

Abstracts

The 24th Annual Meeting of the “Deutsche Gesellschaft für Klinische Mikrozirkulation und Hämorheologie” DGKMH E.V. paralleled by the Annual Meeting of the Society for Microcirculation and Vascular Biology GFMVB

17–19 September 2005, Rostock, Germany

Local Congress Organisation: Prof. Dr. P. Schuff-Werner and Dr. Birgit Holdt-Lehmann (Rostock)
Scientific Committee: Prof. F. Jung (Hoyerswerda), Prof. Dr. R. Angelkort (Dortmund), Prof. Dr. R. Bauersachs (Darmstadt), Prof. Dr. Dr. R.P. Franke (Ulm), Prof. Dr. M. Juenger (Greifswald), Prof. Dr. H. Landgraf (Berlin), Dr. C. Mrowietz (Hoyerswerda), PD Dr. J. Koscielny (Berlin), Prof. Dr. K.P. Schmitz (Rostock)

V – oral presentation, C – oral presentation in common lectures with the annual meeting of the GfMVB, P – poster presentation

V1. Influence of contrast media (iopromide, oxalate, gadolinium-DOTA) on blood viscosity, erythrocyte morphology and platelet function

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The influence of contrast media on blood viscosity, erythrocyte morphology and platelet function was studied. *In vitro* blood was incubated with iopromide (Ultravist[®]), oxalate (Hexabrix[®]) or gadolinium-DOTA (Dotarem[®]). Plasma viscosity and whole blood viscosity were measured and the mean erythrocyte volume and morphology were assessed. Platelet aggregation was measured with a PFA-100[®] instrument. In an *ex vivo* study on patients receiving these contrast media the same measurements were taken. All contrast media increased blood viscosity at a high shear rate in a dose-dependent manner (e.g., with oxalate: from 4.9 ± 0.2 mPa.s to 8.6 ± 0.5 mPa.s at 160 mg I/ml), decreased low shear viscosity (for oxalate: from 44.9 ± 2.5 to 27.7 ± 4.8 mPa.s), increased plasma viscosity (oxalate: from 1.2 ± 0.1 to

2.8 ± 1.3 mPa.s), decreased the mean erythrocytic volume (oxalate: from 89.7 ± 1.4 to 79.7 ± 2.0 fl), and decreased platelet aggregation. Iopromide induced an echinocytic transformation of erythrocyte shape. *Ex vivo*, a decreased hematocrit and a consecutively decreased whole blood viscosity were found with iopromide and oxalate. We conclude that contrast media influence blood rheology, erythrocytes and platelet aggregation *in vitro* and *ex vivo*.

V2. The effect of various contrast media on the morphology of human erythrocytes

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The association of echinocyte formation with a rigidification of blood cells potentially affects capillary perfusion and the tissue oxygen tension. Therefore, this study examines how pronounced echinocyte formation is with admixture of varying amounts of contrast media (CM) (iodixanol 320 mgI/ml, iohexol 350 mgI/ml, iopromide 370 mgI/ml, iomeprol 350 mgI/ml, iomeprol 400 mgI/ml) when compared to an isotonic saline solution and whole blood.

The plasma of $n = 6$ healthy subjects was admixed with NaCl, iodixanol, iohexol, iomeprol 350 and 400, and iopromide in concentrations of 10%, 20% and 40%. The cells were then resuspended in the mixture, incubated for 5 minutes at 37°C and assessed under the microscope.

The medium itself with the different contrast media as well as the varying medium concentrations have a significant effect on the number of remaining normal erythrocytes (= discocytes) ($p < 0.0001$). The percentage of discocytes significantly depends on the suspension medium for all concentrations ($p = 0.0097$).

Of all radiographic contrast media, the percentage of discocytes after iodixanol most resembles that of autologous plasma. In this regard, iodixanol markedly differs from all other contrast media while the latter do not differ from each other with respect to the percentage of remaining discocytes.

V3. Effects of radiographic and MRI contrast media on rheological properties of red and white blood cells

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Original radiographic contrast media (RCM) are hyperosmolar and known to cause red blood cells (RBC) to shrink, distort their shape and change their flow properties. Newer generation RCM cause fewer effects. However, there has been little information on effects of MRI contrast media, or on the effects of any agents on flow properties of neutrophils (the most numerous white cells). We first compared effects of different contrast media on the properties of normal RBC and sickle RBC (donors homozygous for haemoglobin S). The RCM Visipaque (290 mOsmol/l), Hexabrix (600 mOsmol/l), Omnipaque (840 mOsmol/l) and RenoCal-76 (1940 mOsmol/l) caused changes in cell volume, shape and flow rate through 5 µm pores, which varied in severity according to their osmolarity. In the extremes, Visipaque had little detectable effect, while RenoCal-76 caused major increases in RBC flow resistance. The effects on sickle cells were stronger than on normal RBC and it is particularly important to avoid dehydration of these abnormal cells. The MRI contrast media Omniscan and Magnevist were compared. Again, the medium with greater osmolarity, Magnevist caused greater increases in flow resistance, but neither agent

caused marked changes in cell morphology. Based on comparisons of media and time courses of shape changes, morphological changes could not be attributed to changes in osmolality alone. Finally, we compared effects of RCM (Visipaque, Iomeron and Urografin) on morphology and flow resistance of isolated neutrophils. Neutrophils were surprisingly resistant to hyperosmotic media, and we only observed an increase in flow resistance at the highest concentration (30% v/v) of Urografin. Interestingly, this response was maintained after washout of the RCM and neutrophils. They then developed pseudopodia suggesting that they were activated. Recovery took at least 30 minutes. Thus, hyperosmolar media can modify the behaviour of red and white blood cells. While effects are largely due to the osmolality itself, additional morphological effects of an undefined nature are also evident and not easily reversible in neutrophils.

V4. Comparison of the influence of an ionic versus a non-ionic X-ray contrast agent on platelet and coagulation function during diagnostic cardiac catheterisation

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The effects of ionic (oxalate) and non-ionic (iopromide) contrast media on platelet and coagulation function were evaluated *ex vivo* in the present study. In 40 patients undergoing coronary angiography, platelet reactivity, serotonin concentration, thrombin-antithrombin, prothrombin fragments and D-dimers were measured. The use of an ionic X-ray contrast agent (oxalate) in diagnostic cardiac catheterisation angiography is associated with minor thrombin generation and lower platelet activation compared to the non-ionic X-ray contrast agent (iopromide). These data confirm the results of various *in vitro* studies and animal investigations.

V5. Influence of X-ray contrast media on blood fluidity and microcirculation

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Through their characteristic viscosity and osmolality, radiographic contrast media (RCM) can influence plasma viscosity, erythrocyte aggregation and deformability. These rheological changes may lead to subsequent microcirculation impairments. To investigate this effect, a bolus injection of 20 ml RCM was administered into the axillary artery of a total of 130 patients who had to undergo heart catheter angiography. The blood flow in the nail fold capillaries was analysed by intravital microscopy. The RCM iomeprol, iopentol, iodixanol, iopromide, iobitridol and oxalate were compared to a NaCl solution. While the injection of NaCl led to a small but significant increase of capillary erythrocyte velocity (+0.04 mm/s), all RCM induced a decrease (between -0.086 mm/s for iomeprol and -0.425 mm/s for iopromide). The multivariate analysis (with respect to viscosity and osmolality) shows that the osmolality does not influence capillary blood flow. The decrease in mean erythrocyte velocity correlates with the viscosity and accounts for 45% of the change in the capillary blood flow ($r = 0.68$, $p < 0.0001$), thus leaving a residual 55% change that cannot be clarified within this model. This may be due in part to the differential influence exerted by RCM on erythrocyte deformability, e.g., by echinocyte formation. This may be the reason for impaired microcirculation, which can last for minutes following the injection. Also, a chemotoxic effect on the endothelium and, therefore, on capillary blood flow cannot be ruled out. In conclusion, it can be said that RCM influence the microcirculation with varying intensity and duration in relation to rheological effects. While the effect of the RCM's viscosity is certain, the effect of its chemotoxicity cannot be estimated at this time.

V6. The influence of various radiographic contrast media on myocardial oxygen tension

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This study examined whether a one-time bolus injection of 10 ml of radiographic contrast medium (iopromide 370, iohexol 350, iodixanol 320 or iomeprol 350) into the left coronary artery affects the tissue oxygen tension (pO₂) in this artery's supply area as compared to a 0.9% sodium chloride (NaCl) bolus. The radiographic contrast media and NaCl boli were randomly assigned. The study was performed in two parts in 2 sets of 6 domestic pigs with identical sequential design.

241 ± 44 s after the injection of the iopromide bolus, the myocardial pO₂ in the supply area of the left coronary artery (pO₂LAD) had dropped by 44.2% from an initial 40.3 ± 10.9 mmHg to 22.5 ± 8.9 mmHg ($p = 0.0003$). After 576 ± 113.5 s, the pO₂ had returned to its initial value. 171.7 ± 11.9 s after the injection of the iohexol bolus, the pO₂LAD of 34.5 ± 14.6 mmHg had dropped by 14.8% to 29.4 ± 13.9 mmHg ($p = 0.0003$). After 321 ± 47.1 s, the initial pO₂ was restored. The decline of the pO₂LAD after iopromide was significantly greater than after iohexol ($p = 0.0001$), and the time required to return to the initial pO₂ was much longer ($p = 0.001$). 26.7 ± 16.4 s after iodixanol injection, the pO₂LAD declined by 3.5% from 42.2 ± 5.6 mmHg to 40.7 ± 5.9 mmHg ($p = 0.0357$). The initial value was restored after 53 ± 16.7 s. The pO₂LAD was 41.9 ± 7.4 mmHg before iomeprol injection. 303.3 ± 58.9 s after the injection, the pO₂LAD declined by 13.1% to 36.4 ± 7.5 mmHg ($p = 0.0001$). After 577 ± 22 s, the initial value was restored. The bolus application of an isotonic NaCl solution did not result in a pO₂LAD decrease. Immediately after the injection, it increased by a maximum of 3%. In the supply area of the right coronary artery and the peripheral skeletal muscle, no effect of the radiographic contrast media or the NaCl on tissue oxygen tension was observed. Furthermore, tissue temperature, heart rate, systolic and diastolic blood pressure and cardiac output per minute were unaffected. The injection of a radiographic contrast medium in a coronary artery can result in a significant local contrast medium-induced microcirculation disorder in this artery's supply area. The increased viscosity of a radiographic contrast medium leads to a very short-term insignificant effect on the microcirculation. Red blood cells can be affected by the osmolality of contrast media. A relevant microcirculation disorder can, however, occur if an additional rheological defect is triggered, e.g., through echinocyte formation. This is associated with a considerable erythrocyte rigidification and a consecutive obstruction of the capillary passage leading to a measurable microcirculation disorder.

V7. Drug delivery by angiographic contrast media: Restenosis inhibition

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Coronary arteries are occasionally coated with contrast media forming a thin film on the endothelium. We assumed that such a persistent layer may be useful as a delivery medium for anti-proliferative agents such as paclitaxel to prevent restenosis. The aim of our studies was to test the efficacy of taxane compounds added to the contrast agent iopromide-370 in cell culture experiments and the porcine coronary stent model.

In cell culture experiments, iopromide paclitaxel inhibited vascular smooth muscle cell (VSMC) proliferation in a concentration-dependent manner. Exposure times ranging from 3 to 60 minutes were tested. The impact of paclitaxel on the proliferation of vascular smooth muscle cells was of equal magnitude for all exposure times tested with an almost complete inhibition of cell proliferation.

For *in vivo* investigation in a first animal trial, 16 stents were implanted into the coronary arteries of eight pigs. A control group received iopromide-370 alone while the treatment group was injected with a iopromide-protaxel formulation at a dose of 74 $\mu\text{mol/l}$, which is far below protaxel levels inducing systemic toxicity. Quantitative angiography and histomorphometry of the stented arteries established statistic equality of the baseline parameters between the control and treatment groups. After 28 days, the treatment group showed a marked reduction of the parameters characterizing in-stent restenosis, especially a 34% reduction of the neointimal area.

In a second animal trial, 34 stents were implanted into the LAD and CX coronary arteries of 17 pigs. Iopromide-370 was used in group I (control). The treatment groups were injected either with 80 ml intravenous iopromide plus 13.6 mg paclitaxel (group II) in addition to the intracoronary plain CM, or 80 ml intracoronary iopromide plus 6.8 mg paclitaxel (group III), or 80 ml intracoronary iopromide plus 13.6 mg paclitaxel (group IV). Quantitative coronary analysis (QCA) and histomorphometry were used to evaluate measures of restenosis at follow-up on day 28. In a separate experiment, paclitaxel concentration was measured using HPLC in the coronary arteries. Paclitaxel containing preparations were well tolerated. QCA documented no differences between the baseline parameters of the four groups. At follow-up, however, there was a marked reduction of all parameters relevant to in-stent restenosis in favor of the intracoronary iopromide-paclitaxel administration whereas at the same dose intravenous paclitaxel did not affect intimal hyperplasia.

	Controls I and II	Paclitaxel 100 μM in iopromide-370	Paclitaxel 200 μM in iopromide-370	<i>p</i>
Number of vessels	16	10	6	
Diameter stenosis (QCA)	57 \pm 13%	29 \pm 18%	13 \pm 12%	0.001
Area stenosis (histology)	30 \pm 14%	26 \pm 14%	14 \pm 7%	0.038

About 5 min following the last of several coronary injections of paclitaxel 200 μM in iopromide-370 (total dose 80 ml), paclitaxel reached 5–15 μM in the injected LAD and CX.

These studies provide evidence that contrast media may serve as carriers in local drug delivery. CM enhances the solubility of lipophilic drugs. Paclitaxel concentrations in the arteries were high enough to achieve persistent inhibition of cell proliferation. Efficacy does not depend on stent implantation and is not limited to the stented vessel segment. A first clinical trial has been initiated.

V8. Contrast induced nephropathy using sodium bicarbonate or sodium chloride and isosmolar iodixanol

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Contrast media induced nephropathy (CIN) is a complication after coronary angiography. For prevention of CIN, hydration with sodium chloride solution is recommended. With regard to CIN rates, a recent study showed a benefit of sodium bicarbonate hydration versus sodium chloride when using the non-ionic monomeric low-osmolar contrast medium iopamidol (Merten G.J. et al., *JAMA*, 2004).

The aim of this study is to determine the effects of sodium bicarbonate versus sodium chloride on CIN rates in a large randomized cohort of 200 patients using the non-ionic dimeric isosmolar contrast medium iodixanol. We present the results of an interim analysis of the data from 91 patients.

Patients and methods: The study is a prospective, randomized, single-center, double-blind trial with 91 CAD patients (age: 72.6 ± 6.7 yrs, 18f/73m) with elevated baseline serum creatinine levels (SCr) (mean SCr 132.6 ± 29.3 $\mu\text{mol/l}$). Eligible patients were randomized to receive either a 154-mEq/l infusion of sodium bicarbonate ($n = 45$, group A) or one of sodium chloride ($n = 46$, group B) as a bolus of 3 ml/kg per hour for 1 hour before and as an infusion of 1 ml/kg per hour for 6 hours after the angiography/iodixanol administration. The mean (range) of the baseline prehydration SCr value for group A was 129.7 ± 26.0 (84–212) $\mu\text{mol/l}$ and for group B 135.4 ± 32.2 (96–236) $\mu\text{mol/l}$ ($p = 0.70$). The primary end point of this study was a SCr increase of 25% or 44 $\mu\text{mol/l}$ on the first or second day following diagnostic contrast medium application.

SCr, serum cystatine C (CysC), plasma viscosity (PV) and urinary enzymes (alaninaminopeptidase (AAP), N-acetyl- β -D-glucosaminidase and α_1 -microglobuline as indicators of early tubular impairment were measured at baseline and on days 1 and 2 after contrast medium administration.

Results: The CIN rate (in total 6 patients) was equal in both groups (group A 6.7%, group B 6.5%). The SCr increases were in group A between 28% and 68% and in group B between 25% and 48%. All 6 patients showed a decline of their SCr values during control 10 to 14 days after angiography. No patient required any additional therapy.

Before and after iodixanol application in all 91 patients, we found differences in the following parameters: AAP ($p < 0.0001$), CysC ($p = 0.036$) and PV ($p = 0.004$) were significantly increased.

The comparison of these parameters between the two hydration groups on the first day after angiography showed no tendency towards a difference. However, on the second day most parameters had higher values in group A as compared to group B. AAP was distinctive (3.92 ± 3.0 U/mmol urine Cr in group A versus 2.76 ± 3.73 in group B; $p = 0.004$).

Conclusion: This interim analysis revealed low incidences of CIN rates after diagnostic procedures with i.a. administration of the non-ionic dimeric isosmolar contrast medium iodixanol. The control measurements after 10 to 14 days again showed prediagnostic values in the 6 CIN patients. In contrast to (Merten et al., *JAMA*, 2004), we could not find a benefit in using bicarbonate sodium versus sodium chloride with regard to the CIN rates observed.

V9. Importance of osmolality of contrast media in high risk patients: Nephroprotection and clinical consequences

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Contrast media induced nephropathy (CMIN) is one of the most important complications following the use of contrast media. Patients developing CMIN after coronary intervention and requiring dialysis are 35-times more likely to die than patients without CMIN. As a consequence, necessary clinical investigations requiring contrast media are frequently not offered to high risk patients, who otherwise would have received investigations with contrast media. More specifically, patients with coronary artery disease and a high risk of CMIN are at the same time those patients who benefit most from angiography and subsequent coronary intervention.

Different approaches have been investigated to minimize the risk of CMIN in high risk patients. Adequate hydration, use of acetylcysteine and avoidance of nephrotoxic medication are useful measures. Their absolute benefit, however, is rather small and is not confirmed by some studies.

Until recently, the osmolality of all contrast media was higher than human plasma, thus inducing a high osmotic load into the kidney tubules, increasing intratubular hydrostatic pressure and decreasing filtration pressure in the glomeruli. Recently, an isosmolar contrast medium, iodixanol, was developed and tested in clinical trials. CMIN in high risk patients could be reduced to a much larger degree than with all other hitherto known protective measures. The mean peak increase in serum creatinine was reduced from 48.2 to 11.2 $\mu\text{mol/l}$ with iodixanol. Most importantly, the clinically relevant increase in serum creatinine of 88.4 or more was reduced from 15.4% to 0%. At the same time, isosmolar contrast media provoked less adverse events than regular contrast media, supporting the concept that microcirculatory and antithrombotic effects may be involved as well.

In summary, with the identification of high risk patients and the consequent application of nephro-protective measures, including isosmolar contrast media, the incidence of CMIN can be dramatically reduced and investigations requiring contrast media may be offered to all patients who need it.

V11. Cutaneous vasomotion in chronic venous insufficiency (CVI)

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Introduction: Our goal was to find out, if the vasomotion of the skin is different between healthy volunteers and patients with CVI of various degrees of severity.

Methods: We examined laser Doppler flux in 16 healthy volunteers and 34 patients with chronic venous insufficiency. Patients with venous insufficiency were divided in Widmer stages. Laser Doppler flux was measured at the inner ankle area, resp. ulcer edge. Flux data were analyzed by wavelet analysis, Statistical test: non-parametric *u*-test.

Results: Highest laser Doppler flux values were found at the ulcer edge in severe venous insufficiency according to Widmer stage 3. Patients with chronic venous insufficiency showed significant differences in laser Doppler flux signals in comparison with healthy volunteers. Further more significant differences were found by dividing patients with venous insufficiency in Widmer stages (healthy volunteers – Widmer stage 1: $p = 0.001$; Widmer stage 1 – Widmer stage 2: $p = 0.005$, Widmer stage 2 – Widmer stage 3: $p = 0.000$). Wavelet analysis confirmed these results.

Conclusion: Vasomotion of the skin seems to be different between CVI Widmer stages. In addition, nonlinear analysis of laser Doppler flux has the impact to indicate the severity of venous insufficiency, which corresponds to the degree of cutaneous microangiopathy.

V12. Effects of CO₂-gas on microcirculation of the skin in patients with chronic venous insufficiency

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The skin perfusion on the distal lower leg was measured in 19 patients with advanced chronic venous insufficiency (CEAP stage 4 and 5) under treatment with CarboWet[®] (CO₂-releasing wound dressing = verum) in comparison to the treatment with TenderWet[®] (control group = placebo). The trials were an intraindividual controlled comparison of the application of verum and placebo wound dressing which was applied and measured at two different times of investigation. Atrophy of the skin was found in 89.47% of the test persons, hyperpigmentation in all while hypopigmentation was found in 52.63% additionally. Capillaritis alba occurred only in 21.05% of the test persons, induration was assessed in 42.11% and in

5.26% in terms of dermatosclerosis. 31.58% had had one or more (by example reoccurring) ulcers before (CEAP stage 5 or Widmer stage 3a) of whom 15.79% were triggered by a trauma. Patients with an active ulcer did not participate.

Results: Laser Doppler Flux (LDF) as a gauge of skin perfusion increased under the application of CarboWet[®] significantly and also differed significantly from the LDF measurements under TenderWet[®] application. The amplitude of the signal of LDF was changed significantly in both wound dressings, whereas the higher amplitudes were in direction of the application of CarboWet[®] not TenderWet[®].

Conclusion: In this controlled study the hypothesis was verified that CO₂ gas (released out of the prototype of a wound dressing) would lead to a distinct increase of LDF as a gauge for skin perfusion in patients with advanced chronic venous insufficiency.

V13. Microvascular blood flow and skin thickness are influenced by changes in environmental temperature in diabetic and non-diabetic subjects

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In recent years, glucose measurement at various test sites and, for practical reasons, especially at the lower forearm became more popular. Environmental confounders, such as temperature and humidity, might have an impact on skin structure and microvascular blood flow and, therefore, influence glucose measurements at these sites.

In this study, we investigated the effect of environmental temperature on skin thickness (ST) and microvascular blood flow (LDF) in diabetic (D) and non-diabetic subjects (ND).

Thirteen D (6 type 1, 7 type 2; 7 male, 6 female; age 46.3 ± 16.4 years, mean \pm SD; duration of diabetes 4.8 ± 4.9 years, HbA1c $6.5 \pm 1.1\%$); and seven ND (5 male, 2 female; age 38.2 ± 11.3 years) subjects participated in the study. The investigations were performed in a temperature and humidity controlled climatic chamber (EMPA, St. Gallen, Switzerland). Starting at 25°C, the chamber temperature was reduced in 4°C steps every 40 minutes until a minimum temperature of 9°C was reached and, thereafter, reversed to the initial temperature. Microcirculation was measured by laser doppler fluxmetry (LDF; Moor Instruments, TTC-45, Devon, GB), and skin thickness (ST) was determined by ultrasound in reflection technique at the lower forearm (Krautkraemer, USD 10, Germany). The study participants underwent the entire procedure on up to four separate experimental days.

At baseline, diabetic subjects showed a significantly reduced microvascular blood flow (D: 95 ± 55 vs. ND: 119 ± 35 AU; $p < 0.05$) and skin thickness (D: 1.30 ± 0.18 vs. ND: 1.38 ± 0.16 mm; mean \pm SD; $p < 0.05$) compared to the non-diabetic controls. During decreasing room temperature a significant reduction in microvascular blood flow (D: -41 ± 49 AU; ND: -46 ± 51 AU; $p < 0.05$) and skin thickness (D: -0.09 ± 0.13 mm; ND: -0.06 ± 0.11 mm; $p < 0.05$) could be observed. In both groups, the changes over the time in ST and LDF were comparable.

Although ST and LDF at the lower forearm are reduced in diabetic subjects, both groups showed similar dynamics in microvascular blood flow and skin thickness during changes in environmental temperature. The results of this study might provide information for the further development of blood glucose measurement techniques by pointing towards the need for individual considerations with regard to compensation of temperature induced parasitic effects in glucose monitoring.

V14. Effect of antithrombin (AT) on the permeability of postcapillary venules

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Aim: The contribution of the hemostatic system to inflammation is well documented. The disturbance of microvascular permeability is part of a local or systemic inflammatory reaction and may lead to multiple organ failure. By means of intravital microscopy we investigated the effect of substitution of AT on endotoxin-(LPS) induced microvascular leakage in the rat mesentery.

Methods: After exteriorisation of the mesentery, male CD rats (300–400 g bw) were infused with 0.5 mg/kg LPS (*E. coli* O55:B5) over 80 minutes. Two treated groups received 500 U/kg of AT (iv bolus, 10 min) either 20 min prior (pretreatment) or 20 min after (posttreatment) the beginning of LPS-infusion. Further LPS-treated groups received either 500 U/kg of tryptophan⁴⁹-modified AT (Trp⁴⁹AT, lack of heparin binding) or heparinase 0.3 or 3.0 U/kg, respectively. Another group of animals received an equal amount of the carrier solution of the Trp⁴⁹AT preparation which contains about 10% of native AT activity. Groups of animals not infused with LPS, untreated or treated with placebo (albumin), respectively, served as controls. Vascular leakage was detected with FITC-marked rat serum albumin by fluorescence microscopy and evaluated by grey value analysis with a computer assisted image processing system. The observation period was 3 hours after the beginning of LPS infusion.

Results: Infusion of LPS led to a significant increase of microvascular permeability. This effect was reduced to the level of unstimulated controls by substitution of native AT. Treatment with Trp⁴⁹AT or its carrier solution could not prevent the increase of permeability. Heparinase given prior to LPS-challenge completely blocked microvascular leakage.

Conclusion: Substitution of AT, even when it is given after the inflammatory stimulus, ameliorates vascular leakage on postcapillary venules as a consequence of LPS infusion. Glycosaminoglycan structures on endothelial surface play a pivotal role within the inflammatory process and the effect of AT in the microcirculation.

V15. Vasomotion for cutaneous non-melanocytic skin melanoma

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For $n = 39$ patients with BCC the Laser Doppler Flux (LDF) on the lesion resp. a healthy reference skin area and additionally ECG and respiration signals were measured for 3–5 minutes.

Methods: We examine differences between both LDF and ECG resp. respiration by means of symbolic analysis. To this end the original signals will be transformed section wise (17 non-overlapping time sections of 512 data values) to symbolic time series. The symbols arise by comparing an actual signal value with some (for example 10) of the past values. The symbols are then vectors consisting of zero and ones. If the actual value is larger as the past value we write a one and otherwise a zero. We use a kind of symbolic wavelet transformation to find a symbolic multiresolution analysis for both symbolic LDF time series. The symbolic wavelet analysis is based on the mathematical group structure of the symbols. The multiresolution gives us in our setting 6 new symbolic time series for both symbolic LDF series. They represent the differences of 6 resolution step of the symbolic input signal. We evaluate their influence on ECG and respiration with mutual information (MI). To calculate MI we replace the symbols by their norm sum, i.e., the number of one's. $MI = 0$ means no influence resp. stochastic independency. For

the statistical inference we aggregate the MI of all 17 time section to a common value for each patient (median, mean and standard deviation as useful aggregation functions).

Results: We have found a significant higher dependence for the finest (i.e., first) resolution difference on the lesions ($p = 0.004$ for respiration, $p = 0.012$ for ECG). The 95 percent confidence intervalls for lesion resp. healthy skin are non-overlapping. This result can be interpreted as follows: local fine microcirculation at the lesions fails at least partially.

V16. Analysis of human hepatic microcirculatory changes following portal reperfusion and delayed arterialization versus simultaneous portal/arterial reperfusion in living-donor liver transplantation

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In clinical liver transplantation portal reperfusion is preferentially performed before arterial reconstruction to reduce the anhepatic period. Experimental and clinical data have raised doubts upon this concept. However, visualization and quantitative analysis of sinusoidal perfusion comparing simultaneous arterial/portal with portal/delayed arterialization reperfusion are missing up to now. Therefore, the present study aimed to analyze sinusoidal perfusion during sequential and simultaneous graft reperfusion in patients undergoing living-donor liver transplantation ($n = 27$). Microvascular observation was performed using the orthogonal polarization spectral (OPS)-imaging technique. Baseline microcirculation was assessed in donors livers directly following laparotomy. In group 1 ($n = 14$) the reperfusion was performed sequentially compared to group 2 ($n = 13$) with simultaneous arterial/portal reperfusion. Sinusoidal perfusion was analyzed 5 and 30 minutes following rearterialization. The mean cold ischemia time was 76 ± 32 min in group 1 and 95 ± 42 min in group 2 ($p = 0.1$). The mean portal clamping time was 40 ± 16 min in group 1 and 65 ± 21 min in group 2 ($p = 0.07$). Quantification of the microcirculatory parameters was performed off-line by using a computer-assisted image analysis system as were the sinusoidal diameter (D), red blood cell velocity (RBCV), sinusoidal volumetric blood flow (BVs), functional sinusoidal density (FSD), and inter-sinusoidal distance (ISD). Deteriorations of sinusoidal perfusion were significantly less pronounced in group 2. Typically, manifestation of red blood cell sludging and sinusoidal perfusion stasis was observed in group 1. In group 2 the FSD was significant increased compared to group 1. In contrast, the RBCV, D, and BVs in group 1 were significantly higher compared to group 2, indicating a reactive postischemic hyperemia. Delayed arterialization, i.e., portal reperfusion may cause rapid graft rewarming without adequate oxygenation, resulting in warm ischemia, which may lead to the more pronounced postischemic reaction. The lack of *vis a tergo* during initial reperfusion, when sinusoids have to be cleared of hepatocellular blebs and endothelial cells detached during cold storage, may be responsible for sinusoidal no-reflow phenomenon, particularly in areas receiving predominantly arterial inflow and could explain the improvement of FSD following simultaneous reperfusion.

V17. Myocardial oxygen tension during extracorporeal circulation

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Introduction: Experimental data have shown the potential risk of cellular damage of the myocardium during extra corporeal circulation (ECC). The influence of ECC on myocardial microcirculation however

remained unclear. In this animal study influence of ECC on myocardial microcirculation measured by myocardial oxygen tension in a beating heart was investigated.

Methods: In a pig animal model flexible pO₂ microcatheters were positioned in the midmyocardium of the left ventricle and the skeletal muscle and tissue oxygen tension during ECC was monitored and compared with a control group without ECC. Myocardial oxygen tension was continuously measured in both groups with the microcatheters. CMP. Arterial pO₂ was kept stable at 150 mmHg. Body temperature was maintained at baseline value.

Results: Continuous measuring of tissue oxygenation in the myocardium was feasible in all animals. Baseline value in group 1 was 26.5 ± 0.46 mmHg. In group 2 the baseline value was 20.73 ± 0.97 mmHg. There was no significant difference between baseline values. In group 1 a steady increase over time with a value of 41.4 ± 0.47 mmHg at 90 min was observed. In group 2 a rapid increase to a value of 40 ± 0.42 mmHg occurred after 5 min and increased steadily over time to a value of 56 ± 0.42 mmHg at 90 min. During the observation period myocardial oxygen tension remained significantly higher in group 2 compared to group 1. The myocardial oxygen tension at 5 min after unloading the heart in group 2 was significantly higher compared to the control group.

Conclusion: Our findings show the beneficial effect of ECC on myocardial pO₂. This may support the use of ECC in coronary artery bypass grafting because the potential myocardial injury due to ECC is not related to myocardial ischemia. On the contrary, myocardial pO₂ was even increased during extracorporeal circulation in this study.

V18. Alveolar microcirculation after lung transplantation using modified preservation solutions assessed by OPS imaging

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Objectives: Transplant failure due to ischemia/reperfusion injury is one of the major risks during solid organ transplantation. Beside other reasons a severed microcirculation might be the cause. In previous studies we established the observation of the pulmonary microcirculation using OPS imaging and used this method to assess the graft function after 24 hours ischemia with phosphodiesterase-V inhibitor (Sildenafil) modified perfusion in the early phase of reperfusion after lung transplantation (LuTX).

Methods: We performed single LuTX on 21 pigs after 24 h graft-ischemia preserved with buffered low-potassium-dextran solution (I), with addition of 20 µg Epoprostenol (II) and with 5 mg Sildenafil intravenously before start of perfusion (III). Pulmonary microcirculation was assessed by direct intraoperative intravital microscopy with the OPS imaging system and was monitored hourly up until 6 hours after reperfusion. Vessel diameter (VD), red blood cell (RBC) velocity and functional capillary density (FCD) were acquired. Hemodynamics and blood gases were monitored hourly up until 6 hours after reperfusion. Lung tissue was taken after experiment for wet/dry assessment.

Results: VD was significant increased in group III after reperfusion beginning (5.3 ± 0.8 vs. 5.1 ± 1.1 and 4.9 ± 0.9 µm, III vs. II and I; mean ± SD; $p < 0.05$) and slightly decreased during the observation period. Mean of RBC velocity was lower in group III but didn't reach significance (256 ± 93 vs. 263 ± 85 and 283 ± 66 µm/s, III vs. II and I; mean ± SD). FCD recovery reached significant difference in group III during the first hour (2.9 ± 0.4 vs. 2.5 ± 0.5 and 2.2 ± 1.1 µm/µm², III vs. II and I; mean ± SD; $p < 0.05$). Pulmonary circulation showed improvement in group III with lowered pulmonary pressure and pulmonary vascular resistance values over the whole observation time when compared to the other

groups. Oxygenation was also improved in Group III (507 ± 45 vs 440 ± 180 and 293 ± 195 mmHg, III vs. II and I; mean \pm SD).

Conclusions: Sildenafil modified perfusion allows for a better graft function after 24 h ischemia as evidenced by the macro-/microhemodynamical data and the better oxygenation. Further studies to evaluate right dosage and application timing of Sildenafil are necessary.

V19. Preconditioning with nitric oxide inhalation influences. Early cytokine response and cellular infiltration in pulmonary ischemia/reperfusion injury

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Background: Recently, we could demonstrate the improvement of lung function and reduced liberation of reactive oxygen species after preconditioning by nitric oxide (NO) inhalation. Using an *in vivo* pig model of normothermic lung ischemia with NO inhalation as preconditioning serum levels of active transforming growth factor beta 1 (TGF β 1), interleukin 1 beta (IL 1 β), interleukin 6 (IL 6) and tissue infiltration with T-lymphocytes (CD 3) and macrophages (MA) were analyzed.

Methods: After left lateral thoracotomy normothermic lung ischemia was maintained for 90 min, followed by a 5 h reperfusion period (group I, $n = 7$). In group II ($n = 6$) NO inhalation (10 min, 15 ppm) preceded ischemia. Group III ($n = 7$) underwent sham surgery. Serum levels of TGF β 1, IL 1 β and IL 6 were analyzed by enzyme immunoassay from arterial blood. Tissue infiltration with CD 3 cells and MA were immunohistochemically stained using lung tissue harvested before ischemia (H1) and after reperfusion (H2).

Results: Animals in group I showed a significantly increased liberation of IL1 beta and IL 6. Density of tissue CD 3 and MA infiltration was also significantly higher compared to controls at H2. A biphasic TGF β 1 response with lowest levels during ischemia showed no significant differences compared to controls. In group II IL 6 response and CD 3 and MA infiltration were also significantly increased while IL 1 β response was suppressed. The course of TGF β 1 response also showed a biphasic pattern with highest levels immediately after NO administration. After 5 hours of reperfusion there was a slight but not significant reduction.

Conclusion: NO inhalation before lung ischemia leads to decreased serum levels of IL 1 β during early reperfusion. IL 6 liberation was not influenced. Its release seems to be an IL1 β -independent phenomenon suggesting a possible anti-inflammatory aspect in IL 6 release. Density of tissue infiltration of T-lymphocytes and macrophages were comparable in groups I and II. NO inhalation induces an immediate increase in serum levels of active TGF β 1, which is also immediately reversed by ischemia providing further evidence that a short-term regulation of TGF β 1 by nitric oxide exists.

V20. In vitro intravitalmicroscopy after clinical lung transplantation

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Introduction: Transplant failure due to ischemia/reperfusion injury is one of the major risks during solid organ transplantation. Responsible for the so called no reflow phenomenon is a severed microcirculation. Orthogonal polarization spectral (OPS) imaging is a new method to visualize the microcirculation *in vivo* without use of dyes, allowing clinical investigation of human organs. It was the aim of our study to analyze the microcirculation of human transplanted lungs during the early phase of reperfusion.

Methods: 14 patients (7 women and 7 men, mean age 38 years \pm 11, all bilateral lung transplantation) were enrolled in this study. Donors graft met all standard criteria; mean ischemic time was 245 \pm 56 min for the right and 334 \pm 66 minutes for the left lung. Pulmonary microcirculation was assessed by direct intraoperative microscopy from the visceral surface. Images were obtained at timepoints – 15, 30 and 45 min after reperfusion with the help of a orthogonal polarization spectral (OPS) imaging system (Cytoscan™) and processed computer assisted. Capillary diameter, red blood cells (RBC) velocity, capillary blood flow and functional alveolar capillary density (FACD) were evaluated off-line.

Results: 2700 microvessels were analyzed. 15 min after reperfusion capillaries on the lung surface were only minimally perfused. After 30 min we found initialization of the microcirculatory blood flow, with RBC velocity reaching 119.6 \pm 46.7 μ m/s (mean \pm SD) up to 290.4 \pm 7.9 μ m/s ($p < 0.05$) 45 min after reperfusion. FACD increased from 1.7 \pm 0.5 to 8.1 \pm 4.1 μ m/ μ m² ($p < 0.05$). In two cases we observed local intracellular edema direct after implantation, as well as an increased amount of leukocytes in not perfused vessels.

Conclusion: Our study describes the microcirculation of human lungs in the early phase after transplantation. We demonstrated a significant delay of the reperfusion process in the microcirculation compared to the macrocirculation in large vessels after organ reperfusion. Whether or not OPS imaging is a reliable instrument to detect early pathological events after lung transplantation shall be evaluated further.

V21. Vascularization of liver tumors – Preliminary results with Coded Harmonic Angio (CHA), phase inversion imaging, 3D-power Doppler and contrast medium-enhanced B-flow with second generation contrast agent [Optison®]

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Aim: To investigate the dynamic value of contrast-enhanced ultrasonography with Optison® in appraising the vascularization of hepatic tumors using harmonic imaging, 3D-/power Doppler and B-flow.

Materials/methods: 60 patients with a mean age of 56 years (range 35–76 years) with 93 liver tumor, including histopathologically proven hepatocellular carcinoma (HCC) [15 cases with 20 lesions], liver metastases of colorectal tumors [17 cases with 33 lesions], metastases of breast cancer [10 cases with 21 lesions] and hemangiomas [10 cases with 19 lesions] were prospectively investigated by means of multislice CT as well as native and contrast medium-enhanced ultrasound using a multifrequency transducer (2.5–4 MHz, Logig 9, GE). B scan was performed with additional color and power Doppler, followed by a bolus injection of 0.5 ml Optison®. Tumor vascularization was evaluated with coded harmonic angio (CHA), with pulse inversion imaging with power Doppler, 3D power Doppler and in the late phase, (>5 min) with B-flow. In 15 cases with HCC, i.a. DSA was performed in addition. The results were also correlated with MRT and histological findings.

Results: Compared to spiral-CT/MRT, only 72/93 (77%) of the lesions could be detected in the B scan, 75/93 (81%) with CHA and 93/93 (100%) in the pulse inversion mode. Tumor vascularization was detectable in 43/93 (46%) of lesions with native power Doppler, in 75/93 (81%) of lesions after contrast administration in the CHA mode, in 81/93 (87%) of lesions in the pulse inversion mode with power Doppler and in 77/93 (83%) of lesions with contrast-enhanced B-flow. Early arterial and capillary perfusion was best detected with CHA, particularly in 20/20 (100%) of the HCC lesions, allowing a 3D reconstruction. 3D power Doppler was especially useful in investigating the tumor margins. Up to

20 min after contrast medium injection, B-flow was capable of detecting increased metastatic tumor vascularization in 42/54 (78%) of cases and intratumoral perfusion in 17/20 (85%) of HCC cases. All 19 hemangiomas were correctly classified by phase inversion imaging.

Conclusions: Contrast medium-enhanced ultrasound investigation of liver tumors with Optison® allowed reliable detection of tumor foci and, in most cases, appraisal of tumor vascularization. The time available for evaluating of tumor margin vascularization was substantially longer in B-flow.

V22. Objective control of rheopheretic treatment age related macular: Pilot experiments allowing to distinguish responders from non-responders

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Background: age related macular degeneration (AMD) is the most frequent cause of blindness in the developed world. Until recently, AMD was refractory to therapy: other than so called “Drusen” as unspecific lipoprotein deposits no easily quantifiable biochemical abnormality had been identified. Based on initial fortuitous observations subsequent to extracorporeal molecular filtration techniques by Brunner and Borgerg, there have been a number of controlled prospective clinical studies in Germany and the USA corroborating clinical improvement after repetitive extracorporeal plasma filtration: enhanced visual acuity and prevention of complications (wet form of AMD) are promising results.

Insight from *in vitro* studies: as in a large variety of chronic degenerative diseases, the AMD-patients showed the unspecific combination of moderate to strongly enhanced red cell aggregation, enhanced plasma viscosity, slightly rigidified RBC and normal to enhanced Hct-value.

Insight from choroideal fluorescence angiography: Using indocyanin-green (coupled to albumin) as fluorochrome, the inflow of the complex (which can be excited to fluoresce in the near infra red, the principle of Computerised Cardio-Green-Perfusography can be applied to AMD patients. Our pilot studies indeed substantiated the suspicion of perfusion anomalies in the choroideal microvascular bed when compared to the extremely rapid and homogenous perfusion found in healthy controls. Currently, we can report about successful therapy in 4 cases, where data about visual acuity, *in vitro* rheology and rehomogenisation of choroidal are in congruence. However, one patient did not respond: In this case, the rheology was not abnormal, but he had a circumscribed defect in the submacular choroicapillaries which was detected before and several times after rheopheresis.

Preliminary conclusion. Future studies and patient selection might hopefully be guided better by the results of simple rheological tests on the one hand, and perfusographic control of responsiveness to initiating rheopheresis.

V23. Microbubble contrast agent enhanced ultrasound for differentiating breast tumors – First results

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Purpose: To investigate in how far fibroadenoma and carcinoma of the breast can be differentiated by injection of a new contrast agent (Optison®) in the “early” (up to 5 min) and in the “late” (8–20 min after injection) phases.

Materials and methods: 50 patients with 53 impalpable breast tumors (20 fibroadenomas, 33 carcinomas) were examined by means of contrast agent enhanced ultrasound techniques. Three of the cases had two carcinomas in the same breast. The tumor diameters varied between 4 and 15 mm. Histological confirmation was obtained in all cases. Ultrasound was performed using an equipment with a multifrequency linear probe (5–10 MHz, Logiq 9/700, GE, Milwaukee, Wisconsin, USA). Low values of 0.2 to 0.4 for the mechanical index (MI) and a low transmitter output of <50% were specified as machine parameters. B-mode and tissue harmonic imaging mode (THI) both using power Doppler imaging without and with intravenous contrast agent administration (0.5 ml of Optison[®]) were employed. Contrast agent behavior in the tumor was observed for over 20 min.

Results: In plain ultrasound, marginal, penetrating or central vessels were found in 24 of 53 lesions (18 carcinomas, 6 fibroadenomas). After contrast agent injection, size and number of vessels increased and in both benign (17/20) and malignant tumors (30/33) marginal and/or intratumoral vessels were identified in the “early phase” within 2 min after injection. In the “late phase”, a diffuse parenchymal contrast agent accumulation was observed in 8 to 20 min after injection in the cases of 30 of the 33 malignant tumors (Sensiti. No such accumulation occurred in benign tumors. For the carcinoma, the sensitivity was 93% and the specificity 100% in the “late phase” compared with a sensitivity of 100% and a specificity of 25% the “early phase”, when all findings were combined.

Summary: A change of ultrasound equipment settings and the use of Optison[®] as contrast agent allows the detection of tumor enhancement for up to 20 min. Whereas a differentiation between fibromas and carcinomas is difficult in the early phase, contrast agent is only taken up by carcinomas in the late phase.

V25. Antithrombin inhibits sprouting angiogenesis of microvascular endothelial cells *in vitro*

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Background: Antithrombin (AT) has recently been shown to inhibit endothelial cell proliferation, angiogenesis and tumor growth *in vitro* and in animal models. The anti-angiogenic potential may differ between several conformational AT variants. The effect of these variants, including latent AT, a heat-denatured form with low heparin and protease affinity due to a conformational change in which the reactive site, on endothelial cell sprouting in a three dimensional assay was evaluated.

Methods: A microcarrier-(MC)-based *in vitro* assay for quantification of three-dimensional cell angiogenesis was used. Rat pulmonaty endothelial cells (RAPMEC) were cultivated on MC beads until they nearly reached confluence. The beads were embedded into a three-dimensional fibrin matrix, a fibrinogen solution polymerized by addition of thrombin. The matrix was overlaid with culture medium which served as a negative control. Fetal calf serum (FCS) at concentrations of 0.1 of 1.0%, respectively, were added to the supernatant as positive/intra-assay controls. Basic fibroblast growth factor (bFGF, 2 ng/ml) was added for stimulation of angiogenesis with or without alpha-, beta-, Trp⁴⁹-modified- or latent AT (LAT) or a commercially available AT preparation (Kybernin[®], KYB) at different concentrations (all AT-preparations were a generous gift of Dr. U. Kalina, Aventis-Behring, Marburg). Angiogenic activity was quantified by determination of the average number of capillary-like formations per MC (cap/MC). The assay was stopped with 3% paraformaldehyde and evaluated when sprouting in FCS 1.0%-control reached a cap/MC = 1.0.

Results: Upon stimulation with FCS or bFGF RAPMEC migrated from the monolayer and extended long filopodia which closely resembles sprout formation *in vivo*. KYB or alpha-AT dose dependently,

ranging from 0.4 to 3.4 mg (no effect for 0.2 mg), inhibited bFGF- or ECGF-induced sprout formation, whereas no inhibition was found for IAT at a dose ranging from 18 to 300 μ g.

Conclusion: These results raise the question, whether the anti-angiogenic properties, like other cellular effects of AT, depend on the native structure with normal heparin or protease affinity.

V26. Effect of Antithrombin (AT) on the permeability of postcapillary venules

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Aim: The contribution of the hemostatic system to inflammation is well documented. The disturbance of microvascular permeability is part of a local or systemic inflammatory reaction and may lead to multiple organ failure. By means of intravital microscopy we investigated the effect of substitution of AT on endotoxin-(LPS) induced microvascular leakage in the rat mesentery.

Methods: After exteriorisation of the mesentery, male CD rats (300–400 g bw) were infused with 0.5 mg/kg LPS (*E. coli* O55:B5) over 80 minutes. Two treated groups received 500 U/kg of AT (iv bolus, 10 min) either 20 min prior (pretreatment) or 20 min after (posttreatment) the beginning of LPS-infusion. Further LPS-treated groups received either 500 U/kg of tryptophan⁴⁹-modified AT (Trp⁴⁹AT, lack of heparin binding) or heparinase 0.3 or 3.0 U/kg, respectively. Another group of animals received an equal amount of the carrier solution of the Trp⁴⁹AT preparation which contains about 10% of native AT activity. Groups of animals not infused with LPS, untreated or treated with placebo (albumin), respectively, served as controls. Vascular leakage was detected with FITC-marked rat serum albumin by fluorescence microscopy and evaluated by grey value analysis with a computer assisted image processing system. The observation period was 3 hours after the beginning of LPS infusion.

Results: Infusion of LPS led to a significant increase of microvascular permeability. This effect was reduced to the level of unstimulated controls by substitution of native AT. Treatment with Trp⁴⁹AT or its carrier solution could not prevent the increase of permeability. Heparinase given prior to LPS-challenge completely blocked microvascular leakage.

Conclusion: Substitution of AT, even when it is given after the inflammatory stimulus, ameliorates vascular leakage on postcapillary venules as a consequence of LPS infusion. Glycosaminoglycan structures on endothelial surface play a pivotal role within the inflammatory process and the effect of AT in the microcirculation.

V27. Hemorheology and coagulation: Changes after discontinuation of long-term Vitamin K-Antagonist (VKA) treatment

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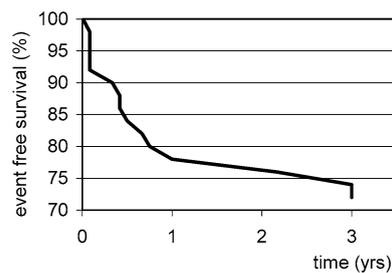
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Background: Patients receiving long-term oral anticoagulation (OAC) for secondary prevention of deep vein thrombosis (DVT) often hesitate to discontinue OAC, as they fear a relapse. Reports of a temporary activation of coagulation in the initial phase of termination of OAC may support this. Therefore, this prospective study investigated coagulation and rheological parameters after discontinuation of VKA during the initial 6 weeks, and attempted to identify whether there is a period of hypercoagulability after discontinuation of VKA.

Patients and methods: In this prospective study one hundred consecutive patients were included in whom long-term OAC for VTE was electively discontinued. Exclusion criteria were presence of hereditary deficiency of antithrombin, protein C or S, patients with lupus anticoagulans or APS. Rheological and haemostaseological parameter were monitored weekly for 6 weeks after discontinuation of OAC including D-dimer levels, fibrinogen, erythrocyte-aggregation, plasma viscosity and hematocrit. Colour coded duplex sonography was performed immediately before discontinuation of OAC and again after 6 weeks. Long-term follow-up was scheduled for one year.

Results: 15 recurrence occurred after a follow-up of 14 ± 1 months. One patient died due to pancreatic neoplasm. The characteristics of the recurrency-group and the no recurrency-group were not different with respect to age. The duration of previous OAC was 22 ± 4 mts in the recurrency-group and 15 ± 2 mon in the no recurrency-group. The recurrency-group had significantly higher D-dimer levels ($p = 0.037$) and also significantly higher hematocrit levels ($p < 0.00001$). Fibrinogen and plasma viscosity normalised in the no recurrency-group, but the levels in the recurrency-group remained higher after discontinuation of OAC.

Summary: One third of the recurrences occurred within the first weeks after withdrawl of OAC. D-dimer levels and hematocrit values were significantly higher in the recurrency-group. Prothrombin-time normalised after 2 weeks in both groups. Fibrinogen and erythrocyte aggregation showed a decrease in the no recurrency-group but remained high in the recurrency-group.



Conclusion: A large number of recurrences occur early after discontinuation of OAC. Elevated D-dimer levels and a high hematocrit seem to be a promising indicator to predict recurrences. A close monitoring of rheological and coagulative factors should be recommended after the discontinuation of coagulative therapy.

V28. A practical concept for pre-operative identification and improved management of patients at risk for bleeding

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Total of 5649 unselected adult patients were enrolled to identify impaired hemostasis prior to surgical interventions. Each patient was asked to answer a standardized questionnaire of bleeding history. Activated partial thromboplastin time (aPTT), prothrombin time (PT) and platelet counts (PC) including PFA-100 (platelet function analyser): collagen–epinephrine (C/E) and collagen–ADP (C/ADP) were routinely done in all patients. Additional tests, bleeding time (BT) and von Willebrand factor (vWF: Ag) were performed only in patients with a positive bleeding history and/or evidence of impaired hemostasis, e.g., drug ingestion.

The bleeding history was negative in 5021 patients (88.8%) but positive in the remaining 628 (11.2%). Impaired hemostasis could be verified only in 256 (40.8%) of these patients. The vast majority were identified by PFA-100: C/E ($n = 250$; 97.7%). The vast majority were identified by PFA-100: C/E ($n = 250$; 97.7%). The only abnormality found among patients with a negative bleeding history was a prolonged aPTT due to lupus anticoagulant in 9 patients (0.2%). The sensitivity of the PFA-100: collagen–epinephrine was the highest (90.8%) in comparison to the other screening tests (BT, aPTT, PT, vWF: Ag). The positive predictive value of the PFA-100: collagen–epinephrine was high (81.8%), but the negative predictive value was higher (93.4%). We identified 254 out of 5649 unselected patients scheduled for surgery at our hospital as having either acquired ($n = 182$) or inherited ($n = 72$) impaired primary hemostasis (platelet dysfunction including von Willebrand disease). All patients were initially pretreated with desmopressin (DDAVP) and further anti-bleeding drugs in case of DDAVP-non-response.

The administration of DDAVP led to a correction of platelet dysfunction in 229 of the 254 patients treated (90.2%). The frequency of blood transfusion was lower, but not statistically significant (9.4% vs. 12.2%: $p = 0.202$) in preoperatively treated patients with impaired hemostasis than in patients without impaired hemostasis. In a retrospective group, the frequency of blood transfusion was statistically significant higher (89.3% vs. 11.3%: $p < 0.001$) in patients without preoperative correction of impaired than in patients without impaired hemostasis.

Preoperative identification and correction of impaired primary hemostasis is possible in nearly all patients affected, and results in a reduction of homologous blood transfusions.

V29. Antithrombotic therapy for venous thromboembolic disease – Present and future

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Venous thromboembolism is a common and potential fatal disease. If properly used, anticoagulant therapy is effective in preventing thromboembolism and in improving survival. Evidence Based Guidelines, Grade 1 recommendations will be presented and strongly indicate that the benefits do, or do not, outweigh risks and costs. A diagnostic decision making tree using laboratory data, clinical data und ultrasound imaging is useful. New score systems reflect on the probability of venous thromboembolism. Due to their favorable pharmacological properties and beneficial effects low molecular weight heparins have become the standard of the prevention and therapy of venous thromboembolism, but new classes of synthetic antithrombotic substances show promising results.

V30. Perioperative management of patients under oral anticoagulation with vitamin K antagonists (VKA) – Role of low molecular weight heparins (LMWH)

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There are numerous indications for a long-term or permanent oral anticoagulation (OAC) with coumarin derivatives. However, there are situations requiring interruption of long-term anticoagulation, e.g., planned diagnostic or surgical interventions. There is a dilemma: Because of the bleeding risks associated with the procedure, continuation of OAC is not possible, but simply stopping the medication brings about a considerable thromboembolic risk. Therefore, an alternative anticoagulation has to

be initiated instead of OAC. This was achieved frequently by an overlapping continuous i.v. infusion of aPTT-adjusted dosages of heparin. Frequent aPTT monitoring had to be performed while the INR (International Normalized Ratio) or PT, respectively, approached the target level adequate for the intervention. Subcutaneous, discontinuous application of low molecular heparin (LMWH) is an alternative to the periinterventional intravenous, continuous application of unfractionated heparin (UFH). The main advantage of low molecular weight heparins versus conventional heparins when administered subcutaneously is their far better bioavailability (LMWH > 90% vs. UFH 20–30%) and their longer elimination half life. The anticoagulant effect resp. the antithrombotic efficacy thus becomes more predictable. Clinical experience with periinterventional anticoagulation of patients with OAC are still limited (no existing guidelines). Somewhat more data are available for the application of LMWH (Certoparin, Dalteparin, Enoxaparin, Nadroparin) as an alternative treatment of patients with contraindications or incompatibility to OAC. A stringent risk/benefit assessment is to be performed in any individual case before the intervention.

After the patient has proved to be eligible, oral anticoagulation should be withheld for as short a time as possible. The target INR (PT) has to be determined in cooperation with the intervening doctor with respect to the patient's individual hemorrhagic and thromboembolic risk. Together with the patient, the prospective intervention date is fixed. Special attention should be paid to the detailed disclosure of risks for the patient.

V31. The “Reverse Flow Viscometer” – a new method for the determination of the dynamic viscosity by capillary viscometry

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A variety of studies demonstrate an association of parameters related to blood viscosity with human pathology of varying origin. Whole blood and plasma viscosity are considered to be clinically useful indicators in the diagnostic work up and therapy monitoring of certain diseases. Thus, viscometry is an often applied method in clinical chemistry.

Many types of common capillary viscometers determine the kinematic viscosity, and it is necessary to measure the density of the probe additionally for the calculation of the dynamic viscosity. This disadvantage was the rationale to develop a new viscometer.

We introduce a new capillary flow viscometer, which is based on the following principle. The sample is sucked up by reduced pressure through the vertical arranged capillary into a closed hollow space. Due to the reduction of the gas filled space by the intruding sample the pressure changes. The pressure (p) in the hollow space is measured. It increases as a function of time (t) in a manner, which depends on the dynamic viscosity of the sample. The viscosity can be calculated from the slope of the plot $d(1/p)/dt = f(p)$. Since the direction of flow is reverse in contrast to common viscometers, the device was named “Reverse Flow Viscometer” (RFV).

The measuring principle is applicable to Newtonian as well as non-Newtonian liquids. The shear rates range from approximately 500 s^{-1} to zero, which is especially of interest for the investigation of whole blood.

The accuracy of the measurements of the viscometer, requiring less than 1.0 ml sample volume, is superior to most conventional methods. The major distinction between the functionality of common capillary viscometers and the RFV is that common capillary viscometers measure the kinematic viscosity whereas the RFV directly provides the dynamic viscosity without any additional density measurement.

V32. Determination of oxygen metabolism in tissues by combined white light spectrometry and laser spectroscopy – An overview about method and study results

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For the assessment of tissues in surgery, intensive care (ICU) or science, the blood flow in the micro-circulation, the tissue pO₂ and other methods like temperature- or pH-measurements have been used. The necessity to assess tissues and organs is obvious in surgery where local perfusion and oxygen situation matters most (e.g., ulcera, transplant, anastomosis). In ICU the regional monitoring is of increasing importance due to regional affect of therapy (e.g., catecholamines, sepsis, organ function).

A new method, called O₂C (oxygen to see) combines white light spectrometry and laser spectroscopy to enabling non-invasive measures in tissues of various depths of about 100 μm to 16 cm. The blood flow in the capillaries, the post-capillary oxygen saturation and the amount of haemoglobin in tissue can be detected pre- intra- and post-operatively. White light in the range of 500 to 800 nm is applied at same time as laser-light of the wavelength 830 nm via flexible fibre optical probes.

An overview about study results of the following fields of application are presented.

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V34. Colloid osmotic pressure – The newest approach, its measurement and clinical value

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Plasma colloid osmotic pressure (COP) is an important determinant and a major physical factor that determines fluid balance at the microcirculatory level. Its measurement on a routine basis would make possible to characterize the overall state of water balance of an organism or an organ by testing blood samples. Until recently however, problems concerning the accuracy and meaning of such measurements have not been fully examined. This uncertainty is in part due to a basic difference between the goals of the well – established osmometer methods in physical chemistry and informations sought by the cardiovascular physiologist. To fill this gap – a handy and realible instrument is needed – which allows the replication of measurments without any kind of teasing handling, like tensioning of membrane versus several hours and any kind of conditioning! The COP 2010 fullfills these demands and presents an appropriate tool in clinical application and economical needs. Therefore the intensified application of such oncometric instruments in various diciplines of med comes into to sight.

V35. Haemocompatibility of stents in interventional neuroradiology

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Haemoincompatibility induced by contact with artificial surfaces and materials is a major obstacle to further development of artificial organs and accounts for much of morbidity in circumstances when the blood circulates through intracerebral and extracranial vessels or a catheter is placed. Materials' testing was performed using a dynamic Chandler System and results were transformed in a dimension free score system. Our models can discriminate *in vitro* hemocompatibility of 8 commercially available stents used during neuroradiologic procedures. Flow characteristics and preanalytical effects may not be neglected. Compared to the results of 50 investigated coronary stents further improvements are necessary to optimize haemocompatibility of stents used in neuroradiology.

V36. Stent-artery interaction of a novel poly(L-lactide) coronary stent and a stenotic coronary vessel: A finite element study

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Background: Atherosclerotic coronary artery disease leads to a progressive luminal narrowing of the diseased vessel. In order to restore luminal patency and blood flow, coronary stents can be introduced into the stenotic vessel in a minimally invasive procedure. A major limitation of stent therapy, however, is in-stent restenosis, which is mediated by vascular injury due to the stent. Finite element analysis (FEA) can be used to simulate stent-artery interaction and support the development of atraumatic stents. In this study, we have investigated the interaction of a stenotic coronary vessel with a biodegradable poly(L-lactide) (PLLA) coronary stent.

Materials and methods: A 3D-model of a stenotic (circular concentric, 50% area, 30% diametral stenosis) coronary vessel was created with the CAD package Pro/Engineer. Geometric and hyperelastic material data were adapted from literature sources. The stent model used in this study represents a biodegradable PLLA coronary stent, which is a proprietary development by our institute. The finite element pre-processor MSC Patran was used to discretize the geometric models with hexahedral elements. The following load cases were modelled: (i) pre-stretch ($\lambda = 1.2$) and pre-inflation (100 mmHg) of the vessel, (ii) balloon expansion of the stent from I.D. = 1.4 mm to I.D. = 3.5 mm. 3-dimensional nonlinear static FEA of these load cases was performed with the FEA software ABAQUS.

Results and discussion: The FEA showed that the PLLA stent is able to withstand the radial forces exerted by the stenotic vessel. The resulting stent oversizing was between 5 and 10%. The maximum radial retraction of the stent was 0.29 mm. Maximum tissue prolapse was observed within the healthy tissue adjacent to the stenosis (70–200 microns). Von Mises stress in the artery was highest within the calcified plaque (38 MPa). *In vivo*, this stress level is very likely to cause plaque rupture during the stenting procedure, since it exceeds the tensile strength (1.6 MPa) of the plaque. In the surrounding healthy tissue (tensile strength = 1–1.5 MPa), the von Mises stress was between 0.2 and 3 MPa. This indicates, that the stent would only cause mild trauma in the adjacent vascular tissue.

V37. Novel polymeric biomaterials for tissue engineering applications in head and neck surgery: Establishment of primary epithelial cell cultures and their biochemical characterization

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The availability of biodegradable polymeric biomaterials which are highly specific adaptable to anatomical, physiological, and surgical requirements gains increasingly significance in the development of new therapeutical options in several medical fields. The biomedical application of biomaterials *in vivo* requires a regular wound healing. The delicate balance between a group of endogenous enzymes, Matrix Metallo-proteinases (MMPs), and their inhibitors (Tissue Inhibitor of MMPs, TIMPs) have a decisive function in the remodeling of the extracellular matrix during processes like wound healing or the integration of biomaterials in surrounding tissues after implantation. In this study the expression of MMPs and TIMPs of primary cell cultures of the pharynx was investigated after cell seeding on the surface of a novel, biodegradable polymer. Primary cell cultures of the pharynx of Sprague-Dawlex rats were seeded on the surface of a thermoplastic block copolymer and on a polystyrene surface as control. Conditioned media of the primary cells were analyzed for MMPs and TIMPs on Day 1, 3, 6, 9 and 12 of cell growth. The MMP and TIMP expression was analysed by zymography and a radiometric enzyme assay. A linear increase of the total cell number of the pharyngeal cell cultures was found after adhesion of the cells on the polymer and the control surface. No statistically significant differences in the appearance and the kinetic of MMP-1, MMP-2, MMP-9 and TIMPs were detected between cells grown on the polymer surface compared to the control. An appropriate understanding of the molecular processes that regulate cellular growth and integration of a biomaterial in surrounding tissue is the requirement for an optimal adaptation of biodegradable, polymeric biomaterials to the physiological, anatomical, and surgical conditions *in vivo* to develop new therapeutic options in otolaryngology and head and neck surgery.

V38. Cell and blood compatibility of polyacrylnitrile (PAN) and PAN-copolymers

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Most polymers used in medical applications today are materials that were not developed originally for this application area. Different biomedical applications are demanding different specifications to the properties (i.e., mechanical properties) and the functionality of the biomaterials. Today polymer systems are developed which allow a tailoring of properties by varying the molecular parameters including a functionalization with regard to their application.

New membrane-forming copolymers were synthesized, based on acrylonitrile (AN) copolymerized with hydrophilic N-vinylpyrrolidone (NVP) monomer, in different percentage ratios, such as 5, 20, and 30% w/w NVP, and with two other relatively highly polar comonomers. All these copolymers were characterized for their bulk composition and number average molecular weight, and used to prepare ultrafiltration membranes. Water contact angles and water uptake were estimated to characterize the wettability and scanning force microscopy to visualize the morphology of the resulting polymer surface. Cytotoxicity was estimated according to the international standard regulations, and the materials were found to be non toxic. The interaction of the membranes with human skin fibroblasts was investigated considering that these cells are among the first to colonize membranes upon implantation or with prolonged external contact. The chemical composition of the polymer membranes was also tested for the response of the blood defence system and the attachment, morphology and functional activity of kidney epithelial cells. The overall cell morphology, formation of focal adhesion contacts, and cell proliferation were estimated to characterize the cell material interactions.

Based on these results novel therapeutic options in the area of tissue- and organ replacement can be expected.

V39. First results after mucosa reconstruction by a polymeric biomaterial in an animal model

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The availability of novel polymeric biomaterials with degradation characteristics, stability and elasticity according to the anatomical, physiological and surgical requirements is the basis for the advance in the field of Tissue Engineering. The mucosa reconstruction of the upper aerodigestive tract by biodegradable, polymeric biomaterials would be a complete new therapeutical option in Head and Neck Surgery. In this study a polymer network was used for the reconstruction of a defect of the gastric wall in Sprague Dawley rats. The aim was to investigate the biomaterial under the extremely chemical, enzymatical, bacteriological and mechanical conditions of the stomach. The animals of the control group underwent a sham operation without biomaterial implantation. The period of implantation time was 1 week, 4 weeks and 6 months. The decisive clinical parameters were the tight connection between the polymer and the surrounding tissue as well as the investigation of the local and systemic inflammation parameters. The surgical procedure and the postoperative period was without complications in all animals ($n = 63$). No statistically significant differences in wound healing as well as in inflammation parameters (haptoglobin, acid α_1 -glycoprotein) were found between the implantation and the control group. These first results suggest that the novel polymeric biomaterial did not influence the wound healing in this animal model. The biomedical application of this polymeric biomaterial will be used for the reconstruction of the mucosa of the upper aerodigestive tract in head and neck surgery.

C1. Pathophysiology of stroke: Lessons from animal models

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Our current understanding of stroke pathophysiology is mainly derived from experimental models. It is currently believed that a focal reduction of cerebral blood flow below a critical threshold induces a spatially and temporally highly complex sequence of events. Excitotoxicity may lead to early necrotic cell death, in particular in the core of the evolving lesion. In the surrounding zone ('penumbra') peri-infarct depolarizations, inflammation, and apoptosis lead to an expansion of the lesion over time [2]. The brain may counteract these deleterious mechanisms by spontaneous reperfusion as well as by endogenous neuroprotection [1]. While mechanisms of ischemic damage have been traditionally studied in models in which a major brain artery is acutely occluded, endogenous neuroprotection, which can be investigated in models of ischemic preconditioning, has only recently come into focus. Adding to the complexity of stroke pathophysiology, recent research has found that stroke has profound effects on the immune system, and that this interaction has important consequences for lesion volume as well as for general outcome [3].

Despite the tremendous progress over the last decades in understanding stroke pathophysiology, and in the development of neuroprotective strategies, the translation of these findings into clinically successful therapy has been disappointing. To overcome the pressing problem of bench to bedside translation in this field, we need to identify the reasons for the clinical failure of so many promising compounds. I will therefore also discuss potential shortcomings of experimental stroke research.

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C2. Mechanisms of inflammatory angiogenesis and lymphangiogenesis in the cornea

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The cornea is one of the few normally avascular tissues of the body. Avascularity is important for corneal transparency and good vision and therefore actively maintained against minor inflammatory insults in most species. Nevertheless, strong inflammatory stimuli can incite parallel ingrowth of both blood vessels (angiogenesis) and lymphatic vessels (lymphangiogenesis) into the normally avascular cornea. The cornea therefore is an excellent model system to study the mechanisms of inflammation-induced angiogenesis and lymphangiogenesis and the role of inflammatory cells contributing to these processes. Using this model system and cytokine traps specific for VEGF-A we could recently show a novel and essential role of VEGF-A in mediating both angiogenesis and lymphangiogenesis. Furthermore, specific local depletion of macrophages demonstrated an essential role for VEGF-A recruited macrophages in an immune-amplification cascade leading to inflammation-associated neovascularization. Finally, the role of angiogenesis and lymphangiogenesis for the induction of immune rejections after (corneal) transplantation are briefly discussed.

C3. Shear stress insensitivity of NOS expression and coronary heart disease

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Shear stress maintenance of endothelial nitric oxide (NO) synthesis effectively helps to retard atherosclerosis. Polymorphisms in the endothelial NO synthase (NOS-3) gene may thus contribute to the development of coronary heart disease (CHD). In endothelial cells isolated from umbilical cords genotyped for the ⁻⁷⁸⁶C/T single nucleotide polymorphism (SNP) of the human *nos-3* gene promoter, shear stress-induced NOS-3 expression (mRNA and protein) was detected in wild-type TT and in heterozygous CT genotype cells but not in cells with the mutant CC genotype. Pre-treatment of the latter cells with a decoy oligonucleotide comprising position –800 to –779 of the C-type *nos-3* promoter effectively restored shear stress-induced NOS-3 expression. In a separate series of experiments, NO-dependent relaxation was monitored in segments of saphenous vein isolated from genotyped patients undergoing aortocoronary bypass surgery. In vein segments from CC genotype individuals, the NO-mediated relaxant response to acetylcholine was significantly attenuated when compared to the CT or TT genotype. Moreover, in patients 64 years old or younger subjected to quantitative coronary angiography, the CC genotype was significantly more frequent in CHD-positive patients (19.0%) as compared to the general population (11.8%, Caucasians, odds ratio 1.8) and in particular the CHD-negative patients (4.4%, odds ratio 5.1). Comparable findings have recently been reported for two Italian cohorts.

The presence of CHD was defined as at least one 50% stenosis in a relevant coronary artery, however most affected individuals presented with a 2 or 3-vessel disease. Both the decreased relaxant response to acetylcholine and the increased CHD risk were independent of other primary risk factors for atherosclerosis such as diabetes, dyslipidemia, hypertension or cigarette smoking. Moreover, no such correlation was found for the ⁸⁹⁴G/T SNP of the human *nos-3* gene affecting the coding region (exon 7). The ⁻⁷⁸⁶C/T SNP of the *nos-3* gene thus constitutes a genetic risk factor for CHD, presumably due to binding of an inhibitory transcription factor to the C-type promoter, blocking shear stress-dependent maintenance of NOS-3 expression.

C4. Basic mechanisms of hypertension: New vascular aspects

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Though hypertension, in most cases, seems to be the result of multiple influences, alterations of microvascular tone and structure play always an important role. Changes have been described in the endothelial vasomotor function as well as in smooth muscle function and reactivity. Clearly, hypertension is associated with endothelial dysfunction as reflected by a reduced formation or efficiency of NO and EDHF. In contrast, the increased release of endothelial vasoconstrictors such as endothelin plays only a role in certain forms of hypertension. Only recently it has been shown that other endothelial defects might also be an important cause of hypertension: the lack of endothelial communication via gap junctions containing connexin 40 has been shown to be a new reason for hypertension, possibly due to exaggerated vasomotion of resistance vessels. In accordance with the clinical finding that calcium entry blockers are not always efficient in treatment of hypertension, recent research revealed Ca⁺⁺-independent pathways in the control of vascular tone, among them regulatory pathways in which small G-proteins like Rho-A and CDC42 play an important role. Stimulators of the Rho pathway are oxLDL and the sphingosine 1-phosphate (S1P). Though little is known so far about the activation of this pathway in human hypertension and the exact role of the receptors involved, the present data suggest a potential role of it in hypertensive diseases. Interestingly enough, S1P seems to be an important mediator of the myogenic constriction (Bayliss effect) which has been shown to be an enhancer of peripheral resistance during pressure increases. Further understanding of these pathways may help to develop new strategies in the treatment of hypertension.

C5. Effect of C-peptide on vascular function in diabetic patients

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Microcirculation in patients with diabetes mellitus type 1 is affected by functional and structural changes of the microvessel itself and by alterations in the rheologic properties of the blood.

Recent investigations provide increasing evidence that impaired C-peptide secretion in type 1 diabetic patients might contribute to the development of microvascular complications. C-peptide has been shown to stimulate endothelial NO secretion by activation of the Ca²⁺ calmodolin regulated enzyme eNOS. NO were shown to increase cGMP levels in smooth muscle cells, to activate Na⁺K⁺ATPase activity and to evolve several effects in micro- and macrovascular blood flow. In type 1 diabetic patients, supplementation of C-peptide was shown to increase endothelial NO secretion and to stimulate microvascular blood flow in several tissues. In addition, C-peptide administration in type 1 diabetic patients leads to a redistribution of skin blood flow in the lower extremity by increasing nutritive capillary blood flow in favour to

subpapillary blood flow. Impaired Na^+K^+ ATPase activity is another feature of diabetes mellitus found in many different cell types and might impact the regulation of various cell functions. C-peptide supplementation in type 1 diabetic patients has been shown to restore Na^+K^+ ATPase activity *in vitro* and *in vivo*. A linear relationship between plasma C-peptide levels and erythrocyte Na^+K^+ ATPase activity could be found in several studies. By decreasing the diameter of the vessel in small capillaries, microvascular blood flow is increasingly affected by the rheologic properties of blood cells. Using laser-diffractoscopy a huge improvement in erythrocyte flexibility could be observed after C-peptide administration in type 1 diabetic patients. This improvement in erythrocyte flexibility can be completely abolished by obain, further demonstrating the role of Na^+K^+ ATPase activity in the biological effects of C-peptide.

In conclusion, C-peptide improves microvascular function and blood flow in type 1 diabetic patients by interfering with vascular and rheological components of microvascular blood flow. Ongoing studies will evaluate the effect of C-peptide administration on the progression of microvascular complications in type 1 diabetic patients.

C6. Primary cutaneous microangiopathy in patients with cardiac allograft vasculopathy and severe lipid disorder

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In the study we investigated whether a disturbance in microcirculation as a result of endothelial dysfunction is detectable in patients with cardiac allograft vasculopathy and severe hypercholesterolaemia ($n = 31$) in comparison to patients with severe coronary artery disease ($n = 49$), and age-matched apparently healthy subjects ($n = 100$). For this purpose, the flow velocity of erythrocytes through cutaneous capillaries at the nail fold of the finger was measured under resting conditions. In addition the reactive hyperaemia in the same capillaries after a three minute ischemia was determined.

Patients with cardiac allograft vasculopathy and severe lipid disorder showed a pathological reduction in mean capillary erythrocyte velocity under resting conditions with $\text{vRBC} = 0.11 \pm 0.08$ mm/s. The latter was significantly and relevantly lower than in patients with coronary three vessel disease ($\text{vRBC} = 0.46 \pm 0.35$ mm/s). It was notable that under resting conditions temporary cessation of flow occurred in 27 out of the 31 patients, which did not occur in healthy subjects and rarely in patients with three vessel disease (1 of 49 patients). In comparison to age-matched healthy subjects ($\text{vmax} = 1.46 \pm 0.52$ mm/s), the patients with three vessel disease showed a significant reduction in post-ischaemic maximum erythrocyte velocity ($\text{vmax} = 0.85 \pm 0.55$ mm/s), with a considerable shortening of the duration of reactive hyperaemia. Patients with cardiac allograft vasculopathy demonstrated a total loss of dilatory ability of the upstream arterioles and no post-ischaemic reactive hyperaemia occurred (only one of the 11 patients presented a weak reactive hyperaemia in the nailfold capillaries).

Since no macroangiopathy was detectable in the upstream arm arteries, primary cutaneous microangiopathy can be assumed in patients with cardiac allograft vasculopathy and severe hypercholesterolaemia.

C7. Impact of rheological variables in cancer

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Rheological alterations are commonly found in malignant disease being most pronounced in advance stage cancer. Although most of these changes are caused by cancer unspecific mechanisms, it has been shown that extent of which is related with the stage of cancer, prognosis of disease and the patient's risk for thrombosis in some cancer types. Monitoring of rheological variables during follow up of patients has been use full in gynecologic cancer, whereas a significant increase of the main determinants of blood viscosity was found when metastasis became clinically apparent. The most frequent constellation in newly diagnosed cancer is an increase in plasma viscosity (PV) and Red Blood Cell (RBC) aggregation that produces hyperviscosity and is compensated for by anemia. Unconditional raise of the hematocrit in cancer can deteriorate microcirculatory flow properties and may plunge the patient in an undesirable hemorheological condition that limits effectiveness of cyto-reductive treatment, favors dissemination of cancer cells, and the development of thrombosis. Modification of hyperviscosity – most likely at the plasmatic level – may represent a concept for cancer treatment and prevention of thrombosis. Anticoagulants and anti-inflammatory substances seem most suitable at this point, since high fibrinogen turn over is an important determinant of hyperviscosity in malignancy.

C8. Platelet adhesion and activation – Implications for atherosclerosis

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Background: Rupture of the advanced atherosclerotic plaque initiates platelet adhesion and activation at the exposed subendothelium leading to thrombotic occlusion of diseased arteries with subsequent ischemia of vital organs. Following activation platelets release a variety of proinflammatory cytokine and chemokines. In this manner, platelets might contribute to atherosclerotic lesion formation; however, the exact role of platelets in the inflammatory processes that initiate atherosclerosis remains unclear.

Results and discussion: We have demonstrated recently that platelets adhere directly to the dysfunctional endothelium of mice lacking *ApoE* in the absence of endothelial disruption. Interestingly, we found that platelet-endothelial cell interactions are present in large, atherosclerosis-prone arteries of *ApoE*-deficient mice at very early stages of atherosclerosis prior to the development of atherosclerotic lesions. Platelets are recruited to the diseased vessel wall in a multi-step process: The initial platelet tethering at the site of endothelial dysfunction of the endothelial monolayer stringently depends on the interaction of *GPIb-V-IX* with von Willebrand factor (vWF) bound to the surface of dysfunctional endothelial cells. During platelet tethering *GPIb α* leads to platelet activation and triggers firm *GPIIb*-dependent platelet adhesion. Importantly, platelet adhesion to the endothelium coincides with inflammatory gene expression and precedes atherosclerotic plaque invasion by leukocytes. Prolonged blockade of *GPIb α* -dependent platelet adhesion in *ApoE*^{-/-} mice profoundly inhibits atherosclerotic lesion formation in the common carotid artery, the aortic arch, and the coronary arteries. In addition, mice lacking *GPIIb* are protected from atherosclerosis.

Conclusions: Together this indicates that platelet adhesion in fact contributes to atherosclerotic lesion formation. These findings establish the platelet as a major player in initiation of the atherogenetic process.

P1. Effects of imipenem on intestinal microcirculation and cytokine release in septic rats

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Background: Antibiotic treatment represents a key component of therapy for severe sepsis. Apart from the sensitivity of the causing microorganisms, when choosing antimicrobial agents in septic conditions, possible effects of antibiotics on the microcirculation and inflammatory mediators should also be taken into account.

Objectives: Aim of this study was to evaluate the effects of imipenem (IMI) on the intestinal microcirculation in septic rats using intravital microscopy (IVM) and on the release of the cytokines TNF-alpha, IL-1 β , IL-6 and IL-10.

Methods: We induced sepsis in the animals (Lewis rats) by using Colon Ascendens Stent Peritonitis (CASP) model 16 hrs prior the experiments. IMI was given i.v. (20 mg/kg) and intravital microscopic examination was performed 2 hrs later. We evaluated intestinal functional capillary density (FCD) and leukocyte-endothelial interaction by IVM. Cytokine release was estimated at the end of the experiments.

Results: In the CASP model we observed a reduced functional capillary density in the muscular and mucosal layers of the intestine and an increase of temporary and firm adhesion of leukocytes to the microvascular endothelium. Acute treatment with IMI did not affect this response. Similarly, cytokine release was not significantly changed in IMI treated animals.

Conclusion: Although various antibiotics exert, in addition to their antimicrobial action, effects in the microcirculation and on cytokine release, IMI did not show this behavior.

P2. Oxyglobin (hemoglobin glutamer 200) reduces Reperfusion injury after cold liver preservation

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Introduction: Microcirculatory failure after cold liver preservation and reperfusion impairs tissue oxygenation and causes additional organ damage. Oxyglobin (Biopure, Cambridge, MA, USA) is a hemoglobin-based oxygen carrying solution of bovine origin capable to improve organ oxygenation. Aim of this study was to evaluate its potential to decrease reperfusion injury after cold liver preservation.

Material & methods: Rat livers were stored at 4°C in Euro-Collins solution for 24 hr and reperfused at 37°C in the isolated perfused rat liver (IPRL) with a sanguineous perfusate for 180 min. The perfusate consisted of Wistar rat blood and Krebs–Henseleit solution (Group A, $n = 6$), supplemented by either HES 6% (Group B, $n = 6$) or Oxyglobin (Group C, $n = 6$). Group C contained 3.9 g/dl polymerized hemoglobin. AST, ALT, blood count, blood gases as well as bile and portal flow were repeatedly determined at intervals of 30 min. As index of oxidative stress malondialdehyde (MDA) was measured after 180 min of reperfusion.

Results: Oxyglobin supplementation increased the perfusate hemoglobin by 3.3 g/dl. Perfusate transaminases increased in all groups during reperfusion. Lowest values for ALT and AST were found in the oxyglobin containing perfusate which were significantly ($p < 0.01$) reduced compared to Group A and B. After 180 min reperfusion ALT and AST levels were in group A 140 ± 28 and 170 ± 37 u/l, in Group B 203 ± 62 and 225 ± 47 u/l and in Group C 61 ± 28 and 126 ± 28 u/l, respectively. Portal flow was also significantly ($p < 0.05$) reduced by oxyglobin compared with control Group A. Bile production showed no difference between all groups after 180 min reperfusion, but in Group C the bile turned into a dark brownish colour as early as 5–10 min after beginning of bile secretion. Perfusate MDA reached 72.4 ± 20.4 nM in Group C and was significantly higher than Group A and B (7.6 ± 0.3 nM and 10.8 ± 0.5 nM, respectively).

Conclusion: Oxyglobin is known to scavenge nitric oxide, which explains the decrease of portal flow during reperfusion in this study. Accompanied by improved liver oxygenation this might also limit the formation of toxic molecules like peroxyxynitrite. Despite increased lipid peroxidation, Oxyglobin has been proven to be effective to reduce reperfusion injury after cold liver preservation.

P3. Obstructive sleep apnea syndrome in patients with acute stroke – Microhemorheological changes

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Background: Previous investigations prove a high incidence of stroke among persons with obstructive sleep apnea syndrome (OSAS). At the same time high frequency of sleep apnea episodes have been mentioned in the acute stage of stroke. The goal of present study was investigation of the microhemorheological disorders during the apneatic episodes in patients with progressive stroke.

Subjects and methods: 40 patients (25 men, 15 woman, mean age 62.0 year) with ischemic stroke accompanied by OSAS were investigated. The following blood parameters were evaluated in while awake and during sleep: erythrocyte aggregability index (EAI), blood plasma viscosity (BPV) and hematocrit (Hct). Body mass index and number of apneas per hour as well as oxygen saturation and EEG sleep structure were registered.

Results: We found, that prevalence of microhemorheological changes among the patients with acute stroke was significantly higher, than in the control group (patients with stroke but without OSAS). It was especially elevated EAI by 20% ($p < 0.001$) within several minutes after the apneas episodes. BPV was also elevated, but less significantly, by 8% ($p < 0.01$). Reliable changes of Hct were not in evidence. Blood rheology disturbances revealed no correlation with REM sleep EEG patterns.

Conclusion: In the acute stage of stroke, the exhibited changes of the hemorheological parameters may be triggered by OSAS, which therefore can be involved in development of the stroke-related complications of the acute phase in persons with chronic cerebrovascular insufficiency.

P4. Simultaneous splenectomy during extended liver resection and hyperperfusion syndrome in small-for-size livers

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Background: Portal hyperperfusion in small-for-size livers might seriously impair postoperative liver regeneration. Using an experimental model, we investigated splenectomy as a measure to reduce portal blood flow and its impact on postoperative recovery following extended liver resection.

Method: Wistar rats underwent partial (90%) hepatectomy with/-out splenectomy under temporary inflow occlusion (30 min). In addition to 10-day survival rate, laser doppler flowmetry of hepatic blood flow and fluorescence microscopic analysis of hepatic microcirculation were performed to assess the effect of splenectomy on initial microvascular reperfusion of liver remnants.

Results: While posts ischemic perfusion failure was comparable between both groups, portal blood flow was significantly reduced after simultaneous splenectomy (3.5 ± 0.4 vs 5.4 ± 0.4 ml/min). Moreover, red blood cell velocity and volumetric blood flow were reduced in splenectomized animals. These animals

experienced lower AST levels (421 ± 36 vs 574 ± 73 U/l) and a significantly increased survival rate, reaching 6.6 ± 1.3 vs 2.6 ± 0.8 days.

Conclusion: Simultaneous splenectomy significantly reduced the risk for postoperative hyperperfusion syndrome in small-for-size livers. Shear stress induced liver injury was diminished due to a significant reduction of portal venous blood flow, which positively influenced postoperative regeneration resulting in significantly higher survival.

P5. Hyperhomocysteinemia in the diabetic foot syndrome. Is homocysteine influenced by the acute phase response?

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Introduction: McCully for the first time in 1969 described the association between homocysteinuria and arteriosclerosis. Since then the importance of mild hyperhomocysteinemia as a cardiovascular risk factor has been investigated. Many epidemiological data, retrospective studies and experimental investigations seem to support a causal connection.

Prospective randomized studies however have not always led to uniform results. We conjecture that this discrepancy can partially be explained by increased levels at the acute phase response. There is an association between homocystein and thrombin (TAT, F1 +F2) in acute coronary syndrome and also after venous thrombosis. In bacterial infections und sepsis increased levels of homocysteine are observed.

We have investigated patients with diabetic foot syndrome in order to observe variations of the individual homocysteine levels following the treatment of the local infection and the C-Reactive- Protein (CRP).

Patients and methods: In 29 hospitalized patients with diabetic foot syndrome the CRP and the homocysteine on admission and discharge were determined.

We did not substitute folic acid, vit. B6, or vit. B12. The average time of hospitalization was 36 days. The average CRP was 2.7 mg/dl on admission and 1.2 mg/dl on discharge the hospital. The average homocysteine level was 20.1 $\mu\text{mol/l}$ and 17.5 $\mu\text{mol/l}$ respectively and the average creatine level was 1.8 mg/dl on admission and also when leaving the hospital. We used the sign-test for two connected samples.

Results: As expected all but 3 patients showed lower or normal CRP-levels on discharge. 22 patients had reduced and 7 patients increased homocysteine levels. This reduction is significant on the 5% level. ($p = 0.013$).

Conclusion: The paper suggests that the level of homocysteine varies depending on the acute phase response. If this can be confirmed, then homocysteine-levels in case-control-studies are systematically measured too high.

P6. Vascularisation and perfusion of hepatocellular carcinoma: Assessment with contrast-enhanced ultrasound using perflutren protein-type A microspheres

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Purpose: To assess the vascularisation of and the perfusion within hepatocellular carcinoma (HCC) including treatment-related changes with contrast-enhanced (CE) ultrasound (US).

Materials and methods: Twenty-six biopsy proven HCC lesions (size range 2.5–8 cm) in 20 patients were examined with unenhanced and CE vascular US modalities immediately before selective angiography for transarterial chemoembolization (TACE) as well as immediately after TACE, using all of the following modalities: color-coded Doppler sonography (CCDS), power Doppler imaging (PDI), CE pulse inversion harmonic imaging with PDI (PIHI + PDI), and CE coded harmonic angio (CHA). In CE US studies, perflutren protein-type A microspheres were administered as contrast agent in a single i.v. bolus of 0.5 ml, diluted in 20 ml 0.9% NaCl. The selective arteriograms and CE computed tomographies were taken for reference purposes. The Wilcoxon test was used for statistical analysis.

Results: Intratumoral vessels could be visualized before TACE in 11/26 lesions (42%) using CCDS; in 15/26 (58%) using PDI; in 23/26 (88%) using CE CHA; in 26/26 (100%) using CE PIHI + PDI. Following TACE, the sensitivities were calculated as follows: CCDS 33%; PDI 55%; CE CHA 77%; and CE PIHI + PDI 100%. The corresponding negative predictive values were for CCDS 74%; for PDI 81%; for CE CHA 89%; and for CE PIHI + PDI 100%. During the capillary phase, contrast enhancement was observable in the CHA mode only.

Conclusion: CE US by means of PIHI + PDI and CHA allows for reliable visualization of residual tumor following TACE equivalent to angiography and contrast-enhanced, if perflutren microspheres are used as contrast agent in a single low dose bolus.

P7. Objective quantification of non-homogeneous percolation of the choroidal microcirculation in a new animal model (albino rats)

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New interest in the pathophysiology of choroidal microvessels stems from the tractability of age related macular degeneration by rheoapheresis (see accompanying Abstract). The pathophysiology of age related macular degeneration of the human eye is unsettled, largely owing to the conventional ophthalmoscopes inability to detect events behind the pigment epithelium. Our group has earlier introduced the method of perfusing an intact microcirculation of experimental animals with rheologically controlled suspension of human erythrocytes (“missing link” between *in vivo* and *in vitro* rheology); this approach was now applied to an animal preparation using anterograde catheterisation of the carotid artery and perfusography: video-fluorescence angiography of the retinal and choroidal bed was applied, novel parametric images (as first applied by the late Andreas Scheffler (1955–2000) could in these cases be generated from the “inflow kinematics” in its dependency on arterial pressure (100 mmHg, 40 mmHg) and defined rheologic abnormalities. Strongly elevated continuous phase viscosity (1.2 to 4.8 mPa s) was compared to RBC-aggregation (Ficoll induced) and non aggregating artificial bloods (isotonic Thomadex solutions).

Two methods for graphically representing the indicator inflow experiments were utilised, namely sequential histograms of the fluorescence intensity and parametric images of the entire network, using the parameter “absolute fluorescence threshold” as base for depicting early and/or late indicator inflow into the volume element represented by a video-pixel. Under normal conditions (100 mmHg, normal plasma or blood viscosity) the well known choroidal perfusion in segments separated by watersheds

were depicted perfusographically. This “normokinetic filling mode” is contrasted by highly inhomogeneous filling either by reducing pressure (40 mmHg) or by using aggregating RBC on the one hand, but by a hitherto unknown type of shunting between the choroidal arteries and veins when using hyper-viscous artificial plasmas. These findings are reminiscent of abnormal percolation patterns in so called DARCY systems, i.e., a fluid displacement in short conduits surrounding impediments.

P8. Non-invasive photometric measurements of peripheral blood flow for characterization of circulation patterns by an analysis of time series

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The absorption of whole blood in the visible and near infrared range is dominated by the different haemoglobin derivatives and the blood plasma. It is well known that pulsatile changes of blood volume in tissue can be observed by measuring the transmission or the reflection of light. This diagnostic method is called photo-plethysmography PPG. The pulsatile change of blood volume is caused by the heart-circulation system. The measured PPG time signals and the ratio between the peak to peak pulse amplitudes at different wavelengths and its dependence on the optical absorbability characteristics of human blood yields information on the human health status. A photometric device PMD will be described which is based on the realisation of a photoplethysmography measurement device developed for the German Space Agency DLR. The non-invasive in vivo multi-spectral method is based on the radiation of monochromatic light, emitted by laser diodes, through an area of skin on the finger. Deferrals between the proportions of haemoglobin and water in the intravascular volume should be detected photo-electrically by signal-analytic evaluation of the signals. The computed coefficients are used for the measurement and calculation of the relative haemoglobin and haematocrit concentration change. First results with this photometric method to measure changes in the haemoglobin concentration will be shown during measurements with healthy subjects. A wigner-wille and a wavelet analysis of the non-stationary PPG time series allows an access to information about the human health status. The wigner-wille distribution is a specific, sensitive method for the identification of heart-circulation patterns. The PPG time series contains information of microcirculation patterns, pulse rate and variability, breathing rate and vasomotion, auto-regulation and thermoregulation frequencies.

P9. Sensor for measuring the red blood cell velocity (RBCV) with high temporal resolution

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For the examination of the microcirculation and in particular the blood flow conditions, cutaneous capillary microscopy is an approach that has gained in significance in the last number of years. It is a non-invasive method, gentle on patients, which makes the capillary shape visible in the microcirculation area of interest. A convenient area for observation is the nail fold of a finger. A improved method for the examination of the microcirculation by measuring the blood cell velocity in the capillaries at the nail fold is presented. The new sensor technique performs in-vivo measurements at the nail fold in realtime. To this an adapted picture tracking is used to eliminate the unavoidable finger movements. To found a correlation between surface temperature and flow rate, the temperature distribution at the nail fold is measured simultaneously.

P10. Analysis of the tissue microcirculation using OPS-imaging

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Introduction: Presently the analysis of the terminal microcirculatory network is based on mathematical models which describe combined effects of network hemodynamics, mass transport, and vascular responses to hemodynamic and metabolic stimuli. Orthogonal Polarisated Spectral (OPS) imaging allows to obtain intravital microscopical data of the morphological tissue structure, representing a non-invasive method to assess the microcirculation. The aim of this study was to develop a new reliable universal algorithm which could be used to quantify microvascular dysfunction.

Methods: 26 healthy volunteers and 15 patients with coronary artery disease (CAD) were studied. To challenge vessel reactivity a standardized orthostatic stress test was performed in three steps: base line (BL) 5 min rest in a horizontal position, (I) standing up 5 min, (II) 5 min rest in a horizontal position. Microcirculation was assessed in the sublingual mucosa and analyzed offline to determine vessel diameter and red blood cell velocity (RBC). After analysis of the obtained data an algorithm containing the studied parameters was developed in order to describe the individual microcirculatory status.

Results: 1692 sublingual microvessels in the CAD group and 1220 in the control group were evaluated. During stress test, vessel distribution depending on their diameter was unchanged in contrast to the CAD group demonstrating a decreased amount of vessels with diameter under 11 μm and an increased amount with higher diameter above 12 μm . This shifting-effect was used to describe the microcirculatory response, facilitating differentiation between pathological or normal reactions. The polynomial curves of RBC velocity and vessel diameter were calculated and integrated in an algorithm.

Conclusions: A standardized method and protocol to evaluate the human microcirculation was established and the microcirculatory response to stress in healthy humans and CAD patients was described. A mathematical algorithm describing the microcirculatory status of the observed tissue was developed. Its informational value shall be further evaluated in future studies. OPS imaging in a central position of the body might be a reliable, non-invasive tool to diagnose clinical relevant disturbances of the microcirculation.

P11. Effect of PDF-V inhibitor on pulmonary microcirculation

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Objectives: Inhibitors of phosphodiesterase V inhibitor (PDF-V) have been proposed for the treatment of pulmonary hypertension because of their dilatative effect on the pulmonary vessels. However, effects on the microcirculatory network have never been observed up until today. It was therefore the aim of our study to evaluate the effect of a PDFV inhibitor on the pulmonary microcirculation in a big animal model.

Methods: 7 pigs (30 kg) underwent median sternotomy. The pulmonary microcirculation was assessed in vivo by orthogonal polarized spectral (OPS) imaging microscope (CytoscanTM). Microcirculatory images were obtained from the parietal surface of the lung before (BL) and continuously up to 30 min after injection of 0.15 mg/kg/body weight i.v. PDF-V into the pulmonary artery. Alveolar capillary

diameter (ACD), red blood cell (RBC) velocity and functional capillary density (FCD) were assessed, as well as hemodynamic and blood gas exchange.

Results: 5 min after application PDFV mean pulmonary pressure (21 ± 2.2 vs. 18.8 ± 1.5 mmHg, BL vs. PDFV, $p < 0.022$) and mean pulmonary vascular resistance (156 ± 32 vs. 85 ± 25 dyn·s·cm⁻⁵, BL vs. PDFV, $p < 0.001$) significantly decreased. ACD (4.21 ± 1.7 vs. 4.86 ± 2.5 μm , BL vs. PDFV, $p < 0.001$) as well as FCD significantly increased (3.4 ± 0.3 vs. 4.7 ± 1.1 $\mu\text{m}/\mu\text{m}^2$, BL vs. PDFV, $p < 0.001$). RBC velocity decreased and reached statistical difference at timepoint 10 minutes after application (244.1 ± 63.5 vs. 205.7 ± 74.3 $\mu\text{m}/\text{s}$, BL vs. I, $p < 0.001$).

Conclusions: Our study demonstrated for the first time the effect of PDFV on the pulmonary microcirculation. We observed a significant dilatation of the terminal capillaries with a recruitment of capillaries as evidenced by the increased FCD. OPS imaging proved again to serve as a reliable tool to assess solid organ microcirculation *in vivo*.

P12. Do adherent leukocytes move?

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Introduction and aim: Leukocyte–endothelial-interactions are a prerequisite for the leukocytes recruitment during acute inflammation. We utilized time-lapse intravital microscopy and digital-based video processing to investigate leukocyte adhesion and migration.

Methods: Intravital microscopy of mesenteric venules was performed in Wistar-rats using digital video recording and time-lapse image compression. Leukocyte-endothelial-interaction, migration and extravasation were recorded over 60–90 min. Adherent leukocytes were divided into rollers (adhesion for less than 1 sec), transient stickers (adhesion for 1 to 30 sec), and permanent stickers (adhesion for more than 30 sec). Additionally, the effects of intravenous anti-ICAM-1 and anti-CD18 antibodies on leukocyte locomotion were analysed.

Results: Most permanent stickers ($84 \pm 13\%$) moved (crawled) actively on the intraluminal site of venules. Baseline measurement of leukocyte crawling velocity yielded an average 9.0 ± 4.5 $\mu\text{m}/\text{min}$ (mean \pm SD) which was not significantly different from crawling velocity of extravascular leukocytes (8.9 ± 4.5 $\mu\text{m}/\text{min}$). Intraluminal crawlers traveled over a mean distance of 35 ± 17 μm with the average duration of 4.5 ± 2.3 min. The maximum distance of leukocyte crawling observed was 150 μm . The maximum time of crawling was 15 min. Under unstimulated conditions, crawling leukocytes detached from the endothelium and did not migrate through the vascular wall. Intravenous injection of anti-ICAM-1 and anti-CD18 antibodies reduced significantly the number and velocity of crawling.

Conclusions: Leukocyte–endothelial-interactions are an active and dynamic process under unstimulated conditions. This process involves long-time (several minutes) interactions of leukocytes with the endothelium, intraluminal leukocyte migration and, finally, detachment from the endothelium. Intraluminal leukocyte crawling is controlled by an expression of adhesion molecules on endothelium and leukocytes.