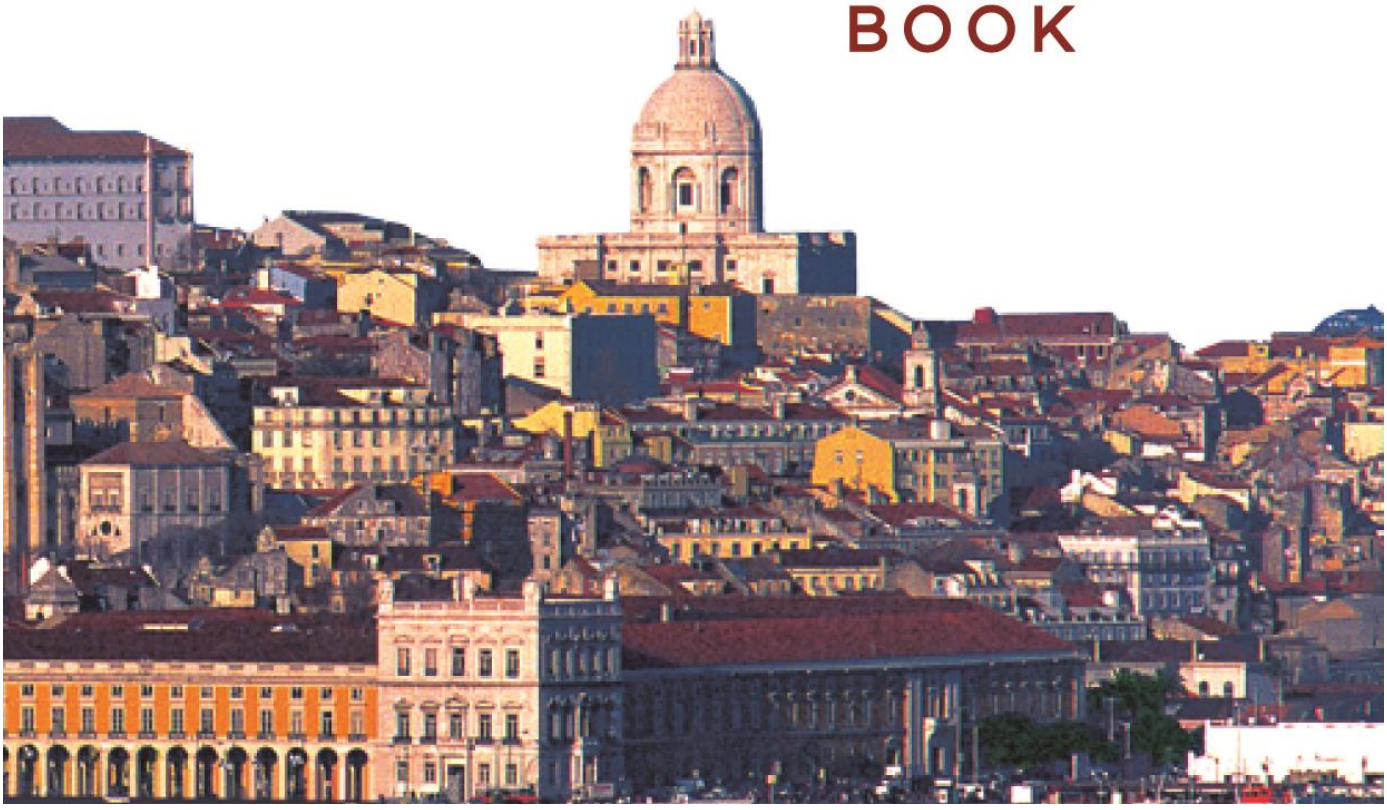
18th Conference of the European Society for Clinical Hemorheology and Microcirculation

5-8 June 2016 | LISBOA | PORTUGAL

Faculty of Medicine, University of Lisbon, Edifício Egas Moniz

ABSTRACT BOOK



Methods in Hemorheology and Clinical Applications

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The study overviews the electrorheological method developed and used recently for quantification of blood microstructural changes due to RBC aggregation-desaggregation and deformation processes at different shear rates and at different local structure of the flow field. A concurrent measurement system with Contraves LS30 rotational rheometer, a device with data acquisition system and the program unit Rheoscan 100 were used to quantify in parallel rheological and electrical properties of whole human blood, RBC suspensions and nanoparticle solutions at a steady and different unsteady flow conditions: rectangular (step-wise), trapezium-liked and triangular regimes of changes of shear rates. The relationship between the whole blood/RBC suspensions conductivity and time/shear rates was studied in parallel with the changes in the rheological behaviour under transient flow conditions. The time variation of blood/RBC suspensions conductivity at unsteady flow conditions and the typical responses of the shear stresses under electric field of 2 kHz are shown. The kinetics of conductivity-time dependences follow the transition from dispersed single RBCs to the formation of RBC aggregates of larger size with respect to the initial state. The results show that the blood conductivity is strongly dependent on the considered blood flow regimes, shear rate, hematocrit and temperature. The results are interpreted related to potential benefits of using presented method in clinical practice.

Seminal contributions of Oguz Baskurt to hemorheology - a personal view

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The outstanding contributions of Oguz Baskurt to the advance of modern hemorheology between 1988 and 2013 cannot be easily summarized. Most of this work was done in close collaboration with the team of HJ Meiselman but Oguz fruitly collaborated with many researchers from all the parts of the world. Probably the most important was to investigate in vivo relevance of blood rheology, RBC aggregation and RBC mechanics to renal hemodynamics, RBC distribution through the myocardium, and pressure-flow relations in isolated tissues or organs. He evidenced the influence of white blood cells on these processes. His studies on the effects of nitric oxide (NO) deserve also a special mention. He also wrote seminal papers demonstrating that NO was released by RBC exposed to shear stress and that this NO could affect vascular tone as well as RBC deformability. He investigated evolutionary aspects of blood rheology in mammals. His contribution to methodologic research and standardization of measurements is also important. Beside all this a careful reading of Oguz's papers is an opportunity to gather new ideas that may stimulate hemorheological research. For example, the report of effects of thyroxine on mechanical properties of erythrocytes, the effects of hardened red cells on plasma renin activity, and many other topics. I think that an in-depth reading of this rich scientific production can be really fruitful for hemorheologists, and represent the best tribute to our late friend.

Red blood cell aggregation- does it matter in the microcirculation?

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Background: Red blood cell (RBC) aggregation has been a major scientific interest of Oguz Baskurt[1]. Unanswered questions for him were: Is RBC aggregation "good" or "bad" for tissue perfusion and should enhanced RBC aggregation in inflammation be corrected therapeutically?

Methods: We analyzed the impact of RBC aggregation on the perfusion of an artificial microvascular network (AMVN) at driving pressures ranging from 2.5 to 60 cmH2O. RBCs were suspended in either 46.5 g/L dextran 40 (non-aggregating) or 35 g/L dextran 70 (aggregating) with a hematocrit of 30%. Both dextran solutions had the same viscosity (2.1 ± 0.2 mPa·s).

Results: Aggregation was observed in 5 μm "capillaries" of AMVNs for RBCs suspended in dextran 70 at driving pressures up to 40 cmH2O. AMVN perfusion rates were similar for aggregating and non-aggregation RBC suspensions. The "capillary" hematocrit was higher for dextran 70 then dextran 40 suspensions (i.e., the Fahraeus effect was reduced by RBC aggregation).

Conclusions: RBC aggregation did not affect the rate of perfusion of an AMVN. A higher hematocrit in "capillaries" of the AMVN indicates a better oxygen transport capacity in the presence of RBC aggregation. This suggests a beneficial effect of enhanced RBC aggregation in disease, which should not be corrected therapeutically.

Reference: [1] O.K. Baskurt and H.J. Meiselman, Erythrocyte aggregation: Basic aspects and clinical importance, Clin Hemorheol Microcirc 53 (2013), 23-37.

Biological Evaluation of polymer-based Biomaterials

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Polymer-based-biomaterials envisioned for the implantation in patients have to be evaluated in a consequent testing regime according to the EN ISO 10993-4/5 norm. After appropriate sterilization, cytotoxicity and endotoxin load are tested in vitro with material extracts or in assays which allow cells - such as immortalized fibroblasts - to contact bulk material surfaces directly. Contamination of the materials with microbial products has to be excluded and is tested in vitro within the immunological evaluation using reporter cell lines or different populations of primary human immune cells. Only sterile, non-toxic, and endotoxin low (according to FDA-limits) polymers will be further tested. First in vitro studies with region-specific primary human cells are designed in view of the designated implantation site. In case of implants in the vasculature, endothelial cells (venous or arterial type), smooth muscle cells or fibroblasts are applied. Prior animal tests, the biocompatibility (e.g. effects of polymers on bleeding, thrombotic processes, vasodilation/vasoconstriction, angiogenesis, etc.) can be tested using the hen´s egg chorio-allantoic membrane test (HETCAM).

Evaluation of the polymers in vivo comprises the implantation of samples subcutaneously in small animal, mostly mice or rats. Integration of the polymers into tissue, inflammatory response, degradation, encapsulation as well as local or systemic toxic effects are studied. Final in vivo studies focus on the implantation of prototypes of the complete devices in a big animal model (pigs, sheep, etc.) to examine its functionality.

Passing all these studies the implants are tested in a clinical trial (first in men study).

Stem cells and vascular regenerative medicine

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Most human tissues do not regenerate spontaneously, which is why “cell therapy” are promising alternative treatments. The Principe is simple: patients’ or donors’ cells are collected and introduced into the injured tissues or organs directly or in a porous 3D material, with or without modification of their properties. This concept of regenerative medicine is an emerging field which can be defined as “the way to improve health and quality of life by restoring, maintaining, or enhancing tissue and organ functions”. There is an extraordinarily wide range of opportunities for clinical applications: artheropathies, diabetes, cartilage defects, bone repair, burns, livers or bladder regeneration, organs reconstruction (lung, heart, liver…) neurodegenerative disorders, sepsis… Different stem cells (SC) with different potential can be used and characterised (totipotent, mesenchymal of different origins, especially those present in tissues …). Today it is undeniable that mesenchymal stem cells like bone marrow, adipose tissue or Wharton Jelly stem cells, are of potential interest for clinical applications because they are easily separated and prepared and no ethical problems are involved in their use. In this talk different potential clinical applications in the cardiovascular field are considered: peripheral arteriopathy in diabetic patients, cardiac insufficiency, treatment of erectile dysfunction. The regeneration of tissue or organ is and will remain a challenge for the future development of cell therapy. Many problems remain to be solved that could lead to the development of innovative strategies to facilitate cell differentiation, increase the yield of cells and ensure a standardised product, overcome the risks of teratogenic effects and/or immune reactions, enable grafting via direct cell or biotissue transplantation and avoid legal issues involved in national regulations.

Relationship between viscosity and endothelial function

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The traditional view has it that conditions in which viscosity is increased may be associated with cardiovascular disease, and that vice-versa cardiovascular disease leads to hyperviscosity. Whether a cause or a consequence, the role and size of the variability in viscosity has not yet been put in relationship with changes in endothelial function at a population level. Further complicating this issue, direct assessment of viscosity has been performed in a very little number of sufficiently large studies.

More recently, the understanding of the role of the vascular endothelium in the control of vasomotion, and of the importance of shear stress and viscosity in modulating endothelial activity has cast some doubt on the relationship between viscosity and endothelial function. Vascular homeostasis is a dynamic system in continuous equilibrium between vasodilation and vasoconstriction, constantly kept in balance by the release of endothelial autacoids which in turn have an effect on the regulation of the deformability and aggregability of circulating cells, erythrocytes, leucocytes and platelets, thus controlling both arterial tone and blood viscosity. Increases in viscosity might be seen as stimuli for endothelial activation but also as a consequence of impaired endothelial function. In our hands, we found a strong association between risk factors, blood pressure and viscosity, but very limited associations with parameters of endothelial function.

Evidence-based hemorheology. Does it exists? Applying evidence from clinical studies to the individual patient

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Clinicians are used to treat individual patients, and therefore may feel that clinical trials do not give individual information for optimal treatment. However, one should bear in mind that all the diagnostic or therapeutic techniques available for us were developed in groups of patients similar to the ones we wish to manage.

Evidence Based Medicine (EBM) is the integration of research evidence (from clinically relevant studies conducted using sound methodology) with clinical expertise (clinician's cumulated experience) and patient values (personal preferences and unique concerns and expectations).

The practical steps of EBM include:
1) assess the patient
2) ask the clinical question
3) acquire the evidence
4) critically appraise the evidence
5) apply the results to the patient and 6) self-evaluate one's practice.

Clinical studies in clinical hemorheology include – among other – interventions in vascular medicine: coronary disease, stroke, peripheral vascular disease, venous insufficiency and thrombosis, etc.

Of these, we will present some practical steps on how to apply therapy results of stroke studies to the individual patient (this addresses step number 5 in the previous definition of EBM practice).

We will do this by discussing the differences between internal and external validity of clinical trials, and defining the importance of baseline risks to choose therapy using the data from the best and most useful studies available. In the end, clinicians will understand how to use evidence effectively.

Extracellular Matrix Structure in Small Arteries: Significance of Postnatal Structural Development to Vessel Function

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Aim: Vascular extracellular matrix (ECM) is dominated by elastic fibers and collagen. Current knowledge of ECM protein development comes from studies of large vessels while resistance vessel data are lacking. Emphasizing elastin, we examined if postnatal changes in arteriolar ECM expression correlates with development of myogenic and endothelium-dependent mechanisms.

Methods: Rat cerebral and mesenteric arteries were isolated at days 3-19, 2 months and 2 years. mRNA expression patterns were examined for elastin, collagen types I, II, III, IV, fibrillin-1, and -2, fibulin-1, versican, brevican, vitronectin, fibronectin, EMILIN-1, lysyl oxidase, and transglutaminase 2.

Results: Elastin, LOX and fibrillar collagens I/ III mRNA peaked at day 11-14 in both vasculatures. 3D confocal imaging of elastin showed continuous remodeling in the adventitia and internal elastic lamina (IEL). Myogenic responses in cannulated arteries were detectable at day 3 and the myogenic response curve shifted to higher intraluminal pressures by day 19. Functional studies showed endothelial-dependent dilation to be less at day 3 compared to day 19 and at day 3 lacked an EDH(F) component that was evident at day 19.

Conclusion: As EDH-dependent dilation is dependent on cellular communication across the IEL maturation of this local structural environment may affect local function. Thus, both structural remodeling and aspects of functional control evolve postnatally in the walls of rat small arteries.

Dynamic Vascular Smooth Muscle Cell Behavior Evident in Cell-Extracellular Matrix and Cell-Cell Interactions

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AIM: In vascular smooth muscle cells (VSMC) there is increasing evidence that actin-myosin based contraction is coupled with myosin light chain kinase independent remodeling of the cortical cytoskeleton. We hypothesized that VSMC contraction and cytoskeletal remodeling is also coordinated with changes in cell-extracellular matrix (ECM) and cell-cell adhesion.

METHODS: To test this hypothesis we used atomic force microscopy (AFM) to measure VSMC stiffness and adhesion involving ECM proteins or N-cadherin. Coating the AFM probe tip with ECM protein or N-cadherin functionalized the probe. We also utilized isolated arterioles pressurized and fluorescently immunolabeled to identify adherens junctions (AJ) containing N-cadherin. Contraction was induced with angiotensin II or phenylephrine and relaxation induced with adenosine or acetylcholine.

RESULTS: The results indicate that during VSMC contraction there is enhanced ECM and cell-cell adhesion and that these changes in adhesion are reversed during VSMC relaxation. The changes in ECM adhesion occurred coincident with changes in cell stiffness and remodeling of the cortical actin stress fibers. Changes in AJ adhesion were accompanied by changes in the number and size of AJ sites between VSMC.

CONCLUSIONS: Our collective observations provide strong evidence that adhesion sites in VSMC are adaptively tuned to local mechanical forces, whether externally applied or internally generated by cell contraction.

Cell-cell coupling and microvascular function

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Gap junctions between cells within the wall of arterioles facilitate the transfer of homocellular and heterocellular signals. Hyperpolarizing current readily transfers between endothelial cells and smooth muscle cells through myoendothelial gap junctions, and can also pass longitudinally via homocellular gap junctions and underlies in both cases coordinated local and conducted dilation. In addition, Ca2+ signals pass from smooth muscle cells to endothelial cells, most likely via myoendothelial gap junctions, which forms a feedback pathway that opposes vasoconstriction. How these pathways can be stimulated in arterioles from various vascular beds, including the human coronary circulation will be discussed. Cell-cell coupling within the walls of arterioles is not only vital in the electrical control of arteriolar diameter, but also enables intercellular Ca2+ signalling.

Novel targets in treating the microcirculation in obesity

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Obesity is leading cause of vascular disease in Western cultures but treatment of this disease is thwarted by a lack of mechanistic information regarding effects of obesity on the circulation. A key defect in vascular function in this population is the loss of endothelial vasodilation, thought to be secondary to increased oxidant stress. Exercise is well-documented to improve vascular function in obese subjects but links between fitness and improved outcomes are unknown. In this presentation, we offer new evidence that increased muscle mass induced by deletion of myostatin confers significant microvascular benefits in obese mice. These changes occur in parallel with improved metabolism and reduction in oxidant stress driven by activity of NADPH Oxidase 1 (NOX1). Further studies deleting NOX1 demonstrated reduced oxidant stress in obese mice despite continued metabolic deficiencies. Deletion of NOX1 normalized endothelial function, suggesting that NOX1 is the link between the improvement in vascular function observed when muscle mass is increased. These findings suggest that increased muscle may be a contributor to the improved vascular health seen when obese individuals exercise. This benefit occurs via reducing the oxidant stress caused by NOX1. Patients with larger muscle mass may explain the “fit but fat “population in which disease is less severe and conferring such benefits to afflicted populations may provide powerful therapy for vascular disease in obesity.

A Novel Role for Protein Disulfide Isomerase on the Hematological Complications of Sickle Cell Disease

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Protein disulfide isomerase (PDI) is a multifunctional oxidoreductase critical in thrombus formation. We studied the effects of deoxygenation on PDI activity in erythrocytes from humans with Sickle Cell Disease (SCD) and two sickle mouse models, BERK and βSAntilles. We show that deoxygenation of sickle erythrocytes increased surface-associated reductive capacity that was sensitive to antibodies against PDI (mAb PDI). We then studied sickle human erythrocytes and showed that PDI inhibition (quercentin-3-rutinoside [Q3R]) significantly reduced deoxy-stimulated dehydration and Gardos channel activity ($P<0.03$). We characterized erythrocyte density and calculated the D50. Both mAb PDI and Q3R significantly reduced D50 when compared to vehicle ($P<0.0001$) while PDI incubation increased cellular hydration status ($P<0.01$). Consistent with these data, Q3R or mAb PDI reduced hemolysis of human cells exposed to 20 mM 2-2'-azo-bis-(2-amidinopropane)dihydrochloride (AAPH); similar results were observed in BERK and βSAntilles mice. We then studied sickle mice expressing HbF; BERK(<1% HbF), BERKγM(25% HbF), and BERKγH(45% HbF). BERKγH had the lowest circulating and cell associated PDI activity among the three mouse types and mice with high HbF/F-cell ratio had low PDI activity. Thus, we posit that PDI activity is important for erythrocyte stability, survival and that its inhibition in SCD may represent a novel therapeutic target for improving its hematological and vascular complications.

Hemorheological alterations in sickle cell anemia and their clinical consequences – the role of genetic modulators

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Sickle cell anemia (SCA) is an autosomal recessive chronic hemolytic anemia, caused by homozygosity for the HBB:c.20A>T mutation. The disease presents with high clinical heterogeneity, stroke being the most devastating manifestation. This study aimed to identify genetic modulators of severe hemolysis and stroke risk in children with SCA, as well as understand their consequences at the hemorheological level.

Sixty-six children with SCA were categorised according to their degree of cerebral vasculopathy (Stroke/Risk/Control). Relevant data were collected from patients' medical records. Several polymorphic regions in genes related to vascular cell adhesion and tonus were characterized by molecular methodologies. Data analyses were performed using R software. Several in silico tools (e.g. TFBind, MatInspector) were applied to investigate the main variant consequences.

Some genetic variants in vascular adhesion molecule-1 gene promoter and endothelial nitric oxide synthase gene were associated with higher levels of hemolysis and stroke events. They modify important transcription factor binding sites or disturb the corresponding protein structure/function.

Our findings emphasize the relevance of the genetic variants in modulating the degree of hemolysis and development of cerebral vasculopathy due to their effect on gene expression, modification of protein biological activities related with erythrocyte/endothelial interactions and consequent hemorheological abnormalities in SCA.

Sickle cell disease a model of cardiovascular risk and new therapeutic targets

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Aim Sickle cell diseases (SCD), as well as other hemolytic monogenetic anemias are accompanied by endothelial dysfunction and low grade inflammatory state progressing to advanced cardiovascular diseases in several organs and systems (stroke, pulmonary hypertension, proliferative retinopathy and thrombophilia).

SCD represents a unique opportunity to study the role of the interactions and crosstalk's between the erythrocytes, leukocytes, platelets and endothelial cells as an mechanism of disease of complex cardiovascular diseases. The most studied pathway is that of nitric oxide, in its several components of the metabolism. Methods One strategy is to study functional polymorphisms of genes such an HBA, HBB cluster, eNOS, ARG, HMOX-1, MPO, ACE and Hp and relate its genetic variation with hematologic and biochemical parameters in blood components also commonly studied in cardiovascular complex disorders (hypertension, diabetes, preeclampsia, heart failure, chronic kidney disease).

Results With this approach we can discover some mechanistic relationship between those polymorphisms and intermediate phenotypes common to those diseases. This permits pharmacogentic target the variant proteins with precursors of NO (Arginine, citrulline and nitrites).

Conclusion Monogenetic diseases of the erythrocyte can to understand the mechanisms of multifactorial cardiovascular diseases and primarily and secondarily prevent them with nutraceuticals and medicines.

Hematocrit and hematocrit viscosity ratio during exercise in athletes: even closer to predicted optimal values?

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The theory of optimal hematocrit suggests that the best value of hematocrit (hct) should be that which results in the highest value of the hematocrit/viscosity ratio h/v. Trained athletes compared to sedentary subjects have a lower hct, but a higher h/v, and endurance training reduces the discrepancy between the actual hct and the «ideal » hct that can be predicted with a theoretical curve of h/v vs hct constructed with Quemada's model. In this study we investigated what becomes this homeostasis of h/v and hct during acute exercise in 19 athletes performing a 25 min exercise test. VO2max is negatively correlated to resting hct and positively correlated to discrepancy between actual and ideal resting hct which is correlated to the maximal rise in hct during exercise. Predicted and actual values of the h/v were fairly correlated (r=0,970 $p<0.001$) but the actual value was lower at rest and this discrepancy vanished at 25 min exercise. Exercise-induced decrease in discrepancy between actual and theoretical h/v was negatively correlated with the score of overtraining. All these findings suggest that h/v is a regulated parameter and that its model-predicted «optimal» values yield a «theoretical optimal» hct which is close to the actual value and even closer when athletes are well trained. In addition, acute exercise sets h/v closer from its predicted ideal value and this adaptation is impaired when athletes quote elevated scores on the overtraining questionnaire.

Moderate Exercise Training Blunts Inflammation in Severe Transgenic Sickle Cell Mice

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Sickle cell disease (SCD) is characterized by severe systemic and vascular inflammation. There is strong evidence that moderate exercise training reduces inflammation in healthy people as well as in multiple diseases conditions. The aim of this study was to characterize the effects of moderate exercise training in a severe transgenic mouse model of SCD (Townes). After eight weeks of training, SS mice had a lower hematocrit than untrained SS (UT-SS) but there were only moderate changes in red blood cell count or total hemoglobin between the two groups. The spleen/body mass ratio was lower in the trained SS (T-SS) mice compared to UT-SS. The spleen was less congested in T-SS, and the magnitude of congestion in all animals correlated with the relative spleen mass. Exercise training increased the percent of venous oxyhemoglobin in T-SS and there was a corresponding decrease in the percent carboxyhemoglobin. White cell count and the plasma concentration of macrophage inflammatory protein-1â were decreased in T-SS. The kidney nuclear NF-êB protein expression was increased in UT-SS compared to UT-AA control mice while there was no difference between T-SS and T-AA mice. Kidney IL-1â and P-selectin mRNA expression as well as liver heme oxygenase-1 (HO-1) mRNA expression were blunted in trained mice. Taken together, these results suggest that moderate exercise training may reduce sequestration of sickle erythrocytes/congestion, resting blood deoxygenation and inflammation in SCD.

Comparative assessment of methods high-frequency ultrasound Doppler and laser Doppler flowmetry in the study of microcirculation during load test

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The study of quantitative and qualitative parameters of a peripheral blood flow is a current problem, as an outcome and prognosis of many diseases depend on function and compensation abilities of the microcirculation system (MCS). The studies of the human MCS are associated with a number of technical difficulties. The MCS has a complex architectonics and high lability, which complicate obtaining the data on blood flow.

The laser Doppler flowmetry (LDF) and high-frequency ultrasound dopplerography (HFUD) are used for research of the MCS parameters. The very important to carry out functional tests, which makes it possible to assess the reactivity micro vessels.

The work objective is a comparison of results and determination of the data correlation´s degree in the research of blood flow parameters in the MCS, obtained together the LDF and HFUD methods before and during the recovery period (RP) after exercise on a bicycle of 10 subjects.

During the RP it was revealed 3 types of reaction: the first is characterized by well-defined periods of a short-term decrease in indicators of blood flow velocity and increase of a peripheric resistance; the second - by a significant change in blood flow dynamics; the third - by a less variability without expressed blood flow fluctuations, but prevalence of a shunt blood flow is observed after 15 min of the RP.

The study identified a good comparability of the results, obtained by the LDF and HFUD methods.

Hemorheological effects of recombinant human erythropoietin therapy in normal rats

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Recombinant human erythropoietin (rHuEPO) is used to treat chronic kidney disease anemia and, illicitly, in sports doping. Our aim was to study the effect of increasing rHuEPO doses on erythroid cells, blood pressure (BP) and endothelial nitric oxide synthase (eNOS) that might affect hemorheology, using a rat model.

Wistar rats were divided in 5 groups: control (vehicle) and rHuEPO-treated groups (100, 200, 400 and 600 IU/kg bw/wk), 3 times/week, along 3 weeks. Blood was collected for hematological analysis. BP was measured by tail-cuff method. Kidney tissue was collected for protein analysis by western blot.

A dose-dependent increase in erythrocytes, hematocrit and hemoglobin levels was found with rHuEPO therapy, in rHuEPO200, rHuEPO400 and rHuEPO600 groups, after 1 week of treatment that was enhanced at the end of protocol. BP raised in all groups receiving rHuEPO. A reduction in kidney eNOS phosphorylation was observed in the rHuEPO200 and rHuEPO600 groups.

Excessive erythrocytosis increases blood viscosity which led to an increase in BP. Reduced eNOS activity was associated with increased levels of systolic BP.

Financial support by Portuguese Foundation for Science and Technology through national funds; co-financed by COMPETE-FEDER (PTDC/SAU-TOX/114253/2009, Pest/C/SAU/3282/2013), FEDER under Partnership Agreement PT2020 (UID/MULTI/04378/2013-POCI/01/0145/FERDER/007728, UID/NEU/04539/2013) and by POPH/FSE (SFRH/BD/61020/2009, SFRH/BD/79875/2011 and SFRH/BPD/81968/2011).

Effects of exercise training and age on optimal hematocrit/viscosity ratio and hematocrit

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It is well known that aging impairs blood rheology and that exercise training improves it. We previously reported that regular cycling is unable to prevent aging-related increase in red cell rigidity and aggregability. The purpose of this study was to investigate whether this adaptation of viscosity factors due to age and training is related to a regulation of hematocrit setting it closer from its ideal value as can be predicted by a model using Quemada´s equation. Thirty-two subjects [16 middle-aged men: 8 cyclists and 8 sedentary men and 16 young men: 8 cyclists and 8 sedentary men] carefully matched to make 4 groups of comparison, were included in this study. Training was associated with a reduced hematocrit in middle age subjects but not in 25 yr old ones. The model predicts higher h/v values than the actual ones and this discrepancy is lower in young subjects, $p<0.05$. The model predicts the same ideal hct (46 ± 1.2 %), but actual hct is lower than ideal in all groups ($p<0.05$). These results show that both training and younger age are associated with a lower predicted value of h/v, and that actual values of both h/v and hct are lower than their model-predicted ‘optimal value’. The correlations between model-predicted values and actual ones support the validity of the concept of optimal hematocrit and its prediction with this model. The discrepancies may indicate a ´reserve´ preventing hematocrit to increase during exercise above its optimal value.

Influences of two high intensity interval exercise protocols on hemorheological variables in overweight men

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This study determined the effects of two different high intensity interval exercise (HIIE) on the main determinants of blood fluidity. Ten overweight men (age, 26.3±1.7 yrs) completed two HIIE protocols on two separate occasions with one week intervening. The two HIIE encompassed performing: 1) 6 intervals, 2 min activity at 85% of VO2max and 2 min active recovery at 30% of VO2max (ratio 1 to 1), and 2) 6 intervals, 30 s activity at 110% of VO2max and 4 min active recovery at 40% of VO2max (ratio 1 to 8). Each exercise trial was followed by 30 min rest. Venous blood samples were obtained before exercise, immediately after exercise and after 30 min of recovery and analyzed for blood and plasma viscosity, fibrinogen and red blood cell indices. The HIIE protocol with lower intensity and shorter recovery (1 to 1) led to higher reduction in plasma volume changes (9.9% vs 5.7%) and more increases in blood viscosity and hematocrit than higher intensity and longer recover (1 to 8) protocol ($P<0.05$). In addition, irrespective of the intensity, HIIE resulted in significant increases in these variables ($P<0.05$). However, fibrinogen concentration did not change significantly either in response to HIIE or in response to intensity ($P>0.05$). Since the protocol with higher intensity and longer recovery has not induced increments in hemorheological variables, it could be concluded that this protocol is safer than lower intensity with shorter recovery for overweight individuals to perform.

How do red blood cell properties contribute to the exercise performance in camels (Camelus dromedarius)?

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We studied the influence of the camels´ rigid and spindle-shaped erythrocytes (RBC) on whole blood viscosity during exercise and determined the theoretical "optimal" hematocrit (opt.HCT). Blood of 11 Arabian camels (C. dromedarius) was collected before and after a 8 km run, as well as after a 30 min cool down period. Flow curves (11-500 s-1, 37°C; Physica MCR301 rheometer, Anton Paar, Austria) and RBC aggregation (MA1, Myrenne, Germany) were analyzed. 51 samples with packed cell volume (PCV) values between 5% and 85% (RBC in autologous plasma) were used to determine the opt.HCT. Despite a gain in plasma volume of 1-2 L per camel, PCV remained unchanged (median: 31% (pre), 32,5% (end of race) and 31,5%, (cool down)), as did dynamic shear viscosity. Aggregation indices were low and remained unchanged. Camel blood showed low shear thinning. Opt.HCT ranged from 49% at 11 s-1 to 31% at 500 s-1. A strong correlation between WBV and aggregation index (M1) suggests some form of organization within the camel blood in relation to shear flow, despite the spindle-shape, bi-convex surface probably preventing substantial aggregation. Contrary to observations in humans and horses, opt.HCT decreased continuously with increasing shear rates (SR). Resting PCV equaled opt.HCT at high SR, suggesting that camels do not depend on a high circulating RBC count to perform. The lacking WBV-increase in camels in response to exercise could be a physiological buffer to offset the effects of dehydration.

Platelet activation and function in response to high intensity interval exercise and moderate continuous exercise in CABG patients

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The purpose of present study was to compare the responses of platelet activation (CD62P) and function (platelet aggregation) in response to high intensity interval exercise (HIIE) and moderate continuous exercise (MCE) in coronary artery bypass grafting (CABG) patients. Thirty patients who had CABG were randomly divided into HIIE, MCE and control groups. After determining the VO2peak, subjects in HIIE group performed an interval protocol included 8 intervals of 2 min activity (running on treadmill) at 90% of VO2peak and 2 min active recovery at 30% of VO2peak, whereas, the subjects in the MCE group carried out 30 minutes of continuous exercise at 60% of VO2peak, and subjects in control group had rested for similar duration. Two blood samples were collected before and immediately after exercise and were analyzed for markers of platelet activation and function. Data analyzes revealed that responses of CD62P to MCE trial was significantly lower compared to HIIE group ($P<0.05$). In addition, increases in platelet aggregation induced by ADP and corrected for increases in platelet count in response to MCE trial was significantly lower than HIIE group ($P<0.05$). Among the platelet indices only changes in plateletcrit and platelet distribution width were significantly different among the three trials. Based on the findings of the present study it could be concluded that the risk of exercise-induced thrombosis is lower during MCE than HIIE in patients with coronary artery disease.

Examination of exercise induced limb ischemia in peripheral artery disease from a hemorheological point of view

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Aim: Our aim was the investigation of microcirculatory blood flow by angiological as well as hemorheological methods in patients with peripheral artery disease (PAD).

Methods: The patient group was made up of 35 patients diagnosed with PAD (66±2 yrs; 17women, 18 men) and 21 healthy volunteers without PAD were enrolled as control group (60±3yrs; 12 women; 9 men). Transcutaneous tissue oxygen pressure, laser Doppler flowmetry were applied. Exercise test (6-minute walk test or treadmill) was performed as provocation; measurements were done at rest and after exercise. Examination of hemorheological parameters (fibrinogen, plasma and whole blood viscosity, red blood cell (RBC) aggregation) was also studied.

Results: All the measured angiological parameters were significantly worse among patients compared to the control group ($p<0.05$). Higher fibrinogen level ($p<0.001$) and RBC aggregation ($p<0.05$) were detected in patients with PAD compared to the control group. The RBC aggregation and plasma viscosity were more deteriorated in patients with claudication than in asymptomatic patients ($p<0.05$). The maximal and pain-free walking distances of treadmill tests had negative correlation with RBC aggregation and plasma viscosity ($p<0.05$).

Conclusion: These findings suggest that certain impaired hemorheological factors may contribute to the altered functional capacity by the deterioration of microcirculation in PAD.

Platelet aggregation inhibition: Hemorheological aspects

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The mortality of coronary artery disease has substantially decreased in the developed countries, however, cardiovascular diseases and their complications still remain the leading cause of premature death worldwide. Hemorheological parameters have been proved to be independent risk factors of ischemic heart disease. Platelets are vital components of normal hemostasis and key participants in pathologic thrombosis by virtue of their capacity to adhere to injured blood vessels and to accumulate at sites of injury. A variability in patient response in the case of both ASA and clopidogrel therapy is well known. Differences were found in clinical and hemorheological parameters of ASA treated patients with effective platelet inhibition compared to patients with ineffective treatment. Clinical and hemorheological parameters, plasma von Willebrand factor and soluble P-selectin levels of patients with effective platelet inhibition by clopidogrel have been compared to patients with ineffective clopidogrel treatment. A possible connection was found between gender differences in hemorheological parameters and in vitro platelet aggregation in vascular patients treated with widely used antiplatelet agents. We investigated the relation of platelet aggregation and fibrinogen levels to advancing age in aspirin- and thienopyridine-treated patients. Specific changes were found in hemorheological parameters and platelet aggregation following carotid stenting.

Inflammation-induced microvascular dysfunction in obesity - a translational approach

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Obesity is associated with elevated risk of cardiovascular events, diabetes, arterial hypertension and cancer and has become a worldwide problem affecting developed and developing countries. Experimental, clinical and epidemiological data indicate that not the amount but the distribution of fat is an important determinant. In animal models, we have investigated effects of dietary interventions and physical exercise on total body fat, aorta eNOS and iNOS expression, microvascular reactivity in response to acetylcholine and sodium nitroprusside, functional capillary density, capillary recruitment and macromolecular permeability on cheek pouch or cremaster muscle preparations. In obese patients, we have investigated whether functional microvascular parameters were correlated with clinical-anthropometrical data and if these parameters would influence obesity-related metabolic disorders, especially glucose homeostasis, in young overweight/obese women. Our results have shown that high fat diet elicits an increase on visceral fat deposition, microvascular dysfunction and insulin resistance in hamsters. In patients we could find a direct correlation between post-load plasma glucose and the time to reach peak red blood cell velocity linking microvascular parameters with metabolic variables and suggesting a key role for microcirculation in obesity-related metabolic disorders. Oxidative stress, inflammation and renin-angiotensin system are also involved on microvascular dysfunction.

Inflammatory edema propagated via the kallikrein-kinin system fuels intracardiac parasitism in experimental Chagas disease

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Chagas disease is caused by chronic infection with the parasitic protozoan Trypanosoma cruzi and is still an important cause of morbidity and mortality in Latin America. Bradykinin (BK) may enhance T. cruzi infectivity via activation of the Kallikrein-Kinin System (KKS). Intravital microscopy studies in the hamster cheek pouch (HCP) suggested that plasma leakage elicited by trypomastigotes was potentiated via mast cell driven activation of the KKS. We have made direct applications of parasites and used dextran sulfate 500 kDa (DXS) to activate KKS. DXS applied on HCPs caused no visible effects within 30 min. However, a minimal leakage from a single postcapillary venule or an application of hamster plasma caused dramatic increases in vascular permeability that was inhibited by a BK-antagonist, HOE-140, cromoglycate and a prekallikrein inhibitor PKSI-527. Similar observations were made at topical applications of trypomastigotes (TCTs) subsequent to a small dose of histamine providing the extravascular plasma that could be utilized by TCTs to activate MC and KKS. This was evidenced by the inhibition of TCT-enhanced permeability by the same drugs that inhibited DXS-induced KKS-activation. Collectively, our studies suggest that endothelial stabilizing drugs might reduce cardiac parasitism and the associated myocarditis by inhibiting plasma leakage, a microvascular response that amplifies T. cruzi-evoked inflammation through the activation of the MC/KKS pathway.

Impact of diet on microvascular function and proinflammatory metabolites of arachidonic acid

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Arachidonic acid (AA) metabolites have an important role in mediating vascular reactivity to various stimuli, thus changing tissue blood flow and tissue supply. In addition, they have proinflammatory or anti-inflammatory effect on vessels. AA is metabolized by cyclooxygenases 1 and 2 to prostaglandins and thromboxane, by lipooxygenase to leukotrienes; by CYP450-hydroxilase to 20-hydroxyeicosatetraenoic acid (20-HETE) and by CYP450-epoxygenase to epoxyeicosatrienoic acids (EETs).Increased vascular oxidative stress may induce non-enzymatic production of isoprostanes from AA, which, together with vasoconstrictor metabolites of AA underlie to endothelial damage and impaired vascular function.

Dietary kitchen salt intake leads to impairment of the vascular reactivity to physiological stimuli, both in conduit and in microcirculatory vessels, even with normal arterial blood pressure levels. Our results in animal and human studies show that increased salt intake significantly changes type and the amount of produced AA metabolites, leading to impaired vascular function.On the other hand, omega-3 fatty acids (FA) have protective role in cardiovascular system, supposedly due to decreased production or effects of the AA metabolites. Thus, diets rich in omega-3 FA, especially omega-3 enriched foodstuff and low in kitchen salt is the most natural way in cardiovascular health protection.

Autodigestion in Inflammation: Digestive Enzymes on the Prowl

(supported by NIH grant GM 85072, HL 10081 and a gift from Leading Biosciences)

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Many diseases are accompanied by gastrointestinal co-morbidities but the molecular mechanism remains elusive. I will discuss two examples. Our evidence in acute experimental shock indicates, that the powerful pancreatic digestive enzymes, which are usually in the intestinal lumen, escape into the circulation at concentrations sufficient to cause autodigestion. The digestive enzymes activity degrades plasma proteins and membrane receptors and causes cell dysfunctions, organ failure and death. Blockade of digestive enzymes in the small intestine reduces autodigestion and morbidities in shock. In chronic cardiovascular disease unchecked proteases may also be present in the systemic circulation causing cell dysfunctions. We investigated the spontaneously hypertensive rat (SHR) with elevated blood pressure and diverse co-morbidities, e.g. insulin resistance, capillary rarefaction. SHR tissues, cells and plasma exhibit an unchecked extracellular proteolytic activity and extracellular domain receptor cleavage, causing reduced receptor functions. E.g. unchecked proteases cause cleavage of beta-2 adrenergic receptors and elevation of the SHR blood pressure. It also cleaves the insulin receptor with reduced insulin signaling (i.e. insulin resistance) and the VEGFR2s with endothelial apoptosis and loss of capillaries (i.e. rarefaction). The results suggest autodigestion may be a fundamental mechanism for cell/organ dysfunction, disease and death.

The History of the Theory of the Circulation of the Blood

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It is reasonable to assert that the seminal event in the history of haemorheology is Harvey's presentation of the concept of the circulation of the blood. Prior to this, the ideas concerning the movement of blood were based, in Europe and Middle East, largely on the principles laid down by Galen, and these had been, in effect, dogma for about a millennium and a half. These principles were basically that blood is formed in the liver, thence it travels to the bodily organs and is consumed – hence there is essentially one-way flow and no circulation of the blood at all. Harvey's revolutionary idea that blood circulates repeatedly around the cardiovascular system, laid the foundation for haemorheology because once that idea was accepted then the fluidity of the blood immediately became potentially of crucial importance – and haemorheology was born. In this lecture the ideas that preceded Harvey will be presented, i.e. those of Galen, Ibn al-Nafis, Vesalius, Fabricius and Colombo etc. Harvey's awareness of this background, due mainly to time spent in Padua, triggered his many experimental investigations and discoveries. Ultimately, these led to his astonishing insights published in De Mortu Cordis in 1628 and which changed the understanding of the cardio-vascular system forever.

Interrelation of blood coagulation and hemorheology in cancer

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Cancer progression is associated with activation of blood coagulation. Evaluation of blood clotting process by means of low frequency piezothromboelastography in patients with solid tumors (n=21) has demonstrated intensification of contact coagulation phase, the rise of intensity of the proteolytic stage of fibrin forming, the shortening of blood clotting time. Reduced intensity of lysis and retraction of clot was fixed in patients compared to healthy control. Analysis of hemorheological indices in cancer patients revealed the decrease of high shear rate blood viscosity in patients determined by the significant lowering of Hct (by 19%, $p<0.01$) which exceeded the effects of plasma viscosity growth (by 9.5%, $p<0.05$) and the decrease of red blood cell deformability promoting elevation of blood viscosity. These changes of macro- and microrheological blood properties in patients with malignancies caused the evident decrease of the efficacy of oxygen delivery to tissue by blood. It was determined by the notable reduction of the number the main oxygen carrier – erythrocytes, as well as by the rise of red blood cell rigidity and aggregability (by 25%, $p<0.05$). Increased blood fluidity in cancer may to some extent compensate high hemocoagulation activity, preventing thrombotic events; on the other hand impaired blood rheology may induce hypoxia in the microcirculation that favors thrombosis, settlement of tumor cells and thus metastasis.

This work was supported by RSCF grant 14-15-00787.

Surgery effect on hemorheological profiles in patients with colon cancer

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The aim of this study was to estimate the rheological properties of blood in cancer patients before and after surgery.

14 healthy volunteers and 12 patients with colon cancer were enrolled in this study. Viscosity of plasma and whole blood were evaluated by means of Brookfield viscometer DV2T (USA). Erythrocyte deformability and aggregability were determined by using of RheoScan-AnD 300 system (South Korea) and direct light microscopic technique with computer image analysis.

Before surgery whole blood viscosity in patients was markedly lower (up to 24.7%, $p<0.01$) compared to healthy control. Further reduction of blood viscosity (up to 24.5%, $p<0.01$ compared to the initial state) was registered after surgery. This reduction was mainly due to the substantial decay of hematocrit – in patients it was by 16% ($p<0,001$) lower than in controls and after surgery this difference was aggravated. Microrheological blood properties were markedly worsened in patients before surgery compared to healthy control - extent of RBC aggregation was increased by 37% ($p<0.05$), resistance of aggregates was elevated by 23% ($p<0.01$), erythrocyte deformability was reduced (by 2%, $p<0.01$). These unfavorable changes of RBC properties were aggravated after surgery ($p<0.01$).

Fixed alterations of hemorheological properties in colon cancer patients may facilitate tissue hypoxia diminishing tissue oxygenation that favors settlement of tumor-cells and thus metastasis.

This work was supported by RSCF grant 14-15-00787.

Hemorheological profile change after chemotherapy: insight into the microrheological change mechanisms

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The subject of this study was to estimate the effect of neoadjuvant chemotherapy (NACT) on the basis of platinum (cisplatin) inpatients (n = 34) with solid tumors on parameters of hemorheological profile. Before and 21 days after each cycle NACT (1 to 3 cycles) the hemorheological profile included blood and plasma viscosity; hematocrit (Hct), RBC aggregation (RBCA) and deformability (RBCD) was recorded. Blood viscosities (BV) were decreased significantly after each cycle of treatment (from 10 to 20%). These viscosity changes were accompanied by Hct lowering by 18%. Plasma viscosity (PV) was reduced by 9% under these circumstances. The results obtained herein demonstrate that two change mechanisms of the hemorheological profile there are under cisplatin chemotherapy. The first mechanism is connected with a decrease of Hct, in a number of cases,up to anemic values (Hct>30%) and PV alteration. Therefore blood viscosity was decreased because both main factors influencing on it were statistically significant lowed. There were positive correlations between BV and Hct (r=0,480) and PV (r=0.680). The second mechanism is connected with positive changes of RBC microrheological properties after cisplatin treatment. Taken together these positive alterations of hemorheological profile can result in an improvement of blood transport potential for oxygen and drug delivery into the tumor tissue.

This work was supported by RSCF research project No. 14-04-01703.

Role of hemorheological factors in the control of microcirculation in anemic patients with solid cancer

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The blood rheology has a regulating effect on the microcirculation. Blood viscosity (BV) determines the magnitude of the shear stress on the arteriole wall for NO release, and under some conditions red blood cells (RBCs) excrete ATP for vascular tone control. The investigation of the role of hemorheology in the regulation of microcirculation was the study aim. In patients with solid malignancies recorded hemorheological profile, microvascular perfusion and oxygenation of tissues and determined the RBC ATP content before and after three cycles of chemotherapy based on platinum (cisplatin). After 3rd cycle of chemotherapy a decrease in microvascular perfusion (MP) by 18% and tissue oxygenation (TO) by 12% were found. Decrease of Hct in these conditions has two negative effects: 1) a decrease in blood oxygen capacity; 2) decrease in BV. There were also two positive responses: 1) some increase in plasma viscosity (PV) and 2) RBC deformability rise by 9%. PV positive correlation with the MP ($r = 0.520$) and TO ($r = 0,850$) confirms this. The RBC content of ATP was 28% less than the control. It was found that RBC ATP negatively correlated with MP ($r = –0,620$). Therefore, an increase in PV could offset the decline in blood oxygen capacity due to stimulation arteriole vasodilatation and an increase of microvascular perfusion and tissue oxygenation.

This work was supported by RSCF research project No. 14-04-01703.

Hemangiomas in infancy – therapeutic options

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Aim: Complicated infantile hemangiomas need early, safe and effective treatment. The aim of this study was to provide greater insight into systemic and topical propranolol treatment efficacy and side effects.

Methods: We report our retrospective experiences of 207 paediatric patients treated with systemic propranolol and of 148 paediatric patients treated with propranolol gel topically photographed and analysed with a specific hemangioma score.

Results: Propranolol treatment was successful in >99 % of the patients. The hemangioma score showed a significant decrease during systemic treatment (8.3 ± 3.3 at beginning and 1.5± 1.4 at the end of treatment). Systemic treatment did not show any differences when distributed according to different localisations or to the patients' ages. During topical propranolol treatment relevant serum levels were not determined. Relevant side effects that may have made it necessary to discontinue the treatment were not observed. However, there was a statistically significant reduction in heart rate during the first six in-hospital systemic drug applications.

Conclusion: Systemic propranolol treatment is highly effective and nearly always safe. Topical treatment with propranolol gel is suitable for specific hemangiomas in addition to cryotherapy and systemic treatment with propranolol. These findings provide highly valuable information on this drug treatment for complicated hemangiomas in infants.

Hemorheological behavior in sickle cell disease pediatric patients

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AIM: Sickle cell anemia is a pandemic disease responsible for several clinical complications including vaso-occlusive events (VOCs). The mechanisms underlying these complications are still not well understood, especially in children. We investigated the hemorheological parameters of sickle cell disease pediatric patients (SCD-PP).

METHODS: We analyzed 28 healthy controls (6.75±4.2 years-old) and 36 homozygote SCD-PP (11±6.2 years old) in order to study their hemorheologic pattern. We use a Haake Rotovisco CV100-RV20 following the ICSH guidelines. RESULTS SCD-PP show higher high-shear rate blood viscosity (ηB200C) and erythrocyte stiffness (Tk) than healthy controls, while we did not detect ηP differences between the two groups. No correlation between the severity of the anemia and the rheological behavior was detected. However, pathological values returned to a normal range strictly after acute blood transfusion therapy.

CONCLUSION: These results confirm the hypothesis that SCD-PP have altered hemorheological behavior. The increased Tk in SCD-PP could lead to alterations of the microcirculatory flux (flow) which are not influenced by the presence of plasmatic proteins or by the severity of the anemia. Moreover, our results suggest that transfusion therapy may be useful for normalizing hemorheological parameters.

Hemorheological profiles in Thalassemia: a new approach for the study of complications

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Several studies demonstrated the presence of high incidence of thromboembolic events in ß -thalassemia, more common in thalassemia intermedia than in thalassemia major. In these patients a chronic hypercoagulable state is evident partially due to the impairment of the natural endothelial anticoagulant system, where the thrombomodulin is increased and the coagulation inhibitors are diminished. Red blood cells of thalassemic patients exhibit impaired flow properties that facilitate micro-circulatory disorders: enhanced aggregability, reduced deformability, as well as a marked elevation of adherence to endothelial cells. Levels of pro-coagulant microparticles derived from endothelium, platelets, RBC and leukocytes are also elevated in thalassemia intermedia patients. The increase in adhesion proteins and vascular cell adhesion molecules suggest that endothelial injury or activation may be a feature of ß-thalassemia, and may play an important role in the recruitment of leukocytes and erythrocytes and promote thrombosis at vascular inflammation sites. The hemorheological profiles of patients affected by ß-thalassemia major and intermedia have been evaluated and significant differences have been observed between the groups, mainly in relationship with transfusion therapy. These results could give reason of the high incidence of thromboembolic events in b-thalassemia, more common in thalassemia intermedia than in regularly transfused thalassemia major.

Hemorheological pattern in disorders of plasma composition

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AIM: An altered plasma composition can cause an increase in blood viscosity (BV). Plasma viscosity (PV) is mainly determined by the total protein concentration and by the presence of abnormal protein fractions. A polyclonal hypergammaglobulinemia is common in systemic autoimmune disorders and rare conditions of plasma hyperviscosity are cryofibrinogenemia, cryoglobulinemia, dysfibrinogenemia. However, plasma hyperviscosity is most often associated with paraproteinemias, in which an abnormal immunoglobulin is secreted by malignant B-lymphoid cells of monoclonal origin. The aim of this study was to investigate the rheological pattern in patients with multiple myeloma (MM) and monoclonal gammopathy of undetermined significance (MGUS).

METHODS: We measured BV at high and low shear rates, haematocrit, PV at high and low shear rates and some derived indexes; we also evaluated erythrocyte deformability by diffractometry at the shear stresses of 6, 12, 30, 60 Pa.

RESULTS: In MM patients we observed, as expected, a decrease in hematocrit and a marked increase in PV, but also a significant reduction of the erythrocyte deformability. A low erythrocyte deformability was also demonstrated in MGUS subjects, who showed a normal hematocrit and an increase in PV, especially at low shear rates.

CONCLUSIONS: The altered erythrocyte deformability observed in patients with paraproteinemias seems to be related to an alteration of membrane lipids and could be a therapeutic target.

Effects of RAGE blockade on inflammation, oxidative stress and nitric oxide bioavailability in transgenic sickle mice

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Sickle cell disease (SCD) is characterized by increased plasma levels of advanced glycation end products (AGEs). Under oxidative conditions, AGEs are generated by non-enzymatic glycation and oxidation of proteins and lipids that amplify oxidative stress when binding to their receptor (RAGE). The aim of this study was to characterize the effects of RAGE blockade with a specific antagonist (RAP) in a transgenic mouse model of SCD (Townes). Eight week-old AA (normal), AS (sickle cell trait) and SS (homozygous SCD) mice were treated intraperitoneally with RAP or vehicle. After 3 weeks of treatment, red blood cell count and hematocrit were significantly higher in RAP-treated SS mice. Reticulocyte count was lower in RAP-SS compared to their vehicle-treated littermates. RAP-treated SS mice had decreased TNF-á mRNA expression in heart and kidney and decreased IL-1â mRNA expression in lung. In liver, TNF-á and IL-1â mRNA expression was higher in vehicle-treated SS compared to AA mice while the same difference was not significant between RAP-treated SS and AA mice. The lower glutathione peroxidase activity in RAP-SS kidney compared to vehicle SS mice suggest reduced ROS production. Finally, eNOS mRNA expression was increased in kidney of RAP-SS and correlated with eNOS protein expression suggesting higher production of nitric oxide (NO) in RAP-SS mice. These results suggest that RAGE blockade may reduce inflammation, oxidative stress and increase NO bioavailability in sickle mice.

Erythrocyte Nitric Oxide in Patients with Glaucoma – ex vivo study

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Glaucoma is a progressive optic neuropathy associated with vascular dysregulation and increased intra-ocular pressure (IOP). The current treatment in IOP reduction is the application of timolol reducing production of aqueous humour.

Aims: This ex vivo study aims to evaluate timolol effect in NO efflux and its derivatives in erythrocytes from glaucoma patients. Methods: The ex vivo effect of AChE modulators ACh and timolol was studied in venous blood of 15 glaucoma patients. Erythrocyte suspensions were incubated with the modulators at 10uM concentration.

Results: No significant differences were obtained in erythrocyte NO efflux and S-nitrosoglutathione(GSNO) concentration in response to ACh or timolol when compared with the no treated erythrocyte of glaucoma patients. Erythrocyte suspensions from glaucoma patients showed higher amount of NO efflux in presence and absence of timolol than those values verified in healthy subjects under the same experimental conditions. Erythrocyte suspensions of glaucoma patients showed higher values of GSNO when in presence of timolol when compared with the values obtained in erythrocyte suspensions with timolol of healthy persons.

Conclusion: We demonstrated that erythrocyte from patients with glaucoma have more availability to liberate NO from erythrocyte both in absence and in presence of timolol than the erythrocytes from healthy persons. The amount of erythrocyte GSNO in presence of timolol is higher in glaucoma patients than in healthy persons.

Soluble CD40 ligand Profiles in Patients with Septic Shock

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Aim: Soluble CD40 ligand (sCD40L) has been considered as marker of thrombosis and inflammation in several diseases, including sepsis. Recent studies challenge this view and point to a role of sCD40L in vascular and endothelial function. An indication of that association in sepsis has not been obtained so far. Therefore, herein we evaluated the association of sCD40L with hemorheological and inflammatory markers on context of septic shock.

Methods: Time-changes of sCD40L serum levels over 72 hours of Intensive Care Unit (ICU) admission were assessed in 20 patients with septic shock and 22 healthy volunteers. Association of sCD40L levels with erythrocyte deformability and aggregation (as markers of hemorheology), plasma haemoglobin and white blood cells (WBC) count (as markers of low-grade inflammation) was assessed in patients with septic shock.

Results: At ICU admission, sCD40L levels in patients with septic shock (4.82±4.62 ng/mL) were lower than levels of healthy volunteers (5.95±3.86 ng/mL, $p=0.043$). sCD40L significantly change over 72 hours of internment ($F=2.1$, $p=0.137$). Soluble CD40L levels in patients with septic shock correlate with Hb ($r=0.61$, $p=0.00$) and WBC ($r=0.63$, $p=0.00$), but not to erythrocyte deformability ($r\geq0.157$, $p\leq0.235$) and aggregation ($r\geq-0.109$, $p\leq0.192$).

Conclusions: These results seem to highlight the association of sCD40L to endothelial function and inflammation in septic shock context.

Inflammatory and hematological disturbances associated with resistance to recombinant human erythropoietin therapy in CKD anemia in a rat model

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Anemia of chronic kidney disease (CKD) is treated with recombinant human erythropoietin (rHuEPO); however, some patients become hyporesponsive. Our aim was to study the hematological and inflammatory disturbances associated to resistance to rHuEPO therapy using the remnant kidney rat model of CKD anemia.

Wistar rats were divided in 4 groups: Sham, CRF and rHuEPO treated groups (200IU/kg b w/wk) - responders (CRF200) and non-responders (CRF200NR). Blood was collected for hematological and biochemical analysis; kidney tissue was collected for gene and protein analysis.

The CRF200 group corrected anemia, while the CRF200NR group developed anemia after an initial response to rHuEPO therapy. CRF and CRF200NR groups showed a trend to higher CRP levels; CRF200NR showed also high levels of renal inflammatory markers.

Our data suggest that the development of anemia/rHuEPO resistance is associated with a higher systemic and renal inflammation, favoring hypoxia and triggering an increase in renal expression of fibrosis markers, which will enhance the inflammatory response, creating a cycle that promotes disease progression.

Financial support by Portuguese Foundation for Science and Technology through national funds; co-financed by COMPETE-FEDER (PTDC/SAU-TOX/114253/2009, Pest/C/SAU/3282/2013), FEDER under Partnership Agreement PT2020 (UID/MULTI/04378/2013-POCI/01/0145/FERDER/007728, UID/NEU/04539/2013) and by POPH/FSE (SFRH/BD/61020/2009, SFRH/BD/79875/2011 and SFRH/BPD/81968/2011).

Rheologic behavior of blood in some myeloproliferative neoplasms

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AIM: The aim was to investigate rheological behavior of blood in patients with some myeloproliferative neoplasms.

METHODS: The study included 16 adults with Polycythemia vera (PV), 42 young with acute lymphoblastic leukemia (ALL), and 67 healthy donors as control group. Of patients 38% had thrombosis. Using rotational viscosimeter whole blood viscosity (WBV) were measured in order of a decreasing and then of an increasing of shear rates with subsequent calculations for erythrocyte aggregation, and erythrocyte deformability, and non-Newtonian behavior of blood. Hematological parameters and erythrocyte indices and B-type natriuretic peptide (BNP) were analyzed simultaneously.

RESULTS: Increased WBV revealed in PV but not in ALL. In patients WBV had differences when it was measured in order of decreasing and then of increasing of shear rates. Patient's WBV dependent on leukocytes count, on MCH and mainly on MCV. Both neoplasms are accompanied increased erythrocyte aggregation but not impaired erythrocyte deformability. Of patients 40% had elevated BNP assuming subclinical cardiac dysfunction that lead to non-fully reversible erythrocyte aggregation. The residual cells units play role in that in PV-patients non-Newtonian behavior of blood is lost later than in ALL-patients and in control group.

CONCLUSION: Patients with some myeloproliferative neoplasms has abnormal blood flow properties forming non-hemocoagulation conditions for thrombosis development.

Actual vs optimal fetal hematocrit measured with punctures of cord blood in utero: relationship with umbilical artery resistance

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At birth hematocrit is rather high, but pioneering works by O Linderkamp have shown that it is adapted to fetal circulation. In addition this author investigated the issue of optimal hematocrit in fetal blood in vitro in narrow glass tubes, and found with this in vitro approach values of an optimal hematocrit ranging as high as 60% in the narrowest tubes. A theoretical 'ideal' hct can also be predicted with a theoretical curve of h/v vs hct constructed with Quemada's model. We used the database of one of our previous papers on fetal hemorheology to reinterpret its results with this concept. A series of 28 intrauterine cord punctures (between 19 and 33 weeks gestation) with doppler measurements of resistance in umbilical arteries was studied. The theoretical ´´optimal hematocrit´´ was well correlated to actual (r=0,857 p<0,01) but systematically lower (Bland-Altman plot +12.1[8.52-15.7]) than the actual one. Umbilical artery resistance index is correlated with actual hematocrit (r=0.407 p<0.05), the discrepancy between ideal and actual (r=-0,542 p<0.05) but not predicted ideal hematocrit, suggesting that the discrepancy between ideal and actual may reflect an adaptative decrease aiming at reducing vascular resistance. These findings indicate that prediction of ideal hematocrit with Quemada´s equation makes sense in fetal blood, and suggest that a ´viscoregulatory mechanism´ maintains hematocrit below this theoretical value in order to avoid excess vascular resistance.

Effect of Oxidized LDL on Erythrocyte Nitric Oxide Metabolism

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Aims: Oxidized low density lipoprotein (ox-LDL) has been reported as an inhibitor of nitric oxide (NO)-mediated dilatation in microcirculation. Oxidized LDL effect on NO metabolism of erythrocytes is not known. Therefore, this study aims to evaluate the effect of ox-LDL on erythrocytes NO metabolism.

Methods: The effect of different concentrations of human purified ox-LDL (25, 50 and 100 mg/mL) on NO metabolism was evaluated on erythrocytes suspensions of healthy subjects.

Results: An inhibitory effect of higher concentrations of ox-LDL on erythrocyte NO efflux was verified. NO efflux his lower as consequence of treatments with ox-LDL 50 mg/mL (1.6±0.27 nM) and 100 mg/mL (1.3±0.22 nM) concentrations than control aliquot (1.9±0.28 nM). No difference was verified in comparison to positive control, acetylcholine (ACh; 1.7±0.21 nM). By the contrary, ox-LDL incubation has a positive effect on GSNO content of erythrocytes. That effect is proportional to concentrations of ox-LDL (10.8±1.4 nM for 25 mg/mL, 12.9±1.5 nM for 50 mg/mL and 12.1±1.9 nM for 100 mg/mL) and is significant relative to control (8.56±0.76 mM) and ACh (8.9±0.52 mM) aliquots.

Conclusions: Presence of oxidized LDL in erythrocyte NO metabolism induces a decrease of NO efflux amount and an increase on intra-erythrocyte GSNO concentrations. These results suggest a role of ox-LDL in mobilization of NO between NO derivatives molecules in dependence of oxidized LDL concentration.

Hemorheological Parameters in Different Forms of Coronary Heart Disease

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In the coronaries there is a continuous change in blood flow, perfusion pressure and shear rate during each cardiac cycle. It is also the place of the narrowest capillaries in the human body, therefore the role of rheological alterations is of great importance here.

Over the last few decades, we have investigated hemorheological parameters (HP) in over 5,000 patients diagnosed with various forms of ischemic heart disease (IHD). In a prospective study, we measured the HP of patients with acute coronary syndrome (ACS). On admission, all variables were significantly worse than those of control subjects. During the hospital phase, some of the HP showed further deterioration, and HP remained in the pathologic range during the follow-up period. In two other studies we showed that HP were in close correlation with the severity of coronary artery disease which had been detected by coronary angiography and coronary CT. In patients treated with percutaneous coronary intervention, changes in HP were very similar to those observed in subjects with ACS. We also analyzed HP in patients undergoing CABG surgery. Our data suggest a hemorheological advantage of off-pump surgery.

In IHD patients during ambulatory cardiac rehabilitation HP showed a significant improvement during the 1.5 year follow-up period.

Our data indicate that rheological parameters are significantly altered in patients with IHD: the extent of the alterations is in a good correlation with the clinical severity of the disease.

Short-term and repeated shear stress exposure below the classic haemolytic threshold impairs red blood cell deformability and induces haemolysis

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The supraphysiological shear stress (SS) red blood cells (RBC) are exposed to while traversing circulatory assist devices (CAD) impairs RBC deformability and may lead to haemolysis. Although exposure time to supraphysiological SS within CAD is limited, the magnitude of SS is crucial to the amount of damage to RBC [1, 2]. We explored changes in RBC function following exposure to SS below the reported "haemolytic threshold" with a duty-cycle typical of that employed by CAD. Blood collected from 20 male donors, aged 18–38 yr, was suspended in a viscous medium and exposed to a SS protocol of 1 s, at 100Pa, every 60 s for 60 duty-cycles. An ektacytometer was used to measure RBC deformability after each exposure. Haemolysis was quantified via spectrophotometry. The mechanical SS protocol impaired RBC function as indicated by: (1) significant shifts in the RBC dynamic morphological response to SS, after 15 duty-cycles; and (2) increased incidence of haemolysis following 60 duty-cycles. The present study demonstrates exposure of RBC to short-term, repeated supraphysiological SS, impairs RBC deformability, with each duty-cycle causing an increase in RBC rigidity that ultimately precipitates haemolysis.

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Analysis of fluctuating blood flow data using the methods of nonlinear dynamics and Allan variance

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AIM: The aim of this work was to develop a novel technique for digital processing of Doppler ultrasound blood flow sensor data from noisy blood flow velocity waveforms.

METHODS: To evaluate the fluctuating blood flow parameters, we applied some nonlinear dynamics methods and algorithms [1]. Here, for identification of chaotic and noise components in a fluctuating coronary blood flow, the Allan variance technique [2] for the first time was used. Analysis of different types of noises (White, Brownian, Flicker) was carried out and their strong correlation with fractality of time series (the Hurst exponent) was revealed.

RESULTS: Based on a specialized software realizing the developed technique, numerical experiments with real clinical data were carried out. Recommendations for identification of noisy patterns of coronary blood flow in normal and pathological conditions were developed.

CONCLUSIONS: The methodology gives us the possibility for the more detailed quantitative and qualitative analysis of a noisy fluctuating blood flow data.

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Shear-induced platelet adherence and activation in a dynamic multiwell-plate system

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Aim: Cardiovascular devices are prone to different shear stresses after implantation. Here, the influence of different shear conditions on the evaluation of the thrombogenicity of polymers in vitro was studied.

Methods: Poly(dimethyl siloxane) (PDMS), polyethylene terephthalate (PET) and polytetrafluoroethylene (PTFE) films were studied. Polymers were exposed to whole blood from healthy humans. Blood was agitated orbitally at low (50 rpm, wall shear stress: τwall,max= 0.28 N·m2) and high (200 rpm, τwall,max= 2.22 N·m2) agitation speeds. Numbers of non-adherent platelets, platelet activation (CD62P [%] positive platelets), platelet function (PFA100 closure times) and platelet adhesion (laser scanning microscopy (LSM)) were determined.

Results: LSM revealed increasing numbers of adherent platelets with increasing agitation speed. At the high agitation speed, lowest platelet densities were observed on PDMS and the highest densities on PTFE. At low agitation speed, the platelet densities did not differ between the three materials. While numbers of non-adherent platelets decreased, PFA100 closure times and percentages of CD62P positive platelets increased with increasing agitation speed.

Conclusion: Differences in the thrombogenicity of the studied polymers were more pronounced at the high agitation speed due to induced shear stresses and platelet activation. Future studies will focus on the application of higher shear stresses to improve the discriminatory power of the test system.

Force spectroscopy on red blood cells of different species: a comparative approach

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Atomic force microscopy was used to characterize the mechanical properties of adult human, horse, camel and chicken red blood cells (RBC) as well as fetal human RBC.

Force-distance curves were obtained in 0.9% NaCl and autologous plasma at room temperature (RT), 32°C and the species´ body temperature giving elasticity as apparent Young´s modulus (E) as well as adhesion of the RBC. The measurements in plasma were performed on adult human, fetal human and horse RBC only.

A Si3N4 cantilever with a non-coated 8nm tip was used to physically indent the RBC up to a given setpoint of 1nN. The obtained force-distance data were processed by employing the Hertz model at a contact point of 500nm indentation depth.

In NaCl at RT, horse and camel RBC showed the highest E followed by fetal human and adult human RBC. In chicken, the results depended on whether the nucleus or the peripheral region was indented. Indentation of the peripheral region resulted in E values slightly higher than those of adult human RBC.

Temperature and medium played an important role. Generally, in plasma E was lower compared to NaCl. With increasing temperature, E decreased both in NaCl (human RBC: ~200 to 500Pa at RT; ~100 to 200Pa at 37°C), and in plasma (human RBC: ~100 to 200Pa at RT; ~50 to 150Pa at 37°C).

Adhesion could only be observed in NaCl. It tended to increase with temperature. In plasma, adhesion was not present anymore.

Chronobiological investigations of micro-rheological parameters in male and female outbred rats: age-related, gender and seasonal differences

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More and more, but controversial data are available on age- and gender-related alterations of hemorheological parameters in human. For experimental medicine there is a lack of related laboratory animal studies using modern micro-rheological instruments. In this comparative follow-up study male (n=10) and female (n=10) CD outbred rats were followed-up over a one-year period and while they were kept in conventional animal facility. Blood samples were obtained by puncturing the lateral tail vein (each time ~0.5 ml, anticoagulant: K3-EDTA) at their age of 3 months (base value, tested in March), and afterwards 1, 2, 6, 9 and 12 months later. The age of animals at the end of experiment was 15-month. Hematological parameters (microcell counter), red blood cell aggregation (light-transmission aggregometry), red blood cell deformability, osmotic gradient deformability and membrane stability (rotational ektacytometry) were tested. Body weight and temperature, as well as estrous cycle of the female rats (by vaginal smear testing) were also examined. The micro-rheological parameters, mostly red blood cell aggregation, showed age-related alterations with gender differences, and the results also were affected by the current phase of estrous cycle, besides supposed seasonal effects (Spring-Winter) as well. These data could be useful for planning and better evaluating results of rat experiments.

Rheopheresis in vascular diseases

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Aim: In Hungary the first rheopheresis treatment was done at the University of Debrecen, Clinical Center, Division of Angiology in July 2014.

Methods: Rheopheresis is a double cascade filtration system. After the plasma separation the MONET filter retains the high molecular weight proteins from the plasma. According to the ASFA 2014 guideline rheopheresis is recommended first line therapy in age-related macula degeneration. In 2014 a patinent with AMD was treated with rheopheresis. We performed 11 treatments in 6 cycles. We assessed the visual acuity, stiffness parameters, endothel function, plasma and whole blood viscosity, prothrombotic activity. We measured phagocyte activity activated monocyte ratio, erythrocyte aggreggability before and after the treatments. We also performed rheophersis in a diabetic foot patient sugffering from non-healing ulcers.

Results: Rheopheresis treatment lowered the plasma and whole blood viscosity. Endothelial function was restored. Stiffness parameters significantly improved. Plasma and whole blood viscosity normalized. We detected antiinflammatory and antithrombotic effects of rheopheresis as well. In AMD patient the visual aciuty significantly improved. In diabetic foot syndrome we could reach complete wound healing.

Conclusions: Rheopheresis has a complex vascular effect. Our results suggest that rheopheresis could be a new therapeutical option in other vascular diseases as well.

Segmental Bioelectrical Impedance Analysis (SBIA) and blood rheology: reducing the gap between in vivo and in vitro?

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Bioelectrical impedancemetry (BIA) has been used to evaluate hematocrit and red cell aggregability in vitro but whole body impedance measurements are also correlated to some hemorheologic factors, suggesting a relationship between viscosity factors and electric properties of blood. We repeatedly reported correlations with whole body BIA and hematocrit, whole blood viscosity and plasma viscosity, red cell rigidity and RBC aggregation. The SBIA Inbody 770 modelizes body as 5 cylinders and measures impedance at 1, 5, 50, 250, 500, and 1000kHz.With the SBIA we found that hematocrit is best correlated to leg reactance at 50kHz but also to leg impedance at 1 and 5 kHz and trunk reactance. RBC aggregation « M » is best correlated to arm reactance at 5 kHz but also to most measurements of segmental impedance (28 correlations found). RBC aggregation « M1 » is best correlated to arm reactance at 5 kHz and to 19 other impedance measurements. A predictive equation for "M" from the mean between the two arm reactances at 5 kHz (maXc5) is found: M=2,1845 maXc5-23,958 ($r=0,665 p<0,001$) that provides a satisfactory Bland-Altman plot (mean difference: 0.000524 range [-1.6;+1.6]. This study suggests that previously reported correlations between BIA and viscosity factors were not spurious, and that in a narrow cylinder such as the arm the structure of circulating blood (hematocrit, red cell aggregation) may influence the passage of an electric current by increasing reactance.

Red blood cells interaction in interchanging media

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AIM: In this work, we aim to assess red blood cells (RBCs) interaction, using an approach with interchanging suspending media.

METHODS: Optical tweezers (OT) coupled with a microfluidic chamber allow the optically trapped RBCs to be moved between two different solutions. A cell doublet was formed in one of the solutions and then moved to another solution. The resultant change in the interaction forces was quantified. Different combinations of macromolecule solutions (plasma, dextran, fibrinogen, and/or albumin solution) and PBS were studied.

RESULTS: The doublet formed in a macromolecule solution disaggregated immediately as soon as it was moved to PBS. However in the opposite way, when cells were moved from PBS to a macromolecule solution, the cells started to interact immediately. Moving thecells from a solution with lower macromolecule concentration to a solution with higher concentration resulted in an enhanced interaction of the cells. When the cells were moved in the opposite way their interaction almost did not decrease.

CONCLUSION: The study reveals that red blood cells interaction can be further assessed in well-defined and interchanging media using OT coupled with a microfluidic chamber. The underlying cell-cell interaction mechanisms will be discussed.

ACKNOWLEDGEMENTS: This work was supported by SFB grant #1027. K.L. acknowledges the support by University of Oulu Graduate School Infotech Grant.

New potentialities of digital optical capillaroscopy for early diagnostics of arterial hypertension and type 2 diabetes mellitus

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AIM: The aim was to evaluate the digital nailfold capillaroscopy (DNC) potentialities for quantifying microvascular abnormalities in patients with Arterial hypertension (AH) and Type 2 diabetes mellitus (T2DM).

METHODS: The study involved 319 adults including 40 patients with prehypertension (PH), 36 patients with AH (mean systolic BP 153±12 mm Hg), 47 treated patients with AH, 52 patients with compensated T2DM, 68 decompensated diabetics, and 76 healthy volunteers (HV). All underwent DNC using a fast CCD-camera and image-processing software allowing for quantifying the diameters of the arterial and venous segments of capillaries, coefficient of remodeling (CR), capillary blood velocity (CBV), capillary network density (CND), etc.

RESULTS: Significant narrowing of arterial loops was revealed in patients with both AH and PH in comparison with HV. CBV in patients with AH was significantly lower in comparison with HV. The study revealed significant difference in CND and CR in comparison of T2DM patients with non-diabetic individuals. Significant changes were found in the decompensated T2DM group compared to the compensated group of diabetic patients.

CONCLUSIONS: DNC reinforced with the advanced image-processing algorithm opens up new possibilities for obtaining clinically important information on microvascular abnormalities in patients with AH and T2DM.

ACKNOWLEDGMENT: The investigation was partially supported by the Russian Scientific Foundation grant ¹ 14-15-00602.

A comparative study of 337 and 532 nm laser irradiation on Thrombi Formation in vivo

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Aim: In vivo thermal effects of laser-induced endothelial damage were investigated comparatively with pulse UV- laser of 337 nm and continuous diode laser of 532 nm.

Methods: Mesenteric venules of anesthetized Wistar rats, (d=20-30 μm) were irradiated with pulse laser light (LGI-21, Russia, λ=337 nm, 3 mW, pulse duration 10-8s, period 0,02s) and continuous one(DPSS Laser, λ=532 nm, 34 mW, South Korea). Laser- induced microvascular disturbances were analyzed using time-lapse video-microscopy. Local intravascular temperature rise was calculated on the basis relatively simple heat transfer model.

Results: Heat effects within irradiated microvessels are determined by direct absorption of laser radiation by blood and heat losses by lateral conduction ($\tau dif=D2/6\rho$) and by longitudinal convection($\tau con=L/v$). Calculated peak temperature rise during one pulse was about 500C.The irreversible primary damages of endothelium which gave rise to thrombus growth followed by pronounced thromboembolic reaction were observed in experiment after a series (at least 10) pulses. The diode laser irradiation (1 s) resulted in formation of stable thrombus with minimal thromboembolic process. The calculated temperature rise inside the irradiated volume of microvessel did not exceed 31 0C.

Conclusions: Both numerical and experimental studies of laser-induced thrombus growth indicate wide possibilities in modeling of well-controlled endothelium damage in mesenteric microvessels.

Assessment of scalp microvascular function

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The microcirculation plays a pivotal role in metabolic processes essential for optimal organ function. Likewise, scalp perfusion may be essential for scalp health and hair growth. However, well-defined methods to measure microcirculation and microvascular function in the scalp are not available yet.

Aim: To determine whether the methodology to assess skin microvascular function on the forearm by means of a local heat stimulus can be applied to the scalp in subjects with a full head of hair.

Method: On two separate days, scalp (at the parting) and dorsal forearm skin microcirculation with and without a gel-filled heating probe of 44ªC were simultaneously assessed using a Full Field Laser Perfusion Imager in 20 healthy subjects.

Results: Baseline microcirculation (flux) was twice as high in the scalp compared to forearm. Upon heat stimulus, scalp flux increased by 65% during the axon-mediated response (P1) and by 89% during the NO-mediated response (P2) relative to baseline. For the forearm this was 165% and 219%, respectively. The within-subject variation of baseline, P1 and P2 for the forearm was 25.4%, 26.7% and 33.2% and for the scalp 26.7%, 26.1% and 25.6%, respectively. There was no correlation between forearm and scalp microvascular function.

Conclusions: Scalp microvascular function may be assessed by the local thermal hyperemia method in subjects with a full head of hair with similar reproducibility as in the forearm.

Comparison of peripheral vascular resistance based on macro- and microcirculatory responses with Poiseuille's law

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Arterioles, the smallest arteries having a diameter less than 100μm regulate the blood pressure (BP) and blood flow (Q). The relation between BP and Q can be represented as Q = BP/Rt by Darcy's law, in which the total peripheral vascular resistance (Rt) is equivalent to the summation of single arteriolar resistance (Ra). On the other hand, Ra is represented as Ra = 8μL/πr4 by Poiseuille's law (μ: blood viscosity, r: arterilolar radius). However, it is still unclear whether the Poiseuille's law can be applied to in vivo cardiovascular system. The purpose of the present study is to evaluate Poiseuille's law's applicability for in vivo microcirculatory analyses. For this purpose, we tried to quantify Rt value by direct measurements of BP and Q in the rat carotid artery, whereas Ra value is determined by direct observation of microcirculation in cremaster muscle, simultaneously. During L-NAME induced vasoconstriction, systemic BP significantly increased from 91 ± 8 mmHg to 114 ± 9 mmHg, while arterial diameter decreased from 137 ± 31 μm to 129 ± 30 μm. The Rt and Ra values during vasoconstriction increased in 23.9 ± 10.4% and 22.0 ± 9.9%, respectively, in which there were no significant differences between them. These result confirmed the Poiseuille's law is acceptable to apply for in vivo microcirculation.

Effects of repeated whole-body cryotherapy on rheological properties of blood in trained and untrained older men

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The aim of the study was to compare rheological properties of blood resulting from repeated wholebody cryotherapy (WBC) in trained and untrained older men. The study included 10 long distance runners and 10 untrained men of age about 60 years old. There was no difference between compared groups in age, body height and body mass. Subjects were exposed to 24 WBC applications every second day (3 min at -130oC). Venous blood was sampled prior first, after 24 exposure to WBC, one week and two weeks after completion of WBC exposure. After regular WBC exposure mean elongation index (EI) was significantly higher in both groups at shear stress levels 0.58 Pa and from 2.19 Pa to 59.97 Pa. Comparison of trained and untrained men showed significantly higher mean EI in trained men at shear stress levels from 0.58 Pa to 59.97 Pa. We observed no significant changes in mean values of aggregation indices (AI, %), the half time (T1/2, s) and amplitude and total extent of aggregation (AMP, arbitrary units) between both groups and in comparison to baseline level. After regular exposure to WBC the level of fibrinogen (g/l) was significantly lower, and mean hemoglobin concentration (g/l) was significantly higher in untrained men, the level of hematocrit (%) was significantly lower in both groups. The study revealed positive effects of WBC on the rheological properties of blood, manifested by increase in erythrocyte deformability.

Keywords: elongation index, aggregation index, physical activity.

Effect of magnesium supplementation on erythrocyte aggregation in NOS inhibition-induced hypertension model

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This study investigated the effects of magnesium on blood rheological properties and blood pressure in nitric oxide synthase (NOS) inhibition-induced hypertension model. Hypertension was induced by oral administration of the nonselective NOS inhibitor N-nitro-L-arginine methyl ester (L-NAME, 25 mg/kg/day) for 6 weeks and systolic blood pressure measured by the tail-cuff method. The groups which given magnesium supplementation was fed with rat chow containing 0.8% magnesium oxide during the experiment. At the end of experiment, blood samples were obtained from abdominal aorta, under ether anesthesia. RBC aggregation was determined by ektacytometry. Plasma fibrinogen concentration was evaluated by ELISA. Whole blood and plasma viscosity were determined by rotational viscometer. Blood pressure was elevated in hypertensive groups and suppressed by magnesium therapy. Plasma viscosity and RBC aggregation were found to be higher in hypertensive rats than control animals and, these parameters significantly decreased in magnesium supplemented hypertensive animals. These results confirm that blood pressure, plasma viscosity and RBC aggregation increased in NOS inhibition-induced hypertension model and oral magnesium supplementation improved these parameters.

Sickle Cell Anemia - NO related genetic modifiers of hematological and biochemical parameters

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Sickle cell anemia (SCA) is an inherited blood disorder with a broad range of complications, including vaso-occlusion and hemolytic anemia. SCA patients present arginine deficiency that contributes to a lower nitric oxide (NO) bioactivity. The amino acid citrulline increases arginine levels and promotes NO production.

We studied the association between hematological and biochemical parameters with genetic variants from eNOS gene, in 26 pediatric SCA patients. Effects of oral citrulline supplementation in SCA were also considered.

Results from this study show a significant statistical association between some parameters and genetic variants: high levels of neutrophils were associated with the eNOS4a allele and an increased reticulocyte count and high serum lactate dehydrogenase levels were associated with both the rs2070744_TT and the rs1799983_GG genotypes at eNOS gene. A symptomatic improvement was observed in patients with citrulline supplementation.

Our results reinforce the importance of NO bioactivity in SCA. We presume that NO, and its precursors such as citrulline, might be used as therapy to improve the quality of life of SCA patients.

Correlation between fibrinogen levels and thromboelastometry in liver transplantation

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AIM: The comparison of a testing for fibrinogen (Fbg) with a point-of-care assay in the postanhepatic phase of liver transplants (LTs).

METHODS: The Fbg level (turbidimetry - QFA Thrombin HemosIL®) was compared with FIBTEM test by thromboelastometry (ROTEM®). A total of 62 blood samples in sodium citrate were analyzed by each method related to 62 patients, 51 primary LTs and 11 retransplants. Alcoholic and viral cirrhoses correspond to 50% with hepatocellular carcinoma in 45.2% of them. The use of Fbg concentrate (FC) and cryoprecipitate (Cryo) at postanhepatic phase was assessed.

RESULTS: Sample results and patient post-testing FC and Cryo transfusion are presented (table 1).

CONCLUSIONS: A good correlation is obtained between methods in normal ranges of FIBTEM and Fbg levels ≥1.5 g/L. FIBTEM has a poor correlation to Fbg < 1.5 g / L; however, MCF seems more reliable than A10. Clotting/ platelet defects, hyperfibrinolysis, hypothermia and heparin have impact in the patient blood management.

Table 1.

Fbg (normal: 2.0 - 4.0 g/L)	FIBTEM (normal: A10 = 7 - 23 mm / MCF = 9 - 25 mm)			
	A10 <7; MCF <9	A10 <7; MCF ≥9	A10 ≥7; MCF <9	A10 ≥7; MCF ≥9
<1.5	2	0	2	5
≥1.5 <2.0	0	0	1	32
≥2.0	0	0	1	19*
*One sample with Fbg >4.0, A10 >23 and MCF >25				
<1.5	1 Pt (FC) 1 Pt (FC + Cryo)	-	1 Pt (FC) 1 Pt (Cryo)	3 Pts (FC)
≥1.5 <2.0	-	-	-	5 Pts (FC) 1 Pt (Cryo)
≥2.0	-	-	1 Pt (FC + Cryo)	2 Pts (FC)
A10 = Amplitude at 10 minutes; MCF = Maximum clot firmness; Pt(s) = Patient(s)				

Impact of the red-vine-leaf extract AS195 on nitric oxide bioavailability in diabetes patients and its potential role in the wound healing process

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Aim: To investigate the effect of AS195 (Antistax®) on nitric oxide (NO) bioavailability in red blood cells (RBC) of male type 2 diabetes patients (T2DM) compared to healthy controls (HC) and its impact in a wound healing in vitro assay.

Methods: As standard medication of T2DM includes the medical substance Metformin, known to affect NO formation, the T2DM were further divided depending on Metformin intake (T2DM+M vs T2DM-M). Venous blood of T2DM+M/T2DM-M and HC was incubated in the presence of AS195 (100µM) or PBS (control) for 30 min at 37°C to measure free radical (ROS) content, RBC-NO synthase dependent NO production and RBC deformability. In a wound healing approach, human microvascular endothelial cells were incubated under the same conditions before or after injuring an intact cell monolayer by a scratch. Vital microscopy was used to determine recovery of the cell layer in the following 24h.

Results/Conclusion: RBC deformability and nitrite levels were significantly decreased in T2DM-M compared to HC but not in T2DM+M which might be related to NO donor effect of Metformin. AS195 significantly decreased ROS levels and positively affected RBC deformability especially in HC and T2DM-M but not in T2DM+M. Also, AS195 improved wound healing in vitro. These findings suggest that T2DM might benefit from AS195 regarding improvement of NO bioavailability and reduction of medical complications including wound healing deficiencies.

Cannabinoid 2 receptor inhibition reverses immunosuppression following acute CNS injury in mice

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Acute CNS injury leads to disturbance of the normally well-balanced interplay between the immune system and the CNS. This dysregulation has been termed CNS injury-induced immunodeficiency syndrome (CIDS). The endocannabinoid system (ECS) plays a role in homeostatic regulation of the immune system. Cannabinoid 2 receptor (CB2R) activity is associated with immunosuppression. We investigated if CIDS can be reversed by inhibiting CB2R activity using the CB2R antagonist, AM630.

CNS injury was induced in C57Bl/6 mice via an intracerebral injection of endothelin-1. The immune system was challenged with endotoxin 24 hours later. Intravital microscopy was used to study the peripheral immune response within the intestinal microvasculature. Brain tissue was stained with triphenyl tetrazolium chloride to measure the infarct size. In addition to CB2R inhibition, the effect of genetic CB2R knockout on the severity of CNS injury, as well as the severity of CIDS was investigated.

Results showed that animals with CNS injury have a reduced immune response to endotoxin when compared to animals without CNS injury. AM630 administration 15 min prior to LPS challenge, reversed this measure of suppressed immune function and did not have any detrimental impact on the infarct size. Genetic knockout of CB2R revealed that the CIDS was not induced after an acute CNS injury, confirming the involvement of the ECS in CIDS.Further studies should investigate the optimal treatment window for CB2R therapy.

Von Willebrand factor – a potential link between microcirculation and the heart

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Aim: To assess whether high plasma values of von Willebrand factor (vWf) could predict subclinical cardiac damage in patients with arterial hypertension and type 2 diabetes mellitus.

Methods: 52 hypertensive subjects with type 2 diabetes mellitus, 51 without diabetes mellitus, and 25 controls were evaluated by plasma vWf, echocardiography, spectral Doppler and tissue Doppler imaging. Patients with coronary artery disease, heart failure or comorbidities altering endothelial function were excluded.

Results: Diabetes patients had higher plasma levels of vWf(174.5±57.3% in patients with type 2 diabetes mellitus and hypertension vs. 136.8±36.8% in patients with arterial hypertension alone and 107.3±24.3% in controls, $p<0.001$), lower mitral E/A ratios (0.86±0.24 vs. 1.13±0.25 and 1.14±0.24, respectively, $p<0.001$)and higher E/E' ratios (9.5±2.3 vs. 7.7±1.5 and 6.2±0.6, respectively, $p<0.001$). Plasma levels of vWfwere correlated with HbA1c levels ($r=0.533$, $p<0.001$) and parameters of left ventricular diastolic function: mean value of E' ($r = -0.457$, $p<0.001$), mean E'/A' ratio ($r = -0.339$, $p<0.001$), mean E/E' ratio ($r=0.409$, $p<0.001$). Treated patients had lower plasma levels of vWf($p<0.05$).

Conclusions: High plasma values of von Willebrand factor are encountered in hypertensive and diabetic patients and corelate with subclinical cardiac damage and poor diabetes control. Its levels could be decreased by antihypertensive and antidiabetic treatments, thus being a potential therapeutic target.

Regulation of Red Blood Cell Deformability Through Adenylate Cyclase - Cyclic AMP Pathway

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AIM: Red blood cells (RBC) are constantly exposed to shear forces in the circulatory system. Shear forces in normal physiological conditions result in the activation of intracellular signalling pathways that can lead to changes in red cell deformability. The goal of this study is to investigate the regulation of RBC deformability in response to shear forces and the molecular changes in RBC via cAMP mediated signalling pathway.

METHODS: SQ22536, pentoxifylline and H89 are known to inhibit adenylyl cyclase, phosphodiesterase and protein kinase A, respectively. Blood from healthy donors was incubated either with or without inhibitors at 37 ^{o}C. Capillary tube system (0.05 cm radius, 1 m length) was used to provide a pressure gradient to apply 5 Pa shear stress. RBC deformability results were confirmed by ektacytometry (Lorrca) and matched results were included in the study. The membrane proteins were isolated, the changes of tyrosine and serine phosphorylation were evaluated by immunoblotting.

RESULTS: After 5 Pa shear stress exposure, RBC deformability with inhibitors was significantly decreased compared to control ($p<0.05$). Immunoblot analysis showed that tyrosine phosphorylation of membrane proteins was altered by inhibitors, although, serine phosphorylation was unchanged.

CONCLUSION: RBC deformability under shear forces could be improved by inducing intracellular adenylate cyclase-cAMP signalling pathway.

Effect of tempol in vivo on flow-induced dilation in vitro in middle cerebral arteries of healthy Sprague-Dawley rats

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OBJECTIVE: This study aimed to determine if in vivo scavenging of superoxide by TEMPOL influences the mechanisms of flow-induced dilation (FID) of middle cerebral arteries (MCA) in vitro.

DESIGN AND METHODS: 11-weeks old healthy male Spraque-Dawley (SD) rats (N=10-16) were given drinking water (control group) or 1 nM/L of TEMPOL in drinking water (TEMPOL group) for 7 days. FID (response to stepwise increase in pressure gradient (Δ10-Δ100 mmHg)) was determined in isolated, pressurized MCA in the absence/presence of the NOS inhibitor L-NAME, COX-1,2 inhibitor indomethacin (INDO), CYP450 epoxidase inhibitor MS-PPOH. Cu/Zn SOD, MnSOD, EC-SOD, COX 1,2, GPx4 and catalase mRNA levels were determined by real-time qPCR from brain blood vessels (N=5-8). All experimental procedures were approved by the local Ethical Committee and conformed to the EU Directive 86/609.

RESULTS: FID (no inhibitors) was similar between groups ($p>0.05$). FID was similarly affected byinhibitors in both groups. TEMPOL significantly upregulated Cu/Zn- and MnSODs and COX1, while downregulated COX2 genes compared to control.

In control group MnSOD positively correlated to COX1 ($r=0.802$, $p=0.05$) and GPx1 ($r=0.904$, $p=0.01$). In TEMPOL group, Cu/ZnSOD positively correlated to GPx1 ($r=0.979$, $p=0.004$), while MnSOD positively correlated to COX2 ($r=0.859$, $p=0.029$).

CONCLUSIONS: TEMPOL affected the expression of COX and SOD isoforms, which may be accountable for preserved FID.

Blood rheology in women with recurrent/late abortion while receiving intravenous Immunoglobulin

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Introduction: Patients with recurrent abortion in the present gravidity were treated with passive immunotherapy. During the treatment the changes of rheological parameters were investigated.

Methods: Plasmaviskosity was determined using KSPV 1 Fresenius; Erythrocyte aggregation using the MA1-Aggregometer and Erythrocyte deformability by means of Rheodyn, Myrenne.

Results: Between January 2013 and December 2014 a total of 58 patients were included into this open-end investigation. Until the end of the 24 week of pregnancy, patients were treated with 3 g Sandoglobulin i.v., maximally for 7 cycles.

At the end of 2014, 38 of 46 women with immunotherapy (82,6%) had a successful live birth. Pv showed no changes during the therapy. Erythrocyte aggregation showed a statistically significant elevation from the 3rd cycle of treatment, while a striking temporary increase in erythrocyte rigidity until the end of the 1st trimester was noticed that was followed by an increase of deformability from the beginning of the 2nd Trimester with a constant negative correlation with the gestational age.

Conclusion: We believe that the continuously elevation of the erythrocyte aggregation is the print of the physiological hypercoagulability that starts at the same time, while the temporary increase in erythrocyte rigidity followed by improvement of deformability during 2nd trimester is the result of pregnancy induced haemodilution.

Osmotic deformability (Osmoscan) tests in Continuous Ambulatory Peritoneal dialysis (CAPD) patients at the end stage renal failure

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Aim: Peritoneal dialysis is a treatment method in advanced kidney failure. In this method, the abdomen is filled with a liquid called dialysis solution using a soft catheter. Anemia also is a primary problem due to deficiency of erythropoietin. In this study, we measured elongation index (EI) as a function of osmolality at a constant shear stress (SS) of 30 Pa to investigate the optimal osmolality range for the erythrocytes in patients under continuous peritoneal dialysis.

Method: The venous blood samples were collected from 15 patients and 13 healthy controls (mean age: 47.6±13.2 and 43.5±14.3 respectively). RBC deformability in osmotic gradient conditions was determined using osmotic gradient ektacytometry (Osmoscan). Minimal EI at low-osmotic environment (EImin), maximal EI at the given SS (EImax), half of the maximal EI at high-osmotic environment (EIhyper), osmolality at EImin (Omin), osmolality at EImax (Omax), osmolality at EI hyper (Ohyper) and the area under the individual EI, osmolality curves (Area) were measured.

Results: EImin, EImax, EIhyper, area and Ohyper values were found lower in patients with severe chronic kidney disease than controls ($p<0.05$), whereas Omin and Omax parameters were not significantly different.

Conclusion: Our findings point out that peritoneal dialysis results in the impairment of erythrocyte deformability under different osmotic gradient conditions. It should be considered as additional risk for their anemia throughout treatment.

Alterations of Erythrocyte Rheology in Patients with Severe Chronic Kidney Disease: Effect of Peritoneal Dialysis and Oxidative Stress

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Aim: Increased oxidative stress, that occurs with excessive free radical production or low antioxidant levels, is common in patients who undergo peritoneal dialysis (PD) and this level of oxidative stress can cause alterations in erythrocyte deformability and aggregation. The aim of this study was to evaluate RBC susceptibility to oxidative stress in PD patients.

Methods: Blood was collected from PD patients and healthy donors. Enzyme activities of Glutathione peroxidase (GPx), Superoxide dismutase (SOD) and Catalase (CAT) were studied in erythrocytes; lipid peroxidation was studied by measuring the amount of MDA in both erythrocytes and plasma samples. All assays were evaluated spectrophotometrically. Erythrocyte deformability and aggregation were measured by Laser diffraction ektacytometry (LORRCA).

Results: CAT and GPx activities in erythrocytes were decreased in PD patients ($p=0.02$ and $p=0.0008$, respectively) whereas SOD activity was increased ($p=0.013$). MDA was not significantly different in erythrocytes however it was significantly higher in plasma ($p=0.0016$), which also shows correlations with t-half ($r=-0.66$, $p=0.0001$) and Amp ($r=-0.56$,$p=0.002$,). Amp in dextran and AI (%) in both plasma and dextran were significantly different in patients and controls ($p<0.05$). Deformability did not show any difference between groups.

Conclusion: Erythrocytes in PD patients show more tendency to aggregation and this could be influenced by lipid peroxidation activity in patient plasma.

The impact of some manoeuvres on postocclusive reactive hyperaemia in the cutaneous microcirculation

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AIM: to assess the impact of skeletal muscle activity, mental stress, and local anaesthetic on the post occlusive reactive hyperaemia (PORH) of the skin, and elucidate some of the potential mechanisms.

METHODS: In 14 young healthy volunteers, we measured cutaneous laser Doppler flux (LDF) and induced PORH by a 3-min occlusion of the brachial artery in basal conditions, during handgrip exercise and mental arithmetic. In 16 age-matched volunteers, we assessed PORH in basal conditions and after the application of EMLA cream. Simultaneously, we measured local skin temperature (T), arterial blood pressure (BP), and the heart rate (HR).

RESULTS: Handgrip exercise increased the peak LDF after PORH (LDFmax) in the forearm, and showed a trend of smaller area under the curve (AUC) and shorter duration of PORH (tdur), whereas in the pulp, shorter tmax and smaller AUC were shown. During mental stress, HR and BP increased, and in the pulp, trends of shorter tmax and tdur were found. The application of EMLA decreased AUC and showed trends of shorter tmax and longer tdur.

CONCLUSION: Handgrip exercise reduces the overall PORH response, which might imply the ´stealing phenomenon´ of skeletal muscles. Mental stress reduces PORH in areas rich in arteriovenous anastomoses (finger pulp), suggesting the importance of the sympathetic nervous system also in local vascular control. Decreased PORH response after EMLA application emphasizes an important contribution of axon reflex in the PORH phenomenon.

Comparing the lower limb vascular response to a passive leg raising test by Laser Doppler Flowmetry and Photoplethysmography

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AIM: Photoplethysmography (PPG) is a reference for quantifying both macro and microcirculation, but progressively lost interest for laser Doppler flowmetry (LDF), currently the most widely used technique for microcirculatory assessment. Information about the combined use of these techniques is still lacking, so our goal was to assess the bilateral lower limb vascular response to a passive leg raising (PLR) test using both PPG and LDF.

METHODS: A group of 10 healthy volunteers (27.0 ± 5.2 y o) was selected after written consent. The PLR test consisted of three phases: (I) 10 min stabilizing period; (II) 10 min record after raising (30°) a randomly chosen leg, keeping the contralateral leg unmoved; (III) return to the initial position for further 10 min. PPG and LDF signals were recorded on the first and second toes of both feet. The Wilcoxon signed-rank test was used for statistical comparison ($p<0.05$).

RESULTS: The area under the curve (AUC) of the LDF signal decreased significantly on both feet between phases I and II which might be explained by the gravitational transfer of blood from the raised leg and by a centrally mediated vasoconstrictor reflex. No differences were found on the PPG signals' AUCs of both feet between phases.

CONCLUSIONS: The combined use of LDF and PPG allows to quantify macro and microcirculatory contributions to the regulation of peripheral circulation in vivo, and suggests that PLR produces different impacts on different cutaneous vascular networks.

Can leukocyte antisedimentaton rate (LAR) predict septic complications and critical care mortality early in polytrauma and burn victims?

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Aim: To evaluate the predictive power of leukocyte antisedimentation rate (LAR), serum C-reactive protein (CRP) and procalcitonin (PCT) levels regarding mortality risk and development of septic complications in critical care trauma patients.

Methods: In a prospective,observational study,36 patients were followed for 5 days(T1-T5)after admission(T1)to our critical care unit immediately after polytrauma(Injury Severity Score>16)or burn injury affecting more than 20% of body surface area. In 11 patients septic complications developed, their LAR, serum CRP and PCT levels were analyzed before and after 3 days of sepsis was declared. Primary and secondary outcomes were septic complications and critical care death, respectively.

Results: LAR showed increasing tendency($p<0.001$)in the observation period in the whole group. 10 patients died due to secondary infectious complications. In the survivor group LAR at T1($p<0.001$) and T2($p<0.001$)as well as CRP at T1($p<0.05$)were significantly higher compared to controls and deceased group. In the deceased group CRP elevation were detected from T2 only. In septic patients LAR($p<0.05$)and CRP($p<0.05$)showed a significant drop one day before sepsis was declared. PCT levels failed to predict the onset of septic complications.

Conclusions: Simple LAR test can predict septic complications and consequent death. Drop in LAR and serum CRP levels may be warning signs regarding the onset of septic complications which allow early therapeutic interventions.

Insulin effect on erythrocyte NO metabolism in patients with sepsis

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Aims: The objective of this research was to evaluate the effect of insulin on erythrocyte NO efflux and GSNO levels in patients with sepsis and to compare with healthy humans. To accomplish this, was performed an in vitro study with blood samples collected from 21 healthy subjects and 20 patients with sepsis. From each sample, blood suspensions aliquots were obtained and incubated in absence (control) and presence of insulin, acetylcoline (ACh) and with both together. Afterwards, NO efflux was quantified with an electrode and S-nitrosoglutathione (GSNO) by spectrophotometric analysis.

Results: NO efflux increased in suspensions with ACh, insulin and both added (comparing to control) in healthy individuals, but for sepsis patients only in ACh aliquot; although there are no significant differences between patients and healthy individuals. GSNO concentration also increased in sepsis patients in the same aliquots comparatively to control and there were significant variation between patients and healthy humans.

Conclusion: erythrocyte NO efflux increase with insulin addition in healthy individuals and patients with sepsis. Concerning erythrocyte GSNO concentration, it was invariable in healthy humans and increased in patients with sepsis when insulin was added. Summarizing, insulin acts in NO metabolism both in healthy individuals and in sepsis patients in absence or presence of ACh.

Erythrocyte deformability tests by filtrometry, slit-flow and rotational ektacytometry in splenectomy, spleen resection or spleen autotransplantation

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Spleen resection at various degrees or spleen autotransplantation can partly preserve/restore the splenic filtration function, as previous studies have demonstrated. For better evaluation and follow-up the effectiveness of various spleen preserving operative techniques, a composite methodological approach has been applied in a canine experimental model. Beagle dogs were subjected to control (n=6), to splenectomy (SE, n=4), one-third and two-third spleen resection (n=4/each) or to spleen autotransplantation groups (AU, using Furka´s method, n=8). The follow-up period was 18 postoperative (p.o.) months. Erythrocyte deformability was determined in parallel by bulk filtrometry, slit-flow and rotational ektacytometry. By filtrometry, relative cell transit time increasing was seen in the SE group (mostly in animal nr. SE-3), showing the highest values on the 3rd, 9th and in 18th p.o. months. Elongation index values decreased in this group (by both ektacytometers). In general, AU and resection groups´ values were lower versus control and higher than in SE. Since in the circulation both elongation by shear stress and filtration occur, these various erythrocyte deformability testing methods together may describe better the alterations. Considering the possible complications related to asplenic-hyposplenic conditions, individual analysis of cases is highly important. Histology and SPECT-CT examinations would reveal the background of the individual differences.

(Grant: OTKA K-105618)

Erythrocyte mechanical stability changes in splenectomy and related to various spleen-preserving operation types in a long-term follow-up animal study

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Filtration of the red blood cells (RBC) by the spleen is based on their deformability alterations, including mechanical properties. Thus, in following-up splenic function using various spleen preservation techniques (e.g., spleen autotransplantation or resection) micro-rheological investigations can be informative. In this study we aimed to investigate RBC membrane (mechanical) stability related to splenectomy (SE) and various spleen preserving operations. Twenty-six beagle dogs were divided into control (n=6), SE (n=4), one-third and two-third spleen resection (n=4/each) and spleen autotransplantation groups (using Furka's method, n=8). The animals were followed-up for one and a half year. RBC deformability and membrane stability were tested by rotational ektacytometry. Although deformability values showed fluctuating differences among groups, mechanical stability values alone didn't show significant difference over the follow-up period. However, the SE group expressed the largest deterioration in elongation index values against the mechanical stress applied. This worsening was the most obvious in the 3rd postoperative month. When we analyzed the cases individually, one splenectomized animal markedly expressed impaired deformability and mechanical stability in the 3rd and 9th month. We concluded, that erythrocyte membrane stability test can be a useful supplementary tool for enforcing micro-rheological alterations when following-up splenic function.

(Grant: OTKA K-105618)

The role of micro-rheological investigations in musculocutaneous and adipocutaneous flap ischemia-reperfusion follow-up studies

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In reconstructive surgery various pedicled flaps can be used for covering tissue defects. During their preparation, transposition and (auto)transplantation, the flaps may suffer from hypoperfusion and/or ischemia-reperfusion (I/R) that can influence flap survival and wound healing. In the department several models have been used for investigating the microcirculatory and micro-rheological effects of I/R. Here we aimed to overview three flap models. (1) In a canine study the latissimus dorsi muscle (LDM) flap ischemia was 1 hour. (2) In a rat model of latissimus dorsi - cutaneous maximus (LDCM) musculocutaneous flap 2-hour ischemia was provided. (3) In the groin flap (GF) adipocutaneous model on rats the ischemic time was 1 hour. In all cases a 2-week postoperative (p.o.) period was examined. Besides hematological, microcirculatory and morphological investigations, red blood cell (RBC) aggregation and deformability were determined. In the LDM flap model RBC aggregation significantly increased in the first 30 minutes of reperfusion, and in the 1st p.o. week. In LDCM flap models the microcirculatory parameters markedly decreased after ischemia, RBC aggregation and deformability also worsened over the 1st week. In the GF model these alterations were seen mostly on the 3rd-5th days. In conclusion, I/R have different effect on various flaps also depending on ischemic time and tissue ischemic tolerance. Monitoring of micro-rheological parameters can be informative in flap studies.

Effects of cannabinoid receptors and gpr55 modulation in experimental sepsis

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Sepsis is the systemic inflammatory response to an infection and is associated with tissue hypoperfusion, multi-organ dysfunction and a high mortality. Based on accumulating evidence suggesting that the endocannabinoid system is up-regulated in acute inflammation, we investigated the impact of cannabinoid receptor (CBR) and GPR55 modulation on leukocyte-endothelial interactions and capillary perfusion within the microcirculation using intestinal intravital microscopy (IVM) in experimental sepsis induced by endotoxin (5 mg/kg lipopolysaccharide). Endotoxemic male C57BL/6 mice (WT or CBR2 knockouts) were treated using the following substances: endocannabinoid degradation enzyme inhibitor (JZL184); CBR1 antagonist (AM281); CBR2 antagonist (AM630); GPR55 agonists (LPI, O-1602); or GPR55 antagonists (CID16020046, O-1918). Endocannabinoid degradation enzyme inhibition and GPR55 blockade reduced endotoxin-induced intestinal leukocyte activation and improved the capillary perfusion in experimental sepsis in WT mice. Contradictory results were found in the CBR2 knockout animals suggesting alternative molecular targets of endocannabinoids to be further investigated. Taken together, these findings implicate that CBR2 activation as well as GPR55 inactivation would be potential therapeutic targets to regulate the host immune system in the early hyperinflammatory phase of sepsis.

Erythrocyte deformability – a partner of the inflammatory response

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Aim: We aim to establish an in vivo animal model of acute inflammation using PAF (platelet activating factor) as inflammatory agent and to study the erythrocyte deformability changes induced by the inflammatory response.

Methods: Counting the number of rolling and adherent neutrophils to endothelium after 2, 4 and 6h of intrascrotal injection of PAF we show the induction of an inflammatory state. Blood samples are collected in order to measure the erytrocyte deformability and to quantify NO efflux from the red blood cells (RBCs).

Results: The results show increase number of rolling and adherent neutrophils after 2h and 4h of inflammation and after 6h of induced-inflammation a decreased number of neutrophils is recruited. This model confirms that the hemorheological properties are affected by the inflammatory response which was here shown by the decreased RBCs deformability index in all the time-points of the study. This result is in line with the need of a low blood viscosity to the recruitment process of the leukocytes towards the endothelial wall. NO efflux from RBCs is also affected by the inflammatory response at the first hours of inflammation.

Conclusions: This animal model demonstrates in vivo the association between an acute inflammatory response and the rheological properties of the blood. For those reasons we consider this an adequate model to study acute inflammatory responses as well as hemorheological parameters.

Duox1-derived H2O2 modulates Cxcl8 expression and neutrophil recruitment via JNK/c-JUN/AP-1 signaling and chromatin modifications

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Aim: DUOX1-derived hydrogen peroxide (H2O2) and Cxcl8 are two key neutrophil chemoattractants. H2O2 is critical at the early phase, whereas Cxcl8 plays a key role in the late phases of recruitment. Here, our aim was to determine whether and how these two chemoattractants crosstalk to recruit neutrophils to inflamed areas.

Methods: We used the amputation of zebrafish larvae tail fin as an inflammation model. Neutrophil recruitment was imaged on specific transgenic lines. RNAs and morpholino antisense oligonucleotides were microinjected to modulate the expression of specific genes. qRT-PCR and immunostaning were used to evaluate Cxcl8-l2 expression. Signalling pathways were affected via the use of specific pharmacological inhibitors. Chromatin immunoprecipitation was used to address AP-1 binding and chromatin modifications at the cxcl8-l2 gene promoter.

Results: We report that H2O2 also contributes to neutrophil recruitment to injuries at the late phase as it induces Cxcl8-l2 expression in vivo through a JNK/c-JUN/AP-1 signaling pathway. Strikingly, H2O2 also promotes cxcl8-l2 expression through chromatin modifications at the level of its promoter.

Conclusions: These results explain how early H2O2 signal regulates neutrophil recruitment at all phases, directly via Lyn oxidation or indirectly by modulating cxcl8-l2gene expression via the activation of redox-sensitive signaling pathways, and further point out H2O2/DUOX1 as a key drug target for anti-inflammatory therapies.

Upper and lower body interval exercise induce similar changes in the main determinants of blood fluidity in overweight individuals

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This study aimed to compare the changes in hemorheological variables following two acute upper and lower body interval exercise protocols. Twelve subjects (age, 32.9±7.07 yrs; BMI, 30.0±3.0 kg/m2) completed upper (arm cranking) and lower body (bicycle) interval exercise protocols on two separate occasions with one week intervening. After determining VO2max by using the specified protocols, subject performed interval exercise included 5 intervals of 2 min activity at 85% of VO2max and 4 min active recovery at 45% of VO2max (totally 30 min exercise). Two venous blood samples were obtained before and immediately after exercise and were analyzed for blood and plasma viscosity, RBC aggregation, RBC deformability, fibrinogen, and red blood cell indices. Interval exercise irrespective of its type affected on hematocrit, fibrinogen and plasma viscosity, but had no effect on blood viscosity, RBC aggregation and RBC deformability. When the responses to upper and lower body trials were compared only a significant difference was detected for plasma viscosity ($P<0.05$) Increases in plasma viscosity following lower body exercise was higher than that of upper body trial ($P<0.05$). However, for all other markers of blood fluidity no differences were detected ($P>0.05$). Therefore, it might be concluded that despite the differences in the amount of muscle involved in the activity, upper and lower body interval exercise result in similar hemorheological responses in overweight individuals.

Effects of 12 weeks trans-resveratrol supplementation and endurance training on responses of platelet indices to exhaustive exercise in male rats

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The aim of this study was to evaluate the effects of twelve weeks of resveratrol supplementation and endurance training on platelet indices in response to severe exercise. Thirty two male wistar rats were randomly divided into 4 groups including control (C), supplement (S), training (T) and training-supplementation (T+S). Rats in the T+S and T groups performed 12 weeks of running on treadmill,5 days a week, started with 10 min at 10 m/min and increased to 60 min at 25 m/min by the final session. S and T+S groups received 10 mg/kg/day resveratrol 5 days a week. After the last training session, all rats in 4 groups performed an acute exercise trial encompassed running on treadmill at speed of 25 m/min and incline of 10% up to exhaustion which was immediately followed by taking a blood sample. Responses of mean platelet volume (MPV) to exercise were significantly different among the 4 groups (P=0.03), though the other indices were not significantly different. The rates of MPV were 7.20±0.50, 6.90±0.15, 7.48±0.48 and 6.96±0.37 femtolitre for C, S, T and T+S groups, respectively. Pairwise comparisons showed significant differences between S and C (P=0.01), as well as S and T (P=0.01) groups. Based on the reductions in responses of MPV to exhaustive exercise, it could be concluded that long-term resveratrol supplementation either accompanied by training or alone might be recommended for reducing the risk of exercise–induced thrombosis.

Comparison of selected rheological and biochemical parameters of blood at the end of one winter swimming season and at the beginning of another.

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OBJECTIVE: The aim of the study was to examine differences in the morphological, rheological and biochemical blood parameters of physically active winter swimmers during the period between the end of one winter swimming season and the beginning of another.

METHOD: We enrolled 17 healthy winter swimmers aged between 30-60yrs, from Cracow Society of Winter Swimmers ´Kaloryfer´, who immersed in cold waters (3 min. at 2C to 7.2C) once a week. Level of their physical activity beyond the swimming season was estimated using Paffenbarger Physical Activity Questionnaire. We analyzed blood parameters twice: at the end of one winter season in April and at the beginning of the next one in November.

RESULTS: Six months following the end of winter season, the levels of MCHC and MCH turned out to be significantly higher, while erythrocyte count and hematocrit level significantly lower in comparision to the baseline. Moreover, the break in winter swimming was reflected by a significant increase in median erythrocyte elongation index (EI) at all shear stress(SS)>1.13 Pa and a decrease in median SS1/2, EImax and SS1/2/EImax ratio. In addition, we observed significant increase in the concentration of transfferin and reduction in the total protein, albumin and beta-1 globulin concentrations.

CONCLUSION: Seasonal effort of winter swimmers between the end of one winter swimming season and the beginning of another has a positive influence on morphological, rheological and biochemical blood parameters.

Rise in RBC aggregability and concomitant decrease in blood pressure 10 days after injection of the long acting erythropoietin analogue MIRCERA

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Erythropoietin is a major regulator of blood viscosity. Its long lasting action analogue MIRCERA seems to be also employed in modern doping. We took the opportunity of a study aiming at developing a detection of recent MIRCERA injection in the context of doping detection to assess the effects of this EPO analogue on RBC aggregation. A single dose 200 □g of MIRCERA was injected to 10 male volunteers and blood samplings were drawn over 24 days. After injection a decrease in mean corpuscular volume at day 2 ($p<0.01$) and day 10 ($p<0.02$), a rise in reticulocyte count ($p<0.001$) between day 4 and day 17 and a decrease in ferritin a day 5 ($p<0.05$). Hemoglobin decreased at day 4 ($p< 0.005$). Hematocrit was unchanged. There was a drammatic (+67%) increase in RBC aggregation index ´´M´´ (from 9,49±1,01 to 17,66±1,8 $p<0.01$). A decrease in systolic blood pressure was observed during the period from day 4 to day 17 (at day 10: -11,90±2,28 mmHg $p <0.001$; at day 17: -15,80±2,83 $p <0.001$. There was also a decrease in diastolic blood pressure, mean and pulse pressure. Correlations between this decrease in blood pressure and M did not reach significance but pulse pressure was positively correlated to M ($r=0,743 p<0.05$).

These data show that the long acting erythropoietin analogue MIRCERA strongly increases RBC aggregation parallel to a decrease in blood pressure, but a possible causative link between the two events is not clearly evidenced.

The AlaxoStent: Improvement of NO-induced microrheological parameters and oxygen uptake during exercise?

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The aim of the study was to investigate the influence of the intranasal AlaxoStent (Alaxo GmbH) during exercise on NO synthesis, NO exhalation, RBC deformability and oxygen uptake.

The parameters were measured in healthy men before and after a 45 min training on a cycle ergometer. Intensity of the intervention corresponded to individual 2mmol, 3mmol and 4mmol lactate threshold with a duration of 15 min per threshold. Spirometric, microrheological and NO parameter were determined for oral, nasal and stent breathing. RBC deformability was measured by ektacytometry and maximum deformability (EImax) was calculated. RBC, plasma and exhaled NO were determined by chemiluminescence detection. Oxygen uptake and respiratory rate were determined via spirometry.

Exhaled NO significantly decreased after exercise with nasal and stent breathing, whereas plasma and RBC NO remained unaltered. RBC deformability improved at 2mmol lactate during oral breathing, whereas nasal and stent breathing showed no changes. EImax increased after 2mmol lactate with stent, but decreased at 3mmol and 4mmol lactate with nasal breathing, and remained unaffected in the oral breathing setting. Respiratory rate for same oxygen uptake was slightly improved with stent breathing compared to oral and nasal breathing.

The AlaxoStent economizes oxygen uptake during exercise without major effects on RBC deformability or NO synthesis.

Plasma aldosterone levels in patients with resistant hypertension

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Aim: The usual pattern of treated hypertension is high renin and low plasma aldosterone. We considered that aldosterone levels in the upper normal range in patients with resistant hypertension could be a clue for primary hyperaldosteronism.

Methods: 104 hypertensive patients on minimum 3 antihypertensive drugs, with/out type 2 diabetes mellitus (DM), were tested for plasma aldosterone levels. Diet, medication and comorbidities were recorded.

Results: Study group consisted in 53 males and 51 females, median age 49 +/-7 years. 36 patients associated type 2 DM. Patients were treated with angiotensin converting enzyme inhibitors, angiotensin receptor blockers, calcium channel blockers, alpha methyl dopa and indapamid or small amounts of hydrochlorothiazide in various combinations. No hypokalemia was noted. 14 patients (13.4%) had plasma aldosterone level above 24 ng/dl, being in the upper tertile of normal reference values (2.21–35.3 ng/dl). 4 out of 36 diabetics and 1 out of 9 patients with clinical hypothyroidism classified in this upper tertile.

Conclusions: Though in our laboratory plasma aldosterone levels between 24 and 35.3 ng/dl are considered normal, we appreciated that in the clinical setting of treated hypertension, in the absence of potassium wasting diuretics and on mild sodium restriction diet, values in the upper third of normal range are elevated and could reveal primary hyperaldosteronism. All subjects will be exposed to a therapeutic trial of spironolactone 50 mg.

Increased arterial stiffness and depressed systolic myocardial longitudinal velocities - which comes first in type 2 diabetic patients?

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AIM: Type 2 Diabetes Mellitus(DM) implies cardiovascular dysfucntion beyond glycemic control. We studied independent predictors for vascular alterations and their comparative rhythm in arteries and heart.

METHODS: We studied for 5 years 27 type2 DM patients without initial cardiac disease. We performed vascular and cardiac scans.

RESULTS: DM control improved not reaching guidelines' target. HbA1c dropped from 9.2 to 8% (-13%). Despite improvement, complications' number increased. We measured beta index and epsilon modulus for vascular stiffness. Both increased from 9 to 13 (+49%) and 132 to 195 kPa (+47%). Renal dysfunction was noted, serum creatinine increased to 119±110 mmol/l. Vascular stiffness correlated with renal dysfunction (r=0.52). It is also cardiac dysfunction in diabetes, myocardial systolic velocities diminishing over time (-13%) while compensating with radial systolic velocities (+13%). Vascular stiffness correlated with left ventricle (LV) high filling pressures (E/Vp) (r=0.47). Using stepwise regression analysis, we established an independent predictive model for stiffness progression. It consists in renal function and LV diastolic function. No velocities were retained in the predictive model. All p were significant.

CONCLUSIONS: Evolution after 5 years of the DM patients under suboptimal treatment was unsatisfactory. Vascular stiffness was higher, though not appearing sooner. The predictive model for vascular dysfunction included only diastolic cardiac function.

The assessment of diabetes complications in people with uncontrolled type 2 diabetes

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Method: T2DM patients (DM > 3 years) were assessed between Mar 2014 - end Oct 2015.They were evaluated by clinical examination, lab exams (HbA1c, FPG, eGFR), cardiological and ophthalmological examination (ECG, US, ocular fundus).

Results: 146 adult T2DM patients, 48% male, mean age 60.2 yrs, mean duration of T2DM 7yrs, HbA1c 9.5 %, FPG: 224.9 mg/dl included. Patients were treated with Met (88.5%), SUs (81%), DPP4i(7.4%), glinides (2.9%) and alpha GI (9.4%).The microvascular complication were: retinopathy in 16.5% of cases, CKD in 10.3% and neuropathy (distal symmetrical polyneuropathy) in 56.3%. 6.6%of the patients had previous stroke, 5.4% previous MI, 39.9% IHD (ECG changes in almost 65% of them), 9.8% heart failure and 13.4% peripheral arterial disease.

Conclusion: It is well known that most of the complications in diabetes arise from damages to small blood vessels and narrowing of large arteries due to chronic hyperglycemia and hypoglycemic episodes. Our study confirm the observation that microvascular complications affect more diabetic patients than macrovascular ones. The most frequent microvascular complication was polyneuropathy due to nerve damages that results from microvascular injury to the small blood vessels that supply the nerves. It is absolutely necessary to screen more often these patients and to maintain their glycemic control on long term.

Study of microcirculatory impairment in type 2 diabetic patients with symptoms of peripherial neuropathy

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Aim: Microangiopathy and neuropathy are late and severe complications of diabetes mellitus. Our aim was to examine the connection between neuropathy and microcirculatory disorder in type 2 diebetic patients in the mirror of metabolic state.

Methods: In our study 50 patients with type 2 DM and symptoms of neuropathy were enrolled. The microcirculatary impairment was examined by Laser-Doppler flowmetry. Provocation probe, the veno-arterial refex (VA) was implicated. The venoarterial reflex is is the decline in limb blood flow in the dependent position due to an increase in pre-capillary vascular resistance. To reify neuropathy, electroneurography (ENG) has been performed. Laboratory tests included lipid parameters and Hg A1C level. Examinations were done in age and sex matched control group.

Results: During VA, decline in blood flow was sifgnificantly higher in the control group (78% vs. 31,8%; $p<0,001$). The results proved micrirculatory impairment. ENG confirmed peripherial neuropathy in every patient. Significant correlation could be detected between VA and triglyceride ($R=0,35$, $p=0,015$) and Hg A1C ($R=0,322$, $p=0,024$). There was no significant correlation between the VA and avarage disease course or severity of neuropathy.

Conclusions: Our results show that VA is an excellent method to detect both neuropathy and microangiopathy in DM. Because of the relationship between the VA and glycemic control, the VA could be suitable for monitoring the effects of new therapies in DM.

Micro and macro vascular complications of type I and II diabetes in bulgaria

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Aim: Diabetes enhances the chance of development of vascular diseases. The objective of the study is to present the relationships between the therapy of type I and II diabetes in Bulgaria and development of vascular diseases.

Method: The investigation included 433 patients with type I diabetes (n=178) and type II diabetes (n=255). People were separated in two groups depending on the type of diabetes, gender, age and therapy (analogues insulin, human insulin, insulin and oral antidiabetic drugs (OAD)).

Results: The therapy of diabetes type I is mainly with analogues insulin and we compared micro and macro vascular complications after this therapy. The results showed that micro vascular diseases are more than 30% after 31 years independently of the gender, while macrovascular diseases are average more than 37,5%.The group of patients with type II diabetes (D2) obtained human insulin and OAD. In this group has a high prevalence of microvascular than macrovascular complications after a therapy with human insulin after 70 yrs. for men and after OAD therapy for women. It was obtained that 48-53.8% of the male patients with D2 have microvascular complications after 51 years and that 42-53% of women after 61 yrs. have the same complications.

Conclusion: There is an age differences for the development of micro and macro vascular complications for patients with diabetes type II according to genders. In man cohort they are developed earlier (51-60 yrs.) than in women cohort (61-70 yrs.).

The assessment of previous risk factors and major comorbidities in people with type 2 diabetes

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Objective: to assess the presence of previous (beforeT2DM diagnostic) risk factors and major comorbidities in people with T2DM in a cross sectional study

Method: T2DM patients (DM duration > 4 years) were assessed between Jan 2014 - end Jun 2015 in a cross sectional study (retrospective data collected). Were evaluated previous cv risk factors and comorbidities: obesity, smoking, systemic hypertension, ischaemic heart disease, chronic thyroid diseases, dyslipidemia, hyperuricemia.

Results: 180 T2DM patients, age >40years, 49.1% male, mean age 62.4 yrs, mean duration of T2DM 8.2 (3.9) yrs, mean HbA1c 8.5% . 62 patients had a HbA1c >9.5%.The patients were treated with: ACEi(51.8%) , AT1RA(43.4%), new generation of CCB(35.6%), diuretics (HCTZ, indapamide) 62.8% and beta-blockers (18%nebivolol, 21.3%carvedilol, 19.5%metoprololol). 65.3% of the patients were obese, 19.1% smokers, 76.3% had SH and 75.7% dyslipidemia. IHD was present in 29%, chronic thyroid diseases in 9% and hyperuricemia in 26%. Microalbuminuria was determinate in 55.6% of the patients and macroalbuminuria in 49.6%. From these 22.6% had micro- and 5, 7% macro-albuminuria.

Conclusion: The presence of previous risk factors and major comorbidities affects the evolution of type 2 DM. In these subjects the control of the illness is more difficult (mean HbA1c 8.5%) and the microvascular complications seems to appear earlier in most of the patients (microalbuminuria in 22.6% patients and proteinuria in 5.7%).

The significance of capillary density to insulin sensitivity in skeletal muscle

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Aim: The aim of the present study was to elucidate whether an increased capillary density in skeletal muscle induced by α -adrenergic blockade would lead to improved insulin-stimulated glucose uptake.

Methods: Seven inactive subjects with insulin resistance underwent a 4-week period in which they received an oral dose (2 mg) of the α-adrenergic antagonist Terazosin. During the intervention period, blood pressure and muscle blood flow was measured regularly. Before and after the intervention period body composition maximal oxygen uptake and insulin sensitivity (Hyperinsulinemic Euglycemic Clamp) were assessed. Two biopsies were obtained from the thigh muscle (m.v. lateralis) before and after the clamp.

Results: After Terazosin treatment, GIR was significantly improved by 21% ($p < 0.05$) compared to baseline. Maximal oxygen uptake, body composition, resting blood pressure and muscle flow were unaltered. No change in basal or insulin-stimulated phosphorylation of Akt2 at Thr308 or Ser473 was observed. Analysis of capillarization is in progress.

Conclusion: The present study shows that four weeks of an adrenergic blockade in insulin resistant individuals improves insulin stimulated glucose uptake. Although capillary analysis was not yet completed at the time of this abstract, the lack of change in insulin signaling suggests that the improved insulin sensitivity was associated with an increase in muscle capillarization as observed in our previous study on rodents.

First attempt at estimating rheological and haemostatic factors in hypertension from among the Bulgarian population

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AIM: We assess two general approvals of the hypertension research: 1. Increase in whole blood viscosity; 2. Abnormal change in hemostatic factors.

PATIENTS: The draft (n=229) covers healthy subjects (HS), symptomatic hypertension (SH), essential hypertension (EH), healthy pregnant women (HP) and preeclampsia (PP).

METHODS: Measurements of albumin (AB), erythrocyte sedimentation rate (ESR), fibrinogen (Fb), prothrombin time (PT) and activated partial thromboplastin time (aPTT) were acquired by automatic lab analyzers. The readings of Zeta sedimentation ratio (ZSR), plasma viscosity (PV) and leukocyte adhesiveness/aggregation (LAA) took place by own home made devices. Von Willebrand factor (vWF) was an ELISA. Visualization with box plot images (BPI) and nonparametric (Mann-Whitney) test were processed by SPSS15.

RESULTS: All tested hypertensive patients showed significant increase in ESR, ZSR and LAA but PV increases only in EH. While Fb was significantly increased in all tested groups the AB/Fb ratio was significantly decreased. The haemostasis screening tests (PT and aPTT) show tendency of decrement (partly significant) within the reference range (RR). Thereto only the value of PP was true pathological (below the RR). vWF shows explicit significant increment in all hypertensive patients.

CONCLUSIONS: An aggravation of rheological parameters and changed hemostatic potential up to the border range refer to low grade inflammation and thromboembolic risk in all forms of hypertension.

Electrical and rheological properties of blood in patients with type 2 diabetes mellitus

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Aim: The study investigated the kinetics of blood conductivity under transient viscometric flow in parallel with the rheological properties of blood in patients with type 2 diabetes mellitus.

Materials and methods: The measurements were performed by the rotational viscometer Contraves LS30 with the standard and a concurrent measuring system MS 1/1, a device, developed by the conductometric method with a software for measurement of conductivity of biological fluids. The measurements of whole blood conductivity and whole blood viscosity (WBV) in a group of 13 patients with type 2 diabetes mellitus and 9 healthy matched controls were carried out at a temperature of 37◦C. WBV at γ from 0, 0237 s-1 to 128, 5 s-1 and the time variation of whole human blood conductivity were investigated at rectangular and trapezium - shaped Couette viscometric flow under electric field of 2 kHz. The kinetics of conductivity signals were recorded both under transient flow and after the complete stoppage of shearing at γ from 0 to 94.5 s-1. A non-linear curve approximation of the growth and relaxation whole blood conductivity experimental dependences was done.

Results and discussion: It was found that the mean WBV and hematocrit values in the group of patients with type 2 diabetes were higher in comparison to the controls in the entire shear rate range. The results obtained for the blood conductivity in time depends on the shear rate and on the hematocrit of the samples under transient flow.

Apocalypse Now? Challenge of Arterial Disease in the First Quarter of the Century

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Abstract not available

Microcirculation in septic shock – a feasible target?

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Sepsis and septic shock are characterized by a low systemic vascular resistance/high cardiac output state, oxygen delivery is typically elevated. Nevertheless, despite this increased delivery of oxygen, tissue hypoxia persists in sepsis and contributes to organ injury. Microvascular dysfunction in sepsis has been extensively investigated in animals and humans. Sepsis results in derangements of microvascular flow, which can be identified very often in the early stages of this disease. Alterations of microcirculation are more severe in no-survivors, therefore it seems to be logical to target dysfunctional microcirculation. However, there is general failure of microcirculation protective interventions to improve clinical outcomes. Does it mean that we are on the completely wrong way? We do not have clear answer yet. Microvascular-protective strategies might probably be effective only in the patients who demonstrate baseline abnormalities of microvascular function. However, at present, we are not ready to specifically target the microcirculation in clinical routine outside studies. In the future, concepts for individualized hemodynamic optimization of both macrocirculation and microcirculation might constitute a new avenue to improve patients' outcome. Supported by Ministry of Health of the Czech Republic, grant nr. 15-31881A.

Is a Perfused Boundary Region right parameter for endothelial glycocalyx description?

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In vivo visualization of the endothelial glycocalyx (EG) is very difficult, mainly due to its fragility. The state of the EG can be indirectly investigated by measuring plasma levels of its constituents or directly by using a light reflectance microscope in a hand-held device. This principle has long been used for semiautomatic assessment of microcirculation. Newly designed software (SW) and next generation of side-stream dark field imaging camera is capable of fully automatic assessment of EG thickness. This SW automatically counts among others parameters the erythrocyte exclusion zone lining the endoluminal side of the endothelial cells called as Perfused Boundary Region (PBR). PBR describes the amount of the lateral deviation of the medial red blood cells (RBC) column. With damaged EG the RBC penetrate deeper into EG, closer to endothelial cells surface, thus the PBR increases. The cut off value recognised as being associated with significant EG damage is 2,5 μm.

As this SW is automatic it omits inter-individual variability in evaluation and it is able to provide us swiftly dozens of results. But can we fully trust this method and should an automatic process be always required in research?

Our experiences with this method are ambiguous both at the field of experimental and clinical research.

Evaluating PBR seems to be promising parameter of the indirect EG assessment. More studies are needed though to widely adopt this method as unambiguous and reliable.

Microcirculation of the eye – non-invasive window to the microcirculation in acute and chronic disease

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The microcirculation of the eye offers unique options to study acute and chronic vascular pathologies in living organisms. Different ocular tissue layers such as conjunctiva, iris or retina can be assessed non-invasively. Experimental set-ups, such as intravital microscopy, or clinical devices (e.g. side stream dark field – SDF – imaging) are available to visualize the ocular microvasculature.

With the advent of high resolution, non-invasive intravital microscopy equipment, it is possible to explore dynamic extra-, intra- and inter-cellular processes that cannot be reconstituted in vitro or ex vivo, or when a link between cellular events and tissue pathophysiology is being pursued. This is the case particularly if inflammation is involved since inflammatory changes in the microcirculation, such as margination and rolling as well as transition to adhesion and migration of immune cells can only be visualized by intravital imaging techniques.

Another domain of intravital imaging in the ocular microcirculation is the observation, quantification and diagnostics of functional changes in capillary blood flow. Changes of the blood flow in the smallest vessels of the eye can be an early indicator for acute (e.g. sepsis) or chronic (e.g. arteriosclerosis) systemic pathologies.

In the future, miniaturized hardware and automated video analysis software will facilitate routine use of ocular microcirculatory parameters for microvascular diagnostics and applied studies.

Microcirculatory studies in pregnant women - a useful tool for early detection of pregnancy-related complications?

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AIM: To evaluate the clinical utility of side stream dark field (SDF) imaging to evaluate physiological changes in the microcirculation in pregnant women and monitor complications such as preeclampsia.

METHODS: We have used SDF imaging to assess sublingual microcirculation in pregnant women to: (1) compare the microcirculation in pregnant women at term with age-matched non-pregnant women, (2) determine the impact of spinal analgesia and administration of phenylephrine on microcirculation in pregnant women undergoing a cesarean delivery, and (3) compare the microcirculation in pregnancy of at-risk individuals who develop preeclampsia versus those that do not.

RESULTS: Results suggest the microvascular flow index (MFI) is significantly higher in pregnant women at term compared to age-matched non-pregnant women. Spinal anesthesia does not appear to significantly impact the MFI of women undergoing a cesarean delivery. Prophylactic phenylephrine therapy during cesarean delivery had no consequences. Analysis of the microcirculation in women who develop preeclampsia is ongoing.

CONCLUSIONS: SDF imaging has the potential to be a useful clinical tool in evaluating microcirculation in pregnant women and may allow for the early detection of pregnancy-related complications such as preeclampsia.

Microcirculatory studies in developing countries – first results of the MicroScreen study

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Aim: semi-quantitative imaging techniques are currently available to monitor the microcirculation. However, movement artifacts, large analysis time, and observer-related bias represent still existing limitations of the technique. Our aim was to validate the reliability of novel automated software (CytocamTool®, AVA4®) in comparison to standard semi-automated software (AVA3®) to analyze the sublingual microcirculation in developing country.

Design: Observational study.

Patients: septic shock patients.

Methods: Using CytoCam-IDF - a new generation Incident Dark Field imaging device, sublingual microcirculatory videos were recorded as previously described. Functional capillary density (FCD), perfused vessel density (PVD), proportion of perfused vessels (PPV) and microvascular flow index (MFI) were analysed and compared using the 3 software packages.

Results: Similar to previous studies, hypoperfusion and microcirculation heterogeneity were observed using a standard semi-automated software. Results of the automated software analyses confirmed the previous findings but showed in part significantly different numbers.

Conclusions: Automated software is able to reduce human interaction and analysis time and to reproduce the previous reported microcirculation alterations. However, the quality of the recorded videos, video processing and vessel segmentation, are important factors that should be considered in the interpretation of automated software reports in septic shock patients.

Evaluation of organ perfusion with contrast enhanced ultrasound after operative or endovascular treatment of visceral artery aneurysms

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Purpose: To evaluate the organ perfusion after operative (OR) or endovascular (ENDO) treatment of visceral artery aneurysms (VAAs) with CCDS or CEUS.

Materials and Methods: Between April 1995 to January 2016, 168 patients were diagnosed with VAAs. 60/168 patients (36%) fulfilled treatment criteria and had either open (29/60, 48%) or endovascular (31/60, 52%) aneurysm repair. Patients' characteristics were consecutively reviewed. Technical success and organ perfusion were determined by ultrasound/CEUS and confirmed by magnetic resonance imaging / CT angiography (CTA).

Results: 18/60 patients (30%) presented with acute bleeding. 16/18 emergency patients (89%) were treated by endovascular means. Two patients showed a segmental liver malperfusion in CEUS and CTA. One small bowel resection had to be performed.
42/60 patients (70%) were electively treated. 27/60 patients (45%) had OR and 15/60 (25%) ENDO. There were no liver or bowel infarctions in CCDS or CEUS confirmed by CTA. Treatment of patients with splenic or renal artery aneurysms led to partial or complete organ loss in 44% (8/18) after OR and in 56% (5/9) after ENDO ($p > 0.05$).

Conclusion: In emergency case endovascular approach is the preferred therapeutic option to control bleeding. Patients for elective splenic or renal artery aneurysm repair in contrast to hepatic and mesenteric procedures have to be evaluated very carefully because of organ loss demonstrated by CEUS either after open or endovascular aneurysm repair.

The influence of different rewetting procedures on the thrombogenicity of nanoporous poly(ether imide) microparticles

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Aim: Dry porous polymers form micro-air bubbles (MAB) when exposed to fluids. Such MAB at material surfaces are reported to induce platelet activation and thrombus formation. Rewetting prior to implantation is discussed to reduce the MAB formation. Here, we report about the influence of different rewetting procedures on the thrombogenicity of PEI microparticles.

Methods: Steam sterilized dry PEI particles with a diameter of 200±50 µm and a porosity about 85±5% were rewetted with phosphate buffered saline (PBS, 24 h) or by immersion in ethanol-series (EtOH, 50% 1 h, 100% 24h; PBS, 24 h). Thrombogenicity of the particles was studied in vitro using human sodium citrated whole blood (60 min, 5 rpm vertical rotation). Numbers of non-adherent platelets were quantified and adhesion of blood cells was analysed by bright field microscopy. Platelet activation (percentage of CD62P positive platelets, amounts of soluble P-Selectin) and platelet function (PFA100 closure times) were analyzed.

Results: Retention of blood platelets on the particles was similar for both rewetting procedures. PFA closure times were reduced and within the reference ranges, and non-adherent platelets were significantly less activated (CD62P positive platelets, soluble P-Selectin) for PEI particles rewetted with EtOH compared to those rewetted with PBS (P<0.05 each="" br="">Conclusion

Rewetting with EtOH resulted in a reduced thrombogenicity of the studied microparticles, most likely because of a reduction in MAB formation.

Cellular interactions during vascularization of human three-dimensional skin constructs

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AIM: The aim of this study was the establishment and characterization of the vascularized dermal equivalent in preparation for use in a 3D skin construct.

METHODS: Endothelial cells (HDMEC) were seeded on top of a fibroblast monolayer. Cocultures were immunolabelled with the endothelial marker anti-CD31, the fibroblast marker anti-TE-7, and the markers of extracellular matrix (ECM) components anti-fibronectin, anti-laminin and anti-collagen type III. Endothelial tube formation was examined qualitatively and quantitatively over 14 days. Monolayer controls were included.

RESULTS: All stages of the angiogenic cascade were observed in the coculture model. The endothelial cells assembled into 3D tubes that branched and networked. The mean number of tubes peaked at day 5, and decreased to day 14. Length and diameter of the tubes increased steadily. The number of branches per endothelial tube increased significantly ($p=0.001$). In cocultures fibronectin expression increased from day 5 to day 14, while collagen III and laminin expressions decreased between day 10 and 14 day. In the controls, the expression of the extracellular matrix components collagen III, fibronectin and laminin was higher than in the cocultures.

CONCLUSIONS: During their proliferation and organization into a complex 3D architecture, the endothelial cells appear to limit the production of some ECM components. This demonstrates that there is fine tuning of the ECM associated with angiogenesis.

Intravital microscopy to study pancreatic inflammation in type 1 diabetes

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Type 1 diabetes (T1D) onset involves acute inflammation of the pancreas resulting in islet cell destruction and dramatic long-term consequences for the individual. Intravital microscopy (IVM) – in contrast to histological or immunohistochemical methods - can be employed to study early, dynamic stages of inflammation (such as leukocyte rolling and adhesion) and functional consequences, for instance, impact on microvascular blood flow of the pancreas. Our goal was to establish an IVM-based method to study early pancreatic inflammation in non-obese diabetic (NOD) mice, which can be used to screen novel medications to prevent or delay T1D in future studies. This included evaluation of leukocyte-endothelial interactions as well as disturbances of capillary perfusion in the pancreatic microcirculation.

In our pilot study, we observed significantly increased rolling behavior as well as firm adhesion of leukocytes to the endothelium in early T1D and a reduction of the functional capillary density in the pancreatic microvasculature of diabetic NOD mice. Experimental preventive treatment with cannabidiol (CBD), a natural cannabinoid, attenuated pancreatic inflammation and resulted in lower blood glucose levels indicating a delay in the onset of T1D in our model.

Employing pancreatic IVM further studies are facilitated to evaluate changes within the pancreatic microcirculation in early T1D and to screen for novel drugs to control or delay the onset of T1D.

Cell layer formation and shear-resistance of human endothelial cells on LDI-crosslinked gelatin-based hydrogels with tailorable elastic properties

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A functionally-confluent and shear-resistant EC layer to prevent cell detachment by shear forces after implantation shall be generated on gelatin-based hydrogels with tailorable elasticity. [1, 2] The EC layer formation and shear resistance were explored on gelatin hydrogels with Young's moduli between 130 and 230 kPa after 9 days of culturing. [3] Cell morphology, proliferation, confluence, substrate interaction and secretion of vasoactive and inflammatory mediators were analyzed.

Staining of actin and VE-cadherin revealed a strong cell-cell and cell-substrate interaction. The secretion of vasoactive and inflammatory mediators did not differ between the hydrogels and the control, but EC density was independent of their elasticity always lower on the hydrogels. Cell proliferation was not sufficient to generate a confluent EC layer within 9 days. However, the study revealed that EC seeded on the hydrogels were able to resist physiological shear forces and there was no adverse influence on EC functionality visible. Therefore they are a suitable substrate for EC and a promising candidate for biomedical applications.

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Blood-derived angiogenic cells (BDACs) resemble a hematopoietic pericyte population and are a promising candidate for therapeutic angiogenesis

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Pericytes reside within the basement membrane of blood vessels and fulfil important functions during vessel formation and homeostasis. They have been identified as mesenchymal stem cells (MSCs) and represent promising candidates for therapeutic angiogenesis. However, MSC-based treatments of ischemic diseases (especially of myocardial infarction) did not result in significant long-term improvement. Interestingly, a pericyte population expressing leukocyte and monocyte markers was previously observed during angiogenesis in vivo. As MSCs do not express hematopoietic markers, this cell type might represent an alternative pericyte population.

Aim: The aim was to source blood-derived angiogenic cells (BDACs) that closely resemble hematopoietic pericytes.

Methods: BDACs were generated from monocytes and subjected to angiogenic assays. Their therapeutic efficacy was evaluated in a pre-clinical model of critical limb ischemia (CLI).

Results: BDACs enhanced endothelial sprouting in vitro and blood vessel formation in vivo1. In contrast MSC-like pericytes were responsible for blood vessel stabilization2 while decreasing the amount of forming sprouts. A single intramuscular injection of BDACs rescued the affected limb in murine CLI model1.

Conclusion: BDACs closely resemble hematopoietic pericytes and represent an advantageous addition or alternative for cell-based treatment of ischemic diseases.

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[2] Blocki et al. Stem Cells Dev, 2013, 22:2347

Geometry of microwells modulate IL-6 secretion of human adipose derived mesenchymal stem cells via ROCK signaling pathway

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Mesenchymal stem cells (MSCs) are highly sensitive to the microenvironment and their behavior can be largely regulated by micro-structures. As an important pro-angiogenic factor, IL-6 secreted by MSCs plays a critical function in mediating regeneration process. Here, we investigated the regulatory effect of microstructured surfaces on IL-6 secretion of human adipose derived MSCs and the potential involved signaling pathway. Polystyrene inserts comprising arrays of square-shaped or round-shaped microwells with a side length or diameter of 50 µm and a depth of 10 µm as well as inserts with a smooth surface were created by injection-molding. To favor the initial cell adhesion, the inserts were coated with vitronectin prior to cell seeding. The MSC morphology was remarkably modulated by the microwell geometry. An elevated IL-6 secretion was detected in MSCs on the surface with square-shaped microwells as compared to those on the surface with round-shaped microwells. Gene array analysis demonstrated the alternation of gene expression profile in response to microwell geometry. The inhibition of ROCK markedly abolished the difference of IL-6 secretion between the cells on different surfaces. These results demonstrated that microwell geometry can influence the IL-6 secretion of MSCs, which was highly associated with ROCK signaling. Our findings highlight the feasibility to regulate stem cell secretion via combination of surface structures and the manipulation of ROCK signaling.

Dendritic cell responses towards clinically used polydimethylsiloxane and polytetrafluoroethylene

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Aims: Although frequently used as implant materials, both polydimethylsiloxane (PDMS) and polytetrafluoroethylene (PTFE) are often associated with adverse effects including foreign body responses. Dendritic cells (DC) are crucial for the initiation of immune reactions and could also play a role in foreign body associated inflammations. Therefore, the interaction of DC with PDMS and PTFE was investigated regarding their capacity to induce undesired cell activation.

Material and Methods: Medical grade PDMS and PTFE film were embedded into polystyrene inserts via injection molding [1]. DC were generated as previously described [1]. Briefly, from human blood purified monocytes were cultivated for six days in the presence of GM CSF and IL-4. The viability, the expression of co-stimulatory molecules, and the cytokine/chemokine profiles were determined after 24 hours incubation of the DC with PDMS or PTFE.

Results: The viability of DC after incubation with PDMS and PTFE was not influenced. However, both polymers induced a substantial activation of the cells shown by the upregulation of co-stimulatory molecules and release of soluble inflammatory mediators.

Conclusion: This study showed the potential of PFTE and PDMS to activated dendritic cells, which could be an explanation for the often observed inflammatory events associated with the implantation of these polymers.

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Hemorheological disturbances in chronic carotid artery stenosis

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Aim: Prior researches suggest that hemorheological parameters are altered in CAS and in chronic cerebrovascular disorders as well, but it is controversial if hemorheological parameters could be markers of the extent of stenosis or atherosclerosis. We investigated the connection among between hemorheological parameters, stenosis and atherosclerosis both in symptomatic and asymptomatic cerebrovascular patients.

Methods: 107 patients were investigated (mean age 64±6 years), 40% of them had cerebrovascular events in the case history and 48% had CAS (>50% in diameter). Routine lab parameters were determined and hemorheological variables were measured: hematocrit, plasma (PV) and whole blood viscosity (WBV), red blood cell aggregation, and deformability.

Results: In the stenotic group WBV and red blood cell aggregation were higher than in the non-stenotic group ($p<0.05$). WBV and PV were increased and red blood cell deformability was decreased in the symptomatic group compared to the asymptomatic group ($p<0.05$). PV and red blood cell deformability were altered in the evolving atherosclerosis group and the CAS groups compared to patients having no signs of stenosis ($p<0.05$), but there was no difference among the CAS groups.

Conclusion: Although hemorheological factors are altered both in CAS and chronic cerebrovascular disorders, the severity of stenosis cannot be detected based on these parameters, but these factors could refer to the presence of atherosclerosis.

Ambulatory and home-based exercise training program in female patients with high cardiovascular risk

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Aim: The aim of the present study was to determine the effects of regular physical training on hemorheological and angiological parameters in high cardiovascular (CV) risk female patients.

Methods: 30 female patients (mean age: 67.6±5.6 yrs) with high CV risk were involved in ambulatory and home-based rehabilitation exercise training program for 3 months. The inclusion criteria were EF ≥ 55 % and exercise tolerance > 5 MET. All subjects underwent a 12-week training program including 1 hour training held in our out-patient clinic 3 times a week and a home-based walking program, where patients were encouraged to walk 10 000 steps a day. At the beginning and after the training period exercise capacity, hemorheological, laboratory and angiological parameters were measured.

Results: After 12 weeks fibrinogen level significantly decreased. Red blood cell deformability presented a significant increase ($p<0.05$). Metabolic laboratory parameters significantly decreased ($p<0,05$). The treadmill test proved significantly better exercise capacity ($p<0.05$). In angiological parameters no significant differences were observed.

Conclusion: The ambulatory and home-based exercise training program resulted in a significantly better risk profile and exercise tolerance in our female patients. All these beneficial changes may contribute to the reduction of CV mortality in patients participating an ambulatory rehabilitation program.

This study was funded by TÁMOP- 4.2.2. D-15 / 1 / KONV- 2015- 0009.

The hemorheological effects of cardiovascular rehabilitation training program in ischemic heart disease – an analysis of prospective studies

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AIM: The effects of physical activity on hemorheological parameters (HPs) have mostly been investigated in healthy subjects, however only limited data is available of its long-term hemorheological effects in patients with coronary artery disease (CAD). We aimed to estimate the beneficial hemorheological effects of cardiovascular rehabilitation training program (CRP) compared to CAD patients, not participating in CRP and to healthy young subjects not achieving recommended level of physical activity.

METHODS: The data of 3 of our previous studies were analyzed: 71 CAD patients with CRP, 36 out of 159 CAD patients without CRP and 40 healthy sedentary subjects. From the original data, hematocrit (Hct), plasma viscosity (PV), whole blood viscosity (WBV) and red blood cell (RBC) aggregation records were analyzed.

RESULTS: After 6 months Hct, WBV, PV and RBC aggregation became significantly ($p<0.05$) lower in the CRP-participating population compared to those who did not participate. All HPs significantly ($p<0.05$) decreased in the CRP-participants compared to the healthy sedentary subjects after 6 months.

CONCLUSIONS: Our results indicate that CRP is able to reverse the deterioration of HPs in CAD after an acute cardiovascular event, and also to achieve better hemorheological status compared to healthy sedentary subjects. These effects may be part of the cardiovascular risk reduction of CRP.

Hemorheological factors can be informative in comparing treatment possibilities of abdominal compartment syndrome

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Abdominal compartment syndrome (ACS) is a severe, life-threatening condition. However, its pathomechanism has not been completely clarified yet, furthermore, the surgical therapy still needs optimization as well. We aimed to investigate microcirculatory and micro-rheological alterations in ACS, using various temporary abdominal closure (TAC) methods, including three settings of vacuum-assisted closure technique. On anesthetized pigs, by intraabdominally placed and filled-up silicone bags, intraabdominal pressure (IAP) at 30 mmHg was maintained for 3 hours, and then decompressive median laparotomy happened. In different experimental groups the abdominal wall was closed by regular suturing techniques, or, alternatively, TAC was maintained for 2 hours using Bogota-bag, or by applying Vivano-sets at -50, -100, or -150 mmHg vacuum values. IAP was monitored by implanted sensors, hemorheological parameters were determined (hourly sampling from cannulated external jugular vein and femoral artery), and laser Doppler flowmetry tests were performed on the liver, kidney, small bowel and the pancreas before and after the TAC period. ACS resulted in significant impairment of macro- and micro-rheological parameters and microcirculation of abdominal organs. All of the used temporary abdominal closure techniques improved the results, however, applying the Bogota-bag and the -150 mmHg vacuum set showed worse microcirculatory and micro-rheological data than the settings at -100 or -50 mmHg.

Hemorheological and angiological differences in diabetic patients with and without intermittent claudication

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Aim: Diabetes mellitus is an important risk factor of peripheral artery disease. We analyzed the angiological and the hemorheological variables of diabetic patients with and without intermittent claudication.

Methods: 89 diabetic patients were classified into two groups: 20 patients (63.5±8.8 yrs, 53% men, 47% women) had claudication, 69 patients (65.5±9.3 yrs, 61% men, 39% women) were asymptomatic. Hand-held Doppler, transcutaneous tissue partial oxygen pressure (tcpO2) measurement, tuning fork test and 6-minute walk test were performed and hemorheological variables were also investigated including red blood cell aggregation.

Results: The ankle/brachial index ($p<0.02$), the tcpO2 measured at rest and at elevated leg ($p<0.003$) and the 6-minute walk test ($p<0.0001$) were significantly deteriorated in the diabetic population with claudication compared to the group without claudication. Higher red blood cell aggregation index and faster aggregate formation could be observed among patients with claudication ($p<0.02$). Despite the statistically better results of the asymptomatic group, 20% of these patients had characteristic results for a severe limb ischemia.

Conclusion: The intermittent claudication is a typical symptom of oxygen deficiency even in diabetic patients that is confirmed by our angiological tests. In a remarkable part of the asymptomatic patients severe limb ischemia could be detected. The increased red blood aggregation could deteriorate the peripheral circulation.

The rate of complete and partial venous recanalisation by optimal and non optimal oral anticoagulant therapy

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Aim: Our aim was to evaluate how the rate of complete and partial venous recanalisation depended on the optimal or non-optimal oral anticoagulant therapy (OAC).

Method: We treated 100 patients with proximal deep vein thrombosis of the lower limb: 50 pts were on warfarin (group W) and 50 pts were on acenocoumarol (group A). The thromboses were acute in all patients. The 6-month therapy was optimal when the INR value was between 2,0- 3,0 at each lab tests. If only one or more INR value were lower than 2,0 the therapy was considered as non-optimal. Recanalization was evaluated by duplex ultrasound.

Results: Of all measurements, INR values in group W remained in the therapeutic range in 74.5%, while it was 71,2% for group A. The proportion of patients on optimal OAC was 52% in group W, and 46% in group A. The recanalization rate was dependent on the stability of INR and it was independent of the drug type. The complete recanalization rate was 91,9% in the optimally treated patients, but it was 74.5% in the non-optimally treated The healing was dependent on the stabile INR value in therapeutic range. Partial recanalisation was 6,1 % in the optimally and 11,8 in the non-optimally treated group.

Conclusion: The healing process of the deep vein thrombosis is dependent on the efficacy of oral anticoagulant therapy.

Keywords: oral anticoagulants, deep vein thrombosis, recanalization.

Correlation between blood rheological properties and red blood cell indices (MCH, MCV, MCHC) in healthy women

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Aim: The intention of this prospective trial was to investigate the physiological associations between blood rheological parameters and red blood cell indices of mid-age healthy women prior elective gynecological surgeries.

Methods: Red blood cell deformability during exposure to low (RBC 1.2, 3.0), moderate (RBC 6.0, 12.0) and high shear forces (RBC 30.0, 60.0), RBC aggregation during stasis (E0) and low shear (E1; 3 s-1) and plasma viscosity (Pv) were correlated with red blood cell-indices (RBC-I: MCV, MCH, MCHC). Patients with infective, chronic or malign diseases, known pregnancy or extreme BMI were excluded.

Results: In total 286 women were included in this evaluation. A significant correlation was found between Pv (mean ± SD: 1.17 ± 0.12 mPa s) and RBC aggregation (E0: 12.6 ± 6.3; E1: 17.9 ± 7.3) with age and BMI, but not with RBC-I. RBC deformability correlated positively with age, MCV and MCH but inversely with MCHC. The correlation between RBC-I and RBC deformability was most remarkable during moderate shear force exposure. Neither hemoglobin nor hematocrit were correlated with RBC deformability or RBC-I.

Conclusions: In healthy mid-age women, cell volume and hemoglobin content have a high influence on the RBC deformability. Low MCHC und high MCV were correlated with an increase in deformability and vice versa. Age was associated with an increase in all determinants of blood viscosity, while BMI showed no influence. MCV, MCHC or MCH had no impact on RBC aggregability.

Comparison of the different normobaric hypoxic conditions on blood viscosity and HIF 1-α levels

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AIM: Our purpose is to compare blood parameters, viscosity and HIF 1-α levels in acute, chronic, intermittent and sustained hypoxic conditions

METHODS: In our study four experimental groups were formed including seven adult rats in each group. Control group was exposed to normoxic conditions, acute intermittent hypoxia (AIH) group to %10 O2 for 5 minutes (5 times), chronic intermittent hypoxia (CIH) group to %10 O2 (10 times 15 minutes a day for 10 days) and chronic sustained hypoxia (CSH) group to %10 O2 for 10 days. At the end of the hypoxia applications blood samples and brainstem and spinal cord tissue samples were taken. Whole blood and plasma viscosity values were measured by using viscometer and HIF1- α levels by ELISA method.

RESULTS: Plasma viscosity values were higher in both chronic hypoxia conditions, but whole blood viscosity value was only significantly higher in CSH in 45S shear rate. HIF1- α levels were higher in AIH and CIH groups but it was lower in plasma and tissues in CSH group.

CONCLUSION: Both whole blood and plasma viscosity values indicate an increase in chronic hypoxia conditions. Contrarily,HIF1-α levels increased in AIH and CIH conditions but decreased in CSH conditions. This situation indicates that the tissue oxygen levels are arranged within this period. And experimental hypoxic modellings is also important for the measurement of this type of parameters.

This study was supported by Erciyes University Research Fund (No:2014-5311)

Duration-magnitude interactions in red blood cell damage: defining the sub-haemolytic threshold

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Background: When red blood cells (RBC) are exposed to shear stresses (SS) of ~400 Pa for ~1 s, they may rupture (i.e., haemolyse) and release their contents into the surrounding medium. Current generation mechanical assist devices are thus designed to minimise high shears for prolonged durations. However, RBC may have impaired capacity to deform when exposed to SS well below the "haemolytic threshold".

Methods: A Couette-shearing system was used to expose RBC suspensions to discrete magnitudes of SS (1, 4, 16, 32, 64 Pa) for specific durations (1, 4, 16, 32, 64 s), immediately prior to RBC deformability being measured using laser-diffraction ektacytometry. Analyses included exploring the second-derivative of the RBC deformability-SS curve, and comparison of maximal RBC deformation (EImax) and SS required for half EImax (SS1/2).

Results and Discussion: When SS was applied at <16 Pa, limited damage was observed. When RBC were exposed to 32 Pa, mild impairments in EImax and SS1/2 were observed, although 64 Pa caused a more dramatic impairment of RBC deformability. Moreover, when RBC were exposed to 64 Pa, the second-derivative of the RBC deformability-SS curve was atypical with a duration effect, thus indicating impaired RBC sensitivity to SS. A surface-mesh was interpolated onto the raw data, and it was found that a clear relation between SS duration and magnitude could be used to predict RBC damage. The present results provide a novel method to explore haemocompatibility.

Beneficial postoperative micro-rheological effects of intraoperative administration of NSAID in patient underwent lower extremity operations

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Ischemia-reperfusion (I/R) may have an adverse effect on blood rheological parameters that has been demonstrated by clinical and experimental data. Worsening in micro-rheological parameters, such as red blood cell (RBC) aggregation, deformability (including membrane stability and osmotic gradient deformability), and the consequent microcirculatory deterioration might act as factors in postoperative complications. In this study (ethical permission nr.: DEOEC RKEB/IKEB 3848-2013) we investigated these parameters in patients (average age: 54.7 years) with elective knee surgery (total knee replacement or anterior crucial ligament replacement). The average ischemic (tourniquet) time was: 92±15 mins. Seven patients did not receive NSAID (Control group), while 5 patients received 4 mg/bwkg sodium-diclophenac from the beginning of the reperfusion, which was repeated in the postoperative (p.o.) period (NSAID group). Blood samples were collected from the femoral vein of the operated side before the ischemia, in the 5th and the 10th minutes of the reperfusion and on the 1st and 2nd p.o. day. RBC deformability decreased by the 1st and 2nd p.o. day in Control group. RBC aggregation index (AI%) increased by the 2nd day, aggregation half-time decreased. Light-transmission aggregometry indices increased by the 1st, and more expressively by the 2nd day in Control group. Administration of NSAID could diminish the postoperative micro-rheological deterioration after lower extremity I/R.

The Role of Pericytes for Angiogenesis in Skeletal Muscle Tissue

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The aim of the present study was to assess the presence of angiogenic factors in pericytes and to elucidate whether pericytes promote angiogenesis by secreting VEGF and enhancing endothelial cell proliferation. Pericytes, endothelial and skeletal muscle cells were isolated from rat skeletal muscle tissue by use of Dynabeads coupled to specific antibodies and the cells were maintained in culture for experiments. Pericytes were found to contain several angiogenic factors and the expression of Vascular Endothelial Growth Factor (VEGF) and Thrombospondin-1 (TSP-1) was higher ($P<0.05$) in perciytes conditioned with skeletal muscle media. The muscle conditioned pericytes had a higher ($P<0.05$) intracellular VEGF concentration and released a 7-fold greater ($P<0.001$) amount of VEGF to the media than untreated control cells. Media from pericytes pre-conditioned with muscle media induced a greater ($P<0.05$) proliferation of endothelial cells compared to non-conditioned endothelial cells. In conclusion, we have identified a complex pattern of cellular communication in cultures of pericytes, endothelial and skeletal muscle cells whereby skeletal muscle cells activate pericytes and induce an upregulation of several angiogenic factors and increase the release of VEGF and other proangiogenic compounds that induce proliferation of endothelial cells. The study strongly suggests that pericytes are important in angiogenesis.

«Optimal» vs actual hematocrit in obesity and overweight

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Equations of blood viscosity may provide a prediction of the "optimal" hematocrit (hct) as the Hct resulting in the highest value of the bell-shaped curve of hematocrit/viscosity ratio h/v. We investigated if overweight and obesity have an influence on these parameters. We compared 32 normal weight subjects, 40 overweight (BMI 25-30) and 38 obese subjects. There was no difference in the theoretical curve of h/v. The actual h/v is the same in the 3 groups but is always higher than the theoretical h/v in all groups.

The actual h/v is lower in overweight than controls (p=0,011). Modelling yields the same value of theoretical optimal hct across BMI classes. The 3 groups have the same values of actual Hct, but actual is significantly lower than optimal in all cases (p<0.001). Hematocrit is lower than predicted due to a discrepancy between predicted and actual h/v which is due to the inter-subject variability of RBC rigidity… The discrepancy between optimal and actual h/v is negatively correlated to RBC rigidity indexes even if the model uses a fixed value of these indexes. Thus keeping in mind that the optimal Hct should not be the same in the various parts of the vascular bed, its theoretical prediction with Quemada´s equation appears to predict a value higher than actual hematocrit but well correlated to it, and the agreement between optimal and actual Hct is dependent on RBC flexibility.

A new look on blood shear thinning

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Blood is a shear-thinning fluid. At shear rates, its drop of viscosity has been related primarily to the breaking-up of networks of "rouleaux" formed by stacked red blood cells (RBCs). For higher in the range 10-1000 s-1, where RBCs flow as single elements, studies demonstrated that RBCs suspended in a viscous fluid mimicking the viscosity of whole blood, deformed into ellipsoids aligned steadily in the direction of the flow, while their membrane rotated about their center of mass like a tank-tread. Such drop-like behavior seemed to explain shear-thinning. Here, using rheometers, microfluidics and simulations, we show that the dynamics of single RBCs in plasma-like fluids display a different sequence of deformation for increasing shear rates going from discocytes to successively, stomatocytes, folded stomatocytes, trilobes and hexalobes, but never ellipsoids. This result is also identical for physiological hematocrits. We correlate this shape diagram to the different regimes in blood rheology for high shear rates and propose a new-look on the interpretation of blood shear-thinning behavior.

Genetic factor effect on blood rheology in children with sickle cell anemia

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Background: Sickle cell anemia (SCA) is a severe hereditary hemoglobinopathy characterized by abnormal blood rheology, which plays a role in the occurrence of several acute and chronic clinical complications. While beta-haplotypes and alpha-thalassemia modulate SCA clinical severity, their effects on blood rheology have been incompletely described. The aim of this study was to test the effects of these genetic modifiers on the hemorheological properties and frequency of vaso-occlusive crises (VOC) of SCA children.

Materials and methods: Steady-state hemorheological profile (blood viscosity, red blood cell (RBC) deformability and aggregation), biological parameters, beta-haplotype, alpha gene status and VOC frequencies (3 years period) were analyzed in 44 SCA (S/S or S/bêta0-thalassemia) children (aged from 3 to 17).

Results: Alpha-thalassemia patients showed increased RBC deformability and aggregation compared to those without. Mean VOC frequency was higher in patients with 2 deleted alpha-genes compared to those with a normal alpha genotype. The hemorheological profile was not influenced by the beta-haplotypes in our cohort.

Conclusions: Our results suggest that alpha-thalassemia increases the risk for VOC events in SCA children through its effects on blood rheology.

Transcranial Optical Imaging of Blood Microcirculation

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The new insights from the combined use of Laser Speckle technology and Dynamic Fluorescent Imaging modality accompanied with the computational adaptive optical filtering are presented. This Transcranial Optical Vascular Imaging (TOVI) technique is simple to use and a cost-effective methodology that allows color coded mapping of blood flows as shown in the illustration. We apply TOVI for advanced color coded smart microcirculation mapping of cerebral blood vessels of the experimental animals. TOVI wide-field images are revealing clear visual information on blood flow and perfusion in the cerebral cortex and meninges. The developed dual-mode imaging technique together with a new computational filtering approach have a great potential in the studies of blood microcirculation and can significantly expand the capabilities of preclinical and clinical functional studies of brain vasculature disorders.

The optimum hematocrit

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The hematocrit (Hct) determines the oxygen carrying capacity of blood. At the same time the Hct increases flow resistance by an exponential increase of blood viscosity. From this dual role of the Hct, the concept of an optimum Hct for tissue oxygenation has been derived. It has been investigated by different methods in vitro. Using the ratio Hct/viscosity, an optimum Hct of 50-60% was determined. Using the perfusion of an artificial microvascular network, we found a similar optimum Hct of 50-60%. In vivo, a deliberate Hct increase to supra-normal levels improves exercise capacity in athletes (Blood doping). Taken together, these data with normal red blood cells and healthy individuals suggest that the optimum Hct may be higher than the physiological Hct (35-40% in women, 39-50% in men). However, these findings are in contrast to clinical studies in patients. The correction of anemia in conditions such as chronic kidney disease, heart failure, coronary syndrome, oncology, acute gastrointestinal bleeding, critical care, or surgery has worse clinical outcomes with liberal compared to restrictive transfusion strategies. Actual guidelines, therefore, set a transfusion threshold as low as 7-8 g/dL hemoglobin (Hct 20-23.5%) and do not recommend a Hct normalisation. The discrepancy between the optimum Hct in health and disease may be caused by differences in hemorheology (red cell deformability, plasma viscosity) and the microcirculation (endothelial function, vasoconstriction).

Potentialities of Laser Trapping and Manipulation of Blood Cells in Hemorheologic Research

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AIM: Laser trapping and manipulation of blood cells without mechanical contact have become feasible with implication of laser tweezers. They open up new horizons for the hemorheologic researchas they offer new possibilities for studying live cells interactions on individual cell level under the influence of different endogenous and exogenous factors. Here we discuss the basic features of these techniques and some examples of challenging hemorheologic studies.

METHODS: The operation principle of laser tweezers is based on the property of strongly focused laser beam to act on the dielectric microparticles located in the vicinity of the beam waist with a force that drives the particle to the equilibrium location and holds it there. If the beam waist position is manipulated, so is the position of the particle. The displacement of the particle from the equilibrium position by external forces can be calibrated so that these forces can be precisely measured in the range ca. 0.1 – 100 pN.

RESULTS: This is the range of forces of elastic deformation of blood cells, e.g., by shear stresses, and of their interaction with each other, e.g., during aggregation, and with vessel walls.

CONCLUSIONS: We conclude that being able to measure these forces without mechanical contact allows for studying on single cell level the mechanisms of interactions that was impossible earlier.

ACKNOWLEDGEMENTS: This work was supported by RSF grant #14-15-0052.

Physiological role of erythrocyte nitric oxide

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Nitric oxide (NO) produced by endothelial cells interacts with erythrocyte through protein band 3, being scavenged by haemoglobin. A signal transduction mechanism involving protein Gi and protein band 3 stimulates erythrocyte NO efflux when acetylcholine (ACh) binds to erythrocyte membrane acetylcholinesterase. Binding of fibrinogen (Fib), to erythrocyte membrane CD47 decreases the NO efflux. When high Fib concentration and ACh were present the efflux of NO from erythrocytes was compromised. The increased NO efflux from erythrocytes in presence of high Fib concentration and band 3 phosphorylation is reinforced in the presence of 4N1K an agonist peptide of CD47. When both Fib and 4N1K are present the NO efflux from erythrocytes is higher or not affected according lower or high levels of cAMP. Erythrocyte NO efflux in patients with systemic lupus erythematous and rheumatoid attrite was significantly negative associated with carotid intima-media thickness. In patients with amyotrophic lateral sclerosis erythrocyte NO content is preserved and an inverse association between respiratory function and NO efflux from the erythrocyte was verified. Sepsis patients before dead at 24h showed higher efflux of NO from erythrocytes that worsening the blood sub lingual microcirculation observed by high unequal blood flow and high microvascular flow index. The in vivo animal models either of inflammation or of hypertension evidenced that the NO efflux from erythrocyte decrease.

RBC-NOS dependent NO production during RBC aging in healthy volunteers and type 2 diabetic patients

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The aim of the studies was to investigate red blood cell (RBC)-nitric oxide synthase (NOS) activation, NO production and RBC deformability in type 2 diabetics (T2DM) and healthy controls (HC) at baseline and the effects of endurance training on these parameters in T2DM with a special focus on in vivo RBC aging.

The parameters were measured at rest in HC and T2DM and in T2DM before and after a six week training intervention. RBC of all ages and RBC separated by density-gradient centrifugation were analyzed. RBC deformability was measured by ektacytometry, RBC-NOS activation was determined by immunohistochemical staining of the serine 1177 residue. RBC NO was determined by chemiluminescence detection.

Compared to HC, T2DM showed less young and more old RBC. EImax of RBC within the same age group was decreased although nitrite levels and RBC-NOS activation were higher. In T2DM, the proportion of young RBC was significantly higher post-training. EImax of RBC of all ages remained unaltered post-training. Detailed consideration of the different RBC fractions showed reduced EImax post-training especially in older RBC. RBC-NOS activation and NO production remained unaltered in RBC of all ages post-training but analysis of the different fractions revealed decreasing RBC-NOS activation and NO production in old RBC post-training. It remains to be investigated whether these changes in older RBC could lead to more rapid elimination of aged RBC, thus favoring the production of young RBC.

Interactions between oxidative stress and RBC-NOS

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Free radicals, such as superoxide (O2-), when produced in greater concentrations than may be regulated by cellular antioxidant defence systems, lead to a state of oxidative stress. Oxidative stress is known to impair red blood cell (RBC) properties like deformability, although impairments are typically specific to whether O2- is generated intra- or extracellularly. Nitric oxide (NO) is associated with beneficial effects, including regulation of vasoactivity and the level of RBC deformation, but its availability was shown to be reduced during oxidative stress. We investigated the relation between intra- and extracellular generation of O2- in RBC suspensions, and the level of intracellular NO concentration, total free radical levels, nitrotyrosine levels, and the activation of RBC-NO-synthase (NOS). We found that intracellular O2- generation, in contrast to extracellular O2-, significantly influenced the free radical levels within RBC, which was associated with decreased RBC deformability. Also, nitrotyrosine levels were significantly increased but markers for lipid oxidation were not affected. The antioxidant Trolox was effective at limiting RBC damage generated by O2-. RBC-NOS activation and NO production were not influenced by O2-. Subsequent studies have investigated the effects of combined O2- generation in the presence of physiological level of shear stress, and these results will be discussed in the context of mechanical-oxidative stress interactions in the RBC.

RBC-NOS and RBC deformability in sickle cell disease

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It has been demonstrated that red blood cells (RBC) express an active and functional nitric oxide synthase (RBC-NOS). RBC-NOS derived NO is thought to play a major role in the regulation of RBC deformability in healthy individuals. Sickle cell anemia (SCA) is a genetic disease characterized by a reduction of RBC deformability, which is at the origin of chronic anemia and acute vaso-occlusive crises. We aimed to 1) characterize RBC-NOS activity in SCA patients and 2) test the effects of RBC-NOS activity modulation on sickle RBC deformability. We showed that while RBC-NOS expression is not different between SCA and healthy RBC, RBC-NOS activation is increased in the former population leading to higher production of NO in RBC. However, the increased oxidative stress in SCA induced the rapid formation of RBC peroxynitrite, which probably further participates to RBC alterations. The incubation of SCA RBC with sodium nitroprusside failed to improve RBC deformability and lead to a 10-fold increase of nitrotyrosine levels. In contrast, although incubating SCA RBC with insulin increased nitrotyrosine levels, it also increased RBC deformability. This positive effect of insulin is not fully understood but enhanced RBC-NOS dependent NO production could lead to increased S-nitrosylation of the a- and b-spectrins, and consequently improvement of RBC deformability. Stimulating RBC-NOS activity and limiting oxidative stress could be an interesting strategy to improve SCA RBC rheology.

Red blood cell nitric oxide and sickle cell anemia - potential and pitfalls

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Aim: Sickle cell disease (SCD) is a primary rheologic disease with increased erythrocyte (RBC) stiffness. RBC nitric oxide (NO) production is controversial with regard to origin and importance. Multiple pathways for NO production proposed within RBC, one of which being an analogue of endothelial nitric oxide synthase that is shear activated. Hemoglobin is thought to be a nitrite reductase. Is shear activated RBC NO production different between SCD and Healthy RBC?

Methods: RBC from 8 healthy and 7 SCD were washed and incubated with nitrite, a NOS inhibitor L-NAME or both. They were then incubated with NO sensitive fluorescent marker DAF-FM. The RBC were then placed in a poly L-lysine coated flow chamber and exposed to 0.5Pa shear stress. Analysis was done on a single cell basis (ImageJ 1.49s).

Results: There was similar increase in RBC DAF fluorescence under shear stress in healthy and SCD, percent change 19.3% vs 22.0% (P=0.60). There was no significant change in the rate of increased DAF fluorescence upon shear activation; the mean slope difference pre to post flow initiation was -70369±53519.2 u/min (P=0.21) with no difference between healthy and SCD. Incubation with L-NAME or nitrite did not alter the change in fluorescence.

Conclusions: RBC produces NO as measured by DAF fluorescence; however, there is minimal shear activated change on a cell-by-cell basis and no significant increase with the addition of nitrite under oxygenated and deoxygenated conditions.

Nitric oxide generated by red blood cells following exposure to shear stress dilates isolated small mesenteric arteries underhypoxic conditions

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Red blood cells (RBC) possess a functional nitric oxide synthase (NOS) enzyme located in the cell membrane and cytoplasm. It has previously been observed that shear stress acting on RBC activates NOS and causes enhanced NO export. The aim of the present study was to investigate the physiological importance (e.g., in local blood flow regulation) of RBCderived NO stimulated by application of shear stress. Blood samples and arterial vessel segments were obtained from Wistar rats; RBC suspensions were adjusted to a hematocrit of 0.1 l/l using Krebs solution. In order to apply shear stress to the RBC suspensions they were continuously flowed through a small-bore glass tube for 20 minutes at a wall shear stress of 2 Pa. The RBC suspensions were then perfused through endothelium denuded small mesenteric arteries having a diameter of ∼300m under both high oxygen (PO2 ∼130 mmHg) and hypoxic conditions. Perfusion of vessel segments with sheared RBC suspensions caused a significant dilation response under hypoxic conditions but not at high oxygen levels. Incubation of RBC suspensions with the non-specific NOS inhibitor L-NAME (10–3 M) prior to shear stress application abolished this dilation response. Our results indicate that NO released from RBC due to shear stress activation of NOS results in vasodilation of vessel segments under hypoxic conditions, and strongly suggest that NO originating from RBC may have a functional role in local blood flow regulation.

Shear wave elastography of the testis in the healthy man – comparison of standard values using ARFI and VTIQ techniques

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Introduction: Shear wave elastography is a recent technique in the assessment of tissue elasticity. Different elastographic techniques have been described over the years. Acoustic Radiation Force Impulse Imaging (ARFI) uses mechanical excitation of tissue to create detectable shear waves, a higher shear wave velocity being associated with an increased tissue stiffness. The Virtual Touch Tissue Imaging Quantification (VTIQ) generates a mechanical push pulse as well, additionally creating a colour-coded map, in which tissue stiffness can be measured on the stored colour map after measurement. VTIQ has already been used in the evaluation of unclear scrotal masses. Both techniques allow an operator-independent examination without application of mechanical pressure.

Material and Methods: Twenty healthy patients without testicular pathology underwent standard B-mode sonography and additional elastography in the ARFI mode as well as the VTIQ mode of both testes using the Siemens Acuson S2000 ultrasound device. Measurements of shear wave velocity were performed in the upper pole, the central portion and the lower pole separately for each testis. Values were described in m/s.

Results: Shear wave velocities determined by VTIQ were all significantly higher than values gained in the ARFI mode. ($p<0.001$ to $p=0.007$). Values were between 0.22150 and 0.28600 m/s higher when the examination was performed using VTIQ.

Conclusion: Both ARFI and VTIQ elastography modes proved to be feasible techniques in the assessment of testicular tissue elasticity. Consideration of higher values for VTIQ are important when comparing different elastography measurements, especially when applying the devices to clinical fields, e.g. work-up of scrotal masses.

Differences of standard values of Supersonic shear imaging and ARFI technique – in-vivo study of testicular tissue

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Introduction: Elastography is a technique which improved over the past decade and enables to assess tissue stiffness. Amongst others, Acoustic Radiation Force Impulse Imaging (ARFI) is implemented in the ultrasound scanner. As an advantage, there is no need for external compression, which reduces operator dependency. This is achieved by qualitative and quantitative responses which are generated in a selected region of interest (ROI) by short-duration acoustic radiation forces (< 1 ms). In a similar manner Supersonic shear imaging (SSI) produces an acoustic pressure wave which induces slow-moving lateral waves within the tissue. The speed of propagation of the shear wave is proportional to the square root of the tissue's elastic modulus. Only since a short while, it is assumed, that there are differences in standard values according to different techniques. Until now, there is no in-vivo study in this context for the examination of the human testis.

Material and Methods: Fifty-eight healthy male the testes were examined using B-mode sonography and different elastography techniques. Firstly, B-mode sonography was performed in order to scan the testis for pathologies followed by performance of real-time elastography in three predefined areas (upper pole, central portion and lower pole) using the SuperSonic Aixplorer ultrasound device (SuperSonic Imagine, Aix-en-Provence, France). Afterwards a second assessment of the same testicular regions by elastography followed using the Acoustic Radiation Force Impulse Imaging (ARFI) technique of the Siemens Acuson 2000 ultrasound device (Siemens Health Care, Germany). Values of shear wave velocity were described in m/s. Parameters of elastography techniques were compared using multivariate analysis.

Results: The values of SSI were all significantly higher in all measured areas compared to ARFI ($p<0.001$ to $p=0.015$). Quantitatively there was a higher mean SSI wave velocity value of 1,1 compared to 0.8 m/s measured by ARFI.

Conclusion: Both, ARFI and SSI techniques enabled to assess quantitative and qualitative testicular stiffness and proved to be feasible for clinical application. For the definition of standard values in the testicular tissue, it is mandatory to distinguish between the different elastography techniques. This will be important for the examination of e.g. scrotal masses and other testicular pathologies.

Characterization of Benign Periablational Enhancement Following Multipolar Radiofrequency Ablation Using Perfusion CT in an In-Vivo Porcine Liver Model

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Background: Thermal ablation is an important interventional option in the management of liver tumors. Immediate postablational imaging regularly shows periablational enhancement. This peripheral hyperperfusion may induce heat-sink effects which could contribute to incomplete tumor ablation. To reduce the effect of hyperperfusion the feeding vessels source must be known.

Objectives: To dynamically characterize the type of blood supply of the periablational enhancement zone immediately after hepatic radiofrequency ablation (RFA) using perfusion CT.

Methods: We used an in-vivo porcine liver model. Multipolar RFA was performed in healthy pig livers. Immediate post-ablational perfusion CT was acquired. The contrast enhancement over time of the peripheral ablation zone, the aorta and the portal vein were recorded. Time differences of the peak periablational enhancement to the peak arterial perfusion and to the peak portalvenous perfusion were calculated and analyzed.

Results: The perfusion peak of the periablational enhancement zone always occurred in mean 8.1 s after the arterial peak in the aorta and in mean 16.9 s before the peak in the portal vein.

Conclusions: Benign periablational enhancement is a result of primary arterial and not portalvenous hyperperfusion. In order to reduce heat sink effects, peri-ablational arterial balloon occlusion or transarterial chemoembolization may be beneficial during RFA.

Platelet-rich Plasma affects Vitality, Differentiation and Gene Expression of Adipose-derived Stem Cells in vitro

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Platelet-rich plasma (PRP) is a source of concentrated autologous platelets. The growth factors and cytokines that are released by the platelets can facilitate the regeneration of soft and bony tissues, making PRP a promising candidate for future therapies. Numerous clinical studies on the regeneration potential of PRP have been conducted, including the treatment of injured nerves, tendons, cardiac muscle, and bone, amongst others. However, the results are inconsistent. This might be due to the fact that a broad variability in the techniques of PRP production exists, affecting the outcome. The simultaneous use of adipose-derived stem cells (ASCs) might improve the regeneration potential of PRP. However, the optimal ratio has yet to be elucidated. Here, we tested the effects of different PRP concentrations on ASCs in vitro. Cell vitality increases with the PRP-concentrations up to a concentration of 10% to 20%. With a PRP concentration of 30% the cell vitality declines. Adipogenic marker genes are not induced by PRP. Interestingly, receptors for growth factors known to be enriched in PRP were not upregulated, too. Whereas PRP had no influence on the expression of mesenchymal stem cell marker CD73, CD105 was downregulated with low PRP concentrations but showed a normal expression with high PRP concentrations.

Outcome after autologous fat transplantation for correction of congenital Breast asymmetry

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The aim of this retrospective study was to determine if the new surgical technique of autologous fat transplantation is as effective and safe as the breast augmentation technique with silicone implants. This was a study on 36 Patients, who has been operated within the last 7 years. For evaluation of our results we proceeded to biometrical measurements (photo documentation, Image J), ultrasound examination (B-mode image, Doppler, elastography, anomalies) and patient related outcome (SF36, HADS, BreastQ).

New insights into erythrocyte membrane structure and dynamics: the viscoelastic profile

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Aim: In this study the rheological and structural characterization of RBCs affected by Hereditary Spherocytosis (HS) and Elliptocytosis (HE), Sickle Cell Anemia (SCA) and ß-thalassemia (Thal) was compared in order to examine the correlation between the erythrocyte membrane alterations and the viscoelastic properties.

Methods: The electrophoretic analysis of the erythrocyte membrane proteins was performed by SDS-PAGE and quantified by Gel Doc XR+. The rheological profiles of whole blood (at native and normalized Hct) and ghosts were determined by the Rheo-Microscope MCR 301. The viscosity at shear rates 200 and 1 s-1, the erythrocyte aggregation index (EAI), the storage modulus G´ and the loss modulus G´´, as a function of angular frequency ù (range 0.1-10 Hz), and the direct imaging under flow were studied.

Results: Defects of the membrane proteins Spectrin and Band 3 were observed in HE/HS and Thal RBCs, respectively. Increased viscosities at 1 s-1 were observed in native HE, HS, SCA RBCs and in normalized Thal samples, and alteration of EAI was evidenced mainly in SCA. Relevant abnormalities in viscoelasticity were found in HE and SCA RBCs. These results were confirmed by the rheological profiles of the membranes only in HE RBCs.

Conclusions: The comparison between blood and ghost rheological viscoelastic profiles allowed to characterize the different membrane structure-dynamics defects and suggested a new way to evaluate the membrane structure and dynamics relationship.

Small amplitude oscillation (SAOS) technique to characterize blood of species with different RBC aggregability

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Small amplitude oscillation (SAOS) technique is used to characterize the shear stiffness of viscoelastic materials. In blood, it reflects the forces between blood cells and between blood cells and surrounding plasma. We analyzed blood of species with high (horse), medium (man), and low (sheep) erythrocyte (RBC) aggregability and compared the results to dynamic viscosity data obtained in rotational shear flow. Amplitude and frequency sweep tests (linear viscoelastic mode, LVE) were performed from hematocrit (HCT) adjusted (40%, 50%, 60%) samples that were tested at 7°C, 22°C, and 37°C. Storage modulus (G´) increased with HCT and decreased with temperature in each species, but the gradient of this increase was species-specific. The lower dependency of G´ on the equine HCT value could be a benefit during physical performance when high numbers of RBCs are released from the spleen. In sheep, an HCT-threshold must be overcome before the desired quasi-static condition was achieved, suggesting that the binding forces within blood are rather weak. This was confirmed by the reduced shear thinning of sheep blood. Human blood was the easiest to measure. The frequencies for tests under LVE were in a narrow range around the resting heart rate of the species. In horse, time-dependent influences concurred at frequencies lower than 3 rad.s-1 due to sedimentation of RBC aggregates. In conclusion, blood is a fragile suspension that shows its best stability around the heart rate of the species.

An image analysis-based technique for locally characterising erythrocyte aggregation in microfluidic applications

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Blood flows in microfluidic applications have gained much attention in recent years due to the increase of interest in lab-on-chip diagnostic devices. However, certain aspects of erythrocyte aggregation in such flows have been overlooked. The present study describes a new approach for characterizing aggregation in the microscale and at a local level. Image processing algorithms have been developed to exploit certain imaging characteristics of aggregates, overcoming certain limitations of brightfield microscopy. Aggregate sizes are resolved at the cell level and aggregate distributions in the microchannels are studied. We report measurements of local aggregation and size distributions of human erythrocytes flowing in bifurcating microchannels. The RBC suspensions were perfused through a T-junction bifurcating PDMS microchannel (100 x 40 um) using a pressure system. Local aggregation characteristics were determined in the microchannel and the size distribution of aggregates through the flow domain was studied for various flow ratios. The results illustrated the fact that smaller aggregates show a preference in the low flow rate branches, which is explained when considering the aggregate size distribution in the parent branch and the flow split characteristics in the bifurcation region. It was observed that as aggregate size increases so does their distance from the microchannel walls, forming regions which are free of aggregates of certain size.

Comparative study of the influence of glucose on red blood cell aggregation

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High level of glucose concentration in blood is associated with diabetes mellitus which becomes a civilization disease. It is well known that the rheological behavior of blood in diabetes patients has been altered. However, in the literature conflicting results describing the effect of glucose concentration in plasma on red blood cell (RBC) aggregation can be found. The goal of the work is to study the effect of glucose on the RBC aggregation. Four techniques for measurements of RBC aggregation were used: sedimentation curve analysis, syllectogram analysis, light scattering analysis and fractal dimensions of three dimensional RBC aggregates analysis. The blood samples for the study were obtained from healthy volunteers. The RBCs were isolated from the whole blood and incubated in solutions of glucose at concentrations 0, 0.1, 1, 2 and 3g/dl for 1 hour. After incubation the erythrocytes were re-suspended in autologous plasma or in dextran 70 solution at hematocrit 1, 5, 7 and 40%. The above techniques for the study of the aggregation were used for RBCs incubated in a glutaraldehyde solution yielding a control results. The sedimentation curve analysis and the aggregate fractal number analysis showed a decrease of the RBC aggregation with the increase of the glucose concentration of the solutions where the cells were incubated. It was found that the light scattering technique and the syllectograms analysis did not show any effect of the glucose on the RBC aggregation process.

Characterization of red blood cells aggregation in microfluidics environment

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AIM: The goal of this study is to quantify and characterize RBC aggregates and hence comprehend the non-Newtonian behaviour of blood at the micro-scale in a confined environment.

METHODS: For this propose, a microfluidic chip was design to provide a controlled flow condition. The linear velocity engendered from the controlled flow provides constant shear rates used to qualitatively analyze RBC aggregates and simultaneously measure the viscosity. Experiments were performed using a micro-Particle Image Velocimetry (μPIV) system for shear rate measurements coupled with a high-speed camera for the red blood cell visualization. Aggregate size distributions are determined using image processing techniques.

RESULTS: RBC aggregate sizes are quantified in controlled and measurable shear rates environments for 5, 10 and 15% hematocrit. This work establishes a relationship between RBC aggregate sizes and corresponding shear rates as well as one between RBC aggregate sizes and apparent blood viscosity at room and body temperature in a microfluidic controlled flow.

CONCLUSIONS: The results of the investigation can be used to better understand the aggregation in a confined environment, help to design microfluidic lab-on-chip (micro blood flow at room temperature), or develop new numerical models for non-Newtonian blood flow.

Visualized erythrocyte's collapsing and fragmentation in high shear flow

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We prototyped the experimental setup which enable us the blood cells' monitoring under the high shear flow. This experimental setup incorporated the counter rotating mechanism, and then, the middle position between the cone and cup surfaces is theoretically stationary, and therefore our visualization target. The aim of this study is to apply this system to the visualization of erythrocytes under the process to subhemolytic trauma and hemolysis induced by high shear stress. Fresh erythrocytes were exposed to high shear stress of 186Pa within 10wt% of polyvinylpyrrolidone (PVP) contained Phosphate Buffered Saline solution using our newly developed experimental system. As the time elapsed, the erythrocytes, exposed to high shear field, started to irregularly wave their membrane, and then, finally fragmented. The high shear stress related blood cell's subhemolytic trauma and the process leading to hemolysis was successfully visualized through this study. Our experimental system would be useful to reveal the response of blood cells to high shear flow, and the subhemolytic trauma by Mechanical circulatory devices.

Blood rheological status during pregnancy

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The aim of the present study is comparison of changes of hemorheological functional condition responsible for blood flow disorders in the microcirculation in physiology pregnancy and control group (women without pregnancy). The principal factor of hemoreological was monitored RBC aggregation with the ´´Georgian technique´´ that is sensitive and provided us with direct and quantitative data and RBC deformability (filtration methoth). We investigated 25 subjects with mean age 25,5±3,4 (physiological first pregnancy, 21 weeks, n=25). The analysis of the data was performed using statistical programs "Origin 4.1"

(Microsoft. Software, Inc) and Microsoft Excel, evaluated Student and criteria Pearson. Protocol of research was adequate Helsinki Declaration. We found that the RBC aggregability was higher by about 20%, in the blood flowing in women with physiology pregnancy than in the systemic circulation women without pregnancy; RBC deformability was decrease by about 15%.Our previous research provides practical recommendations. Therefore, it is especially important to monitor the agregabilityand deformability of erythrocytes at a pregnancy of any form. A correct diagnosis of hemorheological parameters is particularly important for correcting the violations of separate links of the homeostasis, which is an unavoidable cause of pregnancy.

Biphasic responses of RBC function following repeat exposures to short duration, supraphysiological, subhaemolytic shear stress

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The precise interactions between RBC deformability and the magnitude-duration of shear stress (SS) exposure remain largely undescribed for repeat supraphysiological, subhaemolytic SS duty-cycles of less than 15 s. The present study examined RBC function before, during and after application of preconditioning SS within this domain.

RBC collected from healthy volunteers (n=18) were resuspended in high viscosity polyvinylpyrrolidinone and exposed to each of two SS duty-cycles, 64Pa x 3 s and 88Pa x 2 s, for 10 repeated bouts. SS was applied in an annular Couette shearing system and ektacytometer (LORCA MaxSis, Mechatronics, Netherlands), and changes in RBC deformability were measured using laser diffractometry.

A ‘biphasic’ change in RBC deformability was observed, whereby initial SS exposures (i.e., duty-cycle 1 and 2) significantly decreased RBC deformability, with subsequent exposures returning these values to normal.

This biphasic early damage, followed by subsequent enhancement in RBC deformability, has not been previously reported. As RBC impairment response was rapid, demonstrating recovery within the first 4 s of cumulative exposure, it is possible that previously reported duty-cycles of longer durations would have precluded this observation. Whether the observed alterations in RBC deformability are due to passive or active regulators remains to be determined; however, such findings may improve the haemocompatibility of biomedical devices and subsequently, patient survival.

The elasticity of blood

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Although the viscosity of blood has been explored for approximately half a century, its low shear behavior, i.e. the existence of a yield stress is still controversial, even though its existence has deep repercussions on blood circulation. Similarly, knowledge regarding its viscoelasticity is, at best, marginal.

The aim of the present work is to determine whether blood has a yield stress or not through the determination of its linear viscoelasticity.

The experimental origins of this yield stress controversy together with the lack of viscoelastic data is discussed in relation to effects of sedimentation, denaturation of proteins at interfaces and biological variability. This leads to the use of a novel ultra-fast rheometry method as well as of a whole blood which is carefully tuned to allow quantifying the influence of proteins and hematocrit -in the pathophysiologically relevant ranges- on blood´s viscoelasticity.

Results show that blood is dominantly elastic at low strains under high erythrocytes and fibrinogen concentrations and dominantly viscous in the low and normal ranges. Since dominant elasticity means the presence of a yield stress, this result solves the long-standing controversy concerning the existence of a yield stress in whole blood.

Strikingly, blood is elastic (soft gel phase) under conditions known to favor thrombosis-related pathologies, suggesting a new approach for exploring these pathologies, which may then help to define new therapeutic targets.

Erythrocyte interactions – comparison of the aggregation power of polymer molecules used in medicine – not only size does matter

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AIM: Different colloids are used in medicine as a part of solutions for fluid resuscitation therapy and organ preservation in transplantation: hydroxyethyl starches (HES), dextran (Dx), polyethylene glycols (PEG), polyvinyl pyrrolidone (PVP) etc. Some of the problems associated with their application are addressed to alteration in erythrocyte (ERY) rheology. In this report we present our results concerning the estimation and comparison of the aggregation power (AP) of these molecules related to ERY interactions in vitro.

METHODS: Washed in phosphate buffered saline (PBS) human ERY are used during the study. The zeta sedimentation technique is used for quantification of the extent of cell aggregation. Zeta sedimentation ratio (ZSR) based indices (AI) are calculated. The hydrodynamic radius (Rh) of the polymer molecules is determined using viscometric approach.

RESULTS: For all polymers tested a linear range in the relationship AI - concentration was found. The slope of the calculated line was interpreted as measure of the molecule's AP. The following ranking was obtained: PEG > PVP > DX > HES. Within the same chemical type of polymer, increasing Rh of the molecules leads to intensified aggregation. Comparison of the AP of molecules with similar Rh reveals a significant dependence on their chemical nature.

CONCLUSIONS: Our results show that molecule's AP is significantly dependent of their chemical nature – i.e. not only molecular size does matter, as nowadays accepted.

Non-adsorbing macromolecules induce adhesion of diabetic red blood cells to the endothelium

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AIM: Abnormal adhesion of red blood cells (RBC) to endothelial cells (EC) has been linked to the pathophysiology of several diseases associated with vascular disorders. Various biochemical changes on the outer membrane of RBC as well as plasma protein levels, have been identified as being likely to play a key role, but the detailed interplay between plasma factors and cellular factors often remains unclear. In this work, we identified an alternative pathway by demonstrating that non-adsorbing macromolecules have a marked impact on the adhesion of diabetic RBC to EC.

METHOD: We suspended RBC from patients with Type II Diabetes Mellitus (T2DM), in solutions of dextran to mimic the impact of non-adsorbing macromolecules. Static and continuous flow adhesion assays were used to determine the adhesion behavior of T2DM RBC with EC and the results compared with those of normal controls.

RESULTS & CONCLUSION: We found that the presence of non-adsorbing molecules in T2DM promote an increase in T2DM RBC - EC adhesion. It is concluded that this adhesion-promoting effect originates from macromolecular depletion interaction and thereby presents an alternative mechanism by which plasma proteins could regulate cell-cell interactions. These findings should thus be of potential value not only for a detailed understanding of the pathophysiology of diabetes mellitus but also other diseases associated with vascular complications.

An Ex Vivo study of Nitric Oxide efflux from Human Erythrocytes in Both Gender

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Introduction: Acetylcholinesterase (AChE) is located on the outer surface of the erythrocyte membrane. Sex-related differences in erythrocyte AChE enzyme activity had been verified in young adults. It is also known that the binding of circulating acetylcholine (ACh) with AChE in the erythrocyte membrane initiates a signal transduction mechanism that stimulates nitric oxide (NO) efflux.

Aims: This ex vivo study was intend to compare the NO efflux from erythrocytes between genders in healthy donors.

Methods: We included 66 gender age-matched healthy donors (40-60 years old). We performed quantification of erythrocyte NO efflux from erythrocytes and AChE enzyme activity.

Results: There are no significant differences in NO efflux from erythrocytes between man and women. Regarding AChE membrane enzyme activity values, in this range of age, no differences between genders were obtained.

Conclusions: The erythrocyte nitric oxide efflux from healthy humans do not change with the gender. The absence of difference is in accordance to the absence of acetylcholinesterase enzyme activity obtained between both genders.

Keywords: Gender; Erythrocyte; Nitric oxide; Acetylcholinesterase

Clot Quality that it formed in vitro and ex vivo by stored Platelets

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AIM: We aimed to study thromboelastographically clot quality formed by stored platelets (PLT) in vitro and after platelet concentrates (PCs) transfusion ex vivo.

METHODS: PLT aggregation and clot quality (clot strength and clot elasticity) were assayed from 67 single-donor apheresis and leucoreduced PCs prepared without or with plasma substitution by additional solution up to 70 vol%. Clot quality after single PCs transfusion was tested in 49 patients (fibrinogen >= 1,4 g/L and PLT count >= 50 x10(9)L(-1)) from whom 15 were required second or more PCs transfusions.

RESULTS: The data analysis showed the loss of influence of activated but not intact PLT in final clot quality. During PCs storage clot strength reduced gradual up to 40 -55% from initial, and shear elastic modulus declined from (mean ± SD) 1855.6 +/- 30.1 Pa to 662.5 +/- 11.1 Pa. Unsuccessful PCs transfusion resulted in weak clot despite acceptable fibrinogen level and PLT count. Both clot strength and shear elastic modulus had no different significantly in compare to their values before transfusion.

CONCLUSION: We suppose that good transfusion outcome reflect success in vivo recovery of PLT activity determining their contribution in clot strength.

Cluster of red blood cells in microcapillary flow: hydrodynamic versus macromolecule induced interaction

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Red blood cells can be modeled as soft deformable objects. Their shapes strongly couple to the flow. RBC clustering can compromise proper regulation of oxygen delivery to tissues and organs and it has been shown that well-organized clusters in microcapillaries tend to form. The physical origin of this cluster formation can be either long-ranged hydrodynamic interaction [1,2] or a short-range aggregation mechanism caused by plasma macromolecules [3]. How big is the relative contribution of the hydrodynamic interaction compared to the macromolecule-induced interaction on the cluster formation in a confined flow? In our research, flowing healthy red blood cells through microcapillaries at different velocities and different suspending media, the cluster formation is quantified and distinction among adhering and non-adhering clusters is done. Clustering is enhanced in suspension including macromolecules and cluster-shape differences are significant. Our 2-D numerical simulations capture the transition between the stable adhering and non adhering clusters when the flow velocity is increased.

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Comparative study between microvascular tone regulation and rheological properties of blood in patients with type 2 diabetes mellitus

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Aim: To study the changes of the skin blood flow responses to cold stress in patients with diabetes mellitus type 2 through wavelet analysis of the peripheral skin temperature oscillations and to estimate their relationship with the blood viscosity values.

Materials and Methods: The amplitudes of the skin temperature pulsations (ASTP) were monitored by "Microtest" device ("FM-diagnostics", Russia); the whole blood viscosity and the shear stresses were measured by viscometer Contraves LS30, (Switzerland) at 11 shear rates of 0.0237s-1 to 128.5 s-1 in 9 healthy subjects and in 30 patients with type 2 diabetes mellitus. Power law and Herschel-Bulkley (HB) equations were applied to describe the blood rheology. Both models include consistency (a) and flow index (m), and the HB also gives the yield stress ($\tau 0$). The Spearman rank correlations between these parameters and the ASTP in the frequency ranges, corresponding to the myogenic, neurogenic and endothelial mechanisms were calculated.

Results: The ASTP values decreased when the blood viscosity increased. The correlation analysis revealed good ASTP–m ($r>0.5$) and ASTP–a ($r<-0.5$) relationships in the endothelial range, while the ASTP–$\tau 0$ correlation was weaker ($r\approx-0.4$). These correlations became lower for the ASTP during the cold stress.

Conclusions. The results prompt manifestation of endothelial dysfunction in patients with type 2 diabetes.

This work was supported by the Russian Science Foundation under project No 14-15-00809.

Endothelial phenotypes, shear rate and dyslipidemia: a continuum to atherosclerosis

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Atherosclerosis is a chronic inflammatory disease of arteries that develops preferentially at branches and bends that are exposed to disturbed blood flow. It is seen as a highly intricate disease with cycles of progressive endothelial insult, arterial inflammation, altered hemodynamics, and vascular remodelling, leading to plaque formation, progression, and rupture.

Vascular function is modified by flow, in part, via the generation of mechanical forces that alter multiple physiological processes in endothelial cells. Hemodynamic shear stress is crucial for endothelial homeostasis – and an expression of a particular endothelial phenotype – under normal physiological conditions.

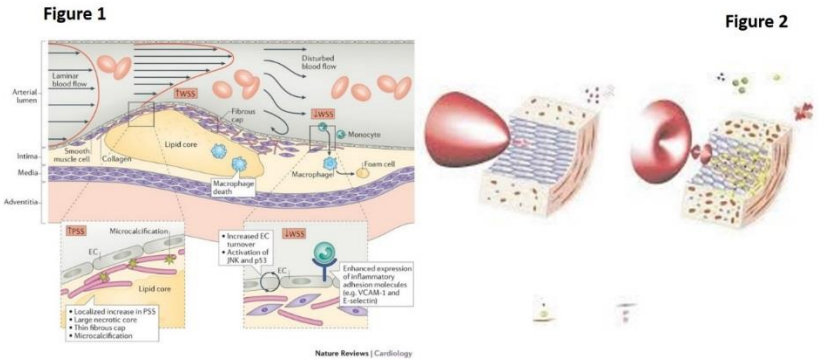
Atheroma develops in areas of low endothelial shear stress (ESS). The high ESS seems to protect against atheroma formation, and the intimal accumulation of lipid and other atherogenic material may be related to ESS. Endothelial cells transduce the fluid shear stress into biochemical signals that regulate many pathways: intracellular enzyme activity, gene transcription, protein and micro-RNA synthesis, and release of bioactive mediators, which can adjust the endothelial cell structure and function, the surrounding cellular environment, and the balance between inhibition and promotion of atherosclerotic processes.

Figure 1. Biomechanical force in atherosclerosis (from Brown AJ, et al. 2016)

In healthy arteries, endothelial structure and integrity remain intact, with normal production of vasodilator, fibrinolytic, and antioxidant mediators, and decrease expression of leukocyte adhesion molecules, inflammatory mediators, vasoconstrictors, and paracrine growth factors. Low ESS increases expression of cell adhesion molecules, growth factors, vasoconstrictors, oxidative species, proteolytic enzymes, and acute inflammatory mediators. The disparity between these 2 phenotypes (figure 2) results in chronic inflammation, extracellular tissue degradation, endothelial proliferation, and apoptosis; intimal lipid accumulation and oxidation; platelet aggregation and thrombosis; plaque neovascularization and intraplaque hemorrhage, contributing to plaque growth, arterial remodelling, and further altered ESS distribution.

In brief, ESS is an important determinant of endothelial function and phenotype. Physiologic ESS induces endothelial quiescence and an atheroprotective gene expression profile, while low ESS motivates an atherogenic phenotype. The functional regulation of the endothelium by local hemodynamic SS provides a model for understanding the focal propensity of atherosclerosis and may help guide future biomarkers and therapeutic strategies.

Figure 2. Hemodynamic shear stress and endothelial phenotypes (from Malek AM, et al. 1999)



Oscillations in cutaneous blood flow in hyperglycemic obese patients

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Obesity and hyperglycemia are known to affect the cardiovascular function and the regulation of peripheral distribution of blood flow. We studied the effects of low-glycemic and hypocaloric diet on skin blood flow oscillations in newly diagnosed hyperglicemic obese patients. Sixty normoglycemic (30 females) and sixty hyperglycemic (30 females) obese adults were recruited and treated with low-glycemic and hypocaloric diet for 6 months. Skin microvascular blood flow(SBF) was recorded using a laser Doppler monitoring apparatus (PeriFlux 4001 System, Perimed) and oscillations in blood flow were determined by wavelet transform. Moreover, reactive hyperemia was investigated after 2 min brachial artery occlusion. Hyperglycemic patients showed lower SBF when compared to normoglycemic subjects as well as total power spectral density(PSD), accompanied by impaired hyperemic response to artery occlusion. The PSD of oscillatory component in the range 0.052-0.15 Hz, related to myogenic activity, was lower in hyperglycemic than normoglycemic people. After dietary treatment,SBF (12.8±0.3vs9.1±0.5 PU,$p<0.01$) and total PSD (333.2±11.2vs116.5±11.4 PU2/Hz,$p<0.01$) significantly increased in hyperglycemic subjects as well as post-occlusive reactive hyperemia. The PSD of myogenic activity-related oscillatory component appeared to be augmented (35.0±0.7vs29.9±0.8%, $p<0.01$).In conclusion, hyperglycemia caused severe alterations in microcirculation, but low-glycemic and hypocaloric diet improve tissue perfusion state.

Microcirculatory dysfunction in obesity: effect of gastric by-pass surgery

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Microvascular dysfunction (MD) may concur to increase the cardiovascular risk in obese patients (Ob-P). Therefore, MD improvement is an important goal in the treatment of Ob-P. We measured forearm skin post-occlusive reactive hyperemia (PORH), using laser Doppler flowmeter (LD), and the normalized spectral power density (N-SPD) of different LD oscillation frequency intervals (which reflect vasomotion), using spectral Fourier analysis, in 37 Ob-P (BMI=47.0±11.8), before Roux-en-Y gastric bypass (RYGB), in 28 healthy, lean control subjects (CS) (BMI=22.7±2.3), as well as in 24 of the same Ob-P, 12 months after RYGB. PORH and N-SPD of the examined LD frequency intervals were significantly lower in Ob-P before RYGB, compared to CS. Twelve month after RYGB, the same Ob-P exhibited a significant weight loss (40 kg on average), together with a significant increase in PORH (by ~50%) and in N-PSD of the different examined LD frequency intervals ($p = 0.0048$, 0.008 and 0.008), as compared to before RYGB, independently of the presence of diabetes. We also observed an inverse correlation between BMI and PORH in the pooled pre- and post-surgery data obtained in Ob-P. These results show a relationship between MD and body fat excess, as well as a beneficial effect of surgically induced sustained weight-loss on MD in Ob-P. Further studies are necessary to verify whether this beneficial effect could halt or slow down the progression of insulin resistance and arterial hypertension in Ob-P.

Blood viscosity in subjects with normoglycemia and prediabetes

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AIM: Increased Blood Viscosity (BV) has been reported to represent a risk factor for the development of type 2 diabetes. However, data in subjects with normal glucose or prediabetes are missing. We have therefore evaluated the relationship between BV and blood glucose in subjects with normal glucose or prediabetes.

METHODS: We have enrolled 264 subjects who were divided in three groups according to blood glucose: Group A (n=74): blood glucose < 90 mg/dl; Group B (n=96): blood glucose ranging 90-99 mg/dl; Group C (n=94): blood glucose ranging 100-125 mg/dl. BV was measured at 37° C with a cone-plate viscometer at shear rates ranging 225–22.5 s-1.

RESULTS: Blood pressure, blood lipids, fibrinogen and plasma viscosity were similar in the three groups. BMI and waist were higher in Group C. Hematocrit ($p<0.05$) and BV (p between 0.01 and 0.001) were significantly higher in Group B and C, compared to Group A. Blood glucose was significantly and inversely correlated with HDL cholesterol and directly with BMI, waist, hematocrit ($r=0.134$), and BV (from 225 sec-1 to 22.5 sec-1, r ranging from 0.162 to 0.131). BV at shear rate 225 sec-1 resulted independently associated with blood glucose.

CONCLUSIONS: The present findings demonstrate a direct relationship between BV and blood glucose in non-diabetic subjects. Furthermore, in subjects with normal blood glucose values, individuals with higher blood sugar levels have increased BV comparable to that observed in subjects with prediabetes.

Metabolic Syndrome: inflammation and hemorheology

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AIM: Metabolic syndrome (MS) is a frequent condition in western societies, on which it imposes a heavy burden of mortality and disability through an acceleration of atherosclerosis. In MS a low-grade inflammation and a state of chronic oxidative stress can be identified. In recent years matrix metalloproteases, including gelatinases, have emerged as markers of atherosclerosis progression. Our aim was to study the role of hemorheological abnormalities in MS and their relation with other biochemical alterations.

METHODS: In groups of MS patients we evaluated the blood rheology pattern, some markers of oxidative stress and the plasma levels of gelatinases A and B and their tissue inhibitors.

RESULTS: We observed an increase in blood and plasma viscosity and a significant association between the rheological alterations and insulin resistance. We also demonstrated an enhanced lipid and protein oxidation, irrespective of whether MS patients were diabetic or not. We found high concentrations of gelatinases and their inhibitors, with gelatinase A prevailing over its inhibitor. The plasma levels of gelatinases and their inhibitors were significantly influenced by diabetes mellitus.

CONCLUSIONS: Plenty of biochemical alterations are demonstrable in MS, but the assessment of their clinical and prognostic relevance needs further investigation. A better knowledge of the overall pathophysiological picture could provide a reliable guide for clinical management of MS patients.

Exploring the role of exercise in conjuction with compression therapy in patients with venous ulcers: initial observations on a feasibility study

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AIM: To assess the microcirculatory effects of an intervention combining exercise and compression therapy in patients with lower-limb venous ulcers.

METHODS: This was an NIHR-funded, randomised-controlled, assessor-blinded, feasibility trial with two parallel groups. Thirty five adults, receiving lower-limb compression for a lower-leg venous ulcer, were randomly assigned to receive usual care (compression only) or usual care plus a 12-week supervised exercise programme. Participants in the exercise group were invited to undertake three, 60-minute sessions of supervised exercise per week. Each session involved a combination of treadmill walking, upright cycling and strength and flexibility exercises for the lower limbs. Participants were assessed before randomisation and 3 months after randomisation. Endothelially-dependent and -indendepent microvascular perfusion and reactivity were assessed using Laser Doppler fluximetry and iontophoresis of acetylcholine chloride (ACh) and sodium nitroprusside (SNP) respectively.

RESULTS: Statistically significant improvements were observed in endothelially-dependent, ACh-induced microvascular reactivity between groups, with measured cutaneous vascular conductance (CVC) being superior in the exercise group.

CONCLUSIONS: Our findings suggest that exercise offers important microcirculatory benefits to a lower-leg, venous-ulcer population. However, the practical challenges of implementing the intervention call for design modifications.

Assessing the evidence: exploring the effects of exercise in diabetic microcirculation

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Diabetes mellitus (DM) is associated with cardiovascular complications. Impairment of glycemic control induces noxious glycations, an increase in oxidative stress and derangement of various metabolic pathways. DM leads to dysfunction of micro and macrovessels, connected to metabolic, endothelial and autonomic nervous system. Thus, assessing vascular reactivity might be one of the clinical tools to evaluate the impact of harmful effects of DM and potential benefit of treatment; skin and skeletal muscle microcirculation have usually been tested. Physical exercise improves vascular dysfunction through various mechanisms, and is regarded as an additional effective treatment strategy of DM as it positively impacts glycemic control, improves insulin sensitivity and glucose uptake in the target tissues, thus affecting glucose and lipid metabolism, and increases the endothelium dependent vasodilation. Yet, not all patients respond in the same way so titrating the exercise type individually would be desired. Resistance training has, apart from aerobic one, been shown to positively correlate with glycemic control, improve vascular reactivity, and has been associated with less negative systemic side effects. It has been prescribed in various forms or in combination with aerobic training. The overview would present recent studies assessing the impact of different types of exercise, some mechanisms involved, and its potential positive and negative effects on treating type I and type II DM.

Study of platelet-activation and –aggregation with stirrer-induced shear generation

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It has known thatshear stimulation in blood stream activate platelets and let them aggregate which can give rise to a thrombus formation. The objective of this study is to examine the effect of shear-inducing stirring on the platelet activation and aggregation in a rotating stirrer-chamber system. The following parameters were investigated: the rotation speed of the stirrer (ù), the gap between the stirrer and chamber (h), the shearing time (ts), and the upper surface area of the stirrer (Astirrer). Platelets were activated and subsequently aggregated by a circulating shear flow in the rotating stirrer-chamber system. We found that the aggregation of platelet was significantly influenced by application of shear flow showing maximum area of the platelet aggregates at a critical shear rate of 3100 s-1, regardless of the stirrer shape. However, the required time to reach the maximum aggregation was significantly shortened to within 30 s for a wide stirrer compared with a narrow one. The wide stirrer generated a strong frictional flow between the stirrer and the fluid in the chamber and induced an effective shear flow. The non-uniform shear field in the stirrer-chamber system was resolved with secondary flow-induced mixing; thus, most of the platelets were homogenously activated. Our findings indicate that the present system consisting of a rotating stirrer and a confined chamber provides effective shear simulation to activate platelets and induce platelet aggregates.

Can Critical shear stress (CSS) be an all-round player? Correlation with yield stress and RBC deformability

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Background: The blood behaves as a non-Newtonian fluid that the fluid viscosity varies with shear rate. Hemorheologic parameters including whole blood viscosity (WBV), RBC aggregability and deformability (EI) have been known as risk factor for atherosclerotic and microcirculatory disease. Disaggregating shear stress (DSS) is used for a representative of RBC aggregability. The yield stress (YS) might be an indirect representative of WBV, because it can be indirectly calculated from a function of viscosity data. While most parameters should be adjusted according as Hct or fibrinogen, there is no need to adjust for DSS, because DSS is a unique value, not effected by Hct or fibrinogen. For clinical compatibility, this study was objected to evaluate the correlation between DSS value and YS, EI.

Methods: 204 subjects were enrolled. Blood viscometer (BVD, Bio-Visco Inc., Jeonju, Korea) for blood viscosity, RBC aggregometer (Rheoscan-AnD 300, RheoMeditech, Seoul, Korea) for DSS and EI were used. YS is calculated with Casson's equation from viscosity data, Hct-adjusted value was used, EI is used as a function with fibrinogen.

Results: DSS is highly correlated with Hct-adjusted YS ($r=0.691$, $p<0.001$), and fibrinogen adjusted EI ($r=0.457$, $p<0.001$). Patients with coronary disease showed higher DSS, YS, and lower EI.

Conclusion: DSS as a single value might be suggested to use in clinical field for evaluating the risk level for atherosclerotic cardiovascular disease.

Characterization of shear stress preventing red blood cells aggregation at the individual cell level and in whole blood samples

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AIM: Red blood cells (RBC) aggregation is an intrinsic feature of blood that has a strong impact on its microcirculation. For a number of years it has been attracting a great attention in basic research and clinical studies. Here,we study a relationship between the RBC aggregation parameters measured at the individual cell level and in whole blood samples.

METHODS: Home-made optical tweezers were used to measure the aggregating and disaggregating forces between the individual interacting RBCs in doublets, in order to evaluate the corresponding shear stresses (τ). The RheoScan aggregometer was used for the measurements of critical shear stress (CSS) in whole blood samples.

RESULTS: The correlation between CSS and SASS (Single cell aggregating shear stress, i.e., τ required to stop an RBC pair from aggregating) was found. SDSS (Single cell disaggregating shear stress, i.e., τ required to disaggregate a pair of RBCs) appeared to be few times higher than CSS and SASS.

CONCLUSIONS: We conclude that (1) SASS can be quantitatively compared with CSS and (2) SASS is significantly lower than SDSS. Therefore CSS should refer only to the shear stress preventing the RBCs aggregation but not to the disaggregating shear stress. The parameters characterizing the disaggregation process might serve as additional sensitive parameters to monitor the state of blood microcirculation.

ACKNOWLEDGEMENTS: This work was supported by RFBR-NRF grant #16-52-51050.

Effect of platelet activation on disaggregating shear stress in red blood cells aggregation

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Red Blood Cell (RBC) aggregation and platelet activation are considered as major determinants for vascular diseases. However, the relation between hemorheological properties of RBC and platelet activity has not been fully understood. Thus, in this study, we investigated the effect of platelet activation on hemorheological characteristics of RBCrelated parameters such as Critical Shear Stress (CSS), Aggregation Index (AI) and Elongation Index (EI). We hypothesized that activated platelet by high shear would affect the strength of RBC aggregation such as elevated CSS. Shear flow was generated by a rotating stirrer which remotely controlled with rotating magnet on the top of a rotor. For experiments, Shear stimulation was applied at 2800rpm for 30sec to both of samples which are whole blood sample and negative blood sample without platelets. As a result, CSS of whole blood was elevated from 181 to 229mPa (SD=±48mPa). In contrast, negative blood sample excluding platelets did not show relevant increase. In addition, AI and EI did not show apparent changes regardless of application of shear stimulation. Consequently, our findings would imply that RBC aggregation should be considered with platelet activation and further study to investigate detailed mechanism among RBC characteristics and platelet activation has to be defined.

Keywords: CSS, Red blood cell, aggregation, platelet, shear

Atherosclerosis: challenges in understanding the pathogenesis through a mathematical modeling approach

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Mathematical modeling and numerical simulations are important tools for a better understanding of atherosclerosis and subsequent development of more effective treatment and prevention strategies. The associated mathematical models include flow, transport, and chemical reactions, interactions of fluid and elastic structures, movement of cells, coagulation and growth processes, and additional complex dynamics of the vessel walls. Several theories have been developed to describe the pathogenesis of atherosclerosis but none of them can explain the whole process due to the large number of factors and different time scales involved. More realistic and comprehensive mathematical models still need to be derived.

In this talk we present a short review of some existing mathematical models for atherosclerosis. Special attention is given to a novel approach that captures essential features of the early stage of atherosclerosis development.

A computational framework to investigate flow dynamics in the superficial femoral artery of a peripheral arterial disease (PAD) patient will also be presented. The patient-specific geometry of the blood vessel and the hemodynamic conditions are derived from magnetic resonance imaging (MRI), performed after stent-implantation. The main goal is to use this MRI-based computational protocol to analyze data from clinical trials exploring possible correlations between hemodynamics and disease progression in PAD patients, and to provide predictive insight into disease management.

Endothelial cell dynamics in vessel regression

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The vascular network undergoes extensive vessel remodelling to become fully functional. Is it well established that blood flow is a main driver for vascular remodelling. It has also been proposed that vessel pruning is a central process within physiological vessel remodelling. However, despite its central function, the cellular and molecular mechanisms regulating vessel regression, and their interaction with blood flow patterns, remain largely unexplained.

We investigated the cellular process governing developmental vascular remodelling in mouse and zebrafish. We established that polarised reorganization of endothelial cells is at the core of vessel regression, representing vessel anastomosis in reverse. Moreover, we established for the first time an axial polarity map for all endothelial cells together with an in silico method for the computation of the haemodynamic forces in the murine retinal vasculature.

Using network-level analysis and microfluidics, we show that endothelial non-canonical Wnt signalling regulates endothelial sensitivity to shear forces. Loss of Wnt5a/11 renders endothelial cells more sensitive to shear, resulting in axial polarisation at lower shear stress levels. Collectively our data suggest that non-canonical Wnt signalling stabilizes forming vascular networks by reducing endothelial shear sensitivity, thus keeping vessels open under low flow conditions that prevail in the primitive plexus.

Adhesive behaviour of mesenchymal stem cells from flowing blood: implications for intravenous therapy

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AIM: Mesenchymal stem cells (MSC) are used in therapy, often by injection into the blood. Adhesion from flowing blood may be a critical step for their recruitment in the microvasculature. We aimed to understand how MSC might ´home´ to injured tissue.

METHODS: MSC from Wharton's jelly (WJMSC), bone marrow (BMMSC) or trabecular bone (TBMSC) were suspended in culture medium or added to whole blood, and perfused through capillaries coated with matrix proteins (collagen or fibronectin) or P- or E-selectin. Adherent cells were observed microscopically and counted.

RESULTS: None of the isolated MSC adhered to selectins even at low shear rate, but all were able to adhere to collagen or fibronectin. However, MSC perfused in whole blood failed to bind to fibronectin, while the fibronectin itself became covered in a single layer of spread platelets. When perfused over collagen, only WJMSC were found to attach, forming aggregates with platelets on the collagen surface. Interestingly, all isolated MSC adhered to a surface coated with platelets, but only WJMSC caused platelets to aggregate in a stirred suspension. When WJMSC or BMMSC were injected systemically via the tail vein in mice, WJMSC caused a decrease in blood platelet count but BMMSC did not.

CONCLUSIONS: MSC show origin-dependent interaction with platelets that may influence their adhesion to damaged vessels, and potentially cause thrombotic complications, depending on the degree of activation of the platelets adhered to the MSC.

Red blood cell rheology and vascular dysfunction in sickle cell disease

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Sickle cell anemia (SCA) is a genetic disease characterized by the presence of abnormal hemoglobin (HbS) that polymerizes under deoxygenated conditions causing a mechanical distortion of red blood cells (RBC). We recently investigated the contribution of blood rheology and vascular dysfunction in vaso-occlusive crises (VOC). Our findings demonstrated that SCA patients have blunted microvascular reactivity during local thermal heating tests compared to controls. In addition, increased blood viscosity and decreased microcirculatory oxygenation were independently associated with a higher risk to develop frequent VOC episodes. Several factors are involved in the vascular dysfunction of SCA, such as enhanced oxidative stress and reduced nitric oxide metabolism, but we recently observed that circulating exosomes, originating mainly from RBCs, were able to alter in vitro endothelial cells barrier permeability and the topographic distribution of the tight junction protein ZO-1 in a SCA severity-dependent manner compared to healthy children. In addition, SCA circulating exosomes promoted monocyte adhesion to endothelial cells, through increases in P-selectin expression. These new data suggest that exosomes originating from RBCs could be one of the sub-cellular elements involved in the endothelial dysfunction associated with SCA. In conclusion, vascular dysfunction and blood hyperviscosity emerge as key factors involved in the severity of SCA and the occurrence of frequent VOC events.

The microvascular behaviour of malaria- and babesia-infected red blood cells – same pathophysiological endpoint but different molecular mechanisms

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The pathogenesis of falciparum malaria and bovine babesiosis are remarkably similar. In both, parasite-infected red blood cells (RBCs) accumulate in the microvasculature causing vaso-occlusive clinical syndromes. Whilst the cellular and molecular mechanisms underpinning the pathogenesis of malaria have been intensely scrutinised, babesiosis has been relatively ignored; despite the fact that babesia parasites offer considerable experimental advantages to relate the function of specific parasite genes to pathological sequelae. We characterised the rheological properties of bovine RBCs infected by B. bovis (BbRBCs) and compared them with human RBCs infected with P. falciparum (PfRBCs). Like PfRBCs, flowing BbRBCs adhere to vascular endothelial cells and form stable interactions that correlate with microvascular sequestration. Intriguingly however, high resolution imaging of BbRBCs revealed structures on their surface (that mediate adhesion) that were morphologically very different to the knob-like structures on the surface of PfRBCs that mediate their adhesion. Using multiple approaches, we have now identified numerous novel proteins at the membrane skeleton of BbRBCs which we believe will be directly involved in the formation of these unique 'ridge-like' structures and hence in pathogenesis and virulence. Linking these novel proteins with physiologically-relevant functions in BbRBCs may also identify future therapeutic strategies to combat both babesia and malaria infections.

Inhibition of Shedding of the Endothelial Glycocalyx and Leukocyte Adhesion with Low Molecular Weight Heparin

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AIM: The endothelial cell (EC) glycocalyx, which consists of a layer of proteoglycans and glycosaminogylcans (GAGs), and adsorbed proteins form the endothelial surface layer (ESL). The ESL acts a barrier between blood and the EC. Shedding of the ESL in response to cytokines (e.g. TNF-alpha) and chemoattractants (e.g. fMLP) exposes ligands for WBC-EC adhesion. Thus, a means of stabilizing the ESL to resist structural changes during inflammation is highly desirable.

METHODS: The infusion of low molecular weight heparin (LMWH) was explored as a means of mitigating shedding of the ESL and leukocyte adhesion during the inflammatory process using techniques of intravital microscopy. WBC-EC adhesion in response to fMLP was observed in post-capillary venules of mesentery (rat) following infusion of varied concentrations of LMWH.

RESULTS: High concentrations of LMWH (1.6 mg/kg) resulted in diminished shedding of glycans and diminished WBC adhesion in response to topical fMLP. Clustering of glycans in the ESL increased with increasing concentration of LMWH, as evidenced by increasing intensity of fluorescently labeled glycans on the EC surface.

CONCLUSIONS: These results appear to result from the ability of LMWH to scavenge heparanase secreted by activated ECs, and ligation of components of the glycocalyx. Thus, the mitigation of pro-inflammatory conditions by LMWH observed in sepsis and ischemia/reperfusion, may be due, in part, to its stabilization of the EC glycocalyx.

Theory and practice of coronary blood flow visualization in open heart surgery

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AIM. The aim was to develop an efficient method for resolution of complex technical issues in open heart surgery and for deeper studying of pathophysiological disturbances of coronary circulation in clinics.

METHODS. Coronary blood flow (CBF) was registered with miniature intraoperative transducers functioning in a continuous wave spectral Doppler mode. Their design features allowed for a high quality signal on the working heart and provided high selectivity and accuracy of epicardial ultrasound due to the use of adaptors allowed for quantification of volume blood flow in coronary grafts.

RESULTS. Physiological peculiarities of spectral characteristics in human coronary arteries (CAs) were noticed. While in most cases in both vascular coronary beds, a normal CBF pattern has a two-phased curve with predominance of a diastolic component, valvular diseases of the left heart often causes significant changes of the curve as well as a redistribution of blood flow in the circulation of right and left CAs. CBF characteristics at presence of severe aortic valve disease before and after its surgical repair were identified.

CONCLUSION. Epicardial echoscopy allows for efficient monitoring of the quality of CBF restoration in the arteries of the heart during surgeries for the ascending aorta grafting with a valvular conduit and reimplantation of the coronary ostia into the graft. Visualization makes it easier to search for superficial heart arteries with the use of ultrasound probes.

Erythrocyte membrane skeleton structure and membrane permeability for oxygen in patients with essential arterial hypertension

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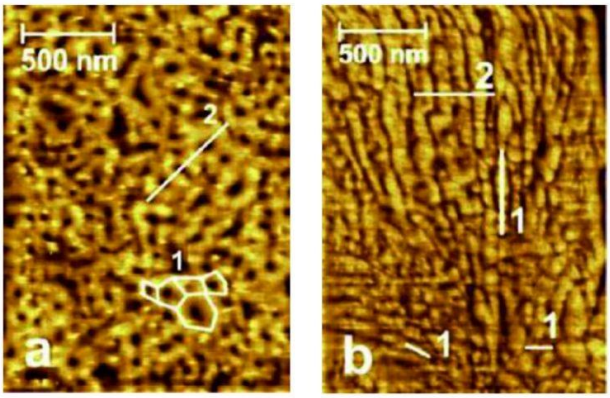
AIM: To search for differences in the erythrocyte membrane skeleton structure and erythrocyte membrane permeability for oxygen between patients with essential arterial hypertension and healthy donors.

METHODS: The erythrocyte topography was mapped by using the atomic force microscope (AFM), while the content of various hemoglobin forms was detected by applying Mössbauer spectroscopy.

RESULTS: In the erythrocytes of hypertensive patients, skeleton connectivity with the membrane proteins via junctional or ankyrin complexes is modified resulting in a transformation of the honeycomb structure of the membrane skeleton network into a corncob structure. This modification has been associated with the impairment of erythrocyte membrane permeability for O2.

CONCLUSIONS: An impaired oxygen release by Hb in RBCs of patients with hypertension may cause hypoxemia and further increase of blood pressure.

Fig. 1. AFM example images of RBC surfaces of a healthy donor (a) and a hypertensive patient (b).



Relation between increased plasma viscosity and increased the body mass index(BMI) in hypertensive patients

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OBJECTIVE: Hypertension and obesity is associated with increased cardiovascular risk(CVR). Our study aimed to investigate possibility of plasma viscosity (PV) as a risk marker in hypertensive obesity patients and a factor that contribute to increase the CVR.

METHODS: We select 324 from 1200 consecutive patients(P) referred from January 2003 to April 2009. CVR factors (blood pressure, lipids, glucose, cigarette smoking, obesity) were evaluated by routine methods. PV were measured by a cone-plate viscometer. We divided the obese hypertensive patients (BMI < 25 Kg/cm2) in three groups according with the BMI: Group 1 – between 25 - 30 Kg/cm2; Group 2 – between 30 - 35 Kg/cm2 and Group 3 – > 35 Kg/cm2

RESULTS: P with elevated BMI values had increased levels of PV (G1: 1.30 ± 0.10 cP, G2: 1.35 ± 0.10 cP and G3: 1.42 ± 0.11 vs.), $p<0.001$, and increased plasma fibrinogen levels (mg/ml) (G1: 287,50 ± 35,48, G2: 321,78 ± 34,29 and G3: 326,96 ± 29,73.), $p<0.001$). There is a relation between the levels of fibrinogen and PV ($p<0,01$). There also are a relation among the PV levels and cardiac parameters (left ventricular mass index) and interface media-intima of the carotid vessel.

CONCLUSION: The present investigation provides evidence that PV is increased in hypertensive subjects with elevated BMI values, independently of other CVR. This finding contributes to explain the high CVR of patients with obesity in hypertension and this market could be important in the determination of CVR.

Role of Red Blood Cells in vascular dysfunction-associated with Alzheimer´s Disease

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Our attention is focused on the study of a new model based on the red blood cell (RBC) and on its interaction with Aß. RBC are highly deformable to assist blood flow in the microcirculation. For this reasons abnormalities in RBC could contribute to AD by obstructing oxygen delivery to brain causing hypoxia. An additional factor, expected to impair the flow of RBC through the microcirculation is their adherence to endothelial cells when Aß is bound to RBC. In our work, firstly we focused on the morphology and nano-properties of RBC´s membrane (i.e. roughness) by Atomic force microscopy, following to soluble Aß peptides exposure at different times, in order to characterize specific alterations induced by Aß. Secondly, considering that RBC membrane contains, among blood elements, higher acetylcholinesterase (AChE) levels, we can assume that there is a mechanism similar to the one which occurs at the neuronal level leading to an increase of Aß toxicity mediated by the binding with AChE. Since mechanical properties of RBC membrane are regulated by a number of molecular components of signalling and/or regulatory pathways, of these, particular interest has been addressed toward protein band 3, protein kinase C isoenzymes, endothelial nitric oxide synthase and caspase 3, due to their possible roles in the modulation of RBC morphology, deformability and metabolic functions.

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Dependence of rheological and biochemical parameters of blood in the group of patients with clinically silent multifocal vascular cerebral lesions

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Hemorheology is a field of science which often becomes interesting to researchers studying impairments related to blood flow disturbances. Clinically silent vascular cerebral lesions (CSVCL) are considered as a problem of great importance in neurology. The aim of this work was the analysis of the interdependencies of rheological and biochemical parameters of blood. The group of patients included persons with clinically silent multifocal vascular cerebral lesions diagnosed by means of neuroimaging. The control group had no such symptoms in the central nervous system (CNS). We analysed hemorheological profiles in a group of 69 patients with CSVCL diagnosed by magnetic resonance imaging (MR) or 64-row computer tomography (CT) in relation to the control group – 17 subjects without such changes. Blood and blood plasma viscosity measurements were performed by means of a rotary-oscillating rheometer Contraves LS-40. For each sample the hematocrit value was measured using the standard method. Analysis of erythrocytes aggregability and deformability was performed with the use of rheological model of Quemada. Biochemical testes of blood were also performed. Received results of rheological and biochemical studies were compared with results obtained in the control group. Special attention was paid to the correlation analysis of rheological and biochemical parameters. Such correlation were found e.g. between the red cells deformability and the fibrinogen level.

Regulation of endothelial recruitment of leukocytes by the local environment

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In inflammation, endothelial cells (EC) respond to cytokines and regulate recruitment of leukocytes through changes in expression of adhesion molecules and activatory signals. However, the responses of the EC themselves depend on local environmental factors, notably the circulatory shear stress and underlying stroma. Both vary between regions of the circulation and in disease. Increasing steady laminar shear stress progressively down-regulates the response to inflammatory stimuli. Thus, we found that conditioning EC by shear stress suppressed their recruitment of leukocytes in response to cytokines. In studies of stromal cells, co-culture of EC with fibroblasts or with mesenchymal stems cells (which are endogenous in tissues in small numbers), again down-regulated leukocyte recruitment. Such homeostatic regulation of the inflammatory response is disturbed in disease. Exposure to disturbed, oscillatory flow predisposes to the formation of atheroma at bifurcations in arteries. Indeed, oscillatory shear or sudden changes in shear may directly induce leukocyte adhesion. Disturbance of normal stroma, that occurs e.g., in rheumatoid arthritis or upon differentiation of MSC, leads to loss of protective effect or even a direct pro-inflammatory milieu. Thus, local environmental factors act as endogenous limiters of the inflammatory response. Their disturbance may be pathogenic, and understanding of the mechanisms may yield approaches to prevent or reverse chronic inflammation.