Aus dem Institut für Vegetative Physiologie

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DISSERTATION

Investigation of the therapeutic effect of External Pneumatic Counterpulsation on the myocardial and cerebrovascular arterial circulation

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Contents

Abstract English: Introduction: Arteriogenesis is the rapid proliferation of pre-existing collateral arteries and is nature´s most efficient rescue mechanisms to compensate for the loss of arterial inflow under conditions of chronic obstructive atherosclerotic disease. It differs from angiogenesis in fundamental aspects: 1) The speed of arterial growth in diameter can compensate for the deficit in blood flow of large conductance arteries, which is not the case in angiogenic sprouting. 2) Arteriogenesis may be located at non-ischemic zones and results in efficient collateral conductance arteries, whereas angiogenesis is located in the region of ischemia 3) The increase in biomechanical shear-rate is currently seen as the key mechanism to stimulate collateral growth. Taking these fundamentals into account our clinical trials in this thesis focused on following question: Is arteriogenesis a potential therapeutic substrate to enhance myocardial tissue perfusion in patients? Which parameters have to be taken into account to evaluate the collateral macro-circulation as well microcirculation? Is it possible to transfer the concept of shear-stress driven arteriogenesis into the cerebral circulation? **Methods:** To detect the capacity of the myocardial perfusion and collateral circulation invasive read-out parameters - pressure derived collateral flow index, CFIp- and the microcirculatory index (IMR) were applied; to assess functional relevance of coronary stenosis, fractional flow reserve was used. Further cerebrovascular blood flow at rest and under ECP-therapy was investigated with transcranial-dopplersonography. **Results and Conclusion:** The results of these trials may be summarized as followed: Upon pre existing coronary stenosis ECP significantly improves collateral conductance and enhances flow reserve (the CFIp improved significantly from 0.08 +/- 0.01 to 0.15 +/- 0.02; $P < 0.001$; FFR-Index improved from 0.68±0.03 to 0.79±0.03; $p=0.001$); while in the control group no change was observed. In patients with severe epicardial stenosis microcirculation can only be assessed reliably if collateral circulation is taken into account (CFIp $r = 0.3$, $p = 0.046$; FFR $r = -0.44$, $p = c0.03$). The findings of ECP treatment in healthy probands are summarized as: ECP does not enhance cerebral blood flow (59±10 vs. 58±13cm/s, n.s.) but flow velocities through-out the ECP therapy are increased compared to rest/baseline (increased shear-rate). Thus this thesis provides 3 important novel findings: 1.External counterpulsation can induce adaptive collateral-growth in patients with CAD and improve myocardial perfusion. 2. Given significant epicardial stenosis microcirculatory indices are efficient in detecting myocardial microcirculatory if collateral circulation in the region of interest is taken into account 3. ECP-treatment increases flow-velocities in the cerebral blood flow - giving rise to the assumption that ECP might induce cerebrovascular arteriogenesis.

Abstrakt deutsch: Hintergrund: Arteriogenese ist ein positives *outward-remodeling* von präexistent angelegten kollateralen Anastomosen. Dieser Prozess zählt zu den effizientesten *Rescue*-Mechanismen des Körpers, einen kompromittierten Blutfluss wiederherzustellen. Arteriogenese unterscheidet sich wesentlich vom Prozess der Angiogenese : 1) Die Geschwindigkeit der kollateralen Proliferation kann einen gestörten Blutfluss in kurzer Zeit wiederherstellen. 2) Arteriogenese findet zumeist in Regionen statt, wo lediglich Blutdruckgradienten vorliegen, nicht aber eine Gewebeischämie. Letztere wiederum ist der wesentliche Auslöser von Angiogenese 3) die Zunahme der intraarteriellen Scherrate ist der wesentliche arteriogene Biomechanismus. Nimmt man diese Aspekte der Arteriogenese als experimentelle Basis von kollateralem Wachstum, haben wir uns in der vorliegenden Dissertation mit folgenden Fragen beschäftigt:1.) Sind Kollateralgefäße ein klinisches Substrat, womit man therapeutisches Wachstum anregen kann? 2.) Welche Parameter eignen sich therapeutische arteriogene Effekte bzw. auch die der Mikrozirkulation zu erfassen? 3.) Kann man das Therapiekonzept der Arteriogenese auf das Gehirn übertragen? 4.) Welche Rolle könnte die Gegenpulsation bei der therapeutischen Erhöhung der Scherrate Rate spielen? Methodisch kamen folgende Techniken zum Einsatz: Kollateraler-Index (CFIp) zur Evaluation der kollateralen Konduktanz, Fraktionelle Flussreserve (FFR) zur Beurteilung der hämodynamischen Relevanz einer Stenose, der mikrozirkulatorische Index (IMR) auf mikrozirkulatorischer Ebene. Neurologisch untersuchten wir den Effekt der Gegenpulsation auf die zerebrovaskuläre Zirkulation mittels Doppler Fluss Analyse. Ergebnisse/Zusammenfassung: ECP verbessert die kollaterale Zirkulation. Dieser Effekt wurde bei gleichbleibender zugrundeliegender Stenose gemessen: Der CFIp verbesserte sich signifikant von 0.08+/-0.01 auf 0.15+/-0.02; $P < 0.001$. In der Kontrollgruppe hingegen keinen Veränderungen. Dazu passend verbessert sich der FFR-Index in der ECP Gruppe von 0.68±0.03 auf 0.79±0.03 ($p=0.001$), aber nicht in der Kontrollgruppe ($p=0.4$). Desweiteren fokussierten wir auf die optimale Detektion des IMR bei Patienten mit einer stabilen KHK. Hierbei zeigte sich, dass je besser die Kollateralisierung war, und je höhergradiger die Stenose, umso mehr war der IMR vom gemessenen CFIp abhängig. Die Überschätzung des IMR korrelierte dabei positiv mit dem CFIp ($r=0.3$, $p=0.046$). Im letzten Teil unserer Versuche untersuchen wir den Effekt der ECP auf die zerebrovaskuläre Zirkulation. Interessanterweise zeigte sich, dass sich die mittlere Blutfließgeschwindigkeit unter ECP aufgrund der zerebrovaskuläre Autoregulation nicht verändert. Analysiert man jedoch die Blutflussgeschwindigkeitsprofile pro Herzzyklus, so zeigte sich eine erhöhte Beschleunigung im arteriellen Einstroms bei gleichbleibender Gesamtgeschwindigkeit. Dieser Befund ist von hoher Bedeutung da durch erhöhte Scherraten zerebrovaskuläre Arteriogenese induziert werden kann.

Introduction:

The causes and consequences of the chronic atherosclerotic disease of the arterial tree remains to be the number one reason for hospital admissions in western countries. Since the first pathological changes within the structural context of the artery do not lead in the majority of cases to clinical symptoms, patients often present to the clinic, once the chronic disease has progressed significantly. At this time-point not only endothelial dysfunction but stenosis or occlusion of conductance arteries can be detected macroscopically and lead to the fatal consequences of coronary heart disease, peripheral arterial disease as well as cerebrovascular disease. Besides optimal medical treatment and active life-style (Boden et al, 2007) the current state-of-the-art interventions is to revascularize occlusive coronary artery disease (CAD) via percutaneous transluminal coronary angioplasty (PTCA) or the coronary artery bypass graft operation (CABG). However in the late nineties novel strategies arose, termed arteriogenesis (the growth of collateral arteries) to therapeutically treat patients with chronic vascular disease. The objective of this approach was the therapeutic stimulation of pre-existing collateral arteries to enhance the tissue perfusion of vascular territories distal to arterial occlusion or stenosis. The physiological basis of this process is the increase in fluid shear stress and fluid shear rates across newly recruited collateral networks and its endothelium respectively. This endothelial cell activation leads to increased expression of nitric oxide synthetase (eNOS) and phosphorylated eNOS (P-eNOS) proteins as well as matrix metalloproteinase-2 (MMP-2). Mononuclear cells invade the collateral arterial tissue from the arterial lumen side as well as the venous flow tract and lead to a controlled inflammatory aspect of arterial tissue remodeling. Under optimal conditions (absence of risk factors such as diabetes or relevant arterial hypertension) mature collateral arteries may be the result of this arteriogenic process. In contrast to angiogenesis - the sprouting of capillaries - arteriogenesis occurs in the absence of tissue ischemia and may restore a compromised blood flow with collateral arterial inflow. This is not the case once capillary sprouting is induced, since an arterial blood flow cannot be compensated be capillary non-contractile endothelial tubes.

In this thesis we focus on three primarily important issues: Is it possible to enhance arteriogenesis non-invasively by means of external counterpulsation ? Is the microcirculatory resistance index a valuable tool to detect changes in myocardial microcirculation independent of collateral flow? Finally we will evaluate ECP as a novel potential clinical treatment strategies in the context of cerebrovascular disease and patients with vascular disease.

Publication 1: Improvement of fractional flow reserve and collateral flow by treatment with external counterpulsation- Original paper

http://dx.doi.org/10.1111/j.1365-2362.2009.02192.x

Introduction:

The chronic atherosclerosis of the coronary arterial tree leads to stenosis and occlusion of the arterial lumen und thus to distal hypoperfusion with the clinical signs of e.g. angina pectoris and infarction. The main causes of morbidity and deaths among patients with CAD are congestive heart-failure and arrhythmias. The therapeutic focus in the field of cardiovascular disease is to prevent ischemic ventricular dysfunction. Invasive strategies like percutaneous coronary interventions and coronary artery bypass grafting are safe and well established clinical procedures in order to revascularize the tissue at risk; but these procedures are also limited by the anatomy of the epicardial coronary vasculature (e.g. too small diameters of coronary arteries for PTCA or CABG, too high risk of dissection or perforation due to atherosclerotic lesions and anatomy) and the clinical condition of the patient in need of therapy (limiting co-morbidities like renal insufficiency, stroke, severe lung disease). Importantly neither PTCA nor CABG lead to an amelioration of the atherosclerosis progression. Both techniques limit or remove the symptoms of CHD, may prevent detrimental effects of tissue hypoperfusion, however they do not attenuate the progression of atherosclerosis. The objective of this clinical trial is based on reports about beneficial effect of External counterpulsation therapy (ECP) in patients suffering from CAD. ECP is a noninvasive ambulant therapy augmenting blood flow volume and blood pressure during diastole in the vascular bed and decreasing systolic pressure. This effect is achieved by three pairs of pneumatic cuffs wrapped around the lower extremities. Triggered by ECG the cuffs inflate at the onset of diastole (diastolic blood flow augmentation) and deflate rapidly at the onset of systole (systolic unloading). The therapeutic effect of ECP has been investigated in numerous published trials: improvement of symptoms, improvement of myocardial perfusion, improvement on endothelial dysfunction (Bonetti e al, 2003). However, no data existed on whether ECP promotes active growth of myocardial collateral arteries (arteriogenesis) or not. Thus the objective of this trial was to investigate the effect of external counterpulsation on coronary artery collateral growth. By using the gold standard invasive method (Seiler et al, 1998) to detect myocardial collateral dependent blood flow, the CFIp (pressure derived collateral flow index), the therapeutic effect of ECP on coronary collaterals in patients with stable angina pectoris and significant coronary stenosis was investigated.

Methodological Part:

Study protocol and patients:

In this principal investigator initiated trial 23 patients (age 61 ± 2 years) with stable CAD and at least one hemodynamic significant stenosis eligible for percutaneous coronary intervention (PCI) were prospectively recruited. Prior to the catheter procedure eligible patients were asked if they were willing to take part in the study. 23 Patients underwent a cardiac catheterization at baseline and were included as soon as the coronary stenosis was proven to be of hemodynamic relevance via pressure derived Fractional Flow Reserve (FFR < 0.8); thenceforth the operators proceeded with the hemodynamic measurements (collateral flow index and microcirculatory index; IMR) and patients were randomized in a 2 : 1 manner to an ECP group (n = 16) and control group (n=8). Medication remained unchanged throughout the study; patients were further instructed not to change their daily activity and document their daily activity in a patient diary. After 7 weeks the invasive measurements of the pressure-derived collateral flow index (CFIp, primary endpoint) and fractional flow reserve (FFR, secondary endpoint) were reassessed and the patients were treated according to the guidelines (FFR $\leq$ 0.8 interventional revascularization, FFR > 0.8 defer and medical therapy).

External counterpulsation and control :

The ECP-group received during seven weeks 35 1-h ECP sessions (5 h / weekly) with an ECP-device. To compensate for the non-therapy related effect of ECP (walk-in, walk-out, regular contact to the doctors) the control group experienced the same protocol over 7 weeks: 4-5 times / week, walk-in appointment (for non-study related diagnostics, nutrition-counseling, psychosomatic counseling and a weekly study team appointment).

Cardiac catheterization and hemodynamic measurements:

As soon as a stenosis (>60% - <90%) was detected in a diagnostic cardiac procedure the operators proceeded with hemodynamic measurements to assess the physiological relevance of the latter lesion. For this purpose pressure derived fractional flow reserve (FFR) was assessed in the region of interest; FFR served as an inclusion criterion as well as a secondary endpoint. Given the FFR-Index < 0.8 patients with written informed consent were included into the study and the investigators assessed the thermodilution curves (IMR) and pressure derived collateral flow index (CFIp, primary endpoint); for hemodynamic measurements mean aortic pressure (P_a mmHg) was detected via the guiding catheter; mean central venous pressure (P_v mmHg) was detected via a catheter placed in the right atrium. Mean distal coronary pressure (P_d mmHg) and

transit mean times (Tmns) were obtained using a 00.14 " flow and pressure-wire positioned distal to the lesion of interest. FFR and IMR were assessed under steady state hyperemia achieved with systemic application of adenosine (140 µg/kg/1min) through the femoral vein.

FFR was calculated as (FFR = P_d-P_v/ P_a-P_v) [Pjils et al, 1996] and thermodilution curves were obtained by injection of 3 ml of room temperature saline within 2 seconds into the coronary artery. The CFIp was obtained as described elsewhere [Seiler et al, 1998]. An adequately sized balloon was placed right proximal to the stenosis in the non-stenotic segment. For measurement of the coronary wedge pressure (P_w mmHg), the balloon was inflated at low pressure until the antegrade coronary flow was interrupted. CFIp was determined at the end of a 60-s occlusion and calculated as (P_w - P_v)/(P_a - P_v) . The IMR was calculated offline as (IMR = P_a * Tmns*(P_d - P_w/ P_a - P_w) by taking the coronary wedge pressure into account as indicated in the presence of a significant stenosis [Aarnoudse et al, 2004].

Statistics:

"Power calculations were based on: One-tailed test for increase in myocardial blood flow in the ROI; significance level of 5%; power to detect change 80%. A mean change of myocardial blood flow respectively collateral blood flow of plus 15% in the ROI. For the ECP group, a sample size of 16 was required (n=12 plus a drop-out rate of 20–30%). In the natural course of the collateral circulation (CFIp) variance is reduced [Meier et al, 2007]; based on this a 2:1 ratio for the control group was planned, resulting in a sample size of 7 (n= 6+1 expected drop-out). Intra-individual comparisons of baseline and follow-up data were performed using paired Student t-test/Wilcoxon test. Between-group comparisons were performed by t-test and ANOVA or by Mann-Whitney test and Friedman test. A chi2 test using Fisher's exact test was applied for comparison of categorical variables among the study groups. 135 patients were screened, 33 patients met all screening criteria and were assessed invasively, ten out of 33 patients did not meet the final inclusion criterion or the pressure wire could not be placed properly, 23 patients were enrolled: 2/16 in the ECP group and 1/7 in the control group had to be excluded for analysis due to protocol violation. Statistical final analysis was performed by a professional statistician (ECP n =14, Control n= 6)" [Buschmann EE et al, 2009].

Results:

1) Clinical baseline characteristics were well based between the groups, there was no significant difference in clinical symptoms, age, sex cardiovascular risk factors and medication.

2) The baseline angiographic and hemodynamic data was also well balanced; the main inclusion criterion - the FFR - did not differ in the groups; so that comparable hemodynamic severity in the stenosis of the vessel of interest was given in both groups. The angiographic degree of stenosis asses via quantitative coronary angiography (QCA) did not differ between the groups. Beside the comparable nature of stenosis the conductance of the collateral vessels in the control group was higher compared to the ECP group [Buschmann EE et al, 2009].

3) Despite the lower baseline collateral perfusion pressure in the ECP group, the CFIp improved significantly (from 0.08 +/- 0.01 to 0.15 +/- 0.02; P < 0.001), while in the control group no change was observed [p. 871, Fig. 1] in http://dx.doi.org/10.1111/j.1365-2362.2009.02192.x

4) In accordance with the CFIp, the FFR-index increased in the ECP-group from 0.68±0.03 to 0.79±0.03 (p=0.001), but not in the control-group (0.68±0.06 to 0.70±0.05, p=0.4) [p. 871, Fig. 2] in http://dx.doi.org/10.1111/j.1365-2362.2009.02192.x

5) The improvement of myocardial blood flow at demand was also strengthened by the fact that the recorded stenosis narrowing (QCA % diameter of stenosis) and the resistance of the microcirculation (IMR) remained unchanged in the ECP group as well as in the control group [Buschmann EE et al, 2009].

6) Clinical benefit was only observed in the ECP group profiting from a reduction of angina and dyspnea according to the Canadian Cardiovascular Society (CCS, P = 0.008) and New York Heart Association (NYHA, P < 0.001) classification [Buschmann et al, 2009].

Discussion:

The Art.Net.2 was the first clinical pilot trial to prove that external counterpulsation leads to a significant increase in collateral conductance and improved in patients with stable angina pectoris. This finding is of high importance as well-developed collateral arteries reduce the size of myocardial infarction [Sabia et al, 1992], and cardiac events [Meier et al, 2007].

Collateral circulation, Coronary Flow Reserve, arteriogenesis: We observed only in the ECP group a significant improvement of the myocardial collateral conductance (ECP p < 0.001, control p= 0.67); further we detected that there was an inter-individual variety of the improvement of the CFIp in the ECP group (12/14 responders,); the close correlation of a low FFF < 0.75 (= high degree of the stenosis) to CFip-responders is in accordance to experimental and clinical data that the degree of stenosis influences the collateral conductance (Schaper W 1971; Pohl et al 2001; Meier et al). In this study patients with poor collateralization and severe epicardial stenosis did benefit the most from ECP therapy.

Fractional flow reserve mirrors the impairment of the myocardial perfusion due to the epicardial resistance since FFR is depending directly on the degree of stenosis under functional vasodilatation (hyperemia) . In our trial FFR improved from 0.68±0.1 to 0.79±0.12 (p = 0.001) in the ECP group (no change in the control group) demonstrating that the myocardial blood flow at demand (hyperemia) was also improved via ECP;

The resistance of the myocardial circulation is the summation of the epicardial and microcirculatory resistance. In order to study the hemodynamic impact of ECP on myocardial perfusion epicardial resistance and microcirculatory resistance were simultaneously assessed . In our study group (ECP and control) there was no change in epicardial stenosis (QCA) and microcirculation (IMR) from baseline to follow up after 7 weeks. Hence, the improvement of the myocardial blood flow at demand (also addressed as myocardial flow reserve) reflected the actual improvement of myocardial blood flow due to adaptive collateral arterial growth (arteriogenesis).

Clinical impact and implications for additional non-invasive therapeutic options

All study patients were qualified and scheduled for PCI at baseline . At the end of therapy 6 /14 patients in the ECP group were - according to the ESC-guidelines- deferred from PCI based on the of FFR > 0.8 and negative non-ischemic testing in the region of interest. 1/7 patients in the control group was also deferred- however in this patient the baseline FFR was close to the cut-off FFR < 0.8. In conclusion external counterpulsation therapy may serve as a valuable complementary treatment strategy in patients suffering from CAD to promote myocardial collateral growth in patients beyond active training and optimal guideline-conform therapy. It is reasonable to speculate that patients, especially those not being capable to perform regular cardiovascular exercise, might profit from such ‘passive collateral coronary training’ and that ECP also may play a future role in cardiovascular rehabilitation [Buschmann EE et al; 2009].

Publication 2 : Influence of epicardial stenosis severity and central venous pressure on the index of microcirculatory resistance in a follow-up study - Original paper

http://dx.doi.org/10.4244/EIJV9I9A180 (free article online)

Introduction:

The clinical outcome of patients suffering from coronary artery disease is significantly determined (beside the nature of the epicardial coronary arteries) by the myocardial microcirculation, which is individual in each patient. Moreover the functional status of the myocardial microcirculation is affected by the presence of hemodynamic relevant epicardial stenosis and coronary risk factors such as insulin-resistance, hypertension, dyslipidemia and

nicotine abuse resulting into an attenuated vasodilatation and loss of terminal vessels [Chilian et al, 1997] Myocardial microcirculatory dysfunction in turn causes constriction of the distal pre-arterioles and arterioles and inadequate sub-epicardial pre-arteriolar dilatation; giving rise to myocardial ischemia which is not necessarily induced or related to the degree of epicardial stenosis [Camici et al, 2007; Pagonas et al, 2014]. Focusing on the endothelial dysfunction of the vasculature - precise the flow mediated vasodilatation- it is well known that laminar shear stress and flow pulsatility improves the endothelial function by increased expression and secretion of endothelial nitric oxide synthetase (eNOS) and decreased expression of vasoconstrictor and pro-inflammatory factors [Vita et al, 2003]; the condition of increased laminar shear rate and flow pulsatility are achieved with physical exercise as well external counterpulsation [Hambrecht et al, 2000; Shechter et al 2003]. Two methods are well investigated for the invasive quantitative measurements of the microcirculatory conductance. Firstly the measurement of coronary flow reserve (CFR); which in fact has lost ground since it is influenced by the flow status of the epicardial arteries and can only be applied to patients without CAD [Pjils et al, 1996]; secondly the microcirculatory resistance (IMR) which is also appropriate in conditions with epicardial stenosis [Aarnoudse et al; 2004]. In our Art.Net-2 Trial [Buschmann et al, 2009] we hypothesized whether ECP can improve the microcirculation assessed invasively with the IMR. Hence the aim of this study was to 1) investigate in patients with stable CAD the influence of the hemodynamic severity of epicardial stenosis and collateral blood flow on the IMR, in the absence of any coronary intervention; 2) the reproducibility of the IMR under different hemodynamic conditions in long-term follow-up measurements and 3) the role of the preload (central venous pressure) on the calculation of the IMR [Pagonas N et al; 2014].

Methodological Part:

Study protocol and patients:

All clinical and invasive data were collected in the Arteriogenesis Network trial 2. Twenty-two patients with stable CAD had undergone twice diagnostic and functional coronary catheterization; at baseline and after 7 weeks. Hemodynamic data were assessed to observe the natural course and the effect of ECP on the collateral conductance.

Hemodynamic measurements of microcirculatory conductance:

The aforementioned issues were addressed by calculating IMR at maximal hyperemia in different ways: (1) Assessment of microcirculation by neglecting the collateral circulation under hyperemia: $IMR_{uncorr.} = P_d * Tmns$; (2) Assessment of microcirculation considering the collateral flow; therefore Pw has to be taken into account; $IMR_{corr.} = P_a * Tmns*(P_d - P_w / P_a - P_w$ (3)

Assessment of microcirculation implementing the effect of hemodynamic loading conditions by considering the central venous pressure; therefore Pv was taken into account; IMR_{cvp}= (P_a- P_v) *Tmns *(P_d - P_w / P_a - P_w) (4) CFR was also calculated to point out the limitation of this methodology CVR= $Tmns_{resting}$/ $Tmns_{hyperemic}$. FFR and CFIp were calculated as described in the Art. Net.-2 Trial [Buschmann et al; 2009].

Statistical analysis:

The data are presented as means ± SD. Regression analysis was used to test the relationship between IMR, CFR, FFR, and CFIp values at each time point. Variability at baseline and during hyperemia was compared by the Wilcoxon signed-rank test. Statistical significance was defined as $p < 0.05$; analyses were performed with SPSS-15.0 [Pagonas N et al; 2014].

Results:

1) By neglecting P_w, IMR was overestimated irrespective of the hemodynamic severity of the epicardial stenosis at baseline ($IMR_{uncorr.}$=15.5±8.9 vs. $IMR_{corr.}$=13.5±8 U, p<0.001) and in the follow-up ($IMR_{uncorr.}$.=16.9±4.9 vs. $IMR_{corr.}$.=13.8±4.6 U, p<0.001) [Pagonas N et al; 2014]

2) The overestimation of IMR (ΔIMR = $IMR_{uncorr.}$ - $IMR_{corr.}$) was positively correlated with the CFIp (r = 0.3, p = 0.046) and negatively correlated with the FFR (r = -0.44, p = 0.03) [Pagonas N et al; 2014]

3) Despite significant changes of the FFR (=0.001) and CFIp (< 0.001) from baseline to week 8 in angiographically stable stenosis (QCA p=0.17), no change of the $IMR_{corr.}$ and $IMR_{uncorr.}$ was found between the two time points [Pagonas N et al; 2014]

4) The IMRcvp did not differ compared to the IMRcorr. at any point of time [Pagonas N et al; 2014]

Discussion:

In this retrospective analysis of the Art.Net-2 study-participants hemodynamic data was assessed A) twice intra-individually ($n_{baseline}$ = 22 and $n_{follow-up}$ = 22) and B) due to alteration of the myocardial perfusion in 15/22 microcirculatory data at 37 different hemodynamic conditions was calculated; the study participants had stable CAD with usual cardiovascular risk factors and significant epicardial stenosis but of divergent hemodynamic relevance. Taking this into account, following results and new findings are derived from this small but representative patient cohort:

1) Reaffirmation that IMR is a reliable functional parameter to assess myocardial microcirculatory resistance in CAD [Pagonas N et al; 2014]

2) De novo description that the IMR may also be applied as endpoint in follow-up studies assessing the effect of different non- interventional therapies on the microcirculation, since all data was obtained prior to PCI [Pagonas N et al; 2014]

3) Novel finding that IMR being calculated merely based on Tmns and P_d the microcirculatory resistance is overestimated irrespective of the stenosis hemodynamic severity. And moreover; that deceptive overestimation of the IMR is provoked the better the collateral conductance and the less severe the epicardial resistance [Pagonas N et al; 2014].

4) We also assessed for the first time that in patients with stable CAD an additional invasive measurement of the systemic preloading (P_v) may be neglected for accurate calculation of the IMR [Pagonas N et al; 2014].

Hence IMR is a reliable methodology of high clinical relevance in the context disturbed myocardial macro-, and microcirculation in patients suffering from CAD. To assess an accurate IMR given epicardial lesions of hemodynamic relevance IMR should be assessed under vasodilatation taking the collateral circulation in the region of interest into account; central venous pressure can be neglected [Pagonas N et al; 2014].

Publication 3 : Does external counterpulsation augment mean cerebral blood flow in the healthy brain? Effects of external counterpulsation on middle cerebral artery flow velocity and cerebrovascular regulatory response in healthy subjects- Original paper

http://dx.doi.org/10.1159/000319891

Introduction: Intra-aortic counterpulsation (IABP) increases in diastole the mean central blood pressure and increases the mean blood flow volume as well blood flow velocities especially in the heart and intra-abdominal organs. In prior findings IABP also improves cerebral perfusion in patients with normal as well impaired cerebral perfusion (stroke) [Uflacker et al 2008]. Since the hemodynamic effect of the non-invasive ECP therapy is very similar to IABP there is growing interest in ECP as a potential therapeutic device in acute as well chronic cerebral ischemia [Han et al,2008]. In impaired cerebrovascular perfusion the improvement and maintenance of cerebral perfusion pressure and oxygen delivery in the area at risk is of imminent importance to avert further irreversible tissue damage. In the brain a constant cerebral blood flow (CBFV) is regulated via a variety of intrinsic processes referred to cerebral autoregulation (CA) by adjusting the cerebrovascular resistance; besides metabolic, neurogenic and vascular myogenic response arterioles are thought to be the main regulator of vascular resistance. [Jungehuelsing et al, 2010] In recent published literature there is evidence that particularly shear stress via an increased flow rate is the pivotal stimulus of cerebral arteriogenesis [Schierling et al 2009]. Our group [Buschmann EE et al, 2009] and others could provide convincing evidence [Bonetti et al,

2003, Schechter et al 2003] that the increase in shear stress as well as shear rate is the driving stimulus for the beneficial effects of counterpulsation. However for the cerebral circulation data are lacking, which provide a direct evidence for a potential pro-arteriogenic effect of ECP on brain collaterals. Since cerebral autoregulation might hamper potential beneficial effects of counterpulsation we set up a clinical trial to test following hypothesis:1) Does cerebral autoregulation eliminate pro-arteriogenic blood flow signals to the periphery of the brain? 2) Does diastolic augmentation of ECP treatment induces constant changes of mean CBFV in the Mid Cerebral Artery (MCA) and 3) finally can trans-cranial doppler waveform characteristics provide information about cerebrovascular response to external counterpulsation [Jungehuelsing et al; 2010]?

Methodological Part:

Subjects:

All subjects gave their written informed consent prior to inclusion. 9 healthy volunteers (age 34.1±11.1; 4 female;) were enrolled; carotid and vertebral duplex sonography as well TCD was performed to rule out large artery occlusive disease.

Study-protocol:

ECP treatment were performed was applied for 20 minutes twice with a pause of 5 minutes in between. The therapeutically cuff-pressure was controlled via the diastolic-to systolic ratio >1.2; blood pressure and 3-lead ECG were monitored continuously. ECP-treatment sessions were well tolerated

Transcranial Doppler Sonography:

Two 2-MHz probes were a fitted on headband and applied properly to the temporal bone so to assess the blood flow volume in the Arteria cerebri media (MCA) on both sides at rest and under ECP therapy. CBFV were measured continuously using a Multidop T2 Doppler device (50Hz, DWL). Continuous real-time MCA blood flow monitoring was registered for 5 min before, during, and 5 min after ECP-treatment periods.

Doppler and Statistical analysis:

"Audio-Doppler signals of the right and left MCA were registered with Multidop T2 in real-time: blood flow velocities and waveforms of each cardiac cycle were analyzed beat by beat (peak systolic=PSV, end-diastolic=EDV, peak diastolic=PDV and mean flow=MFV, mean: n=3609 for each velocity). Data at baseline, rest and post treatment phase were averaged. using Mat lab (©Math Works, Inc.). To guarantee equal pressure status and optimal ultrasound signals during

treatment velocities 3 min after ECP onset were registered until the end and averaged. MFV were calculated as mean of all recorded velocities during a cardiac cycle beat. Distributions of relative velocities per heart cycle were calculated. MFV was set as value 1 with a range of relative flow velocities from 0 to 2. Goslings´ pulsatility index (PI) and Pourcelots´ resistance index (RI) were calculated Nonparametric tests (Wilcoxon ranksum test) were used for all statistical analysis. Data are expressed as means ± SD " [Jungehülsing et al, 2010].

Results:

1) At the onset of ECP-treatment immediate changes of the cerebral blood flow volume and altered doppler waveforms were observed; changes returned prompt o baseline with the stop of the ECP treatment [p. 614; fig 1] in http://dx.doi.org/10.1159/000319891

2) In all subjects during ECP therapy- compared to baseline and resting period- peak diastolic velocities (PDV) were increased (63±11 vs. 76±11cm/s $p<0.001$,); whereas peak systolic (PSV $p<0.001$) and end diastolic velocity (EDV $p<0.001$) decreased significantly (PSV; 87±14 vs. 78±17cm/s; EDV; 40±9 vs. 28±10cm/s), MFV did not increase (59±10 vs. 58±13cm/s, n.s.) [p. 615; fig. 4] in http://dx.doi.org/10.1159/000319891

3) During ECP treatment maximum amplitudes of early diastole were similar to peak systolic velocity in most of the cases. Doppler flow velocity indices as PI (0.79±0.19 vs. 0.89±0.13, $p<0.001$) and RI (0.54±0.06 vs. 0.64±0.1, $p<0.001$) were also significantly increased [Jungehuelsing et al; 2010].

4) Before, in between and after ECP-treatment PDV, PSV, EDV, MVF, RI and PI remained stable [Jungehuelsing et al; 2010].

5) Analyses of velocities per heart cycle revealed accelerated flow velocities through-out the ECP therapy compared to rest/baseline; increased proportions both in the high (1.2-1.3) and in the low (0.3-0.6) velocity sections were seen [Jungehuelsing et al; 2010].

5) There were no significant changes or blood pressure and heart rate at baseline, between or after ECP-treatment [Jungehuelsing et al; 2010].

Discussion:

The increase of shear rate plays a pivotal role in the induction of arteriogenesis. Experimental studies provided recently that also in cerebral arteriogenesis shear stress is the key factor to induce adaptive arteriolar outward remodeling [Schneeloch et al, 2004]. Moreover Zhang et al. [Zhang et al 2007] demonstrated in an experimental ECP model with canines that especially higher velocities and flow rates induce cerebral arteriogenesis [Schierling et al]. Based on this

data therapeutically benefit of ECP in the clinical field of cerebrovascular atherosclerotic disease is to be expected; given that flow velocities and potentially flow rates may be enhanced.
1) The this study for the first time CBFV was continuously examined under ECP treatment in healthy subjects. ECP augments CBFV in the MCA in early diastole in accordance with previous studies ; however, we showed for the first time that the MFV of MCA did not increase but remained constant during ECP. Further analyzes of waveform characteristics revealed higher pulsatility indices (PI) as an indicator for increase cerebrovascular resistance .Here, we also support the hypotheses of Marthol et al that in healthy subjects because of preserved cerebral autoregulation [Marthol, et al. 2005] and that due to regulation of downstream resistance a stable cerebral perfusion in response to diastolic augmentation in the MCA; . 4) The most important insight of this pilot trial was that - although mean cerebral blood flow was not enhanced- ECP induced an increase in flow velocities throughout the low to high flow velocities; resulting in an increases of medium to high velocity percentages during treatment compared to baseline. This is a cutting-edge finding regarding the aforementioned experimental studies wherein cerebral arteriogenesis especially was induced by higher velocities and increased shear stress [Zhang et al 2007; Schierling et al 2009]. Though, further studies beyond ours are needed to investigate the potential therapeutical effect of ECP in the field of cerebrovascular arteriogenesis.

Publication 4: Therapeutic arteriogenesis in peripheral arterial disease: combining intervention and passive training- Review

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In this review we focus on peripheral arterial disease (PAD); in special the benefit of active exercise programs and novel non-invasive strategies - like low-pressure ECP treatment- that is expected to enhance arteriogenesis in the lower limb; moreover we explain the concept of "passive exercise training" in particular in those patients who are unable to do an effective vascular training after PTCA or PTA. We point out, that both – intervention as well as training- are two techniques, which have a close link to each other: Intervention and thus improvement of the arterial inflow will enhance therapeutic shear rates, if after this procedure an efficient exercise training follows, maximal collateral shear rates can be achieved. This also holds true for patients, who cannot join an effective training due to handicaps such as immobility etc. In these cohorts, ECP might be an efficient “passive training” to increase shear rates after endovascular intervention [Bondke Persson et al; 2011].

Summary:

In the late 90ties a vast majority of experimental and clinical trials focused on angiogenesis, the de novo sprouting of capillary vessels in ischemic tissue. This process was thought to be effective in restoring blood flow to endangered territories such as in conditions of coronary heart disease, peripheral arterial disease or cerebrovascular disease. However the physiological pitfall of this angiogenic approach was, that large conductance arteries may not be compensated by small capillaries: First since the Law of Hagen-Poiseuille dictates, that an efficient blood flow is controlled via the change in diameter (to the power of 4) rather than via de novo vessel formation. Secondly de novo capillaries are arranged in a serial coupling, in other words after the Kirchoff Law of electricity, the resistance in these capillaries will increase significantly and will now allow an efficient nutrient blood flow into the periphery. Our group was the first to show, that another process of arterial regeneration is responsible for an efficient blood flow restoration, termed arteriogenesis. This rapid proliferation of pre-existing collateral pathways (in the majority of cases small arterioles) is capable to conduct efficient blood flow into the ischemic zone. Importantly collaterals are often not located in the zone of ischemia. Secondly collateral arteries are circumventing the site of stenosis or occlusion, thus they are located in a parallel coupling, in the words of Kirchhoff, this leads to a drastic reduction of total resistance and thus makes these arteries optimal candidates for blood flow restoration.

The studies of this thesis have contributed to three fundamental clinical question: The first; is it possible to stimulate collateral artery growth in patients. From experimental trials this was shown extensively, largely by occluding large feeding arteries (hindlimb ligation models) and increasing shear forces in the lumen of newly recruited collaterals. However in the clinical scenario this is more complicated, since the stenosis or obstruction of the artery is already existing since several years. The patient has experience angina pectoris in the majority of cases for many months. Hence we identified external counterpulsation as a potential technique to increase shear forces within the arterial lumen of the coronaries. So far it was not shown, that counterpulsation has direct effects on the capacity of the human collateral circulation to grow in diameter by active arteriogenesis. Both the CFIp as well as the FFR increased significantly after 6 weeks of counterpulsation. Noteworthy the degree of the stenosis was not affected by the treatment before and after therapy. These invasive data from patients with stable angina pectoris were the first in the field to document a direct effect of ECP on macrocirculatory arteriogenesis in human subjects with a chronic coronary disease.

In a second set of experiments we addressed the questions, which invasive measurement is the most reliable parameter to detect effects of invasive or non-invasive therapies on the microcirculation. This was of high clinical importance since the individual blood flow conditions in each subject in the myocardial microcirculation depends on the presence of factors such as hemodynamic relevant epicardial stenosis, insulin-resistance, arterial hypertension, dyslipidaemia, nicotine abusus and others. The latter leading to a prolonged or even disturbed vasodilatory capacity or even loss of the terminal vessels in the arterial tree. These pathologies of the distal pre-arterioles and arterioles, may appear independent from the degree of epicardial stenosis. The index can be easily acquired by using a single pressure wire and the thermodilution technique. When the coronary wedge pressure (Pw) is known, IMR enables calculation of vascular resistance independent of the status of the epicardial arteries. Here our clinical data from patients provide substantial evidence, that with chronic coronary artery stenoses IMR is being calculated merely based on Transit mean times and mean distal coronary pressure (PD). Thus IMR is overestimated irrespective of the hemodynamic severity of the epicardial stenosis in the region of interest. Moreover the overestimation of IMR was positively correlated with the CFIp, in other words, changes of the CFIp and FFR reveal the dependence of the index on the severity of myocardial stenosis and collateral blood flow.

In summary, arteriogenesis can be stimulated in human subjects with atherosclerosis and stable coronary heart disease. The effect of counterpulsation is mirrowed by an increase in collateral conductance, thus a direct evidence for collateral growth in these patients. Therefore the application of the enhancement of intra-arterial shear stress open novel avenues in the treatment of vascular disease. Here we show, that a non-invasive application of low-pressure counterpulsation leads to a substantial improvement in patients with Class II Angina pectoris, second that this effect on the macrocirculation can be easily detected via the Collateral Flow Index, whereas changes in the microcirculation remain to be detected with the IMR. Currently this concept is being transferred to the situation of peripheral arterial disease. A rather complex condition of partially very long lasting stenosis or occlusions in the thigh and limb of patients, nevertheless with high potential for collateral recruitment via counterpulsation.

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List of Abbreviations

BP	Blood Pressure
CA	Cerebral Autoregulation
CABG	coronary artery bypass grafting
CAD	Coronary artery disease
CBF	Cerebral Blood Flow
CBVF	cerebral blood volume flow
CFIp	pressure derived Collateral flow Index
CFR	Coronary flow reserve
CVR	Cerebrovascular Resistance
ECP	external counterpulsation therapy
EDV	Enddiastolic Velocitiy
FFR	pressure derived Fractional flow reserve
IABP	Intra-aortic balloon pump
IMR	Index of microcirculatory resistance
MCA	Middle Cerebral Artery
MCBFV	Mean Cerebral Blood Flow Velocity
MFV	Mean Flow Velocity
Pa	aortic pressure
PAD	peripheral arterial disease
PCI	percutaneous coronary transluminal angioplasty
Pd	coronary pressure distal to the stenosis
PDV	Peak Diastolic Velocity
PI	Pourcelot Index
PSV	Peak Systolic Velocity
Pv	Central venous pressure
Pw	coronary wedge pressure
RI	Resistance Index
QCA	quantitative coronary angiography
TCD	transcranial doppler ultrasound

Eidesstattliche Versicherung

„Ich, Buschmann Eva, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: " Investigation of the therapeutical effect of External Pneumatic Counterpulsation on the myocardial and cerebrovascular arterial circulation "selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.

Alle Stellen, die wörtlich oder dem Sinne nach auf Publikationen oder Vorträgen anderer Autoren beruhen, sind als solche in korrekter Zitierung (siehe „Uniform Requirements for Manuscripts (URM)“ des ICMJE -*www.**icmje**.org*) kenntlich gemacht. Die Abschnitte zu Methodik (insbesondere praktische Arbeiten, Laborbestimmungen, statistische Aufarbeitung) und Resultaten (insbesondere Abbildungen, Graphiken und Tabellen) entsprechen den URM (s.o) und werden von mir verantwortet.

Meine Anteile an den ausgewählten Publikationen entsprechen denen, die in der untenstehenden gemeinsamen Erklärung mit dem/der Betreuer/in, angegeben sind. Sämtliche Publikationen, die aus dieser Dissertation hervorgegangen sind und bei denen ich Autor bin, entsprechen den URM (s.o) und werden von mir verantwortet.

Die Bedeutung dieser eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unwahren eidesstattlichen Versicherung (§156,161 des Strafgesetzbuches) sind mir bekannt und bewusst.“

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Datum Unterschrift

Anteilserklärung an den erfolgten Publikationen

Buschmann Eva hatte folgenden Anteil an den folgenden Publikationen:

Publikation 1: http://dx.doi.org/10.4244/EIJV9I9A180
Pagonas N, Gross CM, Li M, Bondke A, Klauss V, **Buschmann EE**. *Influence of epicardial stenosis severity and central venous pressure on the index of microcirculatory resistance in a follow-up study.* Euro Intervention. 2014 Jan; 22;9(9):1063-8. doi: 10.4244/EIJV9I9A180. PubMed PMID: 24457278.
Beitrag im Einzelnen: Ethikantrag; Patientenscreening und Einschluss, Datendokumentation; Erhebung und Dokumentation der invasiven Daten; Datenanalyse; Publikationserstellung

Publikation 2: http://dx.doi.org/10.1111/j.1365-2362.2009.02192.x

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Beitrag im Einzelnen: Entwurf der Studie- Ethikantrag- ISCTRN Registrierung- Patientenselektionierung - Einschluss und Aufklärung- Datendokumentation - Erhebung der invasiven Daten im Herzkatheterlabor Helios Klinikum Buch/Charité - Durchführung und Supervision der Patiententherapie - Datenanalyse - Statistische Berechnungen - Publikationserstellung

Publikation 3: http://dx.doi.org/10.1159/000319891
Jungehuelsing GJ, Liman TG, Brunecker P, Ebel A, Endres M, Buschmann I, Pagonas N, **Buschmann EE;** *Arteriogenesis Network; Center for Stroke Research Berlin. Does external counterpulsation augment mean cerebral blood flow in the healthy brain? Effects of external counterpulsation on middle cerebral artery flow velocity and cerebrovascular regulatory response in healthy subjects.* Cerebrovasc Dis. 2010;30(6):612-7 Epub 2010 Oct 15. PubMed PMID: 20948206.

Beitrag im Einzelnen: Entwurf und Idee zur Studie - Patientenselektionierung - Patiententherapie - Datendokumentation - Datenanalyse- Publikationserstellung
.

Publikation 4: http://dx.doi.org/10.1024/0301-1526/a000092

Bondke Persson A, **Buschmann EE**, Lindhorst R, Troidl K, Langhoff R, Schulte KL, Buschmann I. *Therapeutic arteriogenesis in peripheral arterial disease: combining intervention and passive training.* Vasa. 2011 May;40(3):177-87. Review. PubMed PMID: 21638246.

Beitrag im Einzelnen: Literaturrecherche, Manuskripterstellung
Unterschrift, Datum und Stempel des betreuenden Hochschullehrers/der betreuenden HochschullehrerIn

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Unterschrift des Doktoranden/der Doktorandin

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Unterschrift des Co-Autors mit äquivalentem Anteil

Curriculum vitae

Dr. med. univ. Eva-Elina Buschmann

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

List of publications and original papers:

1. Pagonas N, Gross CM, Li M, Bondke A, Klauss V, **Buschmann EE** *(2014).* Influence of epicardial stenosis severity and central venous pressure on the index of microcirculatory resistance in a follow-up study. *Euro Intervention. 2014 Jan 22;9(9)*:1063-8
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