressure, heart rate and 12-lead ECGs were recorded at rest, at 1-minute intervals during exercise, at peak exercise and for at least 5 min in the recovery phase. The ECG and the changes of ST segment were continuously displayed and measured automatically by a computer assisted system (Cardiovit AT-104 treadmill, Schiller) in all 12 leads. The reaching of age-specific target heart rate or the development of symptoms necessitating termination of the test were taken as end-points of exercise-stress test. The appearance of downsloping or horizontal ST segment depression >1 mm or 0.1 mV 0.08 sec after the J point was taken as evidence of a positive exercise electrocardiography test. The time to >1 mm ST segment depression was defined.
III.3. For comparing of the effects of carvedilol and metoprolol on blood pressure and endothelial function in patients with hypertension and type-2 diabetes mellitus the patient population, the study design and methods used were the following:

### III.3.1. Patient population

The patient population was recruited from two participating outpatient care centers. 36 patients with type 2 diabetes mellitus and mild or moderate essential hypertension (systolic blood pressure > 130 and < 180 mmHg, diastolic blood pressure >85 and < 110 mmHg on repeated measurements) were recruited to the study. Patients were eligible if they met the following inclusion criteria: adequate glycemic control (glycosylated hemoglobin < 7%) with diet or oral hypoglycemic agents, unchanged and constant antihypertensive treatment for 6 months before enrollment (consisted of enalapril, β-antagonists, diuretics), total serum-cholesterol level <6.5 mmol/L, low-density lipoprotein cholesterol level <3.4 mmol/L, triglyceride level <2.3 mmol/L and the flow mediated dilatation of the brachial artery, measured by vascular ultrasound < 5%, confirmed impaired endothelial function. The exclusion criteria were previous myocardial infarction and unstable angina, stroke within the preceding 6 months, stable angina, angiographically assessed coronary artery disease, secondary hypertension, known peripheral artery disease, impaired renal or liver function tests, microalbuminuria, nitrate, lipid lowering, hormone replacement therapy, known intolerance to nitrate, contraindications for receiving β-blockers, smoking, childbearing age in women not using reliable contraception.

### III.3.2. Study design

Our study had a prospective, randomized, open design for parallel study groups. Laboratory tests were done and endothelial function was evaluated by FMD during the initial screening and after the treatment period. The patients were randomized either to carvedilol 2x12.5 mg starting dose daily (n=19) or metoprolol succinate 1x50 mg starting dose daily (n=17). The dose of the study drug was doubled after 4 weeks if the patient had not reached the blood pressure goal of 130/80 mmHg. Side effects, concomitant diseases, blood pressure and
ECG were assessed by interview and physical examination every fourth week during the study. Baseline medications were held constant through the entire study period.

III.3. Blood pressure measurement
Blood pressure and heart rate were recorded in the sitting position, after at least 10 min of rest. Systolic blood pressure was recorded as the pressure noted when Korotkoff sound I appeared and diastolic blood pressure when Korotkoff sound V disappeared. Blood pressure was measured in triplicates with a sphygmomanometer on the same arm and by the same investigator, and the mean of these values was calculated.

III.3.4. Laboratory measurement
Blood samples were analyzed for laboratory measurements (glycosylated hemoglobin, plasma glucose, total cholesterol, low density lipoprotein cholesterol, high density lipoprotein cholesterol, triglyceride, liver enzyme levels, creatinin concentrations) by standard clinical laboratory methods. The tissue plasminogen activator, plasminogen activator inhibitor-1 and vascular cell adhesion molecule-1 levels were measured by enzyme-linked immunosorbent assay (Asserachrom tPA and PAI-1 kits, and h-sVCAM-1 ELISA kit, Roche Inc, France).

III.3.5. Evaluation of endothelial function by flow-mediated dilatation
The evaluation of endothelial function was performed according to the method as described above under III.1.3.

III.4. Statistical analysis
The results are expressed as mean ± SD. Comparisons were made using the paired samples Student’s t-test when assessing the differences at randomization and at study end. To assess correlation between data Pearson correlation was used. Differences were considered statistically significant at the level of $p<0.05$ (two-sided).
IV. Results

IV.1. Noninvasive assessment of endothelial dysfunction in essential hypertension; comparison of the flow mediated dilatation of the brachial artery with forearm microvascular reactivity

Endothelial dysfunction was detectable both with laser doppler flowmetry and flow-mediated dilatation of brachial artery in essential hypertension.

IV.1.1. Laser Doppler flowmetry.

Basal forearm skin perfusion was not significantly different in the patients compared with the control subjects. Iontophoresis of acetylcholine produced a significant dose-dependent increase in cutaneous blood flow both in essential hypertensive patients and control subjects, but the vasodilation to the two doses of acetylcholine was significantly lower in the essential hypertensive patients group than in the normotensive group (p<0.01; p<0.05). The vasodilation to sodium nitroprusside was lower but not significantly different in the essential hypertensive patients group compared with the normotensive subjects group. The three minute occlusion of the brachial artery by a pneumatic cuff produced a significant increase in the cutaneous blood flow after cuff release. The vasodilation was significantly lower in the essential hypertensive patients than in normotensive subjects (p<0.01). Mean biological zero was not significantly different between the two groups.

IV.1.2. Flow-mediated dilation and nitrate-mediated dilation

The FMD of the brachial artery was significantly lower in essential hypertensive patients than in normotensive subjects (3.98±2.4% vs. 9.3 ± 4.9%, p<0.001). The NMD was lower but not significantly different in essential hypertensive patients compared with normotensive subjects (14.46 ± 6.59% vs. 18.21 ± 9.7%, p=ns). There was no significant
relationship either between maximal response to acetylcholine and FMD ($r = 0.28$), or between postocclusive reactive hyperemia and FMD ($r=0.01$). No relation was found between response to sodium nitroprusside and glyceryl trinitrate ($r = 0.31$).

IV.2. Effects of chronic statin therapy on exercise-induced ST segment depression and endothelial function in cardiac syndrome-X patients.

The chronic statin therapy resulted in prolonged time to > 1 mm ST segment depression during exercise stress test, acted beneficially not only on lipid parameters, but also on endothelial function in cardiac syndrome-X patients with mild hypercholesterolemia.

IV.2.1. Lipid levels

Cholesterol levels were not statistically changed in the placebo group. Total serum cholesterol levels decreased significantly by 26 % (5.86 ± 0.13 mmol/L vs. 4.63 ± 0.37 mmol/L, $P< 0.0001$) following 12 weeks of simvastatin treatment and 38 % reduction of low-density lipoprotein levels occurred (4.47 ± 0.12 mmol/L vs. 3.22 ± 0.37 mmol/L, $P<0.0001$). High-density lipoprotein levels increased significantly by 7 % (0.99±0.05 mmol/L vs.1.05 ± 0.05 mmol/L, $P<0.0001$) in the simvastatin group. Triglyceride levels were not significantly modified throughout the course of the study in both groups.

IV.2.2. Flow-mediated dilatation and nitrate-mediated dilatation

Brachial artery flow mediated vasodilation did not change significantly in the placebo group (4.07 ± 0.12 % vs. 4.06 ± 0.15, P= ns), but increased significantly (52%) in the simvastatin group (4.01 ± 0.91 % vs. 6.12 ± 0.79 %, $P<0.0001$). Responses to glyceryl trinitrate were similar during the time course of the study in both groups.

IV.2.3. Exercise stress test

No significant differences were present in the time to >1 mm ST segment depression during stress test after 12 weeks in the placebo group. It was significantly longer by the end of the study in the simvastatin group (4.45 ± 0.39 min vs. 5.33 ± 0.27 min, $P<0.0001$). In 4 patients no inducible ischaemia could be detected during the stress test in the simvastatin
group by the end of the study and the exercise was finished due to reaching of age-specific target heart rate. All patients who had significantly longer time to >1 mm ST segment depression during exercise stress test demonstrated an improvement in flow-mediated dilatation. There was no significant correlation detected between the improvement in percent FMD and the electrocardiogram parameters and fall in total- and low density lipoprotein cholesterol levels.

IV. 3. Comparison of the effects of carvedilol and metoprolol on blood pressure and endothelial function in patients with hypertension and type-2 diabetes mellitus.

Carvedilol treatment significantly improved the endothelial vasomotor function in patients with hypertension and type 2 diabetes, but the effect of metoprolol was not significant. There were no significant differences between the two treatment groups in this respect. The doses of the study drugs were doubled after 4 weeks if the patient had not reached the blood pressure goal of 130/80 mmHg. The mean dose of carvedilol was 35.5±12.7 mg, the mean dose of metoprolol succinate was 67.6±24.6 mg during the study period. There were no significant differences between the flow-mediated dilatation values measured one week prior to randomization, at screening (W-1), and at randomization (W0), in both groups (W-1 2.71±1.12 % vs. W0 2.72±1.11% in carvedilol group, W-1 3.41±0.97% vs. W0 3.42±0.89% in metoprolol group), which supports the reproducibility and repeatibility of the method used. A twelve week treatment with carvedilol significantly improved FMD (W0 2.72±1.11% vs. W12 5.50±2.28%, p<0.0001), while treatment with metoprolol did not change it significantly (W0 3.42±0.89% vs. W12 3.99±1.18%, p=ns). Nitrate mediated dilatation, which is endothelium independent, was not influenced by either treatment (W0 13.35±4.41 % vs. W12
13.31±3.14%, p=ns in carvedilol group, W0 12.96±3.51% vs. W12 12.15±2.61 %, p=ns in metoprolol group.
Carbohydrate or lipid parameters did not change significantly throughout the study period in both groups. The plasma levels of tissue plasminogen activator and plasminogen activator inhibitor-1 were elevated at the randomization, but did not change after treatment in both groups. The vascular cell adhesion molecule-1 levels were in normal range and also unchanged for the study end in both groups.

V. Summary and conclusions
As the flow-mediated vasodilation of brachial artery has emerged as an accessible indicator of endothelial function we aimed to investigate its use for non-invasive evaluation of endothelial dysfunction and assessment of effects of new pharmacologic interventions in clinical settings.
Initially, we designed a comparative study to correlate the ability of two different non-invasive methods, such as flow mediated dilatation of the brachial artery with the laser Doppler flowmetry in the detection of endothelial vasomotor function in patients with hypertension and normotensive subjects. Following, we use the flow-mediated brachial artery vasodilation for evaluation of effects of chronic simvastatin therapy on systemic endothelial function and exercise-induced ST segment depression in cardiac syndrome-X patients with mild hypercholesterolemia. Finally, we compare the effects of carvedilol and metoprolol on blood pressure, endothelial function and metabolic parameters in patients with hypertension and type-2 diabetes mellitus.

On the basis of our results:
1. Endothelial dysfunction was detectable both with laser Doppler flowmetry and flow-mediated vasodilation of brachial artery in essential hypertension, but the endothelium-dependent vasodilation in forearm microcirculation was not related to FMD of the brachial artery.
2. Twelve weeks of simvastatin therapy exerts beneficial effects not only on lipid parameters, but also on endothelial function, detected by FMD, in cardiac syndrome-X patients with mild hypercholesterolemia. The prolonged time to > 1 mm ST segment depression during exercise stress test reflected the improvement of endothelial function.

3. Twelve weeks of treatment with carvedilol significantly improved endothelial (vasomotor) function, assessed by FMD, but had not significant effects on endothelial markers in patients with type 2 diabetes and hypertension compared to metoprolol succinate.

**In conclusion,** the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery is utilisable for the clinical evaluation of endothelial function and effects of appropriate pharmacologic interventions, targeted on the underlying etiological factors, in clinical settings.
List of publications

Full papers:


Abstracts:


