13th Conference of the European Society for Clinical Hemorheology (E.S.C.H.)

June 26th - 29th, 2005 - Siena, Italy

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ABSTRACT BOOK

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ABSTRACTS

LECTURES

FAHRAEUS LECTURE

HEMORHEOLOGY AND VASCULAR DISEASES: RED CELLS SHOULD RUB UP TO THE WALL, LEUCOCYTES SHOULD COPE WITH IT

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It was Alfred A Copley (1910-1992) who said that blood and vessel wall constitute a single organ, i.e. that the content influences the container. It was first thought that high blood viscosity and impaired cell deformability were the main pathological agents, but that is only true in "overt diseases" (examples: Waldenström, polycythaemia for viscosity and hypertension, drepanocytosis for rigid cells, etc). In "covert diseases", mainly vascular diseases, the actual factor is change in the rubbing function of blood, which "shears" the surface molecules as one shears; i.e.cut the wool off sheep and silenced lambs. Normally under the influence of the heart and the elasticity of the arteries, the layers of blood (laminar flow) develop a constant rubbing force, shear stress, constantly acting upon the internal layer of vessels, covered by active endothelial cells. Mechanobiology investigates receptors and transduction across wall cells, showing that thousands of genes are activated in the endothelium, leading to a lot of functions of adaptation. Pulsating and centrifugal forces allow shear to become often unsteady, as well as huge red cell aggregates. So atherosclerosis is due to disturbances of laminar flow in specific areas where low shear allows white cells to adhere and migrate and permeability to increase. Coronary disease, cerebral vascular accidents, aneurisms are thus geometrically local diseases, whose lesions are subsequently enhanced by risk factors. Chronic venous insufficiency is also related to physical forces: hydrostatic and centrifugal pressures, and disruption of shear stress along the wall and around vein valvulae. Here changes in shear and biochemical phenomena are the cause of wall remodelling and varicose. At the level of microcirculation the fall in shear stress induces hypoxia, accumulation of white cells and hemorheological disorders in microvenulae. This leads to lesions of tissue, small vessels and leg ulcers. Numerous treatments in vascular diseases tend to restore flow and blood shearing. However a better understanding in the future should open new therapeutic fields. Particularly genetic approaches could lead to the possibility of identifying patients, in certain families, exhibiting shear sensitivity related to gene polymorphisms.

L1

STUDIES ON BLOOD ELECTRORHEOLOGICAL PROPERTIES

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The phenomenon of electrorheology refers to changes in the rheological behavior due to imposition of electric field. The electrorheological (ER) properties of blood are determined by a variety of factors, contributing in a different way to the overall impedance of the flowing blood. Resulting spatial charge distribution on the interface of the suspended cells induces the electric potential playing a decisive role in determining cell rheological properties and cell interaction. The work identifies and quantifies ER properties of blood at different local structure of the flow field. A concurrent measurement system, using a Contraves Low Shear 30 rotational rheometer was used in the study. It includes a pair of platinum electrodes in cylindrical ring shape, embedded into the wall of a resin made replica of the Couette type flow chamber of the rheometer. The relationship between the blood capacitance and permittivity was studied in parallel with the changes in the rheological behaviour of the samples of whole human blood. The time variation of conductance at different flow regimes and dependence of the apparent viscosity on the hematocrit of whole human blood and plasma under electric field and without electric field were investigated. The results show that the blood conductance is strongly dependent on the considered blood factors. The important implication is that any applications using the blood conductance (the active main component of blood impedance) measurement should take into account the effect of hematocrit and plasma properties.

L2

GLYCATED PROTEINS PROVOKE AN ENDOTHELIAL CELL DYSFUNCTION

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The advanced glycation end products (AGEs) are a heterogeneous class of molecules, including the following main subgroups: bis(lysyl)imidazolium cross-links, hydroimidazolones, 3-deoxyglucosone derivatives, and monolysyl adducts. The Maillard reaction begins with the reaction of the carbonyl (aldehyde or ketone) of the reducing sugar with the aminogroup of the biomolecule. Glycation adducts can also be formed by the reaction of proteins with glyoxal or methylglyoxal and other saccharide derivatives. AGEs are increased in diabetes, renal failure, and aging. Microvascular lesions correlate with the accumulation of AGEs, as demonstrated in diabetic retinopathy or renal glomerulosclerosis. On endothelial cells, ligation of receptor for AGE (RAGE) by AGEs induces the expression of vascular cell adhesion molecules (VCAM), tissue factor, cytokines such as interleukin-6, and monocyte chemoattractant protein-1. A chief means by which AGEs via RAGE exert their effects is by generation of reactive oxygen species, at least in part via stimulation of NADPH oxidase. Diabetes-associated vascular hyperpermeability in vivo can be prevented by blockade of RAGE. Reduced angiogenesis observed in diabetes mellitus may be a consequence of growth factor and matrice glycation. Thus, agents that limit AGE formation, increase the catabolism of these species, or antagonize their binding to RAGE may provide new targets for vascular protection in diabetes.

L3

COMPUTERISED CARDIOGREEN PERFUSOGRAPHY – A TOOL ALLOWING TO QUANTIFY PERFUSION HOMOGENEITY IN PATIENTS

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Pragmatic Background: Clinical hemorheology has been become essential part of therapies in the surgical disciplines and intensive care medicine. Unfortunately, the early aspirations of therapeutic efficacy in chronic degenerative diseases treated in the "conservative" disciplines have not been corroborated, mostly due to lack of controlled studies. The extremely cost intensive biometrical strategies hitherto available make it unlike that the producers of equipment used for extracorporeal hemorheotherapy will be in a position to execute large scale prospective studies. Confronted with this situation at the beginning of the 21st century, the discipline has to develop strategies allowing to "reject" the null – hypothesis of "therapeutic inefficience" on the basis of small patients population. For this purpose, it is of prime interest to identify "non-responders" to demanding (and often invasive) therapeutic interventions associated with the application of extracorporeally applied procedures.

Theoretical Background: in focussing its therapeutic aims at disease states refractory to other means of treatment, it must be accepted that neither trivial concepts concerning "viscosity lowering" nor quantitation of "low flow states" will do justice to their dynamics. The presently known "extents" of rheological abnormalities in these disease states clearly indicates that these can at best be classified as "co-causes" in perfusion deficits well known for their marked spatio-temporal non-homogeneity, stasis manifesting itself in close proximity to normal perfusion. For many decades, our group has attempted to develop techniques allowing to objectively monitor this abnormality in human patients in non-invasive fashion.

Practical Consequences: using the albumin-cardiogreen complex as fluorescence indicator, the kinematics of its entry-passage-exit can be objectively documented by high resolution video-densitometry: In applying computer based pixel-by pixel densitometry, the "geographical" distribution of indicator appearance-disappearance dynamics can be represented by false-colour diagrams, A reliable "parameter" is the time after injection at which the intensities of each of 25 600 pixels exceeds a predetermined "threshold": the diagrams allow to identify areas of delayed and missing perfusion. Whenever this abnormality is tractable by rheoapheretic treatment, it is taken to represent a reversible state of non-perfusion likely to be of rheological origin (see Abstract KIRSCHKAMP). Mathematical strategies allowing alpha-numerical representation of the overall situation and be easily applied, e.g. by calculating the "coefficient of variation" (Standard Deviation/mean) of the entire spectrum of appearance times. With the help of this combination of strategies, we were able to confirm a frequently voiced – yet often refuted – assumption, namely that age related macular degeneration is closely related, often caused by reversible flow impediments in the choriodeal networks, most likely caused by obstruction by red cell aggregates and venular collapse.

Consequences for future Clinical Use: Computer based "geographic" scrutiny and the results of clearly rheological interventions not only corroborate time honoured – but hitherto speculative - pathophysiological concepts, but it can be used for the objective control of rheoapheretic therapies. It can be hoped that under its guidance, not only improved therapeutic strategies can be developed, but – perhaps more importantly – a technique has been made available that separates "responders" from "non-responders". Last but not least, in comparing the alpha-numerical representation of the homogeneity to objectively obtain in vitro data of blood rheology and results of clinical tests, a reliable foundation for trend-analysis as simplest procedure allowing to rejecting the null-hypothesis of non-efficacy is automatically made available to clinicians interested in the success/failure of a therapy.

L4

ISCHEMIA AND ISCHEMIC PRECONDITIONING: THE ENDOTHELIAL POINT OF VIEW

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Multiple lines of evidence emphasize the role of the vascular endothelium in regulating vasomotor, thrombotic and inflammatory mechanisms that are critical in the pathophysiology of tissue injury induced by ischemia and reperfusion (IR). Because of their location at the luminal surface of the vessel, endothelial cells appear to be more sensitive to IR than myocytes. Of importance, during ischemia, a reduced endothelial responsiveness to specific stimuli (a condition termed "endothelial dysfunction") precedes in time, and most importantly contributes to, the appearance of IR-induced tissue necrosis. As well, endothelial dysfunction appears to be the major determinant of the no-reflow phenomenon. Importantly, since 1986 studies have demonstrated that exposure to short-term periods of sublethal ischemia (i.e., ischemic preconditioning) can reduce tissue (as well as myocardial) IR-induced injury. Similarly, multiple studies have demonstrated that a protective phenotype analogous to ischemic preconditioning can be induced by administration of pharmacological stimuli such as adenosine, bradykinin, nitric oxide donors and opioids. Recent studies from our and other groups have demonstrated that a mechanism that is similar to ischemic preconditioning can be induced also at the level of the endothelium. Of importance, we and others were able to show preconditioning using multiple physical and pharmacological ("pharmacological preconditioning") stimuli. Such studies have shown that a crucial step in the complex molecular cascades triggered by these mediators, as well as by ischemic preconditioning, appears to be a free-radical dependent activation of K-ATP channels, and multiple lines of evidence have emphasized the role of endogenous (endothelial) and exogenous nitric oxide in the physiology of both ischemic and pharmacological preconditioning(15) via direct and cGMP-mediated opening of K-ATP channels.

L5

CUTANEOUS microCIRCULATION IN PATIENTS WITH CORONARY ARTERY DISEASE AND ERECTILE DYSFUNCTION

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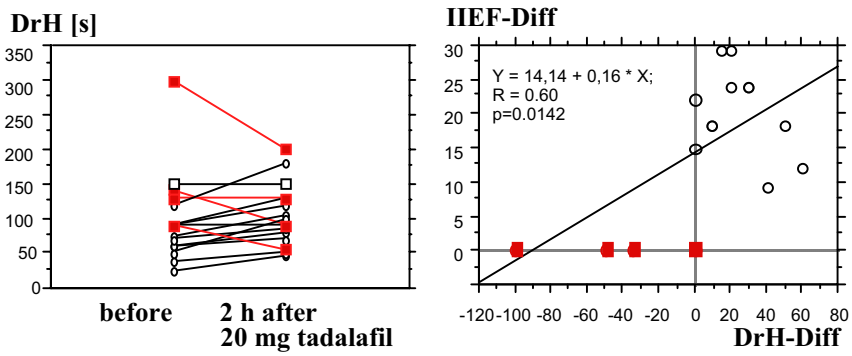
Many patients with cardovascular risk factors or atherosclerotic diseases suffer from erectile dysfunction (ED), so 50% of diabetics, 30% of hypertensives, and 40% of patients with coronary artery disease (CAD). On the other hand, careful studies have revealed that ED-patients suffer from diabetes mellitus (20.2%), from hypertension (41.2%), and from hyperlipidemia (42.4%). Therefore, the symptom ED can be effectively used as early marker of cardiovascular diseases. Because the same risk factors, which lead to ED also lead to cardiovascular diseases, ED and endothelial dysfunction have the same underlying pathophysiological mechanism. PDE5-inhibitors not only improve erectile dysfunction, but are also effective in the treatment of pulmonary hypertension, improve endothelial dysfunction in patients with heart failure, diabetes mellitus, and CAD, increase the coronary flow reserve and prolong the maximal physical activity duration in CAD patients, and increase the maximal oxygen uptake during exercise in heart failure patients. We, recently, studied in a propective, double-blind, placebo-controlled, randomized Cross-Over-trial the acute effect of 50 mg sildenafil on the cutaneous microcirculation in 20 CAD patients. The post ischemic maximal erythrocyte velocity in nailfold capillaries increased from 0.58±0.18 mm/sec to 0.85±0.4 mm/sec in the verum phase significantly (p= 0.0023), while there was no change in the placebo phase (0.59±0.18 to 0.61±0.18 mm/s; p=0.5248). The difference of peak velocities in the sildenafil group was 46.6% versus 4.7% in the placebo phase (p=0.0129). The standardized difference of 0.81 according to Cohen was biometrically relevant. In CAD patients, a single dose of 50 mg sildenafil increased the cutaneous erythrocyte velocity under resting conditions as well as post ischemic. Sildenafil seems not only to be effective in the therapy of impotence due to atherosclerosis, but also to improve the cutaneous microcirculation in CAD patients. In a second study we evaluated the postischemic reactive hyperemia of CAD patients simulateously suffering from ED before and 2 hours after 20 mg tadalafil, another PDE5-inhibitor with a half-life of 18.7 hours. 20 mg tadalafil improved ED significantly (International Index of Erectile Function, IIEF: 7.3±5.6 to 22.4±9.8; p<0.0001). There was a significant correlation between the improvement in microcirculation and the improvement of erectile dysfunction in these PDE5-inhibitors naive patients. Our data indicate that the effect of PDE5-inhibitor on ED in CAD patients can be predicted by studying the cutaneous microcirculation under PDE5-inhibitor influence. In a third study we currently proof the safety of a comedication with 20 mg tadalafil and 5 mg nitrendipin-acute (phiole) in CAD patients, who are under longterm therapy with various anti-hypertensive drugs.

Results: IIEF (International Index for Erectile Function) increased from 7.3±5.6 to 22.4±9.8 after 20 mg tadalafil significantly (p<0.0001). Four patients did not respond to tadalafil clinically (black rectangle in the figures) and presented a shortened or unchanged duration of reactive hyperemia (DrH) while the patients with clinically improved ED (open circles) showed an



increase in DrH. In 5 patients mild side effects were reported (3 back pain; 2 flush).

Conclusion: In ED patients with CAD 20 mg tadalafil improved ED significantly. Tadalafil was well tolerated without major side effects. Post ischemic reactive hyperemia measurement with and without tadalafil predicted the clinical efficacy of tadalafil in naive ED patients.

INTERNATIONAL MEDICAL CONSENSUS MEETING

VENO-ACTIVE DRUGS IN THE MANAGEMENT OF CHRONIC VENOUS DISEASE. AN INTERNATIONAL CLINICAL CONSENSUS

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The Siena International Consensus appears as the first medical consensus meeting, until now, dealing exclusively with the management of veno active drugs (VADs) in the course of chronic venous diseases (CVD). It will propose qualitative and quantitative rules to follow the prescription of VADs by phlebologists and medical practitioners as well. Over the last 50 years veno-active drugs (VADs), e.g. either synthetic products (calcium dobesilate) or plant extracts (coumarin, flavonoïds as oxerutins and diosmin, escin, ruscus extracts, proantocyanidines and Gingko biloba) have been used with great success to relieve pain in the legs of patients with CVD and can be considered as a simple satisfactory treatment. Besides other beneficial effects have been observed, both in patients presenting large varicose veins and even ulcers, as for the improvement of lesions and quality of life. As CVD appears more and more frequent and serious, particularly in western countries, there is a current need for consensus statements related to VADs. Questions have been raised relating to their nature, their precise pharmacological effects and finally their precise indication in the course of the disease. Another important question will remain their respective use facing other treatments, mainly compression therapy. In order to set up an attempt to provide prospects for regulation of VAD indications, a group of International Experts has studied the use of drugs and their actions. First the pharmacological properties of VADs have been studied. Secondly all clinical trials, meta analyses and specific trials have been selected out of a large amount of data, mainly found on Medline. Only works fitting evidence-based medical criteria have been taken into consideration. During the 13th European Conference on Clinical Hemorheology the usefulness of VADs will be submitted to a vote, as well as their indications relative to CEAP stages in patients. final recommendations will be elaborated.

Key words: calcium dobesilate, veno active drugs, chronic venous disease, consensus.

SYMPOSIA

S1.1

HEMORHEOLOGY AND HEMODYNAMICS: DOVE ANDARE?

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Relations between various hemorheological parameters and blood flow behavior in the cardiovascular system continue to be of interest to both basic science and clinical investigators. In general, experimental studies in this area tend to be of three types: 1) use of a vascular bed or an organ as an *in vivo* viscometer; 2) direct or indirect observation of blood distribution and flow dynamics; 3) in vitro rheological testing of blood from subjects whose clinical status may suggest altered blood rheology. The first type is perhaps best represented by the classic 1933 studies of Whittaker and Winton in which differences were noted between *in vivo* and *in vitro* "viscosity". Their publication prompted several studies to resolve this discrepancy, with subsequent reports suggesting the importance of inertial effects and/or the Fahraeus-Lindqvist Effect. General agreement appears to exist regarding the effects of hematocrit on *in vivo* flow resistance, whereas the effects of enhanced RBC aggregation have yet to be fully defined. In the second type, early reports by Knisely of "blood sludge" in the retina have lead to numerous studies in which the effects of various parameters (e.g., hematocrit, RBC aggregation) are observed and quantitated by direct microscopy, with tagged RBC or other tracers employed for indirect measurements of blood flow distribution. Again, literature reports are not always concordant, possibly due to heterophase or temporal effects associated with enhanced red cell aggregation. Clinical hemorheology studies represent the third type: the *in vitro* rheological characteristics of blood, RBC or WBC are determined, and correlations between these results and clinical findings are attempted. Literature reports of this type are numerous, with essentially every major clinical state investigated.

In spite of progress to date, several questions remain unresolved and thus serve as guides for future work. Some are mentioned here in the hope that this ISCH Symposium will elicit several more: a) Is "above normal" RBC aggregation always disadvantageous, and if so at what level?; b) Is plasma viscosity an independent factor, especially when high molecular weight polymers are used to study enhanced RBC aggregation?; c) Are the observed hemodynamic effects of altered blood rheology "specific" or are they indirect via affecting endothelial cell function?; d) To what extent are the observed effects (or lack of effects) of hemorheological changes such as enhanced RBC aggregation related to the existing vasodilatory reserve of the tissue or organ being studied?; e) Are the hemorheological abnormalities noted in several clinical states really involved in the pathology of the disease or are they epi-phenomena….do the rheologic parameters return to control levels consequent to successful therapy of the subject?

S1.2

HEMORHEOLOGICAL PARAMETERS AS DETERMINANTS OF MYOCARDIAL TISSUE HEMATOCRIT VALUES

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It is well known that the hematocrit in microvessels with diameters smaller than 1000 μm is lower than either venous and/or arterial hematocrit, thereby resulting in significantly lower mean hematocrit values for vessels perfusing a given tissue (tissue hematocrit). The mechanisms that underlie this reduction of microvascular hematocrit include axial migration, plasma skimming and Fahraeus effects. It has been previously demonstrated in rats that a linear gradient of hematocrit normally exists through the left ventricular myocardium, and that this gradient is sensitive to alterations of the rheological properties of the circulating blood. The gradient is abolished if the RBC in the perfusate were rigid; fibrinogen infusion and thus an increase of both plasma viscosity and RBC aggregation also affect this gradient. In a new series of studies, we have determined that enhanced RBC aggregation affects the myocardial hematocrit gradient *regardless* of alterations of plasma viscosity. Although the exact mechanisms responsible for the myocardial hematocrit gradient, as well as its physiological significance, are not yet clearly known, it is possible to speculate that increased local hematocrit resulting from elimination of the normal gradient could adversely affect perfusion of sub-endocardial layers of the myocardium.

S1.3

IMPROVEMENT OF TISSUE PERFUSION, BLOOD FLOW AND LOWERING OF BLOOD PRESSURE DUE TO INCREASED BLOOD AND PLASMA VISCOSITY

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Decreasing blood viscosity as a therapeutic procedure is one of the most deeply ingrained medical concepts, established since antiquity, and supported by most studies that link blood rheology and disease. Blood and plasma viscosity, however, are fundamental components of shear stress, the primary determinant of nitric oxide (NO) production by the endothelium. Consequently lowering or increasing viscosity should be in principle have no effect, due to the direct relation between shears

stress, NO production, vasoactivity, and vessel diameter dependant component of vascular resistance. It is well established that lowering blood viscosity by hemodilution is physiologically inconsequential up to about 1/3 of the decrease of blood viscosity due to lowered hematocrit, because of the compensatory increase in cardiac output, an effect is partially mediated by the decrease in NO scavenging due to lesser blood hemoglobin. Hemodilution is pathophysiological beyond this threshold, not due to the loss of oxygen carrying capacity, but because of the inability of the cardiovasculature to transmit sufficient central pressure to the microcirculation for maintain- ing functional capillary density (FCD), which is as critical for tissue survival as oxygen supply. In this situation increasing plasma viscosity with a viscogenic plasma expander restores FCD and normal physiological conditions up to reductions of hematocrit related oxygen carrying capacity of 75%. Increasing blood viscosity beyond normal by increasing hematocrit or other mechanisms is also considered to be potentially pathological. However, in vivo experiments in awake animals show that elevating hematocrit and blood viscosity up to 20 - 30% above baseline causes the decrease of peripheral vascular resistance and blood pressure. Restoring FCD by increasing plasma viscosity and increasing blood viscosity by increasing hematocrit, produce beneficial physiological effects due to mechano transduction in the endothelium, and the increase and/or maintenance in the production of NO due to increased and/or maintained shear stress. These findings provide the basis for understanding the action of diuretics in lowering blood pressure, the application of hyperviscous-hypertonic resuscitation in hemorrhagic shock, and the use of hyperviscous plasma expanders in extreme hemodilution to maintain tissue viability beyond the transfusion trigger.

S1.4

CEREBRAL HYPER- AND HYPOPERFUSION AND ITS LOCAL AND SYSTEMIC HEMORHEOLOGICAL EFFECTS IN A PORCINE MODEL

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Several studies deal with the local and systemic hemorheological alterations under or after ischemia-reperfusion and different hemodynamical conditions, giving valuable information on acutely changed flow properties of the affected and maybe of farther organs and tissues. In recent work we investigated, whether cerebral hyper- and hypoperfusion have an influence on certain hemorheological factors, and if so, in what scale. Nine pigs were anaesthetized, femoral artery and vein cannulation and tracheostomy was performed. A burr hole was prepared on frontal region of the skull and a cannula was inserted into the sinus sagittalis superior. The cerebral perfusion pressure (CPP) was monitored during the whole experiment. After stabilization, periods of controlled CPP increase (up to 170 mmHg, with i.v. noradrenalin) and CPP decrease (till 20-40 Hgmm, using i.v. esmolol) were achieved. Parallel blood samples were collected from femoral artery, femoral vein and sinus sagittalis superior after each period. Hematological parameters (by microcell counter), whole blood and plasma viscosity (by capillary viscosimeter) and erythrocyte deformability (using a bulk filtrometer) were determined. Leukocyte count slightly increased in each samples during the experiment, platelet count increased after hypertension and decreased by hypotension in a non-significant manner. Hematocrit (Hct) significantly increased in arterial and sinus samples by the end of hypertensive period versus baseline, accompanied by similar alterations in whole blood viscosity, which changes were significant after correction for 40% Hct. Plasma viscosity did not change. Erythrocyte deformability significantly worsened in arterial samples after both hypertension and hypotension, and in sinus samples it was impaired by the end of hypotension period versus baseline as well as and arterial and venous values. These results suggest, that cerebral hyperperfusion as well as cerebral hypoperfusion (below the lower autoregulation threshold) in non-injured brain are accompanied by local hematological and hemorheological changes**,** however, further experimental and clinical studies are needed, in the respect of both normal and increased intracranial pressure, too.

S2.1

VISCOSITY, HEMOSTASIS AND INFLAMMATION IN ATHEROSCLEROTIC HEART DISEASES

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Background: In atherosclerotic diseases vascular reserve is impaired and pressure gradient is decreased, therefore reduced blood fluidity can lead to tissue ischemia more rapidly. According to several investigations, classical risk factors are responsible only for 40% of all vascular events; thus research has been focused on the role of hemorheological and hemostaseological factors. In our previous investigations we demonstrated the deterioration of plasma and whole blood viscosities, and other rheological parameters in acute myocardial infarction, different stages of coronary artery disease, and percutaneous transluminal coronary angioplasty.

Methods: Hemorheological variables (plasma and whole blood viscosities, hematocrit, red blood cell aggregation), hemostaseological parameters (plasma fibrinogen and von Willebrand factor (vWf) level), and platelet aggregation were detected in our more recent studies in patients suffered from acute ischemic coronary syndromes (AICS), chronic ischemic heart disease (IHD), and diabetes mellitus. Common risk factors (e.g., lipid profile, smoking habits, glucose level, previous diseases) and medication were also recorded.

Results: Hemorheological variables, fibrinogen, and vWf level of patients with AICS and chronic IHD were significantly higher than those of control subjects ($p<0.01$). Beside the impaired hemorheological characteristics, diabetic patients also showed elevated level of vWf acvtivity, which turned to correlate with hemoglobin A1c concentration ($p<0.01$) rather than fasting glucose level. vWf activity was in positive correlation with the acute phase marker C-reactive protein ($p<0.05$). Effective antiplatelet treatment detected in platelet aggregometry was related to lower plasma fibrinogen concentration and red blood cell aggregation and was also associated with less recurrent vascular events in the follow-up period ($p<0.001$).

Summary: Our studies indicate the active role and interaction of hemorheological and hemostaseological factors in atherosclerotic heart diseases.

S2.2

CONTRIBUTION OF THE -455G/A POLYMORPHISM AT THE BETA FIBRINOGEN GENE AND LEIDEN MUTATION TO HEMORHEOLOGICAL PARAMETERS IN ISCHEMIC STROKE PATIENTS

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The concentration and structure of plasma fibrinogen (FIB) is an important factor in the coagulations cascade and also in the shaping of blood and plasma viscosity depending on both genetic and acquired factors. The -455G/A polymorphism of the -FIB gene is connected with the plasma concentration of FIB but the effect of the Leiden mutation for hemorheological parameters are unclear. It was investigated the prevalence of two genetic polymorphisms in healthy subjects and ischemic stroke patients and the effect for the concentration of the plasma FIB, whole blood and plasma viscosity as well. A total of 278 unreleated ischemic stroke patients and 173 control subjects were enrolled. Polymorphisms were detected by PCR. Plasma FIB concentration (Clauss), whole blood viscosity (90 sec^{-1} shear gradient) and plasma viscosity were measured. The above mentioned parameters were studied also in the subgroup of young(age<50 years) and a subgroup of non smoker patients.

Results: No significant difference was found in the prevalency in H2 alleles between controls and cases (OR:1.08 p=0.85). Similar results were found in conventional risk profile and in the prevalence of vascular diseases in family anamnese between the wild type and H2 genotype of patients. However, the plasma FIB concentration increased both in the total cohort ($p<0.05$) and in the non-smoker subgroup ($p<0.03$) of patients carried H2/H2 as compared to H1/H1 genotype. In spite of aequal hematocrit range, the whole blood ($p<0.03$) and plasma viscosity ($p<0.05$) were increased in the H2/H2 group as compared to other group carriing H1/H1 genotype. Leiden mutation prevalence showed an increased risk (OR:2.00 exact p value 0.08) in patients group as compared to controls. Positive correlation was found in patients with Leiden mutation compared to wild type with highest tertile in plasma viscosity in young patients (p=0.03), which was detectable also in the total group of patients (p=0.03) and in the subgroup of non smoking patients (p=0.05). Conclusions: The plasma FIB concentration depends on ?-FIB gene H2/H2 genotype and this phenomenon has an influence on the plasma viscosity not only in whole patient group but also in the non smoker subgroup of young patients. Leiden mutation can increas the plasma visosity as well. This effect is unfavorable for cerebral micorcirculation produced a prothrombotic state in the chronic state of ischemic stroke.

S2.3

STUDY ON THE HEMORHEOLOGICAL PARAMETERS OF OLDEST OLD RESIDENTS IN THE EAST-HUNGARIAN CITY, DEBRECEN

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Background and programme: According to the forecasts the population of Hungary will decrease by 2025 8,8 millions, by 2050 7,5 millions people. The greatest reduction is expected in the youngest age-group (between 0-14 years) and the most significant increase can be expected in the oldest age-group (over the 90 years). Dealing with this population should be one of the most important social and medical problems in our country and all over Europe. We started a research in September of 2004, under the title: "Complex study of Debrecen residents over 90 years old concerning their cognitive, social and health status." This project is supported by the Medical Research Council (No.: 296/2003). The aim of this study is to give up-to-date information to the social politics and to the medical services in connection with their duty towards the oldest old generation.

Methods of the research: We examine:

17 laboratory parameters

plasma-viscosity (Blood samples are collected into ethylendiamine-tetraacetic acid (EDTA) vacuum tubes, and the measurement is completed within 4 hours after the venipuncture. Plasma-viscosity is measured using a Haake-microviscosimeter)

Results and discussion: In a number of parameters (white blood cell, thrombocyte, GOT, blood sugar, creatinine, HDL-cholesterol, triglyceride, total protein, ɔ-GT, fibrinogen and TSH) the values were in the adult reference range. In spite of this fact, in some cases increasing (fibrinogen HDL-cholesterol) or decreasing (white blood cell, triglyceride, total protein) trends could be recognised as compared with the values found at the age-group (between 60 and 89 years). In some parameters, the values exceeded the adult reference range (collagen cross links, cholesterol, erythrocyte sedimentation rate /ESR/ and plasma viscosity), in other parameters the values decreased (red blood cell and hematocrit). The increase of plasma viscosity has been found at 64% of the residents (average: 1.45 mPas), at 36% the values were in the normal range (average: 1.30 mPas). The plasma viscosity has positive correlation with total protein, fibrinogen and ESR.

S2.4

HEMORHEOLOGICAL PARAMETERS AND AGING

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Background: There is increasing evidence that impaired hemorheological parameters are associated with increased risk of cerebro- and cardiovascular events. The aim of our present study was to examine the relationship of these parameters to the advancing age.

Methods: 6236 cerebro- and cardiovascular patients (3774 males, mean age 59.8 ∂ 13.2 years and 2462 females, mean age 60.9 ∂ 12.8 years) were included in this study. Males and females were divided into three groups, A < 45 years of age (young), B 45-65 years of age (middle-aged), C > 65 years of age (elderly). Hematocrit, fibrinogen, red blood cell aggregation, plasma and whole blood viscosity were determined. To exclude the effect of other parameters except for the increasing age, 623 patients (397 males and 226 females) with similar risk profile, medical history, and medication were selected from the study population for re-evaluation.

Results: All the measured hemorheological parameters significantly correlated with advancing age in the whole population, however, the correlation coefficient was very low. On the other hand, this positive correlation could be revealed only within the middle-aged group; there was not any correlation at young patients. Moreover, hematocrit, red blood cell aggregation, and whole blood viscosity were negatively correlated with age in elderly males ($p < 0.001$). In the homogenous population these parameters did not correlate with the advancing age.

Conclusion: A weak positive correlation of the hemorheological variables and the advancing age could be found in our study population that may be just statistically and not clinically significant. In the homogenous group, these parameters did not correlate with the advancing age. Our results suggest that the hemorheological alterations are independent of aging, increased values are not associated with the older age but the more frequently occurring diseases.

S2.5

HAEMATOCRIT AND BLOOD VISCOSITY RATIO INDICATES RHEOLOGICAL OXYGEN CARRYING CAPACITY AND OPTIMAL HAEMATOCRIT OF HUMAN BLOOD

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It has generally been accepted that a log-linear relationship exists between whole blood viscosity (WBV) and haematocrit (Hct) over the Hct range 0.20 to 0.60. The increase in WBV with increasing Hct is greater as the shear rate decreases. It also seems evident that the higher is the Hct/WBV ratio the higher is the rheological oxygen carrying capacity of the blood. We assumed that there must be an optimal Hct point where the Hct/WBV ratio is higher than at any other points on the Hct scale. Our aim was to examine what is the mathematical relationship between Hct and Hct/WBV assuming a log-linear plot between WBV and Hct. Five ml venous blood samples were drawn from 32 healthy control subjects (CS), from 52 hyperlipidaemic patients (HL) and from 117 Raynaud disease patients (RD) into heparinized tubes. Control subjects were the member of the laboratory staff and patients attended the out-patient clinic of the medical department. Hct was measured using automatic cell counter and WBV values were determined with Hevimet 40 capillary viscometer at 37.0 ºC. WBV values and Hct/WBV ratios were calculated at 3 different shear rates (10, 90 and 200 s^{-1}). Results were expressed as means±SD. The distribution of Hct/WBV ratios along with the Hct scale showed inverted U-shape curves in all 3 groups. The maximum Hct/WBV values of the curves were 10.9, 10.3 and 9.7 mPa^{-1} s^{-1} in CS, HL and RD groups, respectively at the shear rate of 200 s^{-1} ($p < 0.05$ between CS and RD). The peak values of Hct/WBV curves were found at different Hct points: 49%, 45% and 38% for CS, HL and RD ($p < 0.001$ for all pairs). The peak vales of Hct/WBV curves were significantly lower at lower shear rates (9.7, 9.2 and 8.5 at 90 s^{-1} and 5.8, 5.1 and 4.4 at 10 s^{-1} for CS, HL and RD, respectively, $p < 0.05$ between CS and RD for both sets).The differences in locations of peaks along the Hct scale were less marked at lower shear rates than at 200 s^{-1}. This the first report that indeed there is a rheologically optimal Hct (ROH) for a set of blood samples. ROH can be determined by the Hct/WBV ratio that is significantly different in CS and patients groups as well as the location of ROH also depends on the group's characteristics and shear rate applied. After this very first report on ROH further studies are warranted in order to determine clinical usefulness of Hct/WBV ratio.

S2.6

HEMOSTASEOLOGICAL AND RHEOLOGICAL PROPERTIES OF OVERWEIGHT AND OBESE PATIENTS

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Backgroud: It is well known that there is a more frequency of thrombo-embolic events in overweight and obese patients. Searching the causes the investigators suggested some pathological alterations of primary hemostasis and coagulation, but there are some contradictory data too. The frequency of these alterations is not well known.
Patients and methods: Our study was started from 01. 01. 2004 and was ended at 01. 12. 2004. We enrolled 98 consecutive patients. There were 22 overweight (BMI: 28,2±0,9 kg/m^2, mean age: 62,1±9,9 years), 51 moderate obese (BMI: 32,3±1,5 kg/m^2, mean age:60,1±13,7 years) and 25 serious obese patients (BMI: 38,9±4,2 kg/m^2 mean age: 56,8±11,4 years). In the controll group were 60 healthy persons whos have normal body weight (BMI<25 kg/m^2). We did the following blood tests: hemoglobin concentration, number of red blood cells, value of hematocrit, number of platelets, fibrinogen concentration, value of high sensitivity C reactive protein, whole blood and plasma viscosity (by Hevimet 40 viscosimeter), aggregation of platelets (by Born method, with ADP, collagen and epinephrine as inductor). We selected our patients into 2 groups too. In the first (35 patients) group were patients who received antiplatelet therapy, in the second one were the patients without antiplatelet therapy (63 patients).
Results: The hemoglobin concentration was pathological in 31,5% and the value of hematocrit was pathological in 8,6% in these patients. The value of high sensitivity C reactive protein was elevated in 54,3%, the fibrinogen concentration in 48,8%, the whole blood viscosity in 28,2% and the plasma viscosity in 43,8%. The cause of latter alteration mostly was the elevated fibrinogen concentration. The aggregation of platelets was enhanced in the group of obese patient versus healthy persons; p=0.00029 (ADP-5), p=0.0000042 (ADP-10), p=0,0094 (collagen) and p=0,0034 (epinephrine). In the non-treated patients we found enhanced platelet aggregation (value of maximal aggregation > mean+2 SD aggregation value of healthy people) in 17,4% (ADP-5), 38,1% (ADP-10), 19,0% (collagen), 44,4% (epinephrine) consecutively.
Summary: The results of our study suggested that the proportion of polycythemic patients was moderate higher in the group of our obese patients as in the Hungarian population. We found purposeful rheological alterations in the group of obese patients: elevated rate the abnormal fibrinogen concentration and whole blood and plasma viscosity. We detected enhanced platelet aggregation in this group of patients. Above mentioned facts with other rheological alterations maybe the cause of the elevated rate of thrombo-embolic events in the obese patients.
Key words: obesity, fibrinogen, plasma viscosity, platelet aggregation

S3.1

THE INFLUENCE OF VARIOUS RADIOGRAPHIC CONTRAST MEDIA ON MYOCARDIAL OXYGEN TENSION

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This study examined whether a one-time bolus injection of 10 ml of radio-graphic contrast medium (iopromide 370, iohexol 350, iodixanol 320 or iomeprol 350) into the left coronary artery affects the tissue oxygen tension in this artery's supply area as compared to a 0.9% sodium chloride (NaCl) bolus. The radiographic contrast media and NaCl boli were randomly assigned. The study was performed in 2 sets of 6 domestic pigs in two parts with identical sequential design. 241±44 s after the injection of the iopromide bolus, the myocardial oxygen tension in the supply area of the left coronary artery (^{pO2}LAD) had dropped by 44.2% from an initial 40.3±10.9 mmHg to 22.5±8.9 mmHg (p=0.0003). After 576±113.5 s, the pO_2 had returned to its initial value. 171.7±11.9 s after the injection of the iohexol bolus, the pO_2LAD of 34,5±14,6 mmHg had dropped by 14.8% to 29.4±13.9 mmHg (p=0.0003). After 321±47.1 s, the initial pO_2 was restored. The decline of the pO_2LAD after iopromide was significantly greater than after iohexol (p=0.0001), and the time required to return to the initial pO_2 was much longer (p=0.001). 26.7±16.4 s after the iodixanol injection, the pO_2LAD had declined by 3.5% from 42.2±5.6 mmHg to 40.7±5.9 mmHg (p=0.0357). After 53±16.7 s, the ini-tial value was restored. The pO_2LAD was 41.9±7.4 mmHg before the iomeprol injection. 303.3±58.9 s after the injection, the pO_2LAD had declined by 13.1% to 36.4±7.5 mmHg (p=0.0001). After 577±22 s, the initial value was restored. The bolus application of an isotonic NaCl solution resulted in no declining effect on the pO_2LAD. Immediately after the injection, it increased by a maximum of 3%. In the supply area of the right coronary artery and the peripheral skeletal muscle, no effect of the radiographic contrast media or the NaCl on tissue oxygen tension was observed. Furthermore, no effect on tissue temperature, heart rate, systolic and diastolic blood pressure or cardiac output per minute occurred. The injection of a radiographic contrast medium in a coronary artery can result in a significant local contrast medium-induced microcirculation disorder in this artery's supply area. The increased viscosity of a radiographic contrast medium leads to a very short-term insignificant effect on the microcirculation. Red blood cells can be affected by the osmolality of contrast media. A relevant microcirculation disorder can, however, occur if an additional rheological defect is triggered e.g., through echinocyte formation This is associated with a considerable erythrocyte rigidification and a consecutive obstruction of the capillary passage leading to a measurable microcirculation disorder.

Key words: radiographic contrast media, microcirculation, myocardium, tissue oxygen tension, iopromide, iohexol, iodixanol, iomeprol

S3.2

INFLUENCE OF EXTRA CORPOREAL CIRCULATION ON MYOCARDIAL OXYGEN TENSION: RESULTS OF AN ANIMAL MODEL

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Introduction: Experimental data have shown the potential risk of cellular damage of the myocardium during extra corporeal circulation (ECC). The influence of ECC on myocardial oxygen tension however remains unclear. Since the introduction of flexible pO_2 probes, it is possible to measure oxygen tension in a working muscle. Thus, the influence of ECC on the oxygen tension in a beating heart can be investigated.

Methods: In a pig animal model a flexible pO_2 microcatheter was positioned in the midmyocardium of the left ventricle and the skeletal muscle and pO_2 during ECC were monitored and compared with a control group without ECC.

Results: ECC and unloadment of the heart caused a significant higher increase of myocardial pO_2 than in a non ECC control group.

Conclusion: Our findings may support to explain that CABG involving the use of ECC is not inferior to off-pump surgery because the potential myocardial injury due to the pumping is probable not related to myocardial ischemia. On the contrary, myocardial pO_2 was even increased during extracorporeal circulation in this study.

S3.3

ASSOCIATION BETWEEN MYOCARDIAL OXYGEN TENSION AND HEART RATE

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Background: The influence of heart rate on cardiac output, oxygen consumption and myocardial activity has been widely investigated. However, the influence of heart rate on myocardial oxygen tension remains unclear. Since the introduction of flexible pO_2 probes to measure the oxygen tension in a working muscle it is possible to investigate the influence of heart rate on myocardial oxygen tension.
Methods: In a pig animal model a flexible pO_2 probe was positioned in the myocardium and the heart rate was varied via an external pacer. In these animals with a presumably normal vessel regulation the relation between heart rate and myocardial oxygen tension was measured.
Results: In this animal model an optimal oxygen tension was observed at 110 bpm. Within 2 minutes the myocardial oxygen tension adjusted to a change in heart rate. When the heart rate was reaching the level of a fibrillation oxygen tension dropped to zero. In case of a timely unloading of the heart via extracorporeal circulation, myocardial oxygen tension remained unchanged even in a fibrillating heart.
Conclusion: In young healthy pigs with a normal vessel regulation a significant correlation between myocardial oxygen tension and heart rate was observed. A maximum partial oxygen tension was observed at a heart rate of 109 bpm. Each change in heart rate resulted in a corresponding change of pO_2 within roughly 2 minutes. In a working, fully loaded heart fibrillation resulted in a partial oxygen tension of around zero and death of the animal.

S3.4

MYOCARDIAL OXYGEN TENSION DURING FATAL RIGHT HEART FAILURE

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The aim of this study was to evaluate the potential of PFC as blood substitute in case of experimentally-induced ischemia of the left heart ventricle. Myocardial ischemia was induced in pigs via balloon catheter in the left coronary artery. At the same time, a decrease in hematocrit was induced by an isovolumetric hemodilution using PFC as blood substitute. Radiographic contrast media were given to control the positioning of the balloon catheter. The myocardial oxygen tension before, during, and after the injection of both agents (radiographic contrast agent in combination with PFC) were measured. In the first animal the coloration of the skin took on a bluish note 9 minutes after the injection of PFC. The pig died under the clinical aspects of a right heart failure 20 minutes later. The same procedure was performed in a second pig and gave the same results. After these unexpected outcomes, the study was interrupted. The histopathological analysis gave clear evidence that a right heart failure caused the deaths of both pigs. Electron microscopic examinations showed that big particles of foreign body material obstructed capillaries of all organs analyzed, namely the heart, lung, liver, kidneys and spleen. These particles led to a rapid occlusion of pulmonary capillaries, which in turn induced pulmonary hypertension and consecutive fatal right heart failure. Oxygen transfer was remarkably reduced but oxygen consumption increased due to a higher work load of the heart. This mismatch finally resulted in a severe drop of pO2 to zero and finally the death of the animal. Evidently, greatest care has to be taken when PFCs are used in combination with radiographic contrast media. For the type of PFC and the contrast media used here, a pre-, intra- and post-operative contrast medium enhanced x-ray diagnostic must be carefully reflected. Therefore, the day before the use of this PFC, no radiographic contrast medium should be used, because the elimination of these media takes from 24 to 48 hours. Equally, after the use of these PFCs, the use of radiographic contrast media should be avoided for a certain period of time, depending on the type of PFC used.

S3.5

CHANGES IN PLATELET SURFACE-MARKER EXPRESSION DURING HEART SURGERY - COMPARISON OF TWO DIFFERENT HEART-LUNG-MACHINE SYSTEMS

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Background: Platelets contain and release a variety of receptor molecules and molecules which influence platelet adhesion and aggregation. They are also a source of coagulation factors as well as regulators of coagulation and fibrinolysis. Via different

surface molecules they interact with each other as well as different ligands from the vessel wall and the blood plasma. In the present study, the expression of platelet surface proteins was determined in order to compare the effects of two different heart-lung-machines (HLM) on platelet activation.
Methods: The study was performed as prospective, randomized, single-blinded study. Fourty patients were recruited into two groups according to the application of different HLM systems. A standard system (group 1) is compared with a modified HLM which is designed to minimize procoagulatory effects by using a Deltastream pump, surface-modified tubing, and a reduced priming volume (group 2). Blood was collected at different time points before, during, and after surgical intervention (CABG). Platelets were incubated with either CD42b-FITC-/ CD62P-PE- or Factor Va-FITC-/ Tissue Factor-PE-labeled antibodies. Analysis was performed using a FACSCalibur flowcytometric system.
Results: There was no significant change in the CD62P expression during CABG, and no difference among groups. The expression of CD42b decreased significantly at the end of the observation period in group 1 but not in group 2. Factor Va expression was significantly elevated in group 1 between the beginning and 10 min after the end of the surgical intervention, but without differences between groups. Tissue factor expression did not disclose any significant change or difference between groups. In contrast, the expression of Glycoprotein Ib-alpha, a subunit of the von Willebrandt-Receptor-Complex, decreased in group 2 at the end of CABG.
Conclusions: The results exclude a global platelet activation for both HLM systems, but the differences in the expression of Factor Va, CD42b, and Glycoprotein Ib-alpha indicate effects of the HLM modification.
Clinical Implications: Modifications in extracorporeal circulation setups that are in routine use in cardiac surgery influence platelet activation.

S3.6

RED BLOOD CELL AGGREGATION IN SURVIVORS OF ACUTE MYOCARDIAL INFARCTION. INFLUENCE OF PLASMA, ERYTHROCYTE FACTORS AND –455G/A POLYMORPHISM OF THE ß-FIBRINOGEN GENE

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Increased erythrocyte aggregation (EA) has been observed in patients with ischaemic heart disease (IHD), although most of these studies have been performed in the acute phase, when reactant proteins may account for this increase. Little is known about the role played by the erythrocyte itself in this aggregation process as well as about the influence of ß–fibrinogen polymorphisms on this rheological property. To ascertain the contribution of plasma, erythrocyte factors and –455G/A ß-fibrinogen polymorphism to EA in IHD, we investigated the following parameters in 78 survivors of acute myocardial infarction (AMI) and in a well-matched control group of 98 subjects: EA, glucose, total cholesterol (T-Chol), low-density lipoprotein-cholesterol (LDL-Chol), high-density lipoprotein–cholesterol (HDL-Chol), triglycerides, apolipoproteins A_1 and B, protein and functional fibrinogen, plasma sialic acid, membrane sialic acid, the cholesterol and phospholipid content of the erythrocyte membrane and the –455G/A polymorphism of the ß-fibrinogen gene. AMI survivors showed higher glucose ($p<0.001$), a borderline increase in triglycerides ($p= 0.043$), and a statistical decrease in Apo A_1 ($p= 0.003$) relative to controls. EA, functional fibrinogen, and plasma sialic acid were statistically higher in AMI survivors than in controls ($p= 0.001$; $p<0.001$; $p= 0.011$, respectively). Membrane sialic acid content was statistically lower in AMI patients than in controls ($p= 0.026$). No differences were observed in either membrane cholesterol or phospholipid content. No differences were observed regarding the GG or GA fibrinogen polymorphism. Multivariate logistic regression analysis, in which EA was dichotomized as higher or lower than 8.7, demonstrated that triglyceride levels higher than 175 mg/dL (OR= 7.7, $p= 0.001$) and functional fibrinogen levels higher than 320 mg/dL (OR= 3.7, $p= 0.004$) were independently associated with a greater risk of erythrocyte hyperaggregability. Our results suggest that the –455G/A polymorphism of the ß-fibrinogen gene does not seem to be associated with higher plasma fibrinogen concentrations or higher erythrocyte aggregation. Plasma lipids, predominantly triglycerides, and fibrinogen may not only enhance the development of ischaemic events by their recognized atherogenic mechanisms, but also by increasing EA.

S3.7

LEUKOCYTE RHEOLOGY IN STABLE AND UNSTABLE CORONARY ARTERY DISEASE

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Introduction: Leukocytes, in particular polymorphonuclear granulocytes (PMN) play an important role in coronary artery ischemia, especially during reperfusion, via the formation of free radicals, inflammatory mediators or occlusion of capillary networks.

Aim: The objective of this investigation was to investigate PMN rheology in coronary artery disease (CAD), including acute myocardial infarction (AMI) before any reperfusion therapy was commenced.
Methods: PMN filterability was measured after density separation using an oligo-pore filter system that allows measurement of single transit times. 93 patients and control subjects in five groups were studied: 1) 28 patients within 12 hours after the onset of AMI, before reperfusion therapy; 2) 18 with unstable angina pectoris (AP); 3) 13 with stable AP; 4) 13 age matched patients without CAD and 5) 21 healthy volunteers.
Results:Total leukocyte count (10^9/l) was increased in CAD, especially in AMI and unstable angina as compared to control (mean and 95% CI for groups 1 through 5: 12.6 (11.0-14.2), 11.3 (8.5-14.1), 8.5 (7.4-9.6), 8.0 (6.0-10.0), 7.0 (6.1-7.9)). PMN filterability was significantly increased in AMI; this was less pronounced in unstable and stable AP. The longest PMN transit times were found in the control groups. Mean transit times (ms) for groups 1 through 5 were, respectively: 13.6 (11.8-15.4), 16.9 (13.9-19.0), 16.9 (12.8-21.0), 22.0 (19.6-24.4) and 18.6 (15.7-21.5).
Conclusion: The increase of PMN filterability after AMI was unexpected. It may be due to the increased number of PMN after AMI and could indicate that these cells belong to a newly appearing population in the systemic circulation that does not (yet) contribute to microcirculatory disturbance. In comparison to findings from studies of reperfused AMI, this indicates a low level of PMN activation suggesting that a significant washout of activating mediators has not yet occurred. The potential left shift of PMN and a high turnover of WBC suggests that there may be a window for pharmacological interventions, aimed at suppressing leukocyte activation or bone marrow release, especially prior to, during and after reperfusion therapy.

S4.1

POLYMORPHONUCLEAR LEUKOCYTE INTEGRIN PROFILE IN DIABETES MELLITUS AT BASELINE AND AFTER IN VITRO ACTIVATION

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Previously we examined the polymorphonuclear leukocyte (PMN) membrane fluidity in diabetic subjects of type 1 and 2, not only at baseline but also after in vitro activation observing a different behaviour in comparison with control subjects. We recently directed our attention towards the evaluation of the leukocyte adhesion molecules and in particular of η_2-integrins, which are exclusively espressed on leukocytes, also considering that membrane fluidity influences molecular expression over the cell surface. Integrins are usually in a low-affinity state that makes them unable to interact with their corresponding ligands. Activation of integrins results in a conformational variation that modulates not only affinity but also avidity. Up to now in diabetes mellitus great attention has been focused on the soluble adhesion molecules, while data regarding the PMN integrin pattern are few. In this study we examined the PMN integrin pattern in 45 diabetic subjects without macrovascular complications, including 21 subjects with type 1 and 24 with type 2 diabetes mellitus. The PMN adhesion molecules (CD11a, CD11b, CD11c, CD18) were evaluated using indirect immunofluorescence and a flow cytometer, at baseline and after *in vitro* activation with 4-phorbol 12-myristate 13-acetate (PMA) and N-formyl-methionyl-leucyl-phenylalanine (fMLP). At baseline, in diabetic subjects the phenotypical expression of CD11a and CD11b was significantly reduced and CD11c was increased, whereas CD18 was unchanged in comparison with normals. Considering type 1 and 2 diabetic subjects separately, CD11a was reduced and CD11c was increased in both subgroups, CD11b was decreased only in type 1 diabetics and CD18, decreased in type 1, was increased in type 2 subjects. After activation with PMA and fMLP, in normal subjects we observed a significant increase of all PMN adhesion molecules whereas in diabetic subjects only CD11c increased significantly with both activating agents, and CD11b increased only after PMA activation. In type 1 diabetic subjects only CD11c expression was increased, and in type 2 diabetic subjects an increase of CD11b (with PMA) and an increase of CD11c (with fMLP) were noted. In conclusion, we found in diabetic subjects of type 1 and 2 an altered behaviour pattern of PMN integrins both at baseline and, in particular, after *in vitro* activation. These data may help in explaining the role of PMN in the evolution of diabetic vascular complications.

S4.2

INFLUENCE OF LIPIDS AND OBESITY ON HEMORHEOLOGICAL PARAMETERS IN PATIENTS WITH DEEP VEIN THROMBOSIS

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It is not well established whether hemorheological alterations constitute independent risk factors for deep vein thrombosis (DVT). We have determined in 149 DVT patients (86 male, 63 female) aged 42±12 years and in 185 control subjects without a previous history of DVT (89 male, 96 female) aged 42±13 years the hemorreological profile: blood viscosity (BV), plasma viscosity (PV), fibrinogen (Fbg), erythrocyte aggregation (EA), erythrocyte deformability (EEI) and plasma lipids. In the unadjusted mean comparison, DVT patients showed higher BMI, Fbg, PV, EA and triglycerides (TG) (p= 0,001), lower HDL-cholesterol (p= 0,010) and higher apo B (p=0,016). No differences in WB and EEI values were observed. After multivariate adjustment for BMI, TG, Fbg, Apo B and HDL-Chol, PV and EA were not statistically different, and BMI was the only parameter who remained statistically significant (p<0,001), between the two groups. When the above mentioned variables were dichotomized according to their cut-off points which correspond to the mean plus one SD of the control group, a multivariate logistic repression analysis demonstrated that Fbg higher than 320 mg/dL (OR= 3,2; p= 0.004); TG higher than 175 mg/dL (OR= 2.7; p= 0.008) and BMI higher than 30 kg/m^2 (OR= 3.6; p= 0.003) respectively, were independently associated with a greater risk of DVT. The OR associated to BMI> 30 kg/m^2 without adjusting for confounders was 4.6 (95% CI; 2.2-9.5) decreasing when controlled for the mentioned confounders. Therefore both TG and Fb, constitute independent risk factors for DVT independently of obesity, but obesity shows the highest OR. Our results suggest that one of the mechanism by which obesity, hipertriglyceridemia and hiperfibrinogenemia may contribute to increase de thrombotic risk could in part mediated through enhancing EA and PV, thus altering blood flow conditions.

S4.3

INCREASED ADHESION OF ERYTHROCYTES FROM PATIENTS WITH POLYCYTHEMIA VERA IS MEDIATED BY Lu/B-CAM

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Polycythemia vera (PV) is associated to a risk of vascular thrombosis which is related to red blood cell (RBC) abnormalities and platelet activation. We have examined the adhesion of erythrocytes to endothelial cells in culture using a radiometric technique and a flow system connected to a computerized system. We have explored adhesion molecule expression on RBCs in 26 untreated patients with PV using specific antibodies (anti-CD36, CD49d, LW, Lu/B-CAM, CD147, CD47) and flow cytometry analysis. PV RBC adhesion was found to be increased 3.33 folds (2-4.5) compared to normal RBCs ($p<0.001$). The extent of adhesion was correlated to Lu/B-CAM expression on RBCs ($p<0.001$) and was inhibited by monoclonal or polyclonal anti-Lu/B-CAM antibodies. In addition soluble Lu/B-CAM recombinant molecule reduced significantly the adhesion. The increase in adhesion was apparently due to a limited population of RBCs which cannot be detached from endothelium by a shear stress above 2Pa. This abnormal RBC adhesion can be one of the factors responsible for the higher frequency of thrombosis in patients with PV.

S4.4

RED BLOOD CELL AGGREGATION AS AN INDEX OF METABOLIC BALANCE

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The red blood cell (RBC) rouleau formation can decrease a total surface area of the red cells and reduce their glucose consumption. This metabolic change can redistribute the glucose from blood plasma to other body cells. The different red blood cell aggregation (RBCA) was found under various diseases. Moreover the size of the aggregates was proportional to the disease severity (arterial hypertension, CVD, diabetes, heart failure, solid cancers). Our data shown clearly that there are two groups among patients: 1) with initially low RBCA and 2) with initially high RBCA. It has been shown that RBCA was increased after the drug treatments in the group with low aggregation and was decreased in another one. We investigated (*in vitro* study) the effect on RBCA several groups of the drugs and it was found that some of them stimulate aggregation and others – inhibit it. A significant aggregation increasing effect (by 90-150%, $P<0.01$) was shown: in agonists of adrenergic receptors – adrenalin, noradrenalin, phenylephrine, clonidine (mainly - ζ-agonists in concentration of 10^{-5} – 10^{-8} M), thrombin, dexamethason, prostaglandin $F_{2\zeta}$, digoxin, furosemid, inosine, iodoacetamide. On the other hand the drugs (in 10^{-5} – 10^{-9} M): drotaverine, verapamil, PGE_1, euphillin, papaverine, pentoxifylline, insulin, espa-lipon, glucose (glucose: from 3.0 – 5.0 mM) decreased RBCA significantly (by 40-65%, $P<0.01$). The probable intracellular RBCA stimulating mechanism is connected with intracellular Ca^{2+} change. The calcium ionophore A23187 (5.0^{-6} M) increased RBCA by 160%. While the Ca^{2+}channel blockage with verapamil (10^{-5} M) or calcium chelation with EGTA (1.0 mM) was accompanied by a marked aggregation decrease. The same reducing RBCA effect was found when we used "tandems": *verapamil+adrenaline* and *EGTA + adrenaline*. The reduce of RBCA may be connected with an activation of adenylate cyclase. A significant red cell aggregation decrease by 50% ($P<0.01$) was found after incubation RBCs with stable analog of cAMP (dB-cAMP, 50^{-6} M). The similar effect was obtained under cell incubation with the inhibitors of phosphodiesterase – papaverine (10^{-3} M) and drotaverine (10^{-6} M). It is important to note that the addition of glucose (5 mM) in incubation medium led to very significant decrease of RBCA. Thus obtained data make us to suppose: 1) red blood cell aggregation is actively changeable process; 2) The certain level of red cell aggregation corresponds to the organism conditions. In this context the red cell aggregation change it might be considered as an index of metabolic balance in the big cellular population; in red blood cells.

S4.5

EFFECT OF ESPA-LIPON ON MICRORHEOLOGICAL PROPERTIES OF RED BLOOD CELLS IN DIABETIC PATIENTS

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Hemorheological changes associated with diabetes mellitus are widely recognized. Drugs used to treat diabetes may have positive effects on hemorheological parameters. This study was designed to investigate *in vitro* effect of Espa-lipon (alpha-lipoic acid) on the red blood cell macro and microrheological properties. We have evaluated several hemorheological parameters in adult patients with diabetes (n=16), in patients with coronary heart disease and arterial hypertension (n=14) and in healthy individuals (control; n=6). The following hemorheological variables were determined: hematocrit (Hct), plasma viscosity, whole blood viscosity and red cell suspension viscosity (Hct=40%), red blood cell aggregation (RBCA). Blood

viscosity, at both native and at 40% hematocrit, and plasma viscosity were determined at 37 ∀C using a capillary viscometer; blood viscosity was measured at relatively high shear (i.e., >180 s^{-1}, termed BV_1) and at relatively low shear (i.e., <20 s^{-1}, termed BV_2). Red blood cell aggregation (RBCA) in native plasma was assessed by direct microscopic method with computer image analysis. It have been obtained the following aggregation parameters: aggregation rate, the number of erythrocytes per aggregate and aggregation index. To study an *in vitro* effect of alpha-lipoic acid RBCs after 3 washing sessions (in PBS) were incubated for 15 minutes with the above mentioned drug at 37ºC, after which we measured viscosity of this 40% hematocrit suspension at high and low shear and RBCA. Alpha-lipoic acid had no effect on red cell suspension viscosity. While RBCA aggregation was changed significantly under the influence of the drug: aggregation index was decreased by 63% in the group of diabetes patients, by 54% in the group of coronary heart disease and hypertension patients and by 28% in the group of healthy individuals ($p<0.05$ for all groups). Number of erythrocytes per aggregate and aggregation rate were not changed significantly. Thus obtained data showed that the incubation of RBCA with alpha-lipoic acid led to a significant decrease in RBCA *in vitro*. However the effect of alpha-lipoic acid *in vivo* needs to be studied.

S4.6

THE HEMORHEOLOGICAL ASPECTS OF THE METABOLIC SYNDROME ARE A COMBINATION OF SEPARATE EFFECTS OF INSULIN RESISTANCE, HYPERINSULINEMIA AND ADIPOSITY

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The metabolic syndrome which is at high risk for diabetes and atherothrombosis is associated with hemorheologic abnormalities. Initially, insulin resistance was considered as the core of the syndrome. However, it becomes clear that the syndrome is a cluster in which the combined effects of obesity, insulin resistance, and hyperinsulinemia can be inconstantly associated, contributing to a various extent to a global impairment of blood rheology. We previously reported in 157 nondiabetic subjects that both obesity and insulin resistance increase red cell rigidity (Dintenfass's Tk) and plasma viscosity (ξ_p), and that whole blood viscosity at high shear rate (ξ_b1000 s^{-1}) reflects rather obesity than insulin resistance. In this study we aimed at defining the specific hemorheologic profile of insulin resistance and hyperinsulinemia by separating a sample of 81 subjects into 4 subgroups according to quartiles of insulin sensitivity (SI) (measured with the minimal model of an intravenous glucose tolerance test) and baseline insulin.

	highest quartile of SI (n=21)	two middles quartiles of SI (n=39)	low SI and normal insulinemia (n=13)	low SI and hyperinsulinemia (n=7)
ξ_b [1000 s^{-1} mPa.s]	2.65±0.08	2.81±0.07	2.97±0.07	3.06±0.07
Hct (%)	39.2±0.7	39.3±0.6	39.9±1.6	39.2±3
ξ_p mPa.s	1.31±0.02	1.38±0.02	1.37±0.02	1.55±0.04
Tk	0.62±0.02	0.62±0.01	0.65±0.02	0.57±0.04
M	5.3±0.5	5±0.3	7.7±0.9	6.9±1.3
M1	9±0.7	8.4±0.5	13.4±1.6	12.8±1.6

Results show that 1) high SI is associated with low ξ_b due to low ξ_p; 2) low SI regardless insulinemia is associated with increased aggregation indexes ; hyperinsulinemia does not further increase Tk and rather decreases it ; 4) neither SI nor insulinemia modify Hct. Thus hyperinsulinemia and insulin resistance induce hyperviscosity syndromes which are somewhat different, although they are associated most of the time. Low SI increases RBC aggregation while hyperinsulinemia increases ξ_p .

S5.1

DISPOSABLE RBC AGGREGOMETER WITH VIBRATION-INDUCED DISAGGREGATION MECHANISM

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The aggregation of red blood cells (RBCs) is a major determinant of blood flow resistance passing through various veins. Available techniques for RBC aggregation often adopt a rotational Couette-flow using a bob-and-cup system for disaggregating RBCs, which causes the system to be complex and washing required after each measurement. A laser reflection technique has been combined with a vibration-aided disaggregation mechanism, which shows significant advances in aggregometer design, operation and data analysis. The essential features of this design are in its simplicity and a disposable element that is in contact with the blood sample. Using extremely small quantities of blood, the RBCs subjected to vibrations can be quickly and completely disaggregated. This is followed by measuring the backscattered light intensity. The measurements with the present sensor were compared with those of a commercial aggregometer and a strong correlation was found between them. The newly-developed optical aggregometer can measure the RBC aggregability difference for various diseases with ease and accuracy.

S5.2

DEFORMABILITY DISTRIBUTION OF RED CELLS AND THEIR ORIENTATION IN SHEAR FLOW FIELD MEASURED WITH THE AUTOMATED RHEOSCOPE AND CELL ANALYZER (ARCA) EQUIPPED WITH LINKAM CSS

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For routine measurements of both mean red cell deformability and red cell aggregation indices we have developed the Laser-associated Optical Rotational Cell Analyzer (LORCA) which is in use now in many laboratories worldwide {Hardeman M.R. et al Clin. Hemorheol. 14 (1994) 605-618}. In several clinical situations, however, e.g. sickle cell anemia, malaria tropica and treatment with erythropoietin (EPO), the knowledge of the distribution of Red Cell Deformabilities, i.e. the existence of Red Cell subpopulations, is of additional diagnostic importance .The Automated Rheoscope and Cell Analyzer (ARCA), especially developed for this purpose, has been described previously {Dobbe, J.G.G. et al Clin.Cytometry 50 (2002) 313-325}. With the same instrument the orientation of red cells in a flow field (e.g. in elliptocytosis) could be measured. Recently, we were able to incorporate the LINKAM CSS450* plate-plate shearing device. Due to the outstanding small rotational swing (single rotating plate:±2 σm) it was justified to reduce the gap to 30-40 σm maintaining the shear stress variation within acceptable limits (±7.0%). It has been shown that with this smaller gap more cells are in focus and less cells are overlapping. Therefore the performance of image analysis was improved and analysis time reduced. The effect of the following experimental conditions are shown: heat treatment, density gradient centrifugation , known mixtures of rigidified and fresh cells and aging of red cell concentrates under blood bank conditions. Apart from the confirmation of the effect of several clinical conditions described previously (use of EPO during dialysis, elliptocytosis, malaria tropica), new results e.g. sickle cell anemia have demonstrated the LINKAM-ARCA combination to be a rheoscopic analyzer with great potential. The recent introduction of a simplified lens system, in stead of a microscope, resulted in a practical, compact and self-contained instrument.

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S5.3

GUIDELINES FOR THE STANDARDIZATION IN HEMORHEOLOGICAL STUDIES

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An Expert Panel on Blood Rheology formed by the International Committee for Standardization in Hematology set the guidelines for the measurement of some hemorheological parameters in 1985. These guidelines included measurement of viscosity (plasma and whole blood) and assessment of red blood cell (RBC) deformability. New techniques and instruments have been developed since 1985 and thus there is need for the revision of these *recommendations for standardization*. The new set of guidelines should include: 1) techniques for measurement of RBC deformability and aggregation as well as plasma and whole blood viscosity; 2) measurement of white blood cell rheology; 3) sampling and handling of blood samples; 4) recommendations on statistical aspects of hemorheological studies. An international panel formed under the auspices of the International-European Societies for Clinical Hemorheology should work for the establishment of these guidelines.

S5.4

A MULTIGATE DOPPLER SYSTEM FOR INTEGRATED EVALUATION OF BLOOD FLOW VELOCITY PROFILE AND WALL DISTENSION IN HUMAN LARGE ARTERIES

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Background: Arterial Wall Shear Rate (WSR) is an hemorheologic variable modulating endothelial function and vasomotor tone. A multigate Doppler (MGD) system capable to provide an accurate estimation of spectral Doppler components within human arteries and real-time monitoring of WSR has been developed by the Florence group and recently implemented with the facility of estimating arterial diameter and distension in real-time.
Aim of the study was to validate “in vitro” and “in vivo” lumen diameter measurements and distension estimates, with the goal to integrate arterial flow velocity profile and distension measurements for clinical use.
Methods: The system tested consists of an ultrasound front-end (Megas, Esaote, Florence), and a digital signal processing board interfaced to a host PC. Echo signals backscattered from 128 range gates located along the axis of the interrogating beam are acquired from color Doppler overimposed to M-mode. Signals generated from walls and red blood cells are independently processed in real-time. Wall displacement is detected through a modified autocorrelation algorithm, while blood velocity profile is obtained through spectral analysis of signals backscattered by red blood cells. In vitro validation was performed with a device generating a controlled sinusoidal displacement (400 σm) of a plexiglass reflector. In vivo validation was performed by comparing estimates of diameter (D) and systo-diastolic diameter changes (DC) of common carotid artery (CCA) obtained by MGD against a reference system for arterial wall tracking (WTS, Pie Medical, Maastricht) (25 CCA in 17 subjects). Intra- and interobserver variability of measurements with MGD were also evaluated.
Results: In vitro, the error of estimate of wall displacement was always <5% with both systems.
In vivo estimates: Average vessel D was 7.05∂0.8 mm with MGD and 7.16∂1.1 mm with WTS, and DC was 537∂221 σm and 549∂249 σm. D and DC values with the two techniques were tightly correlated to each other ($r = 0.84$ and 0.87, $p<0.001$). Mean differences for D and DC obtained with MGD and WTS were 2.2∂588 and 12.2∂133 σm, with all points within ∂2SD (Bland-Altman analysis).
Reproducibility of MGD measurements: mean differences for D and DC estimates within observer were 170∂640 and 45∂197 σm. Results for interobserver variability were 90∂540 and 41∂194 σm.
Conclusion: A novel US system for measurements of wall displacements in large arteries is presented. Accuracy in the estimates of arterial diameter and distension is comparable to that of reference techniques. Integration with real-time detection of blood velocity profiles may open new perspectives for clinical studies on large artery mechanics.

S5.5

BIOMECHANOPHARMACOLOGY—THE NEW APPROACH FOR STUDYING DRUG ACTION ON INTERVENTION IN ENDOTHELIAL CEEL FUNCTIONS

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The interactions among blood, blood vessel and blood flow will maintain physiological function of blood circulation. However, in abnormal conditions, it may lead to pathological accidents. Biomechanical forces play a central role in the interactions. We believe that research on relations among blood shear stress, secretion of endothelial cell, drug dosage and pharmacodynamics will be the growing point of the new discipline of biomechanopharmacology. Susceptibility to atherosclerosis and stenosis of arteries will lead to abnormal high wall shear stress and shear induced platelet aggregation (SIPA) is likely to happen. Studies show that tetramethyphrazine (TMP) contained in *Ligusticum chuanxiong Hort*, salvianolic acid B (SAB) extracted from *Radix Salviae miltiorrhizae Bge* and diallyl trisulfide (DT) extracted from garlic inhibit SIPA significantly and in a dose dependent manner. TMP and SAB have additive effects on inhibition of SIPA. We studied the secretion of vWF of human umbilical vein endothelial cell. TMP and DT both showed inhibitive effect on vWF secretion. Preliminary clinical investigation showed that SIPA in coronary heart disease was significantly inhibited by TMP. We shall have to consider on the following issues: Should we accept SIPA test as a routine of medical check-ups, especially for the elderly? Should we use pharmacological means as a prevention of SIPA just as we take low-dose aspirin to combat thrombosis? Pharmaceutical goal is to gain homeostasis, the basic physiological concern. In biomechanopharmacology, drugs of protecting endothelial cells to maintain their normal function are expected. We studied influence TMP on apoptosis of cultured rat endothelial cell. The effects of TMP and shear stress were investigated by administration of the drug incorporated with different levels of shear stress. The results indicate that apoptosis may be restrained by a combination of medial level of shear stress with a suitable dose of TMP .Stimulators and inhibitors of angiogenesis are concerns for hopeful prevention and treatment of the life-threatening diseases, such as cardiovascular disease and cancer. To study the influences of shear stress, pressure and TMP on angiogenesis of vascular endothelial cell, cerebral microvascular endothelial cell of rat was pretreated in

a flow chamber with independent adjustment for levels of shear stress and pressure, and then 3D cultured on Matrigel. The results showed that among two combined conditions and the control there was significant difference in angiogenesis ($P<0.01$). The differences were also significant for the time sequence ($P<0.01$). It indicates that combined effects of shear stress, pressure and TMP may influence angiogenesis significantly.

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ORAL COMMUNICATIONS

C1.1

RHEOMETRICAL AND COMPUTATIONAL STUDIES OF BLOOD VISCOELASTICITY DURING COAGULATION

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The results of rheometrical studies of the viscoelastic properties of samples of whole human blood during coagulation can be appropriately modelled using new, modified forms of a viscoelastic nodal network model. The model, which is equivalent in terms of its representation in linear viscoelastic theory to the Gross-Marvin series-parallel model [1], embodies *distributions* of relaxation times and relaxation strengths, and is used in the present work to investigate the interdependence of the high-frequency and low-frequency features of the evolving relaxation time spectra associated with the coagulation of blood, in terms of the evolution of discrete, viscoelastic nodal networks. An analysis of the growth of the networks, in terms of their characteristic wavelengths, is shown to reveal interesting features of the underlying microstructure, and we discuss how the results provide a possible benchmark system for the development of advanced haemorheometrical devices.

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C1.2

THE LOSS OF ERYTHROCTE DEFORMABILITY UNDER OXIDATIVE STRESS IS CAUSED BY PROTEIN DEGRADATION RATHER THAN BY LIPID PEROXIDATION

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The main causative process for the loss of erythrocyte deformability under oxidative stress is poorly studied and still unclear. To investigate that, the effects of hydrogen peroxide (H_2O_2) (10 mM) and different antioxidants including ζ-Tocopherol (vitamin E), Butylated Hydroxytoluene (BHT), vitamin C, N-acetyl-L-cysteine, PNU-101033E and carbon monoxide (CO) gas on erythrocyte deformability, lipid peroxidation and protein degradation were studied *in vitro*. Our findings showed that exposure of erythrocytes to H_2O_2 caused loss of deformability, lipid peroxidation and protein degradation. However, pre-incubation of erythrocytes with vitamin E, BHT, vitamin C or PNU-101033E prevented the lipid peroxidation caused by H_2O_2, but did not prevent the loss of erythrocyte deformability or protein degradation. On the other hand, CO gas prevented both lipid peroxidation and protein degradation, but also prevented the loss of erythrocyte deformability. Vitamin C, unexpectedly caused a significant increase in the loss of erythrocyte deformability that was caused by H_2O_2 . Hence, protein degradation rather than lipid peroxidation could be responsible for the loss of erythrocyte deformability under oxidative stress. This study indicates that the development of drugs that prevent protein oxidation with consequent degradation would be of clinical value in conditions associated with oxidative stress pathologies.

C1.3

EFFECTS OF MACROMOLECULES AND THEIR APHERETIC ELIMINATION ON BLOOD RHEOLOGY: RHEOSPECIFITY OF RED BLOOD CELL (RBC) AGGREGATES DEPENDING ON THE STRENGTH OF AGGREGATING FORCES

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Objectives: The aim was to analyse the rheological properties of strongly aggregating RBC (inducible by alpha-2-macroglobulin) and moderately aggregating RBC (inducible by fibrinogen).

Methods: In vitro, the RBC aggregate geometry was determined for strong and moderate aggregation inducing macromolecules. The two types can be easily differentiated by applying the M and M' mode of the Myrenne aggregometer. In vivo, the flow behaviour of RBC aggregates was analysed by intravital microscopy: Using network scanning, the number of perfused and non perfused microvessels can be determinded.

Results: Higher adhesive forces of strongly aggregating RBC led to a higher deformation and packing density of single RBC within the aggregates. In vivo, only high aggregating RBC persisted in the precapillary bed and led to a non-attendance up to 40% of nutritive capillaries.
Conclusions:Our data support that procedures eliminating alpha-2-macroglobulin in the blood might provide more efficient improvement of overall blood fluidity in microvessels. The filtration procedure using the Diamed Rheopheresis technique has been successfully applied for this aim (s. accompanying abstract: Kirschkamp et al.: "pilot experiments of rheopheretic for age related macular degeneration).

C1.4

CORRELATION OF REDUCTION OF ERYTHROCYTE DEFORMABILITY WITH DIABETIC NEPHROPATHY

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Reduced deformability of red blood cells (RBCs) may play an important role on the pathogenesis of chronic vascular complications of diabetes mellitus and progression of renal failure. However, it has not been known that impaired red blood cell deformability is associated with development of diabetic mellitus and its complications. We studied 72 adult diabetic patients divided into three groups according to diabetic complications. Group I comprised 31 diabetic patients with normal renal function. Group II comprised 21 diabetic patients with chronic renal failure (CRF). Group III consisted of 20 diabetic subjects with end-stage renal disease (ESRD) on hemodialysis. According to the renal function for the diabetic groups, matched non-diabetic groups were served as control (n = 43, 59, and 21, respectively). Red blood cell deformability, measured by disposable laser-diffraction technique, is defined as the elongation index of erythrocyte suspension through a microfluidic channel flown by pressure difference in a physiologic buffer solution. We observed substantially impaired red blood cell deformability in those with normal renal function (group I) compared to non-diabetic control (P = 0.0005). As renal function decreases, an increased impairment in red blood cell deformability was found. Diabetic patients with chronic renal failure (group II) when compared to non-diabetic controls (CRF) had an apparently greater impairment in red blood cell deformability (P = 0.07). The non-diabetic cohort (CRF and ESRD), on the other hand, manifested significant impairment in red blood cell deformability compared to healthy control (P = 0.0001). It is worthy to note that there was a progressive increase in red blood cell deformability impairment, along with progression of renal insufficiency, and thus no significant difference in the degree of red blood cell deformability impairment was observed between diabetic and non-diabetic patients with ESRD (P= 0.66). In diabetic patients, early impairment in red blood cell deformability has been shown in patients with normal renal function, and progressive impairment in cell deformability is associated with renal function loss in all patients regardless of the presence or absence of diabetes.

C1.5

EFFECTS OF HEROIN AND METHADONE MAINTENANCE THERAPY ON BLOOD RHEOLOGY IN HEROIN ABUSERS

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The effects of opiate (heroin) and methadone maintenance therapy on heroin abusers' blood rheology were evaluated in this study. Their influence on the blood viscosity, rheological properties of erythrocytes, the red blood cell count (RBC), mean erythrocyte volume (MEV), hemoglobin (Hb), hematocrit (HCT), mean hemoglobin content of erythrocytes (MCHb), anysocytosis, poikylocytosis, erythrocytes' deformability etc. features of anemia were especially analyzed in chronic opioid users. A group of heroin addicts - active non-treated heroin abusers and heroin users taking methadone, has been compared with a control group of healthy subjects. The high prevalence of changes in erythrocyte morphology, HCT, Hb content and red blood cell deformability in intravenous drug users correlate positively with elevated blood viscosity, estimated by means of LS 30 Contraves rotational viscometer. The results obtained correlate with earlier data [1-3] indicating effects of opiates on blood rheology and erythrocyte opioid receptor levels, as well as higher urinary neopterin concentrations in intravenous drug users under adverse effects of heroin. The study aims also to discuss the question whether the methadone maintenance therapy is able to normalize rheology of heroin addicts. Our data suggest that further studies could be performed to evaluate whether methadone substitution should be useful in the treatment or prevention of hemorheological and blood cell abnormalities in intravenous drug users.
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C1.6

RHEOLOGICAL PROPERTIES OF BLOOD AND PARAMETERS OF PLATELETS AGGREGATION IN ARTERIAL HYPERTENSION

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The development of the diagnostic approach aimed at estimation of the degree of risk of the arterial hypertension (AH) development at the early stage of disease represents an urgent task of modern cardiology. The results of some researches specify the important role of blood rheological properties changes in AH pathogenesis. The goal of our study is to investigate the parameters of hemorheology and platelet aggregation in patients with various grade of AH. After clinical evaluation (medical history, physical examination, blood pressure monitoring, bicycle and information tests with electrocardiography control, echocardiography, blood biochemical and clinical analyses) in the study were included 27 patients with grade 1 hypertension (group 1; 42,3 $\pm$ 3,9 years) and 24 patients with grade 2 hypertension (group 2; 46,3 $\pm$ 3,7 years). In control group were included 29 healthy subjects (39,6 $\pm$ 4,1 years) without any anamnestic and physical data about cardiovascular and other organic diseases. Red blood cell (RBC) deformability was examined by measured time required for 0,5 ml 2% cell suspension to pass through pores of 3μm diameters. Rigidity index (RI) was used to estimate of the erythrocytes deformability. Red blood cell aggregation was assessed by measurement of erythrocyte sedimentation rate (ESR). Blood ($\dot{q}_{b1}$) and plasma ($\dot{q}_{p1}$) viscosity was determined by rotary viscometer at shear rates 20, 100 and 200 s^{-1} at t= 37^{o}C. Degree (D,%), rate (V, %/min), time (T, s) of ADP and adrenalin-induced aggregation of platelets were investigated with using photometer AP-2110. We observed that the rate of ADP (2,5 μM)-induced aggregation of platelets is increased ($p<0,05$) in the group 1 (52,3 $\pm$ 4,6%/min) compared to group 2 (41,3 $\pm$ 3,9%/min) and control (39,1 $\pm$ 3,7%/min). In group 2 RI of red blood cells was significantly higher compared to the group 1 and control (21,3 $\pm$ 1,98 r.u.; 17,4 $\pm$ 1,83 r.u. and 14,7 $\pm$ 1,48 r.u., respectively). Erythrocyte aggregation is increased in group 2 (39,7 $\pm$ 4,1 mm/2h) in comparison with group 1 (31,2 $\pm$ 2,8 mm/2h) and control (13,7 $\pm$ 1,5 mm/2h). The results obtained demonstrate that mild hypertension is associated with a rise of platelets agregability, but moderate hypertension is accompanied by the reduction of red blood cells deformability and increase of erythrocyte aggregation. These data may reflect differences of conditions for formation of microcirculation disorders in AH various grade.

C2.1

THE EFFECTS OF NATTOKINASE, A POTENT PRO-FIBRINOLYTIC ENZYME ON RED BLOOD CELL AGGREGATION AND WHOLE BLOOD VISCOSITY

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The vegetable cheese-like food, Natto is extremely popular in Japan with a history extending back over 1,000 years. It is produced from boiled soybeans by Bacillus Natto via fermentation processes. Besides its well-liked, characteristic flavor, Natto was traditionally utilized as a folk remedy for heart and vascular diseases, to relieve fatigue and to treat the symptoms of beri-beri. In 1980, Sumi extracted and purified a pro-fibrinolytic enzyme from Natto, called Nattokinase. This subtilisin-like serin protease is composed of 275 amino acid residues with the molecular weight of 27.7 kDa. In vitro and in vivo studies have consistently demonstrated the potent pro-fibrinolytic effect of the enzyme and have shown that it suppresses the intimal thickening following endothelial injury in animal models. Also, the oral administration of Nattokinase proved to be beneficial in the treatment of hypertension and was valuable in reducing the risk of thrombotic events among high-risk individuals during long-haul flights. However, no studies have evaluated the value of Nattokinase treatment on various hemorheological parameters to date. Our experiments were thus designed to assess the effects of the enzyme treatment on red blood cell aggregation and whole blood viscosity. Blood samples were obtained from healthy individuals into EDTA and were incubated with Nattokinase (final concentrations: 15.60, 31.25, 62.50 and 125μM) for 30 minutes at 37°C. Hematocrit was adjusted to 40% and RBC aggregation was measured using the Myrenne MA-1 aggregometer. Whole blood viscosity was assessed with a newly developed; computer controlled scanning capillary rheometer (Rheolog®) over the shear rate range of 1-1,000s^{-1}. Our in vitro results demonstrated a significant and dose-dependent decrease in RBC aggregation upon the enzyme treatment. Most importantly, this beneficial effect could be registered in concentrations similar to the serum levels achieved in previous in vivo animal trials. As expected, Nattokinase also evolved a remarkable effect on whole blood viscosity. Our preliminary data reveal the beneficial effects of in vitro Nattokinase treatment on red blood cell aggregation and whole blood viscosity for the first time. Further in vitro and in vivo experiments are essential to clarify the favorable value of this orally administrable, recently extracted and purified enzyme on various hemorheological parameters.

C2.2

RED BLOOD CELL DEFORMABILITY AND AGGREGATION BEHAVIOUR IN DIFFERENT ANIMAL SPECIES

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Comparative animal studies showed the wide variation of whole blood and plasma viscosity, and erythrocyte aggregation among mammalian species. Whole blood viscosity and red blood cell aggregation is influenced by red cell fluidity. To evaluate differences in erythrocyte deformability in mammals, three species were investigated, whose erythrocytes have a different aggregation property: horse, as a species with high, dog with medium, and sheep with almost immeasurable aggregation tendency. Erythrocyte deformability was tested ektazytometrically (Elongation Index (EI), LORCA, Mechatronics, Hoorn, Netherlands) at shear stresses from 0.30 to 53.06 Pa. Although it might be presumed from the aggregation property that horse had the highest EI among the three species, the EI of canine erythrocytes exceeded the value in horses by 10% at high shear stress. Further, equine erythrocytes started to deform at higher shear stresses (1.69 Pa) than did canine and ovine cells. At moderate shear stress (1-5 Pa) deformability was even higher in the sheep than in the horse. We conclude that erythrocyte elongation is different between the animal species, not clearly linked with the aggregation property, and that the degree of deformability at various shear stresses is species-specific.

Key words: Erythrocyte, deformability, fluidity, whole blood viscosity, aggregation, rheology

C2.3

THE IMPORTANCE OF CIRCADIAN RHYTHM ALTERATIONS IN ERYTHROCYTE DEFORMABILITY

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The hormone melatonin, secreted from the pineal gland at night and suppressed during the day, provides a circadian and seasonal signal to the organism. The impacts of pharmacological doses of melatonin on erythrocyte deformability were investigated by our group in several studies in both *in vitro* and *in vivo* conditions. However, the aim of this study was to investigate the effects of alterations in the physiological melatonin levels via the circadian rhythm on the erythrocyte deformability. 50 male rats weighing 250-300g were used in 5 groups. The rats were subjected to 12/12, 24/0, 0/24, 16/8 and

8/16 h of Light/Dark (L/D) cycle, respectively. The elongation indexes (EI) of the erythrocytes were measured by a laser diffractometer (Myrenne Rheodyne SSD) by using 30 µl of whole blood suspended in 2 ml of Dextrane 60.
There was not any significant difference in the EI of the 24/0 h L/D group compared to the control (12/12), whereas the decrease of EI was statistically important in the 0/24h L/D group (p=0.009). This decrease in EI was also significant when this group was compared to the 24/0 h L/D group (p=0.05). Furthermore, the EI was affected significantly from the alterations of the circadian rhythm, compared to the control (16/8, 8/16h L/D; p=0.05 and p=0.007, respectively). As a result, the alterations in physiological melatonin levels via different circadian rhythms have significant impacts on the deformability of erythrocytes, which therefore may cause important cardiovascular implications in the people who are exposed to different light dark cycles. Furthermore, these data represents a new and a quite crucial open-field to be investigated and taken into account in *in vivo* hemorheological studies.

C2.4

THE RED BLOOD CELL SURFACE ACETYLCHOLINESTERASE SUCH AS AN HEMORHEOLOGICAL PATTERN DURING GLAUCOMA TREATMENT.

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Introduction: The normal red blood cell (RBC) deformability has a great value in the perfusion and the oxygenation of peripheral capillaries, especially in peculiar tissues, such as ocular (retina,optic nerve, etc.). There it is also the possibility to interfere with RBC deformability using some drugs influencing the amount of the intracytosolic calcium. Very important it could appear to be the influence of an increase of the RBC surface acetylcholinesterase (AchEs). The AchEs is an enzyme placed on the external surface of the RBC membrane. An increase of the AchEs induces an increase in cytosolic calcium in RBC and a following decrease of RBC deformability, with microcirculation blood flow impairment.
Aim: The aim of this paper was to investigate the possible influence on RBC deformability – via AchEs and intracytosolic calcium (Ca^{++}) level – of several group of drugs, used in the treatment of glaucoma evaluating the influence on AchEs and Ca^{++} of these drugs.
Materials and Methods: We studied 10 healthy control subjects: Group 1 (5 Males and 5 Females aged 42±5 years); 10 glaucomatous patients under therapy with ϐ-Blockers spot-on the eyes: Group 2 (5 Males and 5 Females aged 44±3 years); 10 glaucomatous patients under therapy with a carbonic anhydrase inhibitor (CAI) spot-on the eyes Group 3 (6 Males and 4 Females aged 45±6 years); 10 glaucomatous patients under therapy with prostaglandins (PG) spot-on the eyes Group 4 (5 Males and 5 Females aged 50±3 years). All subjects studied were no smokers and not affected by any metabolic or haematologic or cardiovascular disease. In these patients we evaluated the intracytosolic calcium and the surface acetylcholinesterase level. We considered the mean of three values of three evaluations relating these values with controls using the Student t-test and the linear regression for the statistical analysis.
Results and Conclusion: Our data have shown that it is possible to say that Prostaglandins and carbonic anhydrase inhibitors spot-on the eyes don't change significantly (p=n.s.) the surface acetylcholnesterase activity in glaucomatous patients, if compared to controls. On the other hand the ϐ-Blockers drugs spot-on the eyes in glaucomatous patients are able to induce a significant increase ($p<0,05$) of AchEs and so also an increase of intracytosolic calcium, decreasing finally the RBC deformability and therefore the tissue oxygen supply. So it could be possible to assert that CAI and PG drugs don't interfere significantly with the intraocular microcirculation and hemorheology, especially with the optic nerve blood flow.

C2.5

SELECTIVE RESPONSE OF THE DEGREE OF RBC AGGREGATION TO THE ACTION OF THE CATECHOLAMINES: EFFECT OF ABO BLOOD GROUPS

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The aggregation behavior of human red blood cells (RBC) plays very important role in maintenance of normal blood flow in the microcirculation and continues to be of physiology and clinical interest. More information is needed about hormonal regulation of the rheological blood properties under various physiological and pathological states. It is well known that adrenaline, noradrenaline and dopamine are the main hormones, which are responsible for the acute and chronicle stress adaptation. Catecholamines provide complex integrated metabolic response. The elevated levels of blood catecholamines are registered under intensive physical exercises, emotional stress. Local increase of catecholamines content takes place under cerebrovascular disorders. Our previous studies demonstrated individual differences in RBC aggregative response to catecholamines and alpha- and beta-adrenostimulation. The aim of this study was to investigate possible influence of ABO blood groups on erythrocyte aggregability under high level of catecholamines. Blood was drawn from healthy adult volunteers (men, aged 20±2 years). The following blood samples were studied: O (I) Rh+ (n=10), A(II) Rh+ (n=13), B (III) Rh+ (n=12)

and AB (IY) Rh+ (n=6). Adrenaline, noradrenaline, alpha- and beta-agonists of adrenoceptors (clonidine, phenylephrine and phenoterol) 10^{-6} M were used as aggregating agents. RBC were washed three times and incubated for 15 min at 37∀C with agonists or without ones (control). Incubation solutions were removed by centrifugation and erythrocytes were resuspended in autologous plasma at fixed low hematocrit (Ht=0.05%) for the aggregation measurements. RBCA process was examined by means of direct microscopic technique. The extent of aggregation was calculated as a ratio of a number of aggregates to nonaggregated erythrocytes. Aggregate morphology as well as RBC shape was registered. It was shown that degree of RBC aggregation under catecholamines action depends on the ABO blood group of individual. The more pronounced aggregative effect we registered among blood-group O samples (up to 109.8%, $p<0.01$). Alpha-agonists caused minimal effect on aggregation of erythrocytes having A blood group antigen (A(II) and AB(IY)). In turn, the proaggregative effect of beta-agonist was markedly less for erythrocytes with B antigen (B(III) and AB(IY)). The ABO antigens are carbohydrate structures on red cell glycoproteins and glycolipids. Prof. J. Koscielak considers erythrocyte carbohydrates as largely inert and its basic function as probably protective. So it becomes evident that erythrocyte aggregative properties depend on genetic factors.

C2.6

INHIBITING AND STIMULATING EFFECTS OF SOME DRUGS ON RED BLOOD CELL AGGREGATION

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It is well known that red blood cell aggregation (RBCA) can markedly change under various conditions: stress, physical activity, diseases. Since human erythrocyte membrane contains various types of receptors the aggregation change mechanisms can include *receptor-ligand interaction* and activation of the intracellular signaling pathways - *adenylat cyclase – cAMP* and *Ca^{2+} - calmodulin*. The present study was thus designed to explore the effects of some drugs and chemicals on red blood cell aggregation (catecholamines, prostaglandines, insulin, inosin, glucose, iodoacetamine, dB-cϕ∫ P, ionophore ϕ23187, digoxin). Venous blood samples were obtained from healthy adults. RBCs were separated from the blood by centrifugation at 1,400 g for 15 min and washed 3 times with phosphate buffered saline (PBS). The washed RBCs were then resuspended in PBS at a hematocrit (Hct) of 40%. In each of the research sessions RBC suspension were divided into two aliquots and exposed to: 1) one of the drugs in PBS at 37°C for 15 min; 2) The remaining aliquot (red cell suspension with PBS) was kept at 37°C for 15 min and served as the control. Following the treatment the cells were resuspended in autologous plasma at Hct of 0,5% and then used for aggregation measurement by direct microscopic methods. Alpha-and beta agonists of adrenergic receptors (AAR) (Adrenaline, noradrenaline, phenylephrine, clonidine, metaproterenol) were used in concentrations $10^{-5} – 10^{-8}$ M. Furosemide, inosin, digoxin, iodoacetamine, prostaglandines (PGE1, PGE2, PGF2ζ) – 10^{-8} M. Insulin – from 0.72^{-10} M to 0.72^{-12} M. All analyses were completed within 4 h after blood collection. RBCA was markedly ($P<0.01$) increased after incubation with AAR (from 20 to 100%). The drugs that inhibits glucose intracellular transport and utilization (digoxin, furosemide, inosin, iodoacetamid) stimulated RBCA too (from 44 to 98%; $P<0.01$). It was found that prostaglandin E_1 had very strong RBCA decrease effect (ţ <0.001). While prostaglandin $F_{2ζ}$ increased RBCA by 96% (ţ <0.01). It is important to note that the addition of glucose (5 mM) in incubation medium led to very significant decrease of RBCA. All of the three indexes of aggregation were changed by 24 –70% (ţ <0.001). The effect of insulin on RBCA was lowering and dose-dependant. The most profound red cell aggregation decrease was found under the lowest insulin concentration (0.72^{-12} M). The similar effect (50% of decrease) was obtained under incubation of red cells with stable analog of cAMP (dB-cAMP, 50 σM). The rise of intracellular Ca^{2+} with ionophore A23187 (5 σM) is accompanied by 160% of RBCA increase. Thus obtained data make us to conclude that: 1) red blood cell aggregation is active changeable process; 2) the change of RBCA is connected with the activation both *adenylat cyclase and Ca^{2+}* intracellular signaling pathways.

C2.7

EXERCISE, RED CELLS AND BLOOD DOPING; A CONCLUSIVE ROLE FOR LORCA?

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Red cells are indispensable to muscle oxygen transfer which, on its turn, is indispensable to perform exercise. Both the quantity and quality of red cells are of importance. It is obvious that more red cells and/or cells with a higher MCHC can bind more oxygen. Both aspects however, respectively increase blood viscosity (especially at low shear) and cell rigidity and thus could affect micro-circulatory blood flow negatively. Therefore, with respect to oxygen availability, an optimum hematocrit (Hct) value is established which, however, is directly shear rate dependant. Apart from biochemical characteristics (ATP, 2,3 DPG), mechanical aspects of red cells like their deformability and aggregation indices are of crucial importance for undisturbed oxygen transfer to the muscles and are included in many experimental studies regarding the effect of training, diet and other conditions on exercise performance. The LORCA is the only instrument that can be used for both red cell deformability and aggregation measurement. Meta analysis of the discrepancy found in the literature regarding the effect of

exercise on red cell deformability demonstrates the need for consensus on issues like the status of training, the exercise program and, most prominently, the laboratory measurement techniques.
A separate chapter in sports medicine is the illegal and dangerous use of erythropoietin (Epo), increasing the Hct due to a burst in red cell production. Using the LORCA we were able to demonstrate that use of Epo (in dialysis patients), accompanied by the production of young and flexible red cell population, causes a shift in the mean red cell deformability. During exercise, i.e. at high shear rates, maximal performance may undoubtedly be increased at these high Hct values, but blood viscosity increases exponentially both with decreased shear rate (after the race) and the prevailing high Hct resulting in a dangerous situation. In the continuing struggle against blood doping affecting physical performance, it is suggested to formulate a handicap value inversely related to the starting Hct value of the sportsman.

C2.8

IMPROVEMENT OF THE BLOOD RHEOLOGY PROPERTIES IN EXPERIMENTAL DIABETES BY IMIDAZOBENZIMIDAZOLE DERIVATIVE

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The present study was designed to work out an effective corrector of the hemorheological profile changes in experimental diabetes. Previously, hemorheological screening of 162 bi- and tricyclic benzimidazole and xantine derivatives has been performed. The most active substances were evaluated in class of N9-imidazobenzimidazoles, which activity exceed widely used drugs (pentoxifylline, gliclazide etc.). One of them - AKS-17 has been taken out for further examination. Diabetes was induced in rats by i.v. streptozotocin injection (45 mg/kg). Pathology was verified by changes (regarding to saline group) in body weight, water uptake and blood glucose level (exceed 17 mM/L). AKS-17 (5 mg/kg) or pentoxifylline (4 mg/kg) or saline (control groups – diabetic and healthy) intravenous injections had been performed on 56 day of the study. Blood samples were taken from abdominal aorta 2h after last injection under light ether anesthesia. Blood rheology properties were tested using rotational viscosimetry (shear rates from 3 to 300 sec^{-1}), RBC deformability was evaluated by micropore filtration system (5 μm) and viscosimetry assays, platelet aggregation (ADP 5 μM) was determined by light transmission aggregometer, coagulation parameters were evaluated by hemocoagulometer “SOLAR”, extent of hypotonic and acidic RBC hemolysis were also measured. Fluorescence of DSM+-labeled RBC suspensions were detected with a fluorescence spectrophotometer (Hitachi, Japan) at the excitation wavelength of 480 nm and emission – of 580 nm. Significant increase of blood viscosity at low and high shear-rates (19.3% at 300 and 50.1% - $3s^{-1}$), RBC aggregation index – 17.9% (associated with decrease of DSM+ fluorescence (23.8%) - negative surface charge decrease), increased RBC suspensions viscosity (43%) as well as decreased RBC filtration rate (58.3%), and osmotic fragility (62.96%) were evaluated in diabetic versus normal rats. Platelets aggregation parameters also significantly have changed (aggregation index increased in 79%), as well as hypercoagulable state was observed: plasma viscosity increased, which may be closely related to elevation in fibrinogen level (24.2%). AKS-17 and pentoxifylline reduced blood viscosity at high shear rates (8.6 and 6.1%, respectively) as well as at low shear rates (24.5% and 12.7%, respectively). AKS-17 decreased RBC aggregation index (16.5% versus 10.7% - pentoxifylline), RBC filtration rate (40.1% versus 22.4% - pentoxifylline), viscosity of RBC suspensions (16.1% versus 5.8% - pentoxifylline). Furthermore, they showed hypoglycemic activity (41.96% versus 21.05% - pentoxifylline). AKS-17 administration increased osmotic and acidic RBC fragility (exceed pentoxifylline in 13.2% and 10.4%, accordingly). AKS-17 significantly decreased platelets aggregation (exceed pentoxifylline in 24.6%) as well as improved hypercoagulable state. According to these data it was concluded, that AKS-17 may be useful for further pharmacological investigation.

C3.1

NATURAL ANTICOAGULANTS ARE POSITIVELY CORRELATED TO PLASMA VISCOSITY IN ATHLETES

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Our recent data have shown that sportsmen with higher plasma viscosity have higher levels of factor von Willebrand antigen (vWf) indicating activation of endothelial cell function and prothrombotic state. Natural anticoagulants (antithrombin III (ATIII), protein C (PS), protein S (PS)) also synthesized by endothelial cells play a pivotal role in prevention of thrombin formation and thrombosis. Higher plasma viscosity may play a cofactory role in activation coagulation and the thrombosis. The relationship between two systems (hemorheology and anticoagulant systems) was investigated in athletes with different levels of exercise performance. Blood viscosity, plasma viscosity were measured at high shear stress with a capillary viscometer in 27 male endurance sportsmen and 17 controls. Total cholesterol, HDL cholesterol, total protein and globulins in serum were measured. ATIII, PC, PS, vWf were assayed by the solid-phase enzyme immunoassay. Athletes (n=27) have higher levels of ATIII ($p<0.05$), higher total globulins ($p<0.05$) and lower LDL levels ($p<0.05$) compared to controls. We did not observe significant differences in plasma viscosity, PC, PC, plasminogen activity, however blood viscosity was lower due to lower index Tk in athletes. Subdividing athletes in accordance with level of PWC170, it was found that levels of PS, PC, plasminogen activity, LDL, plasma viscosity were reduced (all $p<0.05$) in higher PWC170 group but PS, ATIII, plasma viscosity, plasminogen activity, vWf (all $p<0.05$), total proteins and globulins ($p<0.01$) were higher in lower PWC170 group compared to controls. In group of athletes index PWC_{170} correlated to PC (Spearman's $r=-0.59$, $p<0.01$) and PS ($r=-0.52$, $p<0.01$) and plasma viscosity ($r=-0.61$, $p<0.001$). PC and PS was also positively correlated with plasma viscosity ($r=0.46$, $p=0.02$ and $r=0.53$, $p<0.01$ respectively) and vWf ($p<0.04$, $p<0.06$ respectively). The positive correlations of the anticoagulants with vWf, a marker of endothelial cell activation, indicate on a significant role activation endothelial cell function in an increase anticoagulant defense in athletes. We suggest that impaired plasma fluidity in athletes with a lower PWC170 may be compensated by an increase of levels of natural anticoagulants (PS, PC). Therefore physiological meaning of the activation of endothelial cell functions in athletes with impaired exercise performance and plasma viscosity may consist in activation of release/synthesis of natural anticoagulants.

C3.2

POLYMORPHONUCLEAR LEUKOCYTE INTEGRIN PROFILE IN VASCULAR ATHEROSCLEROTIC DISEASE WITH AND WITHOUT TYPE 2 DIABETES MELLITUS

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The clinical course of atherosclerosis and diabetes mellitus is strongly influenced by a dysfunction of polymorphonuclear leukocytes (PMN) and we previously described the alterations of some rheological and metabolic PMN parameters, considered by us as markers of this dysfunction. Both leukocyte-endothelium and leukocyte-platelet interactions are involved in the triggering and progression of atherosclerosis and an acceleration of this process characterizes diabetes mellitus (DM). PMN interactions are mediated by integrins and, among them, $beta_2$ integrins are the most important. We examined the PMN integrin expression in subjects with chronic vascular atherosclerotic disease (VAD) and type 2 DM ($n = 21$; mean age 65.4±9.1 years) and in VAD subjects without type 2 DM ($n = 27$; mean age 60.6±7.9 years). We evaluated some integrins (CD11a, CD11b, CD11c, CD18) using flow cytofluorimetry, at baseline and after activation with 4-phorbol 12-myristate 13-acetate (PMA). At baseline VAD subjects with and without DM2 showed an increase in CD11a and CD18 and a significant decrease in CD11b and CD11c. In normal subjects, after activation, an increase in all adhesion molecules was observed; in VAD subjects without DM2 we noted a decrease of CD11a and CD18 and an increase of CD11b and CD11c, while in VAD subjects with DM2 we found a decrease of CD11a and an increase of CD11b and CD11c. In VAD subjects with and without DM2, the basal upregulation of CD11a and CD18 may be related to a PMN spontaneous activation, while the CD11b trend may depend on its self-consumption. The results of this study, besides showing an almost similar trend of the PMN integrin profile in the two groups of VAD subjects, add some elements to an aspect of VAD which has potential therapeutical implications. In animal models, the anti-integrin treatments have been investigated with successful results while, up to now, in humans the same treatments have been used, only in acute ischaemic conditions, without encouraging results.

C3.3

ABOUT THE MECHANISM PRODUCING REVERSIBLE HEMORHEOLOGICAL DISORDERS IN THE BLOOD CAPILLARIES

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Blood is not a real fluid when it is advancing inside the capillaries lumen. This is due to the fact that great part of the blood is made of the cells whose size is commensurable with the capillaries lumen. Therefore the regularities of the blood flow in the capillary cannels do not obey the rules of the fluid mechanics. This is even more pronounced when the blood cells undergo

pathological changes and disturb the blood rheological properties. Proceedings from the foregoing the blood fluidity disorders manifest themselves most obviously in the capillaries and the principal factor disturbing in these latters lumen is the red blood cell aggregation, which causes immediately the blood flow slowing down up to its full stop, i.e. the blood stasis inside their lumen despite a preserved arteriolar-venular pressure difference along their course. This is directly due to derangement of the normal microvascular blood flow structuring under these conditions. The RBC aggregation leads to immediate slowing down up to full stoppage of blood flow and development of the blood stasis in individual capillaries, their groups, or even in the whole microvascular networks. Therefore the RBC enhanced aggregation inside the capillaries should be considered as the most potent factor responsible for the blood rheological disorders in the microcirculation. The RBC aggregation is in turn dependent on the surrounding blood plasma factors, primarily the availability of high molecular weight proteins, such as the fibrinogen and partly globulins in it, which are readily changed under various pathological conditions. The disturbing effect of the RBC enhanced aggregation investigated in details inside the blood capillaries and the disturbing mechanism was appropriately analyzed microscopically under the in vivo conditions in individual microvessels where the anatomical and physiological specificities of the microvascular blood flow were under the strict researchers' control. The specificities of the development and elimination of the capillary stases is also a convenient model for an experimental analysis of the penumbra effects in the microcirculation, when reversible blood stases develop in the boundary region of the normal blood flow, where a slowing down, up to its full stop, develops in individual capillaries or in their groups. This seems to be the most feasible mechanism of the blood no-reflow phenomenon, which inevitably results in reversible or even irreversible microcirculation changes occurring especially in the brain tissue and disturbing the functional state of the cerebral tissue surrounding the individual microvessels.

C3.4

HEMORHEOLOGICAL RESPONSE TO PLASMAPHERESIS

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The general opinion is positive about discrete plasmapheresis (DPA) action onto blood rheological properties. However we have noted time negative hemorheological changes after the first sessions and it has demanded detailed hemorheological research (45 patients with enzymemia, MOF and shock; 3-5 sessions of DPA had to be executed as treatment-and-prophylactic with 1200-1500 ml plasma replacement). After the first DPA session environment media structure change was accompanied by deterioration of microrheological properties which were normalized (or showed the tendency to normalization) after the ending of all DPA course (after 3-5 sessions). To the same term blood viscosity and τ_0 showed improvement of intravascular bloodflow conditions. We conclude that after 1-st DPA session occur dis-coordinated changes which deteriorates microrheological characteristics compensated minimally by macrorheological changes. However adaptable processes result that erythrocytes properties come in conformity with these new conditions. Since this moment all hemorheological parameters become unidirectional and coordinated, and it is traced synergy their actions in the regulation of intravascular bloodfloow conditions. Last circumstance obviously causes the expressed total positive DPA influence on blood rheological properties.

C3.5

EFFECT OF PLASMA LIPIDS ON BLOOD VISCOSITY IN PATIENTS WITH CEREBROVASCULAR DISEASE

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It is known that plasma lipids could increase the cerebrovascular risk through alteration of the hemorheological profile. The aim of our study was to evaluate the relationship between blood viscosity parameters and plasma lipids in patients with cerebrovascular disease. The study included 43 patients with transient ischemic attacks (TIAs), 86 patients with chronic cerebral infarctions (CCI) and 57 subjects with risk factors for stroke. Whole blood and plasma viscosity at different shear rates by Couette rotational viscometer Contraves Low Shear 30, plasma viscosity with capillary viscometer, hematocrit, fibrinogen, cholesterol, triglicerides, HDL and LDL were examined in all patients and in a control group of 56 presumed healthy individuals. The hemorheological results showed increase of fibrinogen, whole blood and plasma viscosity in the patients with TIAs and CCI; it was more pronounced in the CCI patients. Slight increase of fibrinogen in the stroke risk group was also found. Correlation of the indexes of erythrocyte aggregation, erythrocyte deformability and rate of oxygen supply to tissues, estimated from whole blood viscosity measurements with hemorheological determinants was determined. The elevation of triglicerides predominated in the stroke patients` groups. Triglicerides correlated significantly with fibrinogen, whole blood and plasma viscosity in the patients with TIAs. Conclusion is drawn about the significance of plasma triglicerides for decrease of blood fluidity and for impairment of the cerebral circulation in cerebrovascular diseases.

C3.6

MODIFIED ANTIPLATELET ACTIVITY OF ATORVASTATIN IN HYPERCHOLESTEROLEMIC CARRIERS OF LECTIN-LIKE OXIDIZED LOW-DENSITY LIPOPROTEIN RECEPTOR-1 (LOX-1) 3'UTR/T POLYMORPHISM.

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Oxidized-low density lipoproteins (ox-LDL) and the specific receptor Lectin-like oxidized-LDL receptor-1 (LOX-1) are involved in atherogenesis and atherothrombosis. 3'UTR/T LOX-1 polymorphism has been associated with increased risk of acute myocardial infarction (AMI) and coronary artery disease extension (CAD). Suggested mechanisms for vascular risk induction in T carriers are different affinity for ox-LDL and/or increased exposition of the receptor in several cellular lines including endothelial cells, platelets and monocytes. Ox-LDL induced platelet hyperactivity is a relevant mechanisms leading to atherothrombosis. Moreover modified platelet-oxLDL interaction via LOX-1 downregulation is a relevant antiplatelet mechanism induced by atorvastatin. The present study was planned to determine whether LOX-1 genetic variations could affect antiplatelet action of atorvastatin. We studied by platelet P-selectin (P-sel), CD36 and LOX-1 expression (cytofluorimetric detection) whether differences in cellular activation could be suitable in 109 3'UTR/T carriers out of 201 hypercholesterolemic subjects treated with atorvastatin 20 mg/day. PCR technique was employed to determine genetic variations in studied subjects (forward primer: 5'-GCCTGGCACCTTTATGTCAAC-3'; reverse primer: 5'-CTTGGGACAAGCTAGGTGAAATAATA-3'. 3'UTR/C allele MGB probe: 5'-FAM-TTTTTGATTCTAGCTAGCTACCTG-3'; 3'UTR/T allele: 5'-VIC-ATTTTTGATTCTAGCTATCTG-3'). Hyperactivated platelets (P-sel in resting cells and % variation upon thrombin activation, $p< 0.001$) were detected at baseline in patients without significant differences between T or C carriers. P-sel and platelet-associated ox-LDL, were significantly decreased (both $p< 0.001$) in C carriers after one week of treatment before LDL reduction ($r= 0.09$, $p= 0.216$). In 3'UTR/T carriers P-sel was reduced ($p< 0.01$) after 6 weeks of treatment according to LDL ($r= 0.49$, $p< 0.0001$) and ox-LDL ($r= 0.53$, $p< 0.0001$) reduction. No difference of CD36 changes was found between C and T carriers ($p= 0.118$). Moreover a specific detection test shown a different ox-LDL binding affinity in T carriers ($p< 0.001$). In conclusion atorvastatin reduced platelet activity by LDL and ox-LDL lowering and not by rapid CD36 and LOX-1 downregulation in 3'UTR/T carriers as shown in C carriers. Such data show that LDL lowering is needed to achieve antiplatelet action in T carriers. Furthermore present findings suggest to determine whether specific lipid-lowering independent antithrombotic actions described for statins could be genetically influenced.

C3.7

ADVANCED GLYCATION AND GLYCO-OXYDATION ALTERS BLOOD RHEOLOGY AND THE SECRETIONS OF NEUROPROTECTIVE GROWTH FACTORS AND NEUROTOXIC CYTOKINES IN ALZHEIMER'S DISEASE

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Advanced glycation end products (AGEs) and glyco-oxidative mechanisms could induce a pro-inflammatory burst of cytokines from immune and microglial cells also increasing the neurotoxic events induced by beta-amyloid within the brain. On the other hand, increased glycation could potentially reduce the release of vascular growth factors implicated in neuroprotection. Within this context, circulating AGEs and Pentosidine were studied in 27 subjects with mild to moderate Alzheimer's disease (AD) and 22 age-matched healthy subjects. In the two groups of subjects, we also measured blood viscosity (BV), red cell aggregability (RCA), and TNF-alfa, IFN-gamma and Vascular endothelial growth factor (VEGF) released in the supernates of lymphomononuclear natural killer (NK) cells (NK,CD16+/CD56+ purified at final density of $7.75x10^6$ cellule/mL). Both cytokines and the growth factor VEGF were evaluated at baseline (spontaneous secretion) and after 20 h exposure with LPS (1 σg/mL), beta-amyloid 1-42 fragment (A-beta 1-42: 5 σg/mL), and LPS/A-beta co-incubated with Glycated human serum albumin (GHSA: 20 σg/mL). TNF-alfa and IFN-gamma were also determined after GHSA incubated at increased dose-response concentrations: 5,10,20,50 σg/mL . AGEs and Pentosidine were significantly increased in AD than in healthy subjects (15±3 σg/mL and 190±40 pmol/mL vs 8±2.5 σg/mL and 115±28 pmo/mL respectively; $p<0.001$). A parallel pahological increase of BV (19±5 vs 14±2 mPas at SR $1s^{-1}$,$p<0.001$; 4.3±0.3 vs 3.7±0.2 mPas at SR $200s^{-1}$,$p<0.001$) and RCA (4.41±0.4 vs 3.78±0.3; $p<0.001$) was demonstrated in AD than in healthy subjects. A significant dose response increased release of TNF-alfa and IFN-gamma by immune cells was demonstrated merely in AD subjects after incubation with GHSA ($p<0.001$). Moreover, GHSA amplified the overproduction of both cytokines from immune cells of AD subjects induced by LPS ($p<0.001$) and A-beta ($p<0.001$). Finally, a significant reduction of VEGF release by NK cells was found in AD subjects compared to healthy subjects ($p<0.001$); while the co-incubation of NK cells with GHSA determined a further reduction of

VEGF secretion during exposure with LPS. Taken together all these data clearly indicated that advanced glycation and glyco-oxydation are significantly enhanced in AD subjects with mild to moderate dementia. These changes could induce blood rheology disorders and the overexpression of neurotoxic cytokines, also inducing the impairment of neuroprotective growth factors.
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C3.8

ALTERED HAEMORHEOLOGICAL INDICES IN NIGERIAN GERIATRICS

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Background: Elevated plasma fibrinogen concentration is associated with increased risks of cardiovascular diseases while stroke related morbidity in elderlies has been linked with seasonal variations of fibrinogen concebtration especially during winter.
Aim: Our interest was primarily based at establishing Fibrinogen index in Geriatrics especially in the tropics and secondly to assess their rheological parameters which is lacking in the literature.
Materials and Methods: A total of 50 apparently healthy elderly Nigerians with ages between 60 and 85 years comprising of 25 males and 25 females were studied for haemorheologic parameters such as Packed cell volume (PCV), Plasma and whole blood viscosity (PV and WBV respectively), Erythrocytes sedimentation rate (ESR), Plasma Fibrinogen concentration(PFC) and Euglobulin lysis time (ELT) using standard methodologies. They were compared with 50 healthy younger controls with matched age and sex. Student t-test was used to analyse the data.
Results: We observed a statistically significant decreases in PCV and WBV ($P<0.05$ respectively) while PV showed an experimental decrease but not statistically significant. Also, there were significant increases in PFC, ESR and ELT ($P<0.05$ respectively).
Conclusion: Decreased haematocrit, lowered blood and plasma viscosities coupled with slight hyperfibrinogenaemia and hypofibrinolysis are reflections of advanced age. The decreased parameters therefore favours improved haemorheology while the elevated parameters are indicative of abnormal rheology and a predisposition to thrombotic and other cardiovascular diseases at old age.

C4.1

cGMP PROTECTS THE ENDOTHELIUM IN THE SETTING OF ISCHEMIA AND REPERFUSION.

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Background: Animal studies have demonstrated that administration of sildenafil can limit myocardial damage induced by prolonged ischemia, an effect that appears to be mediated by opening of ATP-sensitive potassium (K-ATP) channels. No study has investigated whether sildenafil can also prevent the impairment in endothelium-dependent vasodilatation induced by ischemia-reperfusion (IR) in humans.
Methods and Results: In a double-blind, placebo-controlled, cross-over design, 10 healthy male volunteers (age 25-45 years) were randomized to oral sildenafil (50 mg) or placebo. Two hours later, endothelium-dependent, flow-mediated dilatation (FMD) of the radial artery was measured before and after IR (15 minutes of ischemia at the level of the brachial artery followed by 15 minutes of reperfusion). Seven days later, subjects received the other treatment (i.e., placebo or sildenafil) and underwent the same protocol. Pre-IR radial artery diameter and FMD, as well as baseline radial artery diameter after IR, were similar between visits (P=ns). After placebo administration, IR significantly blunted FMD (before IR: 7.9±1.1%; after IR: 1.2±0.7%, P<0.01). Importantly, Sildenafil limited this impairment in endothelium-dependent vasodilatation (before IR: 7.0±0.9%; after IR: 6.2±1.1%, P=ns; P<0.01 compared to placebo). In a separate protocol, this protective effect was completely prevented by prior administration of the sulfonylurea glibenclamide (glyburide, 5 mg), a blocker of K-ATP channels (n=7; FMD before IR: 10.3±1.5%; after IR: 1.3±1.4%, P<0.05).
Conclusions: In humans, oral sildenafil induces potent protection against IR-induced endothelial dysfunction through opening of K-ATP channels. Further studies are needed to test the potential clinical implications of this finding.

C4.2

EFFECTS OF NO PRECONDITIONING ON THE CYTOKINE-INDUCED SURFACE EXPRESSION OF CELL ADHESION PROTEINS IN HUMAN ENDOTHELIAL CELLS

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Previous investigations in this lab have shown that preconditioning by inhalation of NO for a short period (10 min, 15 ppm) is sufficient to protect the lung against a subsequent, extended ischemia/reperfusion (I/R) episode. The amelioration of the I/R-induced inflammation was among the most prominent results of this treatment. As a first step to investigate the underlying mechanisms, an in vitro cell culture system was developed that allows for studying the effects of NO preconditioning on the cytokine-induced expression of endothelial leukocyte adhesion proteins. A cell ELISA assay was used to detect the surface expression of ICAM, VCAM, and E-selectin in human umbilical vein endothelial cells. Cells were exposed to NO by including a NO-donor (SNAP, 0.3 - 1 mM, 5 - 60 min) into the culture medium, 0 - 16 h before the expression of the adhesion proteins was induced by IL-1η or TNF-ζ for 4h. A NO treatment of 30 min was sufficient to completely prevent the induction by cytokines of the three adhesion proteins, and a significant inhibition was observed after 15 min exposure to SNAP. The protective effect of a 30 min incubation with the NO donor was still detectable after a 8 h incubation in fresh medium. Neither treatment affected the viability of the cells as assessed by Trypan blue staining. By using the specific guanylyl cyclase inhibitor ODQ and the cGMP analogue 8-Br-cGMP, it was shown, that the NO effect was not mediated by cGMP signaling. Since the cytokine-induced expression of the adhesion proteins was completely blocked by the inhibitor of IKK, BAY 11-7082, we suspected that the upstream regulation of NFρB is the primary target of NO. Western blot analysis showed that TNFζ-induced phosphorylation and degradation of the inhibitor of NFρB, IρBζ,was persistently blocked by NO pretreatment for up to 8 h after washout of the NO donor. Inhibition of IKKη by direct S-nitrosylation may be involved in the stabilization of IρBζ, but this effect was restricted to a phase directly following SNAP pretreatment. The time frame of these effects compares well with the duration of preconditioning by NO, but further studies are warranted to evaluate the role of NO-induced IρBζ stabilization in vivo.

C4.3

PLATELET AND POLYMORPHONUCLEAR LEUKOCYTE ACTIVATION MARKERS IN JUVENILE MYOCARDIAL INFARCTION

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In coronary heart disease and especially in acute coronary syndromes and in acute myocardial infarction (AMI), a growing evidence is charged to inflammation mechanisms that involve directly or indirectly platelets and leukocytes. In this regard, our attention has been directed towards some plasma markers of platelet and polymorphonuclear leukocyte (PMN) activation in a group of young adults with acute myocardial infarction (AMI) at the initial stage and after three months. We enrolled 49 AMI subjects aged < 45 years and examined the plasmatic levels of platelet factor 4 (PF4), ϐ-thromboglobulin (ϐ-TG), elastase and

myeloperoxidase (MPO) using ELISA methods. PF4 and ß-TG were increased, compared to control subjects (C), both at the initial stage (PF4 AMI: 44.09±40.54 IU/ml; PF4 C: 11.94±6.92 IU/ml; $p < 0.001$; ß-TG AMI: 107.8±83.34 IU/ml; ß-TG C: 37.52±15.69 IU/ml; $p < 0.001$) and after 3 months (PF4 AMI: 27.80±30.86 IU/ml; $p < 0.05$ vs C; ß-TG AMI: 64.59±35.72 IU/ml; $p < 0.001$ vs C). In control subjects and in AMI patients, at both times of observation, there was a significant and positive correlation between the two platelet parameters, while no correlation was present between each parameter and platelet count. In AMI patients there was an increase in elastase levels in comparison with the control group; this increase was evident at the initial stage (Elastase/ζ1-PI AMI: 101.60±41.90 ng/ml; Elastase/ζ1-PI C: 59.73±17.60 ng/ml; $p < 0.001$) and after 3 months (Elastase/ζ1-PI AMI: 91.65±36.50 ng/ml; $p < 0.001$ vs C). There was no difference in MPO levels between control subjects and AMI patients at the initial stage (MPO AMI: 42.21±20.96 ng/ml; MPO C: 49.63±15.36 ng/ml) and after 3 months (MPO AMI: 48.78±18.66 ng/ml). In control subjects and in AMI patients there was a significant and positive correlation between elastase and MPO level, whereas no relationship was found between each marker and PMN count. Our data show that in young AMI patients the discharge treatment including antiplatelet drugs did not modify platelet activation and suggest the association of molecules able to inhibit PMN activation to the conventional therapy of these AMI patients.

C4.4

CHANGES OF THE MICROCIRCULATION STATE AND THE HEMORHEOLOGY PARAMETERS IN ACUTE CORONARY SYNDROME

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The goal of this study was to investigate the microcirculation state and the parameters of hemorheology in acute coronary syndrome. The studies were carried out in 139 patients with unstable angina and nonQ myocardial infarction, 54 patients with angina pectoris and 67 healthy subjects. Plasma total cholesterol, triglyceride and HDL-cholesterol levels were measured with using standart kits (La Roche - Cormay). Rigidity index was used to estimate the deformability of erythrocytes. Red blood cell (RBC) aggregation was assessed by measurement of erythrocyte sedimentation rate. To observe microciculation directly on the bulbar conjunctiva we have used an intra-vital video-microscopic system. The quantitative evaluation of bulbar conjunctiva images algorithm includes the determination of mean vascular diameters and calculation of the following coefficients: irregular of vessels diameter, vessels wind, capillary density, arterio-venular ratio, arterio-venular anastomosis density, network area, haemorrhages, extravascular oedema and deposits areas, irregular of blood flow, intravascular aggregation, dissemination of aggregates. We have found that in acute coronary syndrome there are tree types of pathological changes: 1^{th} – hemorheological and microcirculation abnormalities; 2^{th} - increase of inflammatory activity accompanied by hypercholesterolaemia; 3^{th} - hemorheological and microcirculation alterations accompanied by increase of inflammatory activity and hypercholesterolaemia. Our findings may lead to definition of additional risk factors of the development of acute coronary syndrome complications.

C4.5

RESPONSES OF PLATELET ACTIVATION AND FUNCTION TO A SINGLE BOUT OF RESISTANCE EXERCISE

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Clinical and pathological studies have suggested that platelet hyper-aggregability and impaired platelet function are linked with the pathogenesis and progression of cardiovascular disease. The acute and chronic effects of endurance exercise on blood haemostasis including platelet aggregation and activation have been previously studied and recently reviewed. Previous studies have demonstrated a rise in platelet activation and function in response to strenuous exercise. Although resistance exercise is nowadays performed, not only by athletes and normal healthy subjects, but also by patients suffering from different illnesses, no information is available regarding the acute effects of this exercise modality on platelet activation and function. Therefore the present investigation was designed to determine the effects of resistance exercise on platelet activation and function in normal healthy individuals. Twenty-one healthy male subjects (mean±SD; age = 27.9±4.8 years) who were non-smokers and free of medication volunteered to participate in this study. Exercise trial was preceded by performing a warm-up consisted 5 minutes of riding a stationary bicycle, and two sets (8 repetitions) of progressive resistance exercises. Exercise protocol consisted of three sets of five to seven repetitions of six exercises including upper and lower body parts at an intensity corresponding to 80% of one repetition maximum (1RM). Venous blood samples (15ml) were obtained before, immediately after exercise and after 30 min recovery and analysed for platelet count (PLT), plateletcrit (PCT), mean platelet volume (MPV), platelet aggregation, and beta thromboglobulin (B-TG). Platelet aggregation was measured using collagen and various final concentrations of adenosine-5'-diphosphate ($2\Delta10^{-5}$, $2\Delta10^{-6}$ and $4\Delta10^{-6}$ M). Plasma volume changes were estimated from haemoglobin and haematocrit readings before and after each exercise trial. Plasma volume decreased 10.1% following resistance exercise and this occurred in parallel with increases of 5.4%, and 6.2% in haemoglobin, and haematocrit,

respectively. Resistance exercise was followed by a significant ($P < 0.01$) increase in platelet count, PCT and MPV. Exercise was also followed by a significant increase ($P < 0.05$) in platelet aggregation, but this only occurred with the high but not with the low concentrations of ADP. Data analysis showed a significant increase ($P < 0.05$) in the concentration of B-TG following exercise. However, this increase was transient and decreased to nearly pre exercise level at the end of recovery. It was concluded therefore that resistance exercise is followed by an increase in platelet count, plateletcrit, and mean platelet volume and this occurred in parallel with an in vivo activation of platelet as manifested by an increase in platelet aggregation and a rise in B-TG.

C4.6

NON-HEMODILUTING DEXTRANE IN CRITICAL LIMB ISCHEMIA: MICROCIRCULATION AND HEMORRHEOLOGY

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Peripheral artery disease (PAD) with severe tissue ischemia is related to rest pain and skin ulcers where ischemia is a consequence of a marked reduction of arterial blood flow with a dramatic decrease of supply of nutrients to the tissue target. The cause of reduced capillary blood flow is a decreased total blood flow of the area below the minimal demand of the cell requirement. Non-invasive evaluation of microperfusion by Laser-Doppler flowmetry (LDF) detect primary information on microvascular sequelae of tissue ischemia. Growing evidence supports a role for accumulation of platelets (PLT) and leukocytes (WBC) in the pathogenesis of ischemia/reperfusion (I/R) injury; PLT in vivo act in similar to leukocyte rolling along and firmly adhere to the microvascular endothelium (secondary to interaction of ICAM1/CD11/CD18) during postischemic reperfusion (with release of proinflammatory mediators and oxygen radicals). This action is mediated by P-selectin, a product of postischemic endothelium, that is rapidly upregulated during reperfusion, while ICAM1 increases over several hours. The aim of this study was to evaluate the effects of Dextrane at a non-hemodiluting dose on PLT and LDF (during postocclusive reactive hyperemia) in PAD. Under I/R conditions, PLT rolling and firm adhesion within postcapillary venules and ICAM1 are increase and LDF parameters depressed but dextrane (at non-hemodiluting concentration) attenuates PLT and ICAM1 and increase LDF parameters during I/R. In this light, dextrane might represent an alternative important drug option to prevent I/R-induced tissue injury via anti-adhesive effects on PLT (and probably leukocyte) and generative on ICAM1.

POSTERS

P1.1

HEMORHEOLOGICAL PARAMETERS IN CORRELATION WITH THE RISK FACTORS FOR CAROTID ATHEROSCLEROSIS

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The study aimed to follow the relationship between some hemorheological variables and the main risk factors for carotid atherosclerosis (CA). The carotid atherosclerosis was approved by color duplex sonography of the carotid arteries in 20 patients with risk factors (RF) for cerebrovascular diseases (CVD), 20 patients with transient ischemic attacks (TIAs) and 20 patients with chronic unilateral cerebral infarctions (CUI). The examined hemorheological variables were whole blood and plasma viscosity, hematocrit and fibrinogen. They were correlated with the intima media thickness (IMT) of the common carotid and the internal carotid arteries and with other main RF for CA: hypertension, diabetes mellitus, coronary heart disease, and hyperlipidemia. Twenty healthy subjects without RF for CA were also examined. The hemorheological investigation showed an increase in blood and plasma viscosity at different shear rates and it was more expressed in the group with CUI. The neurosonographic investigation revealed an increase in the IMT and carotid artery stenoses in the patients` groups with CVD. These were also more often in the patients with CUI. Different correlations were established between the hemorheological parameters, the IMT of the carotid arteries and other RF for CA. In the group with CUI the hematocrit and the whole blood viscosity correlated significantly with the IMT, arterial blood pressure and cholesterol values. These data confirm the influence of the hemorheological parameters on the carotid blood vessel walls and on the blood flow in patients with CVD.

P1.2

POLYMORPHONUCLEAR LEUKOCYTE MEMBRANE FLUIDITY AND CYTOSOLIC Ca^{2+} CONCENTRATION IN SUBJECTS WITH VASCULAR ATHEROSCLEROTIC DISEASE SUBDIVIDED ACCORDING TO THE EXTENT

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In vascular atherosclerotic disease (VAD) and in diabetes mellitus (DM) polymorphonuclear leukocytes (PMN) play a key role in the organ injury. The PMN-mediated tissue damage can be exacerbated not only by an increased PMN count, but also by a PMN functional abnormality. Among the PMN parameters that may reflect this abnormality we include the membrane fluidity (related to the membrane lipidic and proteic components) and the cytosolic Ca^{2+} content (related to the activity of the membrane pumps) and both may be considered also markers of PMN activation. During activation, PMNs release proteolytic enzymes and in fact an elevated level of plasma elastase and myeloperoxidase has been often associated with cardiovascular diseases. In this research we evaluated the PMN membrane fluidity and cytosolic Ca^{2+} content in VAD subjects with and without type 2 DM and examined the association between these parameters and the mono- or polyvascular localization. We enrolled 155 VAD subjects, including 92 non-diabetic (group A: mean age 63.6±9.2 years) and 63 diabetic patients (group B: mean age 65.4±7.8 years). Among group A 63 patients had monovascular and 29 polyvascular disease; among group B 30 patients had monovascular and 22 polyvascular disease. In each patient we evaluated the PMN membrane fluidity labelling the cells with the fluorescent probe 1,4-(trimethylamino)-phenyl-4-phenylhexatriene (TMA-DPH) and the PMN cytosolic Ca^{2+} content marking the cells with the fluorescent probe Fura 2-AM. PMN membrane fluidity did not discriminate normal subjects from diabetic and non-diabetic VAD subjects, while cytosolic Ca^{2+} content was increased in both groups. PMN membrane fluidity did not distinguish normal subjects from mono- or polyvascular VAD patients with and without type 2 DM. PMN cytosolic Ca^{2+} content was increased especially in monovascular VAD patients; both mono- and polyvascular VAD subjects with DM had a PMN cytosolic Ca^{2+} content higher than normals. Our results show the presence of an increased PMN cytosolic Ca^{2+} content in diabetic and non-diabetic VAD subjects but no association between this increase and the mono- or polyvascular localization was observed.

P1.3

CONNECTIVE TISSUE GROWTH FACTOR IS RELEASED FROM PLATELETS UNDER HIGH SHEAR STRESS AND IS DIFFERENTIALLY EXPRESSED IN ENDOTHELIUM ALONG ATHEROSCLEROTIC PLAQUES

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Connective tissue growth factor (CTGF), a potent angiogenic, chemotactic, and extracellular matrix-inducing growth factor, is over-expressed in atherosclerotic blood vessels. It has been shown that CTGF gene expression in endothelial cells is strongly up-regulated by non-uniform shear stress. Furthermore, we have recently shown that CTGF is stored in human platelets, and can be released upon receptor-dependent stimulation. During the initiation and generation of atherosclerotic lesions, platelets adhere to dysfunctional endothelium and release the contents of their granules, among them CTGF, which induces monocyte

chemotaxis and thus recruitment into the vessel wall. To further investigate the role and the regulation of CTGF in atherosclerosis, we examined whether CTGF can be released from platelets by high shear stress, and whether the expression of CTGF protein along the atherosclerotic lesions depends on local hemodynamic conditions. For this purpose, normal human platelets were subjected to (a) low shear stress, 10 dyn/cm^2 for 5 min; (b) high shear stress, 120 dyn/cm^2 for 5 min; (c) alternating 1-min low and high shear stress values for 5 min. The experiments were done at 25°C, using a cone-plate high-shear rheoscope. After centrifugation, the amounts of CTGF in the cell pellet and in the supernatant were analysed by Western blotting. In further experiments, up-stream and down-stream regions of longitudinal sections of 25 human carotid plaques were immunohistochemically analysed for the endothelial expression of CTGF protein. There was a very low CTGF amount secreted from platelets sheared at 10 dyn/cm^2 (11.4±3.9% of total amount of CTGF in platelets). On the other hand, high shear stress resulted in a markedly increased CTGF release from platelets (29±13.8%, p=0.07 vs low shear stress, n=4). In platelets subjected to alternating low and high shear stress, the amount of released CTGF was higher than under low shear stress, but lower than under high shear stress. Immunohistochemical analyses of CTGF expression in the longitudinal sections of carotid plaques showed that the mean numbers of CTGF-positive endothelial cells were significantly higher up-stream as compared with down-stream regions of the atherosclerotic carotid arteries (21.3±3.6 up-stream vs 13.9±2.8 down-stream, p<0.001, n=25). Moreover, in plaques undergoing active intimal neovascularization, the newly formed vessels accumulated particularly in up-stream part of the lesions. In conclusions, this study demonstrated that CTGF can be released from platelets not only by receptor-dependent stimulation, but also by high shear stress. Furthermore, endothelial CTGF protein expression in atherosclerotic vessels was shown to depend on local hemodynamic forces acting on the vessel wall along the atherosclerotic plaque. Disturbed flow at vessel bifurcations may thus induce endothelial CTGF expression and contribute to the progress of atherosclerotic lesions.

P1.4

INVOLVEMENT OF HEMORHEOLOGICAL DISORDERS IN DEVELOPMENT OF CORONARY HEART DISEASE

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Significance of the hemorheological disorders in development of acute vascular syndromes is presently well known, but their role in pathogenesis of chronic coronary heart disease has not been yet sufficiently analyzed. Aim of the present study was investigation of the relationship between the hemorheological, vascular and hemodynemic factors responsible for development of the coronary heart disease. We investigated 50 patients with coronary heart disease of the functional classes I-IV with and without the heart failure. For evaluation of the hemorheological disorders we investigated their most significant symptom, the erythrocyte aggregability, with the "Georgian technique" that provided us with direct and quantitative data. We investigated also the tone of the hand's resistance arteries with an original non-invasive technique based on measurement of the flow velocity changes in the patients and in the healthy controls radial arteries by using the Doppler technique during the standardized postischemic hyperemia. Echocardiographically we studied the standard characteristics of left ventricular function (systolic and diastolic volume, its mass and ejection fraction). ECG by standard leads and the blood pressure were investigated in all patients. We found that the rheological disorders are manifested in the early stages of the disease before its functional manifestation. The most pronounced hemorheological disorders were in evidence in the patients with unstable angina and heart failure. As to the arteriolar resistance index, it was increased only in 45 per cent of all the investigated patients and no significant difference between the patients with and without the heart failure was found. There was a negative relationship between erythrocyte aggregability and the ejection fraction of the left ventricle as well as a positive correlation between erythrocyte aggregability and the left ventricle hypertrophy (p<0.01). We concluded that the blood rheological disorders represent themselves a factor that plays a significant role in pathogenesis of the coronary heart disease. They are predictors of the disease and not only risk factors as it is generally believed. Measurement and correction of these disturbances in its early stages have a high clinical significance.

P1.5

FATTY ACID COMPOSITION IN ERYTHROCYTES OF MAMMALS WITH DIFFERENT DEGREE OF RED CELL AGGREGATION

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Previous studies have shown a wide range of different erythrocyte elongation rate in different mammalian species. The rheologic properties of red blood cells (RBC) consist of humoral and cellular factors. Plasma concentrations of macromolecules like fibrinogen or dextrane are predominant humoral factors for erythrocyte aggregation. The ability of shape regulation and passive reversible shape change of the RBC are important cellular factors for RBC´s fluidity. Many cellular factors affecting RBC aggregation and flexibility have been shown recently. Since fatty acid composition of the RBC phospholipid bilayer is one of the factors influencing membrane fluidity, this could affect RBC aggregation as well. The

present investigation was performed to analyze the fatty acid composition in RBC from animals with high RBC aggregation like horse and pig and from animals with immeasurable RBC aggregation like sheep and to relate the results to the hemorheologic data.

P1.6

RELATIONSHIP OF HEMORHEOLOGICAL ALTERATION TO LIPID PROFILE DURING NORMAL PREGNANCY AND PRE-ECLAMPSIA

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Recent investigations of cardiovascular diseases ascribing an involvement of increased lipid synthesis and lipid metabolism in development of hemorheological decline provide clashing results. Increased erythrocyte aggregation and/or plasma viscosity to be related to hyperlipidemia have been reported, but this is not verified in a number of articles. Normal pregnancy and pre-eclampsia newly considered as transient model of hyperlipidemia and atherogenic lipoprotein profile are commonly found with hemorheological alterations. The goal of the present work was to test for possible relationship of hemorheological indices (HI) to changed lipid profile (LP) in pregnant state. The draft enlisted healthy nonpregnant (NP)- and pregnant (HP) women, pre-eclampsia (PP) and nonpregnants with symptomatic hypertension (SH). Statistics of nonparametric comparisons and linear regressions were processed. Tests for haemorheological- (HI, erythrocyte aggregation (EA), blood plasma viscosity (PV)) and clinico-chemical (blood plasma concentrations of total cholesterol (TC), triglycerides (TG), HDL-C, LDL-C and VLDL) indices were employed. Increase of EA were found in HP, PP, SH vs NP ($P<0.001$, $P<0.001$, $P<0.05$ respectively) and for PP vs HP ($P<0.001$) along with PV increase in PP and SH vs NP (P= nqs in both) and PP vs HP ($P<0.05$). Increased levels of TC, TG, HDL-C and VLDL of HP vs NP refer to $P< 0.01$, $P<0.0001$, $P<0.0001$ and $P<0.0001$ respectively. Thus are the changes of PP vs NP (respective $P<0.05$, $P<0.0001$, ns and $P<0.0001$) but greater in value and not statistical significant for PP vs HP (only for TG $P<0.05$). Similar parallelism in HI and LP is fixed also for SH. Linear regression analyses of summarized data for NP, HP and PP indicate modest relationship of PV and EA vs LDL-C ($r=0.42$ and $r=0.48$ both with $P<0.01$) and appreciable correlation of EA vs TC, TG and VLDL ($r=0.61$ with $P<0.001$, $r=0.69$ with $P<0.001$ and $r=0.68$ with $P<0.0001$). The common tendency of elevated LP inherent for pregnancy turns probably to remodeling with higher adverse values in pre-eclampsia. HI change in parallel. This parallelism corroborate the assumed interrelationships between LP and HI and is underlined by the appreciable correlations prepared by relative small number of tested data.

P1.7

HEMORHEOLOGICAL DISTURBANCESS CORRELATE WITH THE LIPID PROFILE BUT NOT WITH THE NCEP-ATPIII SCORE OF THE METABOLIC SYNDROME

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The metabolic syndrome which is at high risk for diabetes and atherothrombosis is associated with hemorheologic abnormalities, which seem more and more explained by its various symptoms than by insulin resistance which represents theoretically the core of the syndrome. In this study we aimed at defining the specific hemorheologic profile of insulin resistance and hyperinsulinemia by separating a sample of 90 subjects into 4 subgroups according to the clinical score « NCEP-ATPIII » which is the best recognized standardized definition of the syndrome. Results show no significant changes of blood rheology across classes of NCEP score despite a borderline rank correlation between RBC aggregability « M1 » and the score. Whole blood viscosity was mostly correlated to HDL-cholesterol ($r=-0.353$ $p=0.007$) and triglycerides ($r=0.574$ $p=0.0001$). Plasma viscosity was correlated with total colesterol ($r=0.3359$ $p=0.02$) and with LDL-cholesterol ($r=0.357$ $p=0.03$). RBC rigidity « Tk » was negatively correlated to to HDL-cholesterol ($r=-0.430$ $p=0.007$). Aggregability « M » was correlated to total cholesterol ($r=0.356$ $p=0.01$) and « M1 » to HDL-cholesterol ($r=-0.406$ $p=0.006$). Thus, despite previously described correlation with glucose disposal parameters, the hyperviscosity syndrome of the metabolic syndrome is not proportional to its clinical scoring and is strongly dependent upon the lipid profile.

P1.8

EVALUATION OF CIRKAN® IN ACUTE HAEMORRHOIDAL CRISIS: A DOUBLE-BLIND, RANDOMIZED AND PLACEBO-CONTROLLED STUDY

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Background and purpose: The study treatment, a phlebotropic drug consisting of Ruscus extract, Hesperidin methylchalcone and ascorbic acid, had shown its efficacy on pain and congestive state through improvement of venous tone and lymphatic drainage, and reduction of capillary hyperpermeability. The purpose of this randomised, double-blinded, placebo-controlled study was to investigate CIRKAN® in the treatment of acute haemorrhoidal crisis.
Material and methods: This clinical study of 382 patients presenting with acute haemorrhoidal crisis was conducted in France over a 7-months period (November 2003 to May 2004). All subjects received 8 tablets daily for 4 days. Patients (n) were randomly assigned to receive either double dose (n=129) or single dose (n=128) or placebo (n=125) treatment. Primary endpoint (pain intensity) was assessed at the inclusion visit/Day 1 (baseline value) and at the final visit/Day 5 using a Visual Analogical Scale (VAS), along with the functional and local signs. Moreover pain intensity was daily reported in the patient's diary. Safety and compliance to the treatment were assessed throughout the study period.
Results: The mean age was 43±12 years. At baseline, no difference of pain intensity was found among the three treatment groups. At day 5, the mean pain intensity variation was statistically significant (p=0,036) in the double dose group versus the placebo group with respectively - 5,23 mm and – 4,53 mm. In terms of response rate (reduction of pain intensity of 40% from baseline values) the results were statistically significant versus placebo for both treatment groups: 80.6% (p=0,0022) of the subjects showed a positive response to the double dose, 78,1% (p= 0,0191) in the single dose group and 63.2% in the placebo group. The pain intensity decreased most expediently in the double dose group, with a statistically significant difference versus placebo demonstrated at Day 3 (p = 0.0099). Improvement of local signs as inflammation, oedema, pruritus and rectal bleeding were also observed but there were no statistically difference between treatment groups. The treatment was very well tolerated and no serious adverse event was reported.
Conclusion:Results of this study showed safe and rapid therapeutic benefits of CIRKAN® in the treatment of acute haemorrhoidal crisis.
Keyword: haemorrhoidal crisis, ruscus extract
This study was performed in accordance with the principles laid down by the 18th World Medical Assembly (Helsinki, 1964) and its amendments. The study was also conducted in accordance with the Good Clinical Practice guideline (CPMP/ICH/135/95). The study protocol was approved by the CCPPRB Ethical Committee of Ambroise Paré, in Boulogne-Billancourt (France) on October 14th, 2003.

P1.9

THE RED BLOOD CELL DEFORMABILITY ALTERATIONS UNDER DESFLURAN ANESTHESIA IN RATS

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General anesthesia, either with inhalation or nonvolatile anesthetics, is known to affect the overall cardiovascular function as well as the microcirculatory hemodynamics. In this study, the effects of desfluran anesthesia on the red blood cell deformability of young and old rats are investigated. 33 male rats were used in the study and the rats were divided into two groups according to their age (young and old) comprising of two subgroups in each. First group was the young control (n=5), the second was the young group treated with desfluran (n=7), the third group was the old control (n=7) and the last group was the old group treated with desfluran (n=7). %6 of desfluran was applied to the rats with inhalation in an adjustable cage for one hour. The elongation indexes of the erythrocytes were measured by a laser diffractometer (Myrenne Rheodyne SSD). Deformability indexes of red blood cells were significantly increased with desfluran in young rats (p=0.042) whereas it has significantly decreased in old rats (p=0.004) with desfluran application compared with their controls. When we compared the young and old control groups, the deformability indexes were significantly higher in old ones (p<0.001). However, there were not any significant difference between the old and the young desfluran applied groups. A volatil anesthetic agent desfluran has impaired the deformability of erythrocytes in old rats compared to their controls, whereas it had the opposite effects on young ones which may be due to the alterations in membrane structure with age. These results reveal that the inhalation anesthetics like desfluran may cause more serious problems in the elder people during the surgery and may influence their hemodynamic parameters.

P1.10

THE INFLUENCE OF TWO DIFFERENT HYDROXYETHYL STARCH SOLUTIONS (6% HES 130/0.4 AND 200/0.5) ON BLOOD VISCOSITY

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We performed the current study to investigate the influence of 2 different hydroxyethyl starch (HES) solutions, the novel medium molecular weight HES 130/0.4 (6%) and HES 200/0.5 (6%), on plasma and whole blood viscosity *in vitro* and *ex vivo* in patients with severe head injury. For the *in vitro* experiments, blood was incubated with increasing concentrations (0%-50% vol/vol plasma) of either 6% HES 130/0.4 or 6% HES 200/0.5 solution. Plasma viscosity and whole blood viscosity (hematocrit [Hct] 45%) at high (94.5 s^{-1}) and low (0.1 s^{-1}) shear rates were determined. Both HES solutions increased plasma viscosity, but HES 130/0.4 to a lesser extent than HES 200/0.5. Whole blood viscosity was significantly less with HES 130/0.4 than with HES 200/0.5 at concentrations of 37.5% and larger. In the *ex vivo* study on 31 patients with severe cranio-cerebral trauma treated randomly with either HES 130/0.4 or HES 200/0.5 over several days, frozen plasma samples were thawed and plasma viscosity was determined. Blood was reconstituted with normal erythrocytes (0 Rh neg, Hct 45%) for whole blood viscosity measurements. In both groups plasma and blood viscosity tended to increase over time without statistical significance. Although the prominent effects found *in vitro* are not keeping with the *ex vivo* data, they are likely to reflect the true clinical situation during repetitive, large-dose HES administration. We therefore conclude that HES 130/0.4 may have hemorheological advantages over conventional HES 200/0.5 when used in large quantities.

P1.11

ACUTE ADMINISTRATION OF ILOPROST IN PATIENTS WITH RAYNAUD'S PHENOMENON ASSOCIATED WITH SYSTEMIC SCLEROSIS: ELECTROPHYSIOLOGICAL AND MICROCIRCULATORY FINDINGS

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Iloprost is a stable prostacyclin analogue which has been shown to be effective in the treatment of Raynaud's phenomenon (RP) associated with systemic sclerosis (SSc). Several mechanisms of action other than vasodilation and the antiplatelet activity are involved in the clinical efficacy of the drug. In order to find out some possible additive mechanisms responsible for the observed beneficial effects on microcirculation, the effects of Iloprost on sympatho-vagal balance were studied in patients affected with RP secondary to SSc, evaluating, in parallel, changes in peripheral microcirculation with videocapillaroscopy (VCS).

Twenty-one patients (54.9±12.8 years, 17♀, 4♂) with SSc were submitted to Iloprost infusion (2 ng/kg/min for six hours for 3 consecutive days) as a treatment of RP. Heart rate variability (HRV) and VCS were evaluated in all the patients before and after i.v. administration of Iloprost in day 1 of treatment.

HRV: 12-lead ECG was recorded for 10 min. during spontaneous and metronome (12 cycles/min) breathing, by Cardioline ECT WS 2000 (Remco Italy). Algorithm, used for the analysis of HRV, was a spectral method (fast Fourier Transformation). Three main spectral components were distinguished in a spectrum calculated from short-term recordings of 5 minutes:

- a very low frequency component (VLF): <0.04Hz;
- a low frequency component (LF): range 0.04-0.15Hz
- a high frequency component (HF): range 0.15-0.4 Hz.

A Video Cap (MDS Group) was used with a video specific probe (x 200 magnification). The observation sites were: hand nailfold, conjunctiva, antecubitum, gigival edge. VCS observation permit to detect various qualitative-morphological alterations which may be analyzed and quantified. A semiquantitative scale is performed to point capillary flow sludging and capillary incisures as 0=normal, 1=slight, 2=mild, 3=severe.

All the subjects were studied in the same environmental conditions: between 9 and 11 a.m. after an overnight fasting, in a temperature-controlled laboratory (21°C to 24°C) and 20 minutes in supine position.

Statistical analysis: A Student's t test for paired data was performed to compare HRV and VCS parameters before and after treatment.

Total power of HRV increased slightly but not significantly (977∂598 vs 1139∂804 $msec^2$, p=0.3) after Iloprost infusion, whereas LF/HF ratio decreased significantly (4.5∂4.7 vs 3.0∂2.9; p=0.03). In particular, HF spectral component, which is the expression of vagal activity, increased significantly after Iloprost infusion (151∂133 vs 216∂199 $msec^2$, p=0.05). Capillary flow sludging decreased significantly (2.01∂0.57 vs 1.27∂0.35, p=0.05), capillary incisure decreased but not significantly (2.15∂0.38 vs 1.78∂0.28, p=0.3). No other modifications.

Our study show that Iloprost reduces the sympathetic imbalance in RP patients by increasing the vagal tone. This effect may account, at least in part, for the observed beneficial acute effects of the drug on the microcirculatory flow.

P1.12

COMPARISON OF BLOOD RHEOLOGICAL CHANGES IN THE MICROCIRCULATION DURING THE HEMORRHAGIC AND TRAUMATIC SHOCKS IN RATS

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Objective: The aim of the present study was analysis of the specific hemorheological disorders during the experimental traumatic and hemorrhagic shocks.
Methods: Experiments were carried out in adult white laboratorial rats of both sexes. Experimental animals were divided in three groups (control group, with hemorrhage, and a group with severe injury of, hind leg). The following hemorheological properties were investigated: The RBC aggregability, their deformability, and the systemic hematocrit. The RBC aggregability was assessed by using the "Georgian technique"; the RBC deformability was determined with the nuclepore membrane filter method of Reid; the hematocrit was investigated by blood centrifugation. The obtained results were treated statistically by using the "Two sample T test".
Results: We found that in the animals with traumatic shock the erythrocyte aggregability index increased by mean 181%, while in the hemorrhagic group this index was on the contrary, decreased by mean of 68%. The RBC deformability underwent a significant decrease during both the traumatic and hemorrhagic shocks by mean 52% in the first and by 62% in the second case. The systemic hematocrit decreased by mean 45% during the traumatic and by mean 50% during the hemorrhagic shock.
Conclusion: The obtained data provide evidence that the hemorheological disorders represent themselves most significant factors among the microcirculatory disturbances in the pathogenesis of both, the traumatic and hemorrhagic, shocks. The increased RBC aggregability plays the most important role during the traumatic shock, while the decreased RBC deformability and systemic hematocrit are significant in development of the hemorrhagic shock.

P1.13

HEMORHEOLOGICAL AND MICROCIRCULATIONARY DISORDERS DURING SEPTIC SHOCK IN RATS EXPERIMENTS

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Objectives: Despite a remarkable progress in the intensive care medicine, sepsis and shock remain to be major clinical problems in the intensive care units. It is agreed upon that the microcirculatory disorders play an important role in the pathogenesis of shock, but involvement of the specific blood rheological disorders in the microcirculation during it have not been sufficiently analyzed as yet. The aim of the present study was the experimental analyzes of hemorheological disorders in the intestinal mesentery during the septic (endotoxic) shock.
Methods: Experiments were carried out with white laboratory rats of both sexes weighing about 200 g. The animals were divided into two groups: the control and with the septic (endotoxic) shock, which was produced by intravenous injection of endotoxin. We used the lipopolisaccharide from Escherichia coli (serotype 0111:B4, Sigma) in a dose of 10 mg/kg body weight. The microcirculatory changes were investigated with the Leitz microscopical technique in the small intestine mesentery, where the microvessels diameters and the blood flow velocity in their lumen were measeured by using intravital videomicroscopy. The following blood rheological properties were investigated in the same animals: the RBC aggregability, their deformability and the systemic hematocrit. The RBC aggregability was measured by using the "Georgian technique"; the RBC deformability was determined with the nucleopore membrane filter method; the hematocrit was investigated by blood centrifugation.
Results: We found in all animals that the microvessels diameters did not change appreciably, while the RBC aggregability index increased by mean of 136%. As to the RBC deformability it decreased by 71%, and the systemic hematocrit lowered by 31%, as compared to the same parameters in the control group.
Conclusion: It is just the hemorheological disturbances and not the microvessels diameters that undergo the most pronounced changes, which are immediately involved in development of the endotoxic shock of rats.

P1.14

PERSISTENCE OF THE ALTERED POLYMORPHONUCLEAR LEUKOCYTE RHEOLOGICAL AND METABOLIC VARIABLES AFTER 12 MONTHS IN JUVENILE MYOCARDIAL INFARCTION

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Although thirty years ago a relationship between leukocyte count and acute myocardial infarction (AMI) was observed, only in the last decade the specific role played by leukocytes, and especially polymorphonuclear leukocytes (PMN), in AMI has been underlined. This clinical condition is also associated with PMN rheology impairment and in this study we evaluated two major

rheological aspects (membrane fluidity and cytosolic Ca^{2+} concentration) in a group of young adults with AMI. We enrolled 41 young AMI patients (39 men and 2 women; mean age 41.0±4.0 years), who were examined 5-10 days after AMI (T1) and 12 months later (T2). All the subjects were treated with ACE inhibitors, statins and platelet antiaggregating agents (aspirin or ticlopidine or clopidogrel). The membrane fluidity was obtained labelling granulocytes with the fluorescent probe 1-[4-(trimethylamino)phenyl]-6-phenyl-1,3,5-hexatriene (TMA-DPH) and considering the degree of fluorescence polarization, inversely correlated to the membrane lipid fluidity. The cytosolic Ca^{2+} content was obtained marking PMN cells with the fluorescent probe Fura-2AM and considering the ratio between the Fura 2-Ca^{2+} complex and the unchelated Fura 2 fluorescence intensity. Both parameters were evaluated at baseline and after *in vitro* activation with 4-phorbol 12-myristate 13-acetate (PMA) at the concentration of 4.5 σM, prolonged for 5 and 15 minutes. At T1 the PMN membrane fluidity and cytosolic Ca^{2+} content in AMI patients were respectively decreased and increased in comparison with control group. Comparing the two PMN parameters at T1 and T2, at the second time of observation the membrane fluidity was increased and not any more different from control subjects, but there was also a further increase in cytosolic Ca^{2+} content. *In vitro*, PMN activation caused no significant variation of these parameters in the control group, while in AMI patients membrane fluidity significantly decreased and cytosolic Ca^{2+} content increased not only during the initial stage, but also after 12 months. The long-term functional alteration of PMN cells observed in young adults with AMI confirm the role of these cells in the inflammatory response following AMI. In the light of these data, the use of molecules able to modulate granulocyte activity, such as calcium channel blockers or pentoxifylline, should be reconsidered in myocardial infarction, together with the usual pharmacological treatment.

P1.15

OXIDANT/ANTIOXIDANT UNBALANCE AND ENDOTHELIAL DYSFUNCTION IN ADVANCING-AGE

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According to the free radical theory of aging it is well established that the sum of the deleterious oxidative reactions going on continuously through the body contributes to the overall decline in physiological functions. A progressive decay of endothelial vascular function is a well-documented aspect of aging process and contributes to several age-related diseases with an increasing morbidity and mortality. A possible mechanism by which advancing age leads to an impaired endothelial function seems to be ascribed to a decreased plasma nitric oxide (NO) bioavailability. Biological activity of NO is strictly dependent on superoxide anion that compromises its effective half-life and regulating functions. A major defence against superoxide radicals produced during the age-associated oxidative reactions is represented by extracellular superoxide dismutase (EC-SOD). To verify the potential involvement of the age-dependent modifications of EC-SOD activity in the impairment of plasma NO availability with advancing age, 40 healthy men divided into 4 age groups for the purpose of comparison (young: 27.4±1.5, range 20-34, years; middle: 50.8±2.2, range 40-60, years; old: 70.0±1.8, range 62-78, years; very old: 86.1±1.1, range 80-92, years) were enrolled in this study. Plasma samples were used for measurements of the stable end-product nitrite/nitrate (NO_x), as expression of NO availability, EC-SOD activity, thiobarbituric acid reactive substances (TBARS) as marker of lipid peroxidation, low density lipoprotein (LDL) copper-mediated oxidation in vitro and total antioxidant capacity (TEAC). As indicated by our results, a significant progressive decrease of plasma NO_x content and EC-SOD activity were observed with advancing age and their values were positively correlated ($r=0.713$, $p<0.001$). We also observed increased values of TBARS together with reduced lag time for in vitro oxidation of LDL and decreased values of TEAC in aged individuals. EC-SOD values were negatively correlated with plasma TBARS values ($r=-0.855$, $p<0.001$). Thus, it is presumable that the decrease of EC-SOD activity may lead to significant accumulation of superoxide anions in the vascular microenvironment predisposing to peroxynitrite formation able to initiate lipid peroxidation and lower defence strategies. Findings of the present study give further insight into the mechanism involved in age-associated vascular endothelial decay, indicating that the decrease of antioxidant defence strategies play a primary role by compromising NO availability in normally aged individuals, particularly through a progressive decrease of EC-SOD activity.

P1.16

FETAL AND JUVENILE ANIMAL HEMORHEOLOGY

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The study provides information on the blood fluidity in healthy, juvenile sheep and rabbits during growth (n=18), and shows also data from fetal rabbits and cats. In the fetal rabbit (n=3) and cat (n=2), whole blood viscosity (WBV; LS30, Contraves, Switzerland) and plasma viscosity (PV; OCR-D, Paar, Austria) was low (WBV(0.7 s^{-1}): rabbit: 3.28/3.00/2.44; cat: 7.87/10.88. WBV(94 s^{-1}): rabbit: 2.57/2.48/2.39; cat: 2.75/3.73 mPas) (PV: rabbit: 1.10/1.10/1.05; cat: 1.27/1.39 mPas), which was associated with a low plasma protein concentration and a low erythrocyte count despite a high erythrocyte volume. After

parturition, blood viscosity increased in rabbits in parallel with hematocrit, while MCV decreased (WBV(0.7 s^{-1}): 9.28(8.07/10.88); WBV(94 s^{-1}): 3.67(3.62/3.82); PV: 1.15(1.15/1.25) mPas). In contrast, in the sheep, whole blood and plasma viscosity decreased after delivery (WBV(0.7 s^{-1}): 1.31(0.94/1.88); WBV(94 s^{-1}): 2.45(2.43/2.85); PV: 1.24(1.23/1.29) mPas). Hematocrit and MCV decreased, while erythrocyte count increased under these circumstances. In summary, whole blood viscosity was similar among fetal sheep, rabbits, and cats and is diminished compared to adult individuals to guarantee an optimal oxygen supply during a period of life in which the oxygen maintainance of the child depends on the health and the environment of the mother. However, during growth, blood viscosity rose in rabbits, while it continously decreased in the sheep. At an unknown time point this fall in blood viscosity in lambs must reverse, since adult sheep again show a higher blood viscosity than juvenile lambs at the age of 2 months.

P1.17

MEASUREMENTS OF THE PLASMA-VISCOSITY IN THE OLDEST OLD AGE-GROUP

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Background and programme: According to the forecasts the population of Hungary will decrease by 2025 8,8 millions, by 2050 7,5 millions people. The greatest reduction is expected between the 0-14 years old age-group, while the biggest increase will be over the 90 years old age-group. The occupation with this population should be one of the most important social and medical problem in our country and all over Europe. We started a research-work in September of 2004, under the title: Research of Debrecen residents over 90 years old concerning their cognitive status and a complex study of their health. This work is supported by the Health Scientific Committee (No.: 296/2003). The aim of this study is to give a real picture to the social politics and to the medical services in connection with their duty towards the oldest old generation.
Methods of the research: We examine:
17 laboratory parameters
plasma-viscosity (Blood samples are collected into ethylendiamine-tetraacetic acid (EDTA) vacuum tubes, and the measurement is completed within 4 hours after the venipuncture. Plasma-viscosity is evaluated using a Haake-microviscosimeter)
resistance of the plasma against the oxidative stress (1. The measurement of carbonyl content in oxidatively modified proteins 2. The lipid per-oxidation of the plasma 3. The measurement of oxygen radical absorbance capacity)
cognitive functions (Mini Mental State Test)
rate of the depression (Abbreviated Geriatric Depression Test)
social state (Sociological questionnaire, which contains 134 questions about their nutrition, health status, physical activity, identity, relationships, etc…)
general physical state
Results and discussion: By this time, we have experienced the increase of the plasma-viscosity at 64% of our samples in comparison with the adult reference range (1,15-1,35 mPas). We have measured 70 samples, 45 were higher (average: 1,45 mPas), 25 samples were in the normal range (average: 1,30 mPas). Correlation seems to be positive with the total protein, fibrinogen, ESR, cholesterol and triglycerid, and negative with the HDL-cholesterol. We would like to enlarge the number of our samples continuously, and follow the results of plasma-viscosity with attention, especially the correlations with the other laboratory parameters. Our aim is also to compare our results with similar international studies, besides with another laboratory screening test made in 2001 in Debrecen among the 60-90 years old people.

P1.18

PARTIALLY OPPOSITE HEMORHEOLOGICAL EFFECTS OF AGING AND TRAINING AT MIDDLE AGE

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Aging impairs blood rheology while various training protocols improve it. The purpose of this study was to delineate the respective roles of aging and endurance training on blood rheology. Thirty-two subjects [16 middle-aged men: 8 cyclists (MAcy) and 8 sedentary men (MAsed) and 16 young men: 8 cyclists (Ycy) and 8 sedentary men (Ysed)] were compared in this study. Results showed higher red cell rigidity and aggregability (AFFIBIO), lower RBC disaggregability (AFFIBIO) at middle age than at 25 yr, regardless training status. However there was no age-related difference in whole blood viscosity at either native or corrected hematocrit, plasma viscosity, hematocrit, and Myrenne aggregation indexes M and M1. Training was associated with a reduced hematocrit in middle age subjects but not in 25 yr old ones. We evidenced no training effect on red cell rigidity (Dintenfass's Tk index), in whole blood viscosity at either native or corrected hematocrit, and plasma viscosity.

Thus regular cycling at middle age maintains a low hematocrit but does not prevent aging-related increase in red cell rigidity and aggregability. Specific effects of cycling amond other sports may explain this specific pattern.

P1.19

EFFECTS OF PHYTOSTEROLS SUPPLIED IN LOW-FAT MILK, IN THE HEMORHEOLOGICAL PARAMETERS AND PLASMA CHOLESTEROL CONCENTRATIONS IN WISTAR RATS

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Clinical and experimental studies have shown that the use of phytosterol esters as a food ingredient reduces the plasma concentrations of cholesterol and LDL cholesterol, not affecting the HDL cholesterol levels. There are evidences that hypercholesterolemia is a risk factor for the development of atherosclerosis and this is associated with hemorheological alterations. Based on the use of phytosterols as a food ingredient, because of their cholesterol lowering properties, we have conducted a 30-day feeding study with groups of 10 Wistar rats, drinking low-fat milk containing phytosterols, in order to evaluate the plasma cholesterol concentrations and the hemorheological parameters. Thus we have used milk containing the following concentrations in phytosterols: 0 (as the Placebo group), 0.2, 0.3, and 0.4 g of phytosterols per 1dL of milk. Throughout the study, clinical observations, body weights and food and milk consumption were measured. After the 30-day feeding period, blood samples were collected and biochemical and hemorheological parameters were determined.
The fourth concentrations studied were tolerated as evidenced by the absence of clinical changes or major abnormalities in growth, food and milk consumption. For the plasma cholesterol concentration there were no significant differences in the cholesterol and HDL cholesterol levels, relatively to the control group, but there was a decrease of about 70% in the LDL cholesterol levels. In the hemorheological parameters some significant changes were observed in the plasma viscosity and in the membrane fluidity in all the experimental groups, comparing to the Control. The blood viscosity and the erythrocyte deformability have significant changes in the Placebo group that are ameliorated with the ingestion of the phytosterols enriched milk. With these results we have concluded that phytosterols maintain their cholesterol lowering properties when incorporated in milk and can be considered a hypolipemic food component.

P1.20

PHYTOSTEROLS IN MILK AS A DEPRESSOR OF PLASMA CHOLESTEROL LEVELS: EXPERIMENTAL EVIDENCE WITH HYPERCHOLESTEROLEMIC PORTUGUESE SUBJECTS

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Artery-clogging plaques may formed when among others factors, cholesterol levels become too high decreasing artery diameter and at some pathological conditions enabling the blood supply to the heart or brain, triggering a heart attack or stroke. Plant sterols have been reported to decreased plasma concentrations of cholesterol without any side effects. In order to evaluate if milk should be a good vehicle for phytosterols lowering cholesterol plasma levels, we performed a study with hypercholesterolemic patients (n=19), treated with enriched phytosterols milk (2g/day). Another group of Hypercholesterolemic patients (n=15) of match age drinking equal type of milk but without phytosterols were used as a control group. In all cases the subjects were instructed to maintain the same dietary intake during the study. Concentrations of total cholesterol, HDL-C, LDL-C and biochemical, hematological and hemorheological parameters were measured in the beginning, after 15 and 30 days of milk beverage intake. Hypercholesterolemic patients treated with phytosterols enriched-milk shows a significantly diminishes on total cholesterol and LDL-C plasmatic concentrations by 9.62% ($p < 0.05$) and 12.20% ($p < 0.05$), respectively. After 30 days, a little increase in the total cholesterol and LDL-C levels were observed for hypercholesterolemic subjects, 6.69% ($p < 0.05$) and 8.68% ($p < 0.05$) were the values obtained for total cholesterol and LDL-C levels, respectively. In the hypercholesterolemic control group there was no statistically difference between plasma levels of total cholesterol, HDL-C and LDL-C during the study. This trial includes the evaluation of some biochemical, haematological and hemorheological parameters as plasma viscosity, erythrocyte aggregation and □-carotene levels; significantly differences were not observed during the study for both groups during phytosterols-milk intake. The results obtained during the study show a positive effect with the enriched-milk with phytosterols as a good vehicle as plasma cholesterol-lowering as combined treatment for hypercholesterolemia.

P2.1

ADRENERGIC AGGREGATION OF ERYTHROCYTES

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Adrenergic aggregation of erythrocytes is known but mean of this phenomenon is investigated a little. We studied adren-induced aggregation of erythrocytes in low-concentrated suspensions (Hct=0,05%-0,2%) with original buffer and by inductors: epinephrin, ADP and norepinephrin (end-concentration 0,02 dyn/cm^2). η-adrenoreceptors have been blocked by propranolol. Process had to be investigated by microscopy and by aggregometry simultaneously. The study have shown wave character of process and have allowed to calculate such parameters as linear erythrocytes aggregates forming time, three-dimensional erythrocytes conglomerates forming time, degree and rate of aggregation. Thus the preparations stimulating ζ- and η-adrenoreceptors (epinephrin, norepinephrin) possessed high aggregative activity and low rate of aggregation, and blocking of η-adrenoreceptors by propranolol has shown that in absence of fibrinogen the erythrocytes aggregation is mediated mainly by ζ-adrenoreceptors. On the contrary, fibrinogen introduction in system reduced aggregation effect of inductors proportionally to fibrinogen concentration. Also adrenergic aggregation of erythrocytes is convertible. Research has shown that this phenomenon can be only in patients who are under stress (for example, surgical). Thus intensity of erythrocytes stress-aggregation and its presence allows to estimate expressiveness and action of stress-factors.

P2.2

INCIDENCE OF PULMONARY THROMBOEMBOLISM (PTE) AND NEW GUIDELINES FOR PTE PROPHYLAXIS IN JAPAN

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Venous thromboembolism (VTE) has been on the rise in Japan in recent years, together with the Westernization of Japanese eating habits. According to the investigation by Editorial Committee on Guideline for Prevention of Venous Thromboembolism by Japanese Society of Anesthesiologists in 2002, 369 cases of pulmonary thromboembolism (PTE) were registered among 467 General Hospitals and University Hospitals. The rate of perioperative PTE is estimated to be 0.044% (369/837540), and the fatal rate among clinical PTE was 17.9%. 36% of the cases occurred in Orthopedics, 22% in general surgery and 10% in Obstetrics and Gynecology. 59% of the cases did not received prophylaxis, and 52% of the cases were restricted mobility. The incidence of clinical PTE after general surgery was 0.16% from 1994 to 2000 in Japan. Fatal rate among clinical PTE was 21.3%, and fatal rate among all patients was 0.04%. The incidence of clinical PTE after total hip joint replacement surgery among 46 papers between 1990 and 2001 in Japan was 1.1%, and that of fatal PTE was 0.15% among all patients. Patients of PTE were investigated between 1991 and 2000 among 68 University Hospitals and 34 General Hospitals in Japan. 76 cases of PTE occurred in Obstetrics, and 14.5% of them were fatal. The incidence of PTE was 0.003% in vaginal deliveries and 0.06% in cesarean sections. 178 cases of PTE occurred in Gynecology, and 13.5% of them were fatal. The incidence of PTE was 0.03% in benign diseases and 0.42% in malignant diseases. However, the incidence of PTE in Japan is considered to be at least one level lower compared with Western populations according to ACCP guidelines. Furthermore, low molecular weight heparin (LMWH) is not covered by health insurance and is contraindicated for pregnant women still now in Japan. Then, we established Japanese guidelines for VTE prophylaxis according to Japanese clinical evidences of PTE in 2004. We classified four risk groups according to ACCP guidelines. Recommended prophylaxis is early ambulation for low risk group, graduated compression stocking (GCS) or intermittent pneumatic compression (IPC) for moderate risk group, IPC or low dose unfractionated heparin (LDUH) for high risk group, and LDUH + IPC or LDUH + GCS for highest risk group. And, risk group should be raised one rank in cases with any additional risks, such as obesity, advanced age, pregnancy, operation time, and other complications. Recommended prophylaxis in this guideline may be minimum one, because the purpose of this guideline is to penetrate the awareness and education of VTE throughout Japan. Fortunately, the management fee for PTE prophylaxis was established and covered by health insurance in April 2004. However, after accumulation of further evidences and application of pharmacological agents, such as LMWH, we will establish the advanced guidelines in the future.

P2.3

THE INFLUENCE OF HEMATOCRIT ON PRIMARY HEMOSTATIS UNDER HIGH SHEAR CONDITIONS IN VITRO

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Primary hemostatis consists of platelet adhesion to subendothelial collagen, their activation and aggregation and the formation of a platelet plug. Erythrocytes are involved in this process because they tend to flow in the center of the vessel and push platelet towards the site of action on the vessel wall and enhance shear forces, which activate platelets further. In the platelet function analyser PFA-100® (Dade Behring, Düdingen, Switzerland), the in vivo situation is simulated in vitro with blood

being aspirated at high shear rates ($5000s^{-1}$) through a capillary into a membrane pore with a diameter of 150 µm coated with type I collagen and either epinephrine or adenosine diphosphate. Aggregating platelets plug the pore and stop the flow, which is measured as the closure time. We analysed the influence of erythrocytes on platelet function analyser measurements by systematic variation of the hematocrit (20, 30, 40, and 50%) at constant platelet counts of 289±61 k/µl plasma, or 152±30 k/µl blood, 96±9 k/µl blood and 54±5 k/µl blood, respectively. An inverse correlation was found between hematocrit and closure time under all circumstances. A decrease of the platelet count by 50 k/µl could be compensated for by a 10% increase in hematocrit. The hematocrit must, therefore, be taken into consideration for the correct interpretation of PFA-100® measurements. Our data also provide a pathophysiological rationale to reduce the risk of bleeding in patients with thrombocytopenia and anemia by normalizing the hematocrit with erythrocyte transfusions.

P2.4

FOLIC ACID DOES NOT PROTECT ENDOTHELIAL FUNCTION FROM ISCHEMIA AND REPERFUSION. A HUMAN IN VIVO STUDY.

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Introduction: Uncoupling of the nitric oxide synthase (NOS) is a condition associated with increased superoxide anion and decreased NO production by this enzyme. Studies showed that folic acid can prevent and/or reverse NOS uncoupling in diabetes, smoking, hypercholesterolemia and nitrate tolerance, and animal data report a protective effect of this group B vitamin in ischemia and reperfusion (IR) injury. We set out to test whether folic acid administration limits IR-induced endothelial dysfunction in humans.
Methods and results: In a double-blind, parallel study, 20 healthy young male volunteers were randomized to receive folic acid, 10 mg/day, or matching placebo for 7 days. At the end of the treatment, endothelium-dependent, flow-mediated, dilation (FMD) of the radial artery was measured before and after IR injury (15 minutes of ischemia at the level of the brachial artery followed by 15 minutes of reperfusion). There was no difference at baseline between groups in any variable. After placebo, IR significantly blunted FMD (before IR: 6.7±1.0%; after IR: 1.5±1.3%, $P<0.01$). A similar effect was observed in the folic acid group (before IR: 6.3±1.1%; after IR: 2.1±1.0%, $P<0.02$, P=ns compared to placebo).
Conclusions: As opposed to results from animal studies, high-dose folic acid is unable to protect the vascular endothelium from IR injury in humans.

P2.5

POSTCONDITIONING DOES NOT LIMIT ENDOTHELIAL DYSFUNCTION INDUCED BY ISCHEMIA AND REPERFUSION IN HUMANS

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Introduction: Animal studies have shown that exposure to brief periods of controlled ischemia (postconditioning) at the end of a prolonged ischemia limit postischemic tissue injury, an effect that might be mediated by a less abrupt production of toxic oxygen free radicals during reperfusion.This effect has important potential clinical applications in cardiovascular surgery and in the setting of primary angioplasty. We set out to test whether postconditioning can prevent ischemia-induced endothelial dysfunction in a human in vivo model.
Methods and results: 10 healthy young non smoking volunteers were enrolled in this cross-over, controlled, investigator-blinded study. In two separate visits, subjects were exposed to either forearm ischemia and reperfusion alone (15 minutes of ischemia induced by inflation of a pneumatic cuff at the level of the brachial artery followed by 15 minutes reperfusion) or 15 minutes ischemia followed by postconditioning (three periods of 20 seconds of ischemia separated by 10 seconds reperfusion immediately at the end of the 15 minutes of ischemia). Endothelium-dependent flow mediated dilation (FMD) was measured at the level of the radial artery both before and after ischemia (or ischemia + postconditioning) on each visit. There was no difference between visits in baseline parameters. Forearm ischemia blunted FMD by a similar degree on both study visits (ischemia alone: before ischemia: 7.7±1.3, after: 2.5±1.4%; postconditioning: before: 7.3±1.2, after: 2.6±1.6%; $p<0.05$ for both, p=ns between visits).
Conclusions: Postconditioning does not limit post-ischemic endothelial damage in this human in vivo model. Our data appear to contradict those of animal studies where postconditioning dramatically reduced ischemic injury. Further studies are necessary in human models to evaluate the clinical applicability of postconditioning.

P2.6

HEMORHEOLOGICAL DISORDERS DURING ISCHEMIC BRAIN INFARCTS WITH AND WITHOUT DIABETES MELLITUS

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It is well known for the practical medicine that diabetes mellitus complicates significantly the course of brain infarcts in neurological patients. The aim of the present study was to find out whether the hemorheological disorders play a role in these cases. We investigated the blood plasma viscosity, as well as the RBC aggregability (the "Georgian index"), in two groups of the patients with brain infarcts: with and without complications of the disease by diabetes mellitus, and compared the results with the healthy control group of about the same age. The present investigation results have shown that both of the mentioned indices of blood rheological disorders were significantly higher in the patients with ischemic brain infarcts as compared to the healthy control group. As to the other patients group where the brain infarcts were complicated with diabetes mellitus, the both investigated hemorheological disorders underwent significant increase in all the investigated patients. The specific changes of the blood rheological disorders were found to be as follows. During the ischemic stroke the blood plasma viscosity was increased by 9.2 per cent, while in the patients group complicated with diabetes mellitus the increase was significantly more pronounced - by 17.6 per cent. As to the erythrocyte aggregability, it changed considerably more - by 113.8 per cent during stroke without diabetes, while in the diabetic patients the RBC aggregabilty index was found to be increased by mean 147.3 per cent. We concluded, therefore, that the complication of the ischemic brain infarcts by the diabetes mellitus results in a significant increase of the patients' blood rheological disorders in all the investigated cases. This should be taken into consideration by the medical personal treating the stroke patients in the neurological clinics and in the critical care units.

P2.7

COMPARISON OF ERYTHROCYTE AGGREGABILITY CHANGES DURING THE ISCHEMIC AND HEMORRHAGIC STROKE

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The aim of this work was comparative investigation of erythrocyte aggregability changes in the systemically circulating blood during the ischemic and hemorrhagic strokes. Subjects of the present study were patients with ischemic brain infarcts (14 patients) and with hemorrhagic stroke (21 patients) from the Intensive Care Unit of Institute of Neurology and Neurosurgery. The blood samples were obtained from the following blood vessels: the common carotid artery carrying blood to the primarily damaged brain hemisphere, the both jugular veins carrying blood from the primarily damaged and the contralateral hemispheres, as well as from the cubital vein to obtain specimens of the systemically circulating blood. Erythrocyte aggregation was evaluated by using the "Georgian technique". We found that the RBC aggregation indices increased in both the regional as well as the systemic circulation of the hemorrhagic stroke patients: jugular veins of the damaged hemisphere (69,1±1,3), jugular veins of the contralateral hemisphere (66,5±1,2), carotid arteries (63,0±1,4), and cubital veins (59,7±1,2), as compared to the ischemic stroke patients: jugular veins of the damaged hemicphere (62,5±1,5); jugular veins of the contralateral hemisphere (58,7±1,6); carotid arteries (57,6±1,9), and cubital veins (50,3±1,7). Therefore, we suppose that various mechanisms are involved in the development of the hemorrhagic and ischemic stroke. The role of the blood rheological properties changes in their pathogenesis seems also to be different.

P2.8

DISPOSABLE EKTACYTOMETRY: LASER DIFFRACTION IN A SLIT FLOW

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Deformability of erythrocyte is one of the major determinants of blood flow resistance passing through small capillaries of the microcirculation. Available techniques for measuring erythrocyte deformability often require washing process after each measurement, which is not optimal for day-to-day clinical use. A laser diffraction technique has been combined with slit-flow rheometry, which shows significant advances in ektacytometry design, operation, and data analysis. The essential features of this design are its simplicity (ease of operation and no moving parts) and a disposable element which is in contact with the blood sample. Both the laser-diffraction image and pressure were measured with respect to time, which enable to determine the elongation index (EI) and the shear stress. The range of shear stress is 0 ~ 20 Pa and the measuring time is less than 1 min. The Elongation Index (EI) is determined from an isointensity curve in the diffraction pattern using an ellipse-fitting program. With the present device, the deformation of erythrocyte subjected to continuously decreasing shear stress in a microchannel flow can

be quickly measured with extremely small quantities of blood. The measurements with present device were compared with those of LORCA and a strong correlation was observed. The deformability of the hardened erythrocyte was markedly lower than that of the normal erythrocytes. In addition, the young cells showed higher values of the elongation index (EI) than the old cells. The newly developed slit ektacytometer can measure the erythrocyte deformability with ease and accuracy. In addition, the slit ektacytometer can be easily used in a clinical setting owing to the incorporation of a disposable element that holds the blood sample.

P2.9

FRACTAL ANALYSIS OF MONOCYTES IN DIABETES

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Fractal geometry is a very useful tool for describing the irregular and complex shapes of many natural objects. In the field of pathology fractal geometry has been applied with remarkable success to analysing premalignant and malignant tissues, the architecture of trabecular bone in osteoarthrosis and several other lesions. The aim of this study was to evaluate the fractal dimension of pericellular membrane of monocytes observed by transmission electron microscopy in diabetic patients and in control subjects. The results showed that fractal dimension of circualting monocytes is statistically increased in diabetic patients (type 1 and type 2), compared with sex- and age-matched controls ($p<0.01$). The mechanism underlying the observed increased complexity of pericellular membrane may be explained by the *in vivo* activation of the circulating monocyte in diabetes. In effect, fractal analysis of monocytes from healthy human subjects, stimulated *in vitro* with the ionophore A23187 or with the oligopeptide FMLP, showed an analogous significant increase of complexity of pericellular membrane, compared with their controls ($p<0.001$). Our approach is able to quantitatively evaluate the morphological modifications of circulating monocytes in diabetic patients, offering new parameters useful to follow the effects of therapeutical procedures.

P2.10

POLYELECTROLYTE MULTILAYER THIN FILMS USED AS NEW TOOL FOR VASCULAR TISSUE ENGINEERING.

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A method to obtain hemocompatible vascular substitutes consists to recover the luminal surface of the substitutes by a monolayer of endothelial cells. Nevertheless, the surface of current substitutes is not favourable for the adhesion and the growth of endothelial cells. Indeed, it is necessary to improve the interactions of endothelial cells by surface modifications. Recently, a new versatile method of self-assembled architectures based on the alternate adsorption of polycations and polyanions has been developed leading to the build-up of multilayered polyelectrolyte films. This layer-by-layer deposition method is mainly based on electrostatic interactions. The method offers also large possibilities for varying the physico-chemical properties of films such as surface roughness, film stiffness and thickness. In this work, we evaluated the biocompatibility, adhesion and activation of endothelial cells on two type of films ending either by poly(D-Lysine) (PDL), or poly(allylamine hydrochloride) (PAH), and compared to PDL or PAH monolayers, glass and fibronectin. Our results on biocompatibility showed that the polyelectrolyte films improved the initial adhesion and the growth of endothelial cells, without inducing cytotoxic effects after several days of culture. The evaluation of adhesion properties showed that despite a lower expression of β1 integrins, endothelial cells adhered firmly on the polyelectrolyte films. Moreover, the signal of adhesion was not perturbed for endothelial cells on the PAH ending film, confirming the biocompatibility of this films. Finally, PAH ending film did not perturb the activation of endothelial through the ICAM-1 expression in basal condition or under TNFα or shear stress stimulation, contrary to PDL ending film. Consequently, considering the biocompatibility, adhesion and activation of endothelial cells on polyelectrolyte films, PAH ending film could constitute a useful tool for vascular tissue engineering.

P2.11

HEMORHEOLOGICAL DISORDERS IN BRAIN CAPILLARIES (EXPERIMENTAL STUDIES)

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Cerebral hemorheological disorders producing brain tissue hypoxia are the most frequent cause of neurological pathologies. Many clinical studies have shown an availability of hemorheological disorders during brain infarcts. Purpose of the present

experimental study was investigation of the hemorheological disorders producing blood flow disorders in the brain capillaries. The experiments were carried out with the chinchilla rabbits. In the first series of experiments we produced enhancement of the RBC aggregation (the Georgian technique) in the systemic circulation by intravascular introduction of high molecular dextran. In the second group we produced postischemic brain edema in the brain. In the both groups of animals, as well as in the control group, we performed a fast supravital fixation of the brain tissue with subsequent microscopical investigation of the brain capillaries in the unstained thick histological sections. After dextran introduction we found an availability of blood stasis in the majority of brain tissue capillaries, while in the control group the capillary blood flow demonstrated its normal state. The immediate cause of the capillary stases was the intravascular RBC aggregation because of circulation in the blood of high molecular dextran, while in the second group the blood stasis developed because of the enhanced fluid transfer from the capillaries into the brain tissue. In addition, we found a decrease of diameters of those capillaries where the blood flow was preserved but the RBC deformation coefficient was found decreased. Proceeding from the fact that the diameters of brain capillaries are smaller than in other organs of the body, as well as from the obtained experimental results of the present study, we conclude that the capillary blood stases were produced just by the enhanced RBC aggregation, as well as by the disturbance of their ability to be deformed in the flow.

P2.12

LOCAL HEMATOCRIT CHANGES DEPENDENT ON THE INTENSITY OF MICROCIRCULATION IN THE HUMAN SKIN

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The aim of the present study was to find whether there is a correlation between the blood flow rate and the hematocrit changes in human skin microcirculation, which has been earlier demonstrated only in the animal experiments. The experiments were carried out in a group of practically healthy human subjects where the RBC concentration was compared in blood samples taken from the skin warmed to 37°C or cooled to 6°C in the right and left hands. We have regularly found that the hematocrit was always higher in the skin where the blood flow was locally increased as compared to the decreased one. The difference was found to be mean 16.5±9.5 per cent and was statistically significant ($P<0.001$). In another series of experiments we compared the RBC concentration in blood samples taken from the homonymous fingers of both hands without any changes of their temperature. We found that the hematocrit was always higher in blood from the right hand then from the left one while the differences fluctuated from 5 to 14 per cents. Therefore, we concluded that the blood with a higher hematocrit is distributed from the aorta arch to the right brachial artery than to the left one, i.e. as it has been earlier demonstrated by us in the rabbits' experiments. The phenomenon of the local hematocrit changes being depended on the blood flow rate is of a high physiological significance, since the enhancement of microcirculation is usually correlated with increase of metabolic demands of the appropriate tissues, while the increased RBC concentration provides for their better oxygen supply. On the other hand, the present experimental results prove that the standardization of the conditions of blood sampling in patients is necessary in the clinical laboratories. Otherwise significant mistakes, up to one third of the obtained results, might occur.

P2.13

ERYTHROCYTE STIFFNESS IN DIABETES MELLITUS STUDIED WITH ATOMIC FORCE MICROSCOPE

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Stiffness is a very important factor characterizing erythrocytes' mechanical properties during blood flow. Its quantitative evaluation is crucial for studying the mechanisms regulating the red blood cell (RBC) elasticity. We used the Atomic Force Microscope (AFM) to assess the RBC stiffness in blood samples taken from patients suffering from the diabetes mellitus (7 patients). For comparison, the erythrocytes originating from 8 healthy persons (control) have been also investigated. Each blood sample, consisting of 100 µl of the heparinated blood, was dissolved in 200 µl of 10 mM phosphate buffered saline solution (PBS) and then it was deposited on the pretreated (with poly-L-lysine solution, 0.01%) glass coverslip for 30 minutes. Afterwards, glass coverslip was washed in the PBS buffer. The erythrocytes remaining on a glass surface were immediately measured with the AFM – around 20 randomly selected erythrocytes were studied and the stiffness of each erythrocyte was sampled in 20–30 randomly chosen points by recording the force curve for each point. The recorded force curves were used to construct the histograms of the erythrocyte Young's modulus (YM) for each blood sample. The distributions were characterized by a mean value and a distribution width W (where $W = 2\Delta$ *standard deviation)*. The distribution of blood samples taken from healthy persons was characterized by the average value of the Young's modulus of 4.4±0.6 kPa, and the

distribution widths of 3.0±0.6 kPa. In turn, the YM distributions for patients suffering from diabetes mellitus were very broad: the determined average mean value was 14.3±1.4 kPa, and the average width 13.0±2.0 kPa. These values were significantly larger in comparison with those obtained for the healthy subjects. The increase of the erythrocyte Young's modulus for patients suffering from diabetes mellitus correlates well with the reports of observed alterations of the RBC deformability in diabetes mellitus. Most probably, the increased concentration of Ca^{2+} in erythrocytes might be responsible for the observed RBC stiffening. One has to stress that the AFM offers a new and a unique characterization of the mechanical properties of erythrocytes by the distribution of the erythrocyte Young's moduli calculated for a given blood sample. Indeed, many effects related to the impairment of the RBC deformability may be reflected very strongly not only in the increase of the mean value of the erythrocyte YM but also by the changes of the YM distribution shape. These changes can be closely related to the ability of the erythrocytes to alter their shape when passing through capillaries, which is of great clinical importance.

P2.14

OSCILLATING VISCOMETER - EVALUATION OF A NEW BEDSIDE TEST

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A viscometer for bedside blood measurements was developed, consisting of an oscillating resonator probe mounted directly into a disposable vacutainer tube for blood withdrawal. It was tested in vitro on blood samples with variable hematocrits (20-60%), increasing fibrinogen concentrations (0-20 g/l), increasing concentrations of an admixed radiographic contrast medium (iopromide: 0-160 mg Iodide/ml) and erythrocyte concentrates stored at 4°C for 7 weeks. Results were compared with those obtained with a conventional Couette viscometer (Contraves LS 30). Oscillating viscometer data were generally higher. Oscillating viscometry had a good sensitivity for changes in hematocrit and these results correlated well with Couette viscometry ($r=0.96$, $p<0.0001$). An increase in erythrocyte aggregation, as observed with increasing fibrinogen concentrations, however, did not increase oscillating viscometry in contrast to Couette viscometry. Erythrocyte aggregation apparently depleted the boundary layer near the measuring probe surface from erythrocytes, resulting in an unchanged oscillating viscosity despite increasing plasma viscosity. Bedside tests in 17 patients with coronary heart disease and 10 controls confirmed the easy practicability of the test and showed decreased oscillating viscosity in these patients despite higher fibrinogen concentrations. We conclude that oscillating viscometry may become a safe bedside test providing additional biorheological information on blood viscosity near the vessel wall.

P2.15

LASER DIFFRACTOMETRY TECHNIQUE: CLINICAL APPLICATIONS TO VASCULAR PATHOLOGIES

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Isc Hypertension (HTA) and dislipidemy (DLP) represents one the major risk factors for cardiovascular and cerebral-vascular ischemic disease onset. The mechanisms through which it can induce vascular damage are both metabolic and mechanical. Hemorheological alterations in HTA are the results changes affecting both red cell intrinsic structures and their interactions with most important plasmatic components. Several hemorheological determinants (biochemical, ionic, metabolic and rheologic) could influence and produce an impaired erythrocyte deformability rendering an increased flow resistance in the microcirculation. Previous works have demonstrated that in hypertensive patients the aggregate shape parameter (ASP) is increased. This fact represents an increase of aggregating energy and could be a possible mechanism responsible for the rise of arterial pressure in essential hypertension. In a previous work we presented the "Erythrodeformeter" that permit to obtain the stationary and dynamical linear parameters of erythrocyte membrane by laser diffractometry. Laser diffraction produced by human red blood cells (RBC) in suspension is utilized in the Erythrodeformeter to assess cell deformation. Stationary and oscillatory shear-induced elongation of cells leads to an elliptical diffraction pattern, its geometric characteristics being directly related to those of deformed RBC. Erythrocyte stationary parameters (Deformability Index, surface viscosity and elastic modulus) were obtained in stationary regime (Rasia, 1995). Complex viscoelastic parameters (dynamic elasticity, dynamic loss, viscous and elastic components of the complex viscosity) were obtained when operating in oscillating mode (Riquelme et al., 1998). The diffractometric method is sensitive to detect pathological or induced alterations on RBC membrane, which can affect the in vivo blood flow. The behavior of erythrocyte membrane under oscillatory shear stress is very different to the viscoelastic materials usually studied. Therefore, determination of RBC mechanical properties by laser diffractometry is very important in Mechanic Research, likewise of its applications in Clinical Biochemistry and Medicine. The obtained rheological parameters give important information about the erythrocyte membrane and permit detect and characterize erythrocyte alterations in vascular pathologies.

P2.16

STRUCTURE-ACTIVITY RELATIONSHIPS FOR THE PROTECTIVE EFFECTS OF SELECTED FLAVONOIDS AGAINST LIPID PEROXIDATION, PROTEIN DEGRADATION AND DEFORMABILITY LOSS OF OXIDATIVELY STRESSED ERYTHROCYTES

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The effects of eleven flavonoids on lipid peroxidation, protein degradation, deformability and osmotic fragility of erythrocytes exposed *in vitro* to 10 mM H_2O_2 for 60 min at 37 ºC have been studied. The flavonoids quercetin, rutin and morin significantly protected erythrocytes against lipid peroxidation caused by H_2O_2. This inhibition of lipid peroxidation could be explained by the presence of at least two hydroxyl groups in ring B of the flavonoid structure, regardless of their positions. However, the flavonoids quercetin, 3,5,7-trihydroxy-4'-methoxy flavone-7-rutinoside and 3-hydroxy flavone significantly protected erythrocytes against protein degradation. This inhibition could also be explained by the presence of a hydroxyl group at C-3 in ring C of the flavonoid structure. Quercetin and 3,5,7-trihydroxy-4'-methoxy flavone-7-rutinoside significantly protected erythrocytes against loss of deformability and increased osmotic fragility, indicating that the loss of erythrocyte deformability and the increase in osmotic fragility of erythrocytes exposed to H_2O_2 are related to protein degradation rather than to lipid peroxidation. The other flavonoids (chrysin, 2-carboxy ethyl dihydroxy flavone, apigenin, cirsimaritin, Ü-naphto flavone and flavanone) failed to protect erythrocytes against the observed oxidative damages. The results demonstrate the importance of the chemical groups substituted on the basic skeleton of the flavonoids in dictating the type of antioxidant activity, and also demonstrate the hemorheological potentials of flavonoids that have particular protein-antioxidant activities.

P2.17

EXERCISE-INDUCED OXIDATIVE STRESS AFFECTS ERYTHROCYTES IN SEDENTARY AND TRAINED YOUNG MEN

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Intravascular hemolysis is one of the most emphasized mechanism for destruction of erythrocytes during and after physical activity. Exercise-induced oxidant stress has been proposed among the different factors for explaining exercise induced-hemolysis. The validity of oxidant stress following exhaustive cycling exercise on erythrocyte damage was investigated in sedentary and trained subjects before and after antioxidant vitamin treatment (A, C, and E) for two months. Exercise led to statistically significant increase in thiobarbituric acid reactive substance (TBARS) and protein carbonyl content levels in sedentary subjects and resulted in increase of osmotic fragility and decrease in deformability of erythrocytes meanwhile determined intravascular hemolysis (increase in plasma hemoglobin concentration and decrease in haptoglobulin levels). Administration of antioxidant vitamins for two months prevented oxidant stress (TBARS, protein carbonyl content) in sedentary subjects without functional or mechanical alterations in erythrocytes. Trained subjects' erythrocyte responses to exercise were different from sedentary before antioxidant vitamin treatment. Osmotic fragility and deformability of erythrocytes, plasma hemoglobin concentration and haptoglobulin levels were not changed after exercise although an increase in oxidant stress after exhaustive exercise period was observed. After antioxidant vitamin treatment functional and structural parameters of erythrocytes and oxidant stress markers were not different in sedentary group. Younger erythrocytes population was determined in trained subjects by density separation of erythrocytes. These findings suggest that the exercise-induced oxidant stress may contribute to exercise-induced hemolysis in sedentary young men. However this mechanism might not be accounted for trained subjects due to their younger erythrocytes population.

P2.18

EVALUATION OF A TORSIONAL-VIBRATING TECHNIQUE FOR THE HEMORHEOLOGICAL CHARACTERIZATION

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Clinical measurement of blood viscosity is an important parameter in the diagnosis of different diseases (eg diabetes, hypertension, cardiovascular diseases). Furthermore, the significance of the blood viscosity in the microcirculatory flow is of great importance. Thus, a simple and accurate evaluation of hemorheological properties could be an important challenge in clinical practice. Nowadays, validated measurements of blood viscosity is commonly carried out with rotational viscometers by means of the various geometric configurations. An aspect of a certain importance in these experimental conditions is the classification of the blood as Newtonian or non-Newtonian fluid with relation to the shear rates under investigation. Furthermore, red blood cells deform under mechanical force and this aspect could lead to an artificial variation in the apparent viscosity. In this work, an evaluation of the application of a new technique for the viscosity determination is focused. In particular, a torsional-vibrating viscometer was adopted (VM10AL, CBC Europe) in the presence and in the absence of stirring conditions at thermostatted conditions (37±0.1°C). The profile of the rheological behaviour as a function of time was recorded

and compared with that obtained using a cone-plate rotational viscometer (AR500, TA Instrument). The comparison between the data obtained by the two technique has evidenced a correlation in terms of start-points and end-points. Further investigation are in process and the preliminary results are in accordance with the initial hypothesis.

P2.19

A NEW ANIMAL MODEL FOR THE STUDY OF THE PERFUSION HOMOGENEITY OF THE CHORIOIDEA OF ALBINO RATS

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Owing to the difficulties of visualizing the choroidal microcirculation in man, the pathophysiology of age related macular degeneration of the human eye is unsettled. Controlled studies suggesting tractability by rheapheretic therapy makes necessary to test their efficacy in an animal model not complicated by the retinal pigment epithelium.
To this aim we adapted the following techniques to the eye of the rat: intravital microscopy of the perfusion of isolated organs, retrograde catheterisation of the carotid artery and perfusography (a new parametric method of imaging based on the angiography, s. contribution Schmid-Schönbein). To study the perfusion depending on defined rheologic properties we used as artificial solutions: red blood cells (RBC) suspended in solutions of Thomadex to simulate different viscosities of the plasma and solutions of Ficoll to simulate different tendencies of RBC aggregation. These parameters were not only studied under normotension but under hypo- (40 mmHg) and hypertension (200 mmHg) as well. For the whole range between hypo- and hypertension, the experiments revealed that both RBC tendency of aggregation and plasma viscosity have a decisive effect on chorioidal perfusion homogeneity under the experimental conditions simulating “hypoperfusion” (abnormal flow conditions) and when applying strongly aggregating artificial blood”. This amounts to the corroboration of a recent theory of FRIEDMAN: AMD is likely to result from the superpositioning of generalised atherosclerotic and macrohemodynamic anomalies by local factors impeding choreoideal perfusion[1].
References:
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P2.20

PILOT EXPERIMENTS FOR OBJECTIVE CONTROL OF RHEOAPHERETIC TREATMENT AGE RELATED MACULAR DEGENERATION (AMD)

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Background: age related macular degeneration is the most frequent cause of blindness in the developed world, associated with severe loss of life quality in the aging population. Despite many attempts, there was - until recently – no therapeutic approach, primarily owing to the fact that neither classical post mortem signs (other than so called “Drusen” as unspecific lipoprotein deposits) nor any easily quantifiable biochemical abnormality had been identified. In addition, the disease in its early stages is highlighted by a spontaneous variability of symptoms, while there is progressive deterioration in the so called wet form of more advanced cases. Based on initial fortuitous observations subsequent to extracorporeal molecular filtration techniques by BRUNNER and BORGERG, there have been a number of controlled prospective clinical studies in Germany and the USA corroborating clinical improvement after repetitive extracorporeal plasma filtration, manifesting itself in enhanced visual acuity and lack of progression of the so called dry into the so called wet form of AMD. Whatever the cause, the results are related to interruption of the underlying degenerative process involving the system of choroidea, epithelial cells sensory cells and nerve cells.
In vitro studies: as in a large variety of chronic degenerative diseases, the AMD-patients showed the unspecific combination of moderate to strongly enhanced red cell aggregation, enhanced plasma viscosity, slightly rigidified RBC and normal to enhanced Hct-value. However, the analysis of the proteins retained in the filters showed predominance of alpha-2-macroglobulin, the high molecular weight serum protein who had long been identified as cause of a specific type of RCA termed “clump aggregates” (see accompanying poster KIRSCHKAMP plasma proteins).
Combined retinal and choreoideal fluorescence angiography: In extending previous work in den Department of Physiology, the principle of Computerised Cardio-Green-Perfusography was applied to AMD patients, where pilot studies indeed substantiated the suspicion of perfusion anomalies in the choroideal microvascular bed. This is a counterintuitive finding in light of the well known fact that the latter is usually strongly hyperperfused, the haemodynamics primarily serving the purpose of keeping the sclera stiffened from within. More detailed analysis – to be detailed in the lecture by SCHMID-SCHÖNBEIN – substantiated the pathophysiological extrapolation from the retinal (and all other peripheral) vascular bed to the choroidea: there are substantial non-homogeneities in the “arrival” times of the cardiogreen-albumin complex used for fluorescence emission.

Distinct differences between retinal and choroideal haemodynamics were found, as well as preferential re-homogeneisation of the latter after rheo-apheretic therapy.
Preliminary conclusion: It is too early to draw generalisable conclusions concerning the clinical efficacy from the present state of our pilot study: suffice it to say that a rigorous study protocol including 4 repetitive extracorporeal plasma filtration over a period of 1 month is applied. The clinical results are in keeping with the one's found elsewhere in similar studies: however, we will be showing close relationship between visual acuity, blood rheological improvement in vitro and elimination of previously underperfused or non-perfused choroideal subsegments. We wish to stress that negative correlations and/or failure of the therapy are not unexpected: for this reason, we submit that in light of the expense of the procedure, our hope to identify on the basis of objective measurement the individuals failing to respond seems to be fulfilled: we consider this an important achievement relevant for the entire field of clinical hemorheology at the verge of becoming part of curative medicine.

P3.1

EFFECT OF HYDROXYUREA ON THE DEFORMABILITY OF THE ERYTHROCYTE MEMBRANE IN PATIENTS WITH SICKLE CELL ANAEMIA

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Sickle cell disease is characterized by occlusion events in the microcirculation system resulting to tissue ischemia, organ failure, and, occasionally, death. It is a classical case of rheological disorder, of the red blood cells, where the molecular defect being manifest at the cell level as a distinguishing morphologic feature and loss of deformability. The decline of the red cell deformability as a result of the overall rheological abnormality is accompanied by a very high cytoplasmic viscosity, which is a consequence of both cell dehydration and polymerization of sickle haemoglobin. Hydroxyurea is a drug that was proposed to treat some patients with sickle cell disease. Although the clinical beneficial effects of HU have been proven, the direct effect of HU on sickle cells adversely affects their deformability by the reaction with HbS to form methemoglobin. This contradiction led the investigators to postulate that the direct effects of UH on the rheology of red blood cells in sickle cell patients can be masked by other factors. The purpose of this study was to determine the effect of hydroxyurea (HU) on the deformability of the erythrocyte membrane in order to improve the rheological properties of the RBC of patients with sickle cell anaemia (SCA) and to investigate the mechanical and rheological properties of these cells using micropipette as well as filtration techniques. The advantages of the micropipette technique is its accuracy and the fact that it measures the rheological and mechanical parameters of an individual cell instead of the average of these parameters of a population of cells. The advantage of the filtration technique is its simplicity. The rigidity index (IR), which is a measure of cell rigidity and the elastic shear modulus (μ), which is a measure of cell's membrane deformability, of red blood cells from health donors were found much lower (IR=13.15∂0.50, μ=11.0x10^{-3}∂2.0) than those from patients and untreated ones (IR=46.11∂13.08, μ=21.1x10^{-3}∂2.1) ($p<0.001$). For those cells from patients treated with HU, the values of IR and μ were estimated between those mentioned above (IR=31.90∂12.22, μ=15.0x10^{-3}∂1.3) and were significantly lower than the untreated ones ($p<0.05$). In the micropipette aspiration experiments with sickle cells, it was observed that the aspiration from the dimple region (concave side) took place more easily and the μ had a value lower than that calculated from the cells aspirated from the other convex side, which seamed to be more rigid. These observations and the results may imply a different rheological and mechanical behavior of the convex and the concave sides of the sickle cell.

P3.2

BLOOD RHEOLOGY CHANGES IN EXPERIMENTAL RHEUMATOID ARTHRITIS

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Recent therapeutic approaches in patients with rheumatoid arthritis (RA) is known to be based on initial pathogenesis findings in murine models of experimental arthritis (van den Berg W.B., 2005). The present study was designed to investigate the role of hemorheological profile changes in development of experimental adjuvant arthritis. Rheumatoid arthritis was induced in rats by plantar injection of 0.2 ml Freunds adjuvant (Grand Island Biol. Co., USA). Pathology development was verified by changes in body weight, rectal temperature and knee joint circumferences (regarding to saline group). On 28 day of the study blood samples were taken out from abdominal aorta under light ether anesthesia. Hemorheological profiles have been formed according to data obtained in 17 tests. Blood rheology properties were evaluated using rotational viscosimetry (shear rates from 3 to 300 sec^{-1}), RBC deformability was tested by micropore filtration system (5 μm) and viscosimetry assays, platelet aggregation (ADP 5 μM) was determined by light transmission aggregometer, coagulation parameters were evaluated by hemocoagulometer "SOLAR", extent of hypotonic and acidic RBC hemolysis were also measured. Leukocytes adhesion was evaluated according to adhesion index as a ratio of leukocytes number before filtration via nylon to their number after (light microscopy techniques were used). Fluorescence of DSM+-labeled RBC suspensions were detected with a fluorescence spectrophotometer (Hitachi, Japan) at the excitation wavelength of 480 nm and emission – of 580 nm. Significant increase of blood viscosity at low and high shear-rates (11.3% at 300 and 47.3% at $3s^{-1}$), RBC aggregation index – 19.7% (associated with decrease of DSM+ fluorescence (38.6%) - negative surface charge decrease), increased RBC suspensions viscosity (24.3%) as well as decreased RBC filtration rate (43.5%), osmotic and acidic fragility diminution (2.3 times and 24.2%, accordingly) were evaluated in rheumatic versus normal rats. As the result of these changes reduction in oxygen delivery index (Ht/q_{300}) was observed. Platelets aggregation parameters have also significantly changed (aggregation index elevated in 2.03 times), as well as leucocytes adhesion increased in 33.03%. Furthermore, hypercoagulable state was observed: plasma viscosity increased in 14.5%, which may be closely related to elevation in fibrinogen level (44.6%) as well as soluble fibrin monomer complexes increased (55%), activated partial thromboplastin, prothrombin and thrombin times shortenings were observed. According to these data it was concluded, that experimental arthritis produces multiple changes in hemorheological profile, which are associated with increase in blood and plasma viscosities, RBC and platelets aggregation, loss in erythrocytes deformability,

leucocytes activation as well as hypercoagulable state observed. That's why it may be suitable as a model for hemorheological drug testing in experimental pharmacology.

P3.3

THE INFLUENCE OF SEVOFLURAN ANESTHESIA ON THE RAT RED BLOOD CELL DEFORMABILITY

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Alterations in blood rheology under the influence of anesthesia have been observed and discussed among the responsible factors for the deterioration of tissue and organ perfusion related to anesthetic procedures. Sevofluran is one of the volatil anesthetics which is being used very common in surgery. 22 rats were used in the study and the rats were divided into two groups according to their age (young and old) comprising of two subgroups in each. First group was the young control (n=5), the second was the young group treated with sevofluran(n=5), the third group was the old control (n=7) and the last group was the old group treated with sevofluran (n=5) %2 of sevofluran was applied to the rats with inhalation in a adjustable cage for one hour. The deformability indexes of the erythrocytes were measured by a laser diffractometer (Myrenne Rheodyne SSD). Deformability indexes of red blood cells were significantly decreased with sevofluran in old rats ($p=0.028$) whereas it had not any significant effect in young group compared with their controls. When we compared the young and old control groups, the deformability indexes were significantly higher in old ones ($p<0.001$). However, there were not any significant difference between the old and the young sevofluran applied groups. A volatil anesthetic agent sevofluran has impaired the deformability of eryhtrocytes in old rats compared to their controls, whereas it had not any significant effect in young ones which may be due to the flexibility of the young erythrocytes leading them to tolerate to the environmental changes. This results reveal that the inhalation anesthetics like sevofluran may cause more serious problems in the elder people and their hemodynamic parameters should be checked more seriously during the surgery.

P3.4

VIDEOCAPILLASCOPY (VCS) EVALUATION IN DIABETIC AND HYPERTENSIVE MICROANGIOPATHY.

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Videocapillaroscopy (VCP) is a safe, reliable, not invasive method to detect microvascular alterations. Microcirculatory involvement was found in several vascular diseases and it seems to be related with inflammatory and dysmetabolic patterns. In these conditions vascular changes can be considered as a "steady state" modification: microvascular system undergoes local modifications for omeostasis restoring. This long-lasting process induces vessel structure alterations and chronic disruptions which are the morphological expression of the disease and may give clinical symptoms. VCS is able to explore skin and mucosal microcirculation. In peripheral districts (nailfold, hand and foot, gingival edge, labial mucosa) the capillary loops are disposed in different plans, vertically to the skin except for the nailfold where they follow a parallel courseand. In conjunctiva the two main observation sites are the arteriolar-venular district (or para-microcirculation) and the periirideal network that represents the capillary bed. Both qualitative and quantitative evaluations can be perfomed. The aim of this study was to evaluate the systemic microcirculatory damage in hypertension and diabetes. Three age-matched groups were selected. 160 hypertensive non-diabetic patients (93 ♀, 67 ♂ mean age 66.4±14.7), 106 diabetic non-hypertensive patiens (57 ♀, 49 ♂ mean age 66.8±12.5), 60 healthy subjects (38 ♀, 22 ♂ mean age 65.3±10.5). Each subject was studied by VCS (Video Cap, MDS Group, x200 magnification). The observation sites were: hand nailfold, conjunctiva, antecubitum. All the subjects were studied in the same environmental conditions: between 9 and 11 a.m. after an overnight fasting, in a temperature-controlled laboratory (21 to 24°C) and 20 minutes supine position. The hypertensive patients were matched in 3 subgroups (stage 1, 2, 3). The diabetics patiens were matched in 4 subgroups (1: normal level of glicated haemoglobin - HbA_{1c} - no proteinuria, 2: HbA_{1c} > 10%, 3: proteinuria > 3 gr/24 h, 4 undesiderable HbA_{1c} and proteinuria). Four damage stages were detected in conjunctiva of hypertensive subjects: phase 1 (or vasomotion instability), phase 2 (or venulo-capillary), phase 3 (or arteriolar, 3A reversible, 3B not reversible), phase 4 (or desertification). Analogue microvascular damage was evidenced in peripheral circulation. Morphological alterations; distribution changes including loss of normal capillary course and disregulation of linear disposition in different planes and perpendicularity, microaneurisms, pericapillary and tissue oedema, sludiging were found in diabetic patients. In conclusion VCS is able to detect specific microcirculatory damages in diabetes and hypertension. Furthermore these findings are related with severity and lasting of pathological course. Ongoing studies are aimed to evaluate whether microcirculatory patterns changes could be indicators of clinical progression and/or pharmacological effectiveness.

P3.5

RED BLOOD CELL DEFORMABILITY IN IRON DEFICIENCY ANAEMIA

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In patients with iron deficiency anaemia (IDA) it has been suggested that the shortened erythrocyte lifespan may be in part due to decreased erythrocyte deformability. In order to know whether erythrocyte deformability is decreased in IDA patients, we have determined the erythrocyte Elongation Index (EI) by means of ectacytometric techniques (Rheodyn SSD, Myrenne Gmbh, Germany), in 50 IDA patients related to chronic gastrointestinal blood loss or nutritional iron deficiency and 100 well age and sex matched healthy controls, without anaemia and with ferric status within the normal range. At the three shear stresses tested, 12, 30 and 60 Pa, IDA patients show statistically lower EI than controls (37.4±6.7 vs 48.6±2.9; 45.0±6.0 vs 54.5±2.8; 48.7±5.8 vs 57.0±2.9 mPa·s, respectively; $p<0.001$). A statistically significant correlation was found between EI at 12, 30, and 60 Pa and the hematimetric indices MCV, MCH and MCHC ($p<0.001$), suggesting that the alteration in surface/volume ratio (shape) which characterizes this kind of microcytic hypocromic anaemia, accounts in part for the decreased EI. Rheodyn SSD, as an ectacytometric technique, is very sensitive to alterations in red blood cell geometry, for what seems to be a useful tool for detecting diminished erythrocyte deformability in IDA patients.

P3.6

REFERENCE VALUES OF PLASMA VISCOSITY IN A SPANISH MEDITERRANEAN POPULATION

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Plasma viscosity (PV) constitutes an independent predictor of initial and recurrent cardiac events and mortality. It has been suggested that there is a geographical variation in PV values related to coronary event rates. Little information exists regarding PV in Spain. Therefore, our objective was to determine PV in a large sample of randomly selected subjects from the Spanish population and to study which demographic or cardiovascular risk factors (CVRF) influence levels of PV in this population. 1.277 subjetcs (503 male, 774 female) aged 43 $\pm$ 14 years (range: 20-74) were randomly selected from an Eastern Spanish population. These subjects were free of cardiovascular diseases and other major diseases. PV was measured at 37º C by means of the Fresenius GmbH Germany plasma viscosimeter. In addition, plasma total cholesterol, triglycerides, glucose and fibrinogen were measured. No differences in PV were observed regarding gender (male: 1.24 $\pm$ 0.06 cP female: 1.24 $\pm$ 0.06 cP, $p= 0.952$). Subjects older than 50 years showed higher PV: 1.24 $\pm$ 0.06 cP than those aged less than 50 years: 1.23 $\pm$ 0.06 cP, $p= 0.006$. We evaluated the prevalence of the following CVRF: hypercholesterolemia (T-cholesterol > 220 mg/dL), hypertriglyceridemia (triglycerides >175 mg/dL), hypertension, diabetes, tobacco smoking and obesity (BMI > 30 kg/m^2). Prevalence of these factors was: 28%, 4.3%, 8%, 2.3%, 33%, 8% respectively. Only T-Cholesterol >220mg/dL and BMI> 30 kg/m^2 were independently associated with highest PV values when men and women were analyzed together. Finally, in order to discard the potential confounding effect of these risk factors, in a further analysis, only subjects without cardiovascular risk factors (n= 643) were considered. In this subjects, PV showed even lower values (1.23 $\pm$ 0.06 cP) than those observed in a healthy subpopulation without cardiovascular risk factors made up of 653 subjects in Aachen, (Germany) (1.24 $\pm$ 0.05 cP) and measured by the same methodology. In conclusion we found lower VP values in this Mediterranean Spanish population than in other European countries and hypothetize than these may be related to Mediterranean diet consumed in this population and contribute to the low incidence of acute myocardial infarction in our country.

P3.7

DIFFERENT RBC AGGREGATING PROPERTIES OF THE $A\zeta_2B\eta_2\nu_{A2}$ AND $A\zeta_2B\eta_2\nu_A\nu'$ SUBPOPULATIONS OF HUMAN FIBRINOGEN

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The blood plasma fibrinogen (TF) has been fractionated into two pools: F1 – homodimers with respect to the ν chain, and F2 – heterodimers composed of ν_A and ν' polypeptides. TF but neither F1 nor F2 induced aggregation of the red blood cells (RBCs) under standard conditions (observation under a microscope of RBCs, 60 000 cells/σl in 0,8% TF, or in either 0,8% F1 or 0,8% F2, in a haemocytometer). A three-fold higher concentration of RBCs was required for the formation of rouleaux by F1 but F2, under the same conditions, was still ineffective. Only substitution of the haemocytometer for microscopic slides, i.e. setting the

suspension in gentle motion, revealed aggregating properties of F2. However, the rouleaux formed by F2 were definitely less complex then those formed by F1. The most probable structural feature responsible for different properties of F1 and F2 as RBC aggregants are C-ends of their ν polypeptides with ν' being, among others, by 16 amino acids larger than ν_A and bearing in addition two sulfate residues on its tyrosine residues. This assumption was strengthened by the finding that 14 molar excess of the synthetic peptide ν'(414-427) inhibited two-fold the F2 induced RBC aggregation as calculated from the microscopic aggregation index. The peptide scarcely inhibited the rouleau formation in a mixture of F1 and F2 at the physiological ratio. Unexpectedly, the peptide accelerated the F1 induced RBC aggregation. The opposite effects of the synthetic peptide on RBC aggregation – inhibition when in mixture with F2 and acceleration when in mixture with F1 - cannot be explained by concentration gradients of the molecules ("depletion layer" theory) because in either combination they were exactly the same. On the contrary, these effects suggest some degree of specificity of binding of fibrinogen to RBC.

P3.8

THE DIAGNOSIS OF TRANSCAPILLARY FLOW DISTURBANCES IN THE LUNGS OF LUNG CANCER

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We have made the diagnosis of transcapillary flow disturbances in the lungs of lung cancer patients with the available noninvasive method of videobiomicroscopy of eye conjunctiva with further morphometric processing of obtained data using PC. To evaluate the obtained data a comparative morphologic investigation of histologic samples taken from the intact areas of the lung parenchyma has been carried out. Using videobiomicroscopy we calculated the size of venous capillary sacculations, the square of capillary glomerules (S_g), the amplitude (A), the 'length' of the twisted part of a microvessel (L_t), the amplitude-length coefficient (C_a), the frequency-length coefficient (C_f), the size of venous capillaries (V), intravascular conglomeration of aggregated erythrocytes (conglomerate), RBC aggregation coefficient (C_s) and the degree of sludge.
77 patients with lung cancer have been studied. In 45 patients the size of sacculations was 8-22,8 mcm, S_g – 65,2-95,4 mcm, A – 51,8-96,3 mcm, L_t – 77-165,8 mcm, C_a – 44,7-88,9 mcm, C_f – 12-64%, V – 16,3-21,5 mcm, the size of conglomerations – 39,8-64,3 mcm, 0,1ÒC$_s$Ò0,7 sludge I-II. We diagnosed moderate disturbances in the capillary flow that were confirmed by microscopy: the vessels were moderately plethoric, twisted, the walls were occasionally thickened, the endothelium was swollen and perivascular edema and moderate RBC sludge were noted. In 32 patients the size of sacculations was Ó29 mcm, S_q Ó63,5 mcm, A – 92-120,8 mcm, L_t – 150,9-232,1 mcm, C_a – 82,7-116,7 mcm, C_f – 10-14%, the size of venous capillaries V – 23,9-30,5 mcm, the size of conglomeration – 34,9-57,7 mcm, C_s Ó0,9, sludge – III-IV. We determined marked disturbances in the transcapillary flow in the lungs confirmed by microscopic examinations: the vessels were plethoric, the walls significantly thickened, edematous, the endothelium was swollen, there being a marked RBC-sludge and perivascular edema with focal lymphoid infiltration.
Thus, videobiomicroscopy of eye conjunctiva can be used as an available noninvasive diagnostic method for the evaluation of transcapillary flow disturbances in the lungs.

P3.9

THE FLUIDITY OF BLOOD IN AFRICAN ELEPHANTS (*Loxodonta africana*)

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The large cellular volume of erythrocytes and the increased plasma concentration of proteins in elephants are factors which potentially affect blood rheology adversely. To verify blood rheology, routine hemorheologic variables were analyzed in four African elephants (Loxodonta africana), housed in the Schönbrunn zoo of Vienna.
Whole blood viscosity at three different shear rates (WBV at low shear rate: WBV 0.7 s^{-1} and WBV 2.4 s^{-1}; WBV at high shear rate: WBV 94 s^{-1} done by LS30, Contraves) and erythrocyte aggregation (agregation indices AI by LS30; aggregation indices M0, M1 by Myrenne aggregometer) were high (WBV 94 s^{-1}: 5.368 (5.246/5.648); WBV 2.4 s^{-1}: 16.291 (15.6/17.629); WBV 0.7 s^{-1}: 28.28 (25.537/32.173) mPas; AI 2.4 s^{-1}: 0.25 (0.23/0.30); AI 0.7 s^{-1}: 0.24 (0.23/0.28); M0: 7.8 (6.4/8.4); M1: 30.2 (25/31)). Plasma viscosity (PV) was increased as well (1.865 (1.857/1.912) mPas) compared to other mammalian species. These parameters would indicate a decrease in blood fluidity in elephants. However, erythrocyte flexibility (LORCA, Mechatronics) was increased, which in contrast, has a promotive effect on peripheral perfusion. Blood rheology of the elephants was determined by a high whole blood and plasma viscosity as the result of pronounced erythrocyte aggregation and high plasma protein concentration. Thus, in the terminal vessels the resistance to flow will be increased. The large erythrocytes, which might impede blood flow further due to geometrical reasons, however, had a pronounced flexibility. We conclude that the effect of the increased inner resistance to peripheral blood flow was counteracted by the increased flexibility of the erythrocytes to enable an adequate blood flow in African elephants.

P3.10

HEMORHEOLOGICAL DISORDERS DURING THE 1ST AND 2ND TYPES OF DIABETES MELLITUS IN PATIENTS WITH FOOT GANGRENES

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The aim of the present study was evaluation of the blood rheological disorders, in particular the RBC enhanced aggregation, for comparison of these changes in the 1st and 2nd types of diabetus mellitus. For evaluation of the RBC aggregability we applied in the present study the "Georgian technique", which was developed and perennially applied in our laboratory. Its opportunity is that it is direct and quantitative. All the investigated patients suffered from the foot diabetic gangrenes. They were divided into two groups: (a) with the 1st and (b) with the 2nd types of diabetes mellitus. When we matched the RBC aggregability indices in the both groups of the diabetic patients and compared the obtained results with those in the healthy control group, we found that the rheological disorders were considerably pronounced. They increased by 62 per cents ($p<0.001$) during the 1st type and by 57 per cents ($p<0.001$) during the 2nd type in the diabetic patients. The comparison of the RBC aggregability changes the both groups of patients showed that the difference between them was not significant. Therefore we concluded, that the blood rheological disorders are similar during both the 1st and the 2nd types of diabetes mellitus. The disturbed blood fluidity related to the increased aggregability in the microcirculation promotes, in particular, the development of the patients legs gangrene during the both types of diabetes mellitus.

P3.11

DYNAMIC HEMORHEOLOGICAL PARAMETERS IN BETA THALASSAEMIA MINOR

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The η thalassaemia is a hereditary hemolytic disease, in which each clinic phenotype encompasses a heterogenic group of genetic alterations resulting in the decrease or the absence of the η globin chain synthesis in red blood cells (RBC). In heterozygote cases, the synthesis of beta chain is reduced declining the hemoglobin A levels. In this pathology, the continued synthesis of normal amounts of the non-affected globin chain (ζ chains) results in the excessive accumulation of these normal chains, within the erythroid cells. Some rheological abnormalities can appear from the selective interaction of the ζ chain excess with the cytoskeleton of the erythrocyte membrane. The studies that have been done about carrier states for η thalassaemia suggest the existence of decreased red cell deformability. The erythrocyte ability to become deformed when they are driven by the blood flow is a well-known fact in the efficiency of blood circulation. Whole blood and erythrocyte suspensions may be considered to be viscoelastic and their behavior may be described according to complex viscoelastic parameters when they undergo oscillatory stresses. This dynamic behavior is physiologically important due to the "in vivo" pulsative blood flow. The aim of this work was to evaluate complex erythrocyte viscoelastic parameters in patients suffering from heterozigote η-thalassaemia and to compare them with healthy individuals. Blood anticoagulated with Na_2EDTA, was drawn using large bore needles to avoid mechanical cell damage from 15 healthy controls and 15 thalassaemic patients. All thalassaemic patients were η minor thalassaemic diagnosed by conventional methods and classified by methods of molecular biology (PCR_ARMS) being 8 η^0 (η^0 39) and 7 η^+ (3 η^+ Intron 1 nucleotide 6, and 4 η^+ Intrón 1 nucleotide 110). 100 σl of anticoagulated whole blood were resuspended into 4ml of an isotonic viscous suspending medium (polyvinilpirrolidone 360 KDa at 5% in PBS; pH 7.40 ∂ 0.05; 295 ∂ 8 mOsmol/Kg, viscosity 22 cp. at 25°C). Erythrocyte dynamic rheological properties have been determined by applying laser diffractometry of sheared RBC (ektacytometry) using a homemade apparatus called Erythrodeformeter⊇ [8]. For this purpose, the motor is put in oscillatory mode to apply a sinusoidal shear stress at seven different frequencies (0.5, 1, 1.5, 2, 2.5, 3 and 3.5 Hz) [9]. This operation is performed to obtain phase shift between stress and strain for the calculation of the four parameters of the dynamic viscoelasticity: dynamic elasticity, dynamic loss, real and imaginary parts of the complex viscosity. Our results reveal that even though thalassaemic erythrocytes show a decreased deformability in the stationary state, when they are in a dynamic state, hemorheological alterations are only evident at low oscillatory frequencies, i.e., at frequencies which are lower than the normal cardiac frequency (60 cycles/min = 1 Hz), producing no significant alterations at increased cardiac rhythm.

P3.12

MIGRATION MECHANISM OF ERYTHROBLASTIC ISLAND IN RAT BONE MARROW

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The kinetics of erythroid cells into the blood circulation was examined. That is, it has been considered that mature erythroblasts migrate toward the sinusoid along the cytoplasmic processes of macrophages of erythroblastic islands in bone

marrow. However, this hypothesis has never confirmed. As a result of this study, the more mature erythroblasts were not regularly arranged in the peripheral direction of the erythroblastic islands. Immature erythroblasts were populated more in the erythroblastic islands away from the sinusoid than in those islands neighboring the sinusoid, whereas mature erythroblasts were more in erythroblastic islands neighboring the sinusoid. In addition to migration of single reticulocyte through the sinus wall, the reticulocytes anchored to the central macrophages are transported into the peripheral blood circulation. The above findings suggest that the formation of erythroblastic islands occurs in a region away from the sinusoid, that erythroblastic islands migrate towards the sinusoids as erythroid maturation proceeds, and that the transportation of matured erythroid islands into the blood stream occur in addition to the transportation of solitary matured erythroid cells. Furthermore, this finding gives a new insight to the functional relation between endothelial cells and macrophage, so the meaning on clinical medical is important.

P3.13

EFFECTS OF WATER INTAKE ON THE RESPONSES OF HAEMORHEOLOGICAL VARIABLES TO RESISTANCE EXERCISE

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Introduction: Endurance exercise induces significant changes in blood rheological variables and the main mechanism responsible is exercise-induced haemoconcentration. In addition, it has been demonstrated that acute bouts of resistance exercise induce significant increases in blood rheological variables. However, the mechanism responsible for these changes is not understood yet and also controversy still exists over the interaction between plasma volume reductions and alterations in plasma constituent levels. Therefore, the present study was designed to examine whether the blood rheological changes in response to heavy resistance exercise are due to plasma volume reduction during exercise or some other factors associated with exercise.
Methods: Eleven healthy male subjects (mean±SD; 26.6±5 years; stature = 1.72±0.06 m; body mass = 79.3±12.8 kg) participated in this study and signed a written informed consent approved by the University's Human Ethics Committee. After familiarisation and determination of one repetition maximum (1RM), subjects performed three exercise trials in three separate sessions. The aim of the first session was to determine the amount of weight loss following a heavy resistance exercise trial (3 sets of 5 repetitions of 6 exercises at 80% of 1RM). In the second and third trials subjects performed the same resistance exercise protocol both with and with out drinking an amount of water equal to that recorded for body weight loss. Three venous blood samples were taken before exercise, immediately after exercise, and at the end of 30 minutes recovery and were analysed for haematocrit, Haemoglobin blood rheological variables.
Results: Although plasma volume loss was higher in the control experiment (-10.4%) than water trial (-8.4%), this difference was not statistically significant. Plasma viscosity increased from 1.56±0.05 to 1.65±0.08 mPa.s, and from 1.57±0.05 to 1.66±0.08 mPa.s immediately following resistance exercise trials of control and water experiment, respectively. Haematocrit, fibrinogen, albumin, and total protein were significantly increased in response to resistance exercise and returned to pre-exercise level following 30 minutes of recovery. No significant difference between blood rheological responses to both resistance exercise trials (water or control) was found.
Discussion / Conclusions: Plasma volume loss through sweating and respiratory tract during resistance exercise could have contributed to the decrease in plasma volume, but this contribution was negligible and had no significant effect on blood rheological variables. Therefore, it is concluded that the main mechanism responsible for increases in blood rheological variables in response to resistance exercise could be the plasma shifts or redistribution from intravascular space to the extravascular spaces rather than plasma volume loss.

P3.14

IS PLASMA VISCOSITY A PREDICTOR OF OVERTRAINING IN ATHLETES?

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There is a lack of consensus about the biological diagnosis of the overtraining syndrome (OTS). Recently, efforts have been made to standardize its clinical diagnosis (eg, standardized questionnaires like that of the French consensus group on overtraining of the Société Française de Médecine du Sport - SFMS). We previously reported that the early signs of overtraining (= « overreaching ») in elite sportsmen are associated with a hemorheologic pattern (raised hematocrit and plasma viscosity ξ_p) that suggests some degree of reversal of the 'autohemodilution' which characterizes fitness, and that the feeling of heavy legs in overtrained athletes is related to higher ξ_p and higher red cell aggregation. We thus investigated on a sample of

48 athletes (age 24±6 yr), referred for possible diagnosis of overtraining to what extent plasma viscosity is a predictor of OTS. From those 48 athletes 10 had a value of ξ_p in the highest quartile (ξ_p >1.44 pPa.s) and 8 of them had a diagnosis of overreaching, while in the 38 whose ξ_p was <1.44 mPa.s there were 20 cases of overreachings. Overt cases of OTS were found in 1 subject of the highest quintile and two in the lowest. Thus the predictive value of ξ_p for early stages (overreaching) or chronicized stages (overtraing syndrome) is as follows: a) prediction of overreaching: sensitivity 28.57%; specificity 90% ; positive predictive value 80%; negative predictive value 47.37% ; b) prediction of chronicized overtraining: sensitivity 2.70%; specificity 18.18% ; positive predictive value; 10.00% ; negative predictive value 5.26%. These results show that ξ_p is a rather specific, although poorly sensitive predictor of overreaching but has no interest in the diagnosis of the overtraining syndrome itself.

P3.15

DETERMINANTS OF THE HEMORHEOLOGIC EFFECTS OF LOW INTENSITY ENDURANCE TRAINING IN SEDENTARY PATIENTS SUFFERING FROM THE METABOLIC SYNDROME.

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Hemorheologic effects of exercise training («hemorheologic fitness») are very different according to the mode and the intensity of this training. We previously reported that low intensity endurance training in sedentary patients suffering from the metabolic syndrome sumultaneously improved blood rheology, body composition and lipid oxidation at exercise. However the link between these metabolic and the hemorheologic improvements is unclear and our working hypothesis that plasma viscosity is an integrated marker of the metabolic status in these patients and could be useful for their follow-up remains undemonstrated. We thus aimed at further analyzing the effects of this kind of training on blood rheology in 24 patients (55 yr, mean BMI 32 kg/m²) submitted to a 2 months targeted training designed for increasing exercise lipid oxidation. Variations of whole blood viscosity at high shear rate (ξ_b1000 s^{-1}) were explained here by two statistically independent determinants : hematocrit and red cell rigidity. ξ_b (1000 s^{-1}) decreased in 16 subjects, but increased in 8, due to a rise in hematocrit. While changes in RBC rigidity appeared to reflect weight loss and decrease in LDL cholesterol, this unexpected rise in hematocrit had no clear statistical explanation. Besides, plasma viscosity was related at baseline to cholesterol levels (r= 0.4714; p= 0.0483) and its training-induced changes are related to those of the VO_{2max} (r=-0.692) but not to those of the lipid oxidation rate. Red cell aggregability (Myrenne) before training reflected both the circulating lipids (Chol, HDL and LDL) and the ability to oxidize lipids at exercise (r=-0.5142 p= 0.0171). Factors associated to a post-training decrease in aggregability (M1) were weight loss (r=0.471) and more precisely decrease in fat mass, improvement in lipid oxidation rate exercise (r=0.433), rise in HDL-Chol (r=0.595), and decrease in fibrinogen (r=0.886). On the whole the major determinant of a hemorheologic improvement was an increase in cardiorespiratory fitness (VO_{2max}), correlated with a decrease in plasma viscosity, rather than an improvement in lipid metabolism, althought RBC aggregability and deformability exhibited clear relationships with lipid metabolism.

P3.16

ALTERED FIBRINOLYTIC RESPONSE IN NIGERIAN LONG DISTANT DRIVERS

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Background: Exercise has been defined as physical activities designed to develop muscular strength or endurance, joint mobility and cardiovascular fitness. It has been linked with improved rheologic properties and blood flow. However, this is presumed to be deficient in this group of subjects.

Aim: This study was therefore designed to evaluate the rheologic fitness of long distant drivers in relation to their cardiovascular activities.

Methods: total of 100 long distant male motor drivers and 50 apparently healthy individuals (Controls) who drives sparingly were studied. They include drivers covering an average distance of 700 kilometres daily and have been driving for a minimum period of 3 years. Their blood samples were obtained for haemorheological parameters of Haematocrit (HCT),Erythrocytes sedimentation rate (ESR), Whole blood and Plasma viscosities WBV and PV), plasma Fibrinogen concentration (PFC) and Euglobulin lysis time (ELT) using standard methodologies. Their blood pressures (BP) were also estimated while student t-test was used for statistical analysis.

Results: There were statistically significant increase in all the parameters measured (P<0.05 respectively) except ESR, though increase in value but not significant. The increased ELT indicates a hypofibrinolytic activity. Also, there were statistically significant increases in both systolic and diastolic pressures.

Conclusion: Increased haematocrit coupled with hyperviscosities and hyperfibrinogenaemia with a concomitant hypofibrinolytic activity are abnormal rheologic indices and could be dangerous signals for impaired cardiovascular function as evidenced by raised diastolic and systolic blood pressures in long distant drivers.

P3.17

HAEMORHEOLOGICAL ALTERATIONS IN NIGERIAN PULMONARY TUBERCULOSIS PATIENTS (PTB)

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Background: Pulmonary tuberculosis (PTB) is a major infectious disease with very high incidence in developing countries and this is expected to rise with the incidence of HIV infection.
Aim: Despite the known consequences of PTB on blood flow, rheologic properties have not been viewed with any serious research attention. This study was designed therefore, to investigate the possible haemorheological effects of the infection.
Methods: Haemorheological parameters were studied in 40 (17 males and 23 females) and 10 (5males and 5 females) newly diagnosed PTB patients but confirmed to be HIV negative. Their ages ranged between 25 and 45 years. Also, 50 apparently healthy controls with age and sex matched were compared. Haematocrit (HCT), Plasma viscosity (PV) Erythrocyte sedimentation rate (ESR) and Plasma Fibrinogen concentration (PFC) were estimated with standard methodologies while student t-test was used to compare the data.
Results: Patients (treated and untreated) show statistically significant increase in PFC, PV and ESR ($P<0.01$ respectively) while there was a statistically significant decrease in the HCT ($P<0.01$). However, treated patients show an improved rheological values than the untreated ones (i.e reduced ESR and PV with an increased PCV, $P<0.01$ respectively). There was no established male and female differences amongst the patients.
Conclusion: Hyperfibrinogenaemia with hyperviscosity are possible consequences of PTB infection and coupled with reduced PCV and increased ESR indicates chronic signals of altered haemorheology which may predispose the patients to increased cardiovascular risks. The inclusion of haemorheological parameters in the monitoring of patients on treatment is hereby emphasized.

P3.18

USE OF CONFOCAL MICROSCOPY TO STUDY THE KINETICS OF LOW-DENSITY LIPOPROTEIN UPTAKE IN A HUMAN ENDOTHELIAL CELL LINE

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Introduction: All lipoproteins that contain apolipoprotein B (apo-B), such as LDL, VLDL, tend to promote atherosclerosis. Although several hypothesis about atherogenesis have been proposed during the past decades, none can completely explain the whole process of atherosclerosis because this disease is associated with multiple risk factors. The uptake and transport of LDL by the arterial wall is a continuous dynamic process. The increased uptake in the lesion-prone areas can be due to an enhanced permeability through endothelial junctions and/ or LDL receptor (LDLR)-mediated endocytosis. The chemical and physical causes of atherosclerosis include not only hypercholesterolemia and certain changes of plasma protein content, but also shear stress or turbulences due to increased blood pressure. Several studies have demonstrate that: although every part of the arterial tree is exposed to the same concentration of plasma LDL, atherosclerotic lesions preferentially tend to occur at the regions of arterial branching and curvature where shear stress on the vessel wall may be low and flow may be disturbed, which suggests that local shear stress play a significant role in the focal nature of the lesions.
In this work, we report a feasability study to follow-up the kinetics of the uptake of fluorescent labeled-LDL by cultured of an endothelial-like cells line ECV304 under static or dynamic conditions using a Confocal Laser Scanning Microscope (SP2 Leica, Germany).
Materials and methodes: To study the kinetics of native LDL and ox-LDL uptake in endothelial cell line ECV304, LDLs were labeled with two carbocyanine dyes, DiO as the donor and DiI as the receptor. Confluent monolayer cells were incubated with 10µg/ml DiI-LDL or DiO-LDL in static conditions or subjected to a laminar flow under a Confocal Laser Scanning Microscope (SP2 Leica, Germany).
Results and discussion: The results showed: (1) the possibility to follow the kinetics of LDL endocytosis in living cells, (2) shear stress in comparison with control group more effectively enhanced LDL uptake. (3) ox-LDL induce a rapid uptake, followed by a degradation of LDL. This preliminary study, in a methodological manner, allows us to show that the behaviour of LDL was not related to the probes but depends on its biochemical characteristics. It appears that the oxidation of LDL induce many changes in their behaviour. Regarding all available data, we conclude that confocal microscopic study could be a rapid and useful method to study LDL interactions with vascular cells. However, further studies need to be performed to elucidate the molecular mechanism by which shear stress modulates the expression of LDL receptors.

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P3.19

RELATION OF ERYTHROCYTE NITRIC OXIDE WITH HEMORHEOLOGICAL PARAMETERS

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The nitric oxide (NO) is an important vasodilator messenger that interferes with a number of physiological and pathophysiological processes. When the erythrocytes are stimulated with acetylcholine there are an increase of the NO and NO metabolites. In this study we stimulated human erythrocytes obtained from patients with hypercholesterolemia (HC; n=42), renal transplantation (RT; n=18) and hypertension (HT; n=10) with acetylcholine (ACh) and measured the NO production, comparing with the NO levels achieved on erythrocytes of healthy persons (n=27). We used human erythrocyte suspensions in sodium chloride 0.9% pH 7 (hematocrit 0.05%) to measure the NO production with an amperometric NO sensor during stimulation with ACh 10 σM. We also measured the hemoglobin, hematocrit, erythrocyte aggregation, erythrocyte deformability, plasma viscosity and fibrinogen concentration from human blood samples. The erythrocytes NO levels, according to the different studied groups, were of 2.5±0.7 nM (HC; P=0.038, n=42), 2.4±1.1 nM (RT, n=18) and 2.2±0.8 nM (HP; n=15) against the 2.0±0.8 nM (n=27) for the control groups. The erythrocyte aggregation levels measured in 5 seconds, significant increase in 18,5% the values of healthy persons (9.7±1.7) on hypertension disease and 11% on hypercholesterolemia and renal transplantation. The plasma viscosity levels increase 2,4% on hypertension and above 2,0% in the other diseases, with 1.24±0.04 mPa.s as the control value. The fibrinogen levels of the hypercholesterolemic persons increased in 10,9% the control values (274±55 mg/dL). We observed the most significant change on the NO production in hypercholesterolemia erythrocytes samples associated with an increase of the erythrocyte aggregation, plasma viscosity and plasma fibrinogen. The hypertension persons have significant changes in their erythrocyte aggregation and in plasma viscosity but this changes does not seem to be related with changes on erythrocyte NO response. In conclusion, human erythrocytes of different diseases have different physiological responses to ACh stimulation that leads to changes on NO mobilization mechanisms and on hemorheological parameters. The different erythrocytes NO values obtained after ACh stimulation seems to be related with the studied hemorheological parameters, suggested a future target for vasodilate therapeutic action on a microcirculatory network, damaged by different sorts of stimulus.

P3.20

MICROCIRCULATION AND VENOUS DISEASE HISTORY: ROLE OF SULODEXIDE

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The clinical expression of venous disease is the result of the microcirculatory damage. Taking in examination the clinical classification of venous disease (CEAP), it is evident how the progression toward higher stages is caused by microcirculatory ingravescent disease. In fact, up to stage C2 (varices) microcirculation can compensate venous macrocirculatory hypertension, while edema onset, C3 stage, shows microvascular/tissular unit decompensation. The subsequent toxic, inflammatory and degenerative events characterizing advanced microangiopathy, have their macroscopic expression in trophic disorders and ulcer, typical of stages C4-C6. Starting from these presuppositions, we wished to verify if it was possible to slow microangiopathy progression by pharmacological intervention on its pathogenic mechanisms. In this purpose, 292 subjects with previous deep vein thrombosis were examinated and subsequently monitored for 5 years, taking note of their CEAP classification clinical score at the beginning and at the end of the study. 99 patients (group A) received sulodexide 500 U/die continuously, and diosmine-esperidine association limited to summer period, in addition to support stockings, 105 patients (group B) received support stockings alone and, finally, 88 subjects (group C), who refused all previous therapies, were considered as controls. Rational of sulodexide use was represented by its antithrombotic, fibrinolytic and antiinflammatory properties, and by its ability of modulating endothelial permeability and leukocyte adhesion and activation. All these actions antagonize pathologic events cascade underlying microcirculatory damage. At the first control, 60 patients from group A (60,6%), 60 patients from group B (57,4%) and 51 patients from group C (57,9%), were classified as C0-C2, whereas 39 subjects from group A (39,4%), 45 from group B (42,6%) and 37 from group C (42,1%) were classified in higher classes (C3 - C5). After 5 years, 51 patients from group A (51,5%) were classified as C0-C2, in comparison to 44 patients from group B (42%) and 24 from group C (27,3%). In C3-C6 classes 48 patient from group A (48,5%), 61 from group B (58%) and 64 from group C (72,2%) were registered. Then, 9 patients (9,1%) from the group receiving sulodexide, had an evolution towards CEAP classes typical for decompensated microangiopathy, in comparison with 16 patients (15,4%) from the group receiving

support stockings as the only treatment. Evolution toward severe stages was considerably higher in the control group, with an increment of 27 patients (30,6%). Finally, considering the usual SPT evolution, the venous ulcer, it occurs only in one case (1%) in group A, whereas 4 (3,8%) and 9 (10,2%) ulcers developed in groups B and C respectively. In conclusion, this study seems to indicate that pharmacological treatment with sulodexide, a drug active on microcirculation, in addition to traditional elastic compression, is able to slow the evolution of chronic venous failure, with reduction in its socio-economic costs, largely compensating the costs associated to the employment of drugs. Obviously, this is a limited experience requiring further investigation.

P3.21

IN VITRO DEFORMABILITY OF RED BLOOD CELLS FLOWING IN MICROCAPILLARIES IN A GEL MATRIX

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The deformability of red blood cells flowing in microvessels is essential to maintain optimal blood circulation and to allow gas transfer between blood and tissues. From a pathological viewpoint, reduced RBC deformability is involved in a number of blood diseases, such as Thalassemia, Iron Deficiency, Congenital Spherocytic and Non Spherocytic Anemias, Idiopathic Myelofibrosis. In spite of such physiopathological relevance, measurements of RBC deformability are usually of difficult clinical application, being still carried out by approximate methods and under conditions quite different from those occurring in vivo. In this work, we started investigating RBC deformability in microcapillaries embedded in a gel matrix. The microcapillaries may have size similar to that of RBC diameter (down to 5 micron). The gel matrix hosting the microcapillaries is placed in a rectangular flow cell, where a dilute suspension of RBCs, isolated by centrifugation, is fed through a syringe. The flow cell is mounted on a motorized x-y stage of an inverted microscope equipped with a CCD camera. Images of RBCs under flow are perfectly visualized, digitized by a frame grabber, and stored on hard disk for later analysis to measure type and extent of deformation. Experimental variables include flow rate and size of microcapillaries. RBCs both from healthy donors and from patients have been investigated and compared.

P3.22

ATHEROSCLEROSIS AND HEMORHEOLOGY: CHICKEN OR THE EGG?

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Hemoheology is the science of blood flow in relation to pressure, flow, volumes, and resistances in blood vessels, has been associated with cardiovascular disease (CVD). According to Poiseuille, resistance to flow is determined by both vascular geometry and blood viscosity, but in clinical cardiology, the role of vessel size is sometimes overemphasized at the expense of viscosity. The lowering of blood viscosity regardless of changes in vessel diameter, increases microvascular flow to tissues by reducing vascular resistance. The question arises whether rheologic factors initiate atherosclerosis or are the result of the disease. Standard CVD risk factors are associated with mediators of blood viscosity such as low shear stress (tangential force)/rate (blood velocity), red blood cell (RBC) aggregation, RBC deformability and plasma viscosity. Low shear stress, which can increase blood viscosity, is found at most areas of atherosclerosis due to the non-laminar flow of the blood. In a matter of seconds decreased shear stress can impair nitric oxide mediated vasodilator response and over a longer period of time stimulate other markers (endothelin-1, vascular cell adhesion molecule,…) associated with the atherosclerosis cascade in the arterial wall. Treatment of hemorheologic parameters correlates to therapy for CVD risk factors. LDL-apheresis, a method of reducing LDL-C by over 60% in about 3 hours, can immediately reduce blood viscosity by 20% at all shear rates using a scanning capillary rheometer (Rheolog ®) by improving RBC aggregation, RBC deformability and plasma viscosity. Apheresis rapidly enhances endothelial function, nitric oxide activity and vascular blood flow. Studies using LDL-apheresis, for patients with CVD, have demonstrated immediate improvement of coronary blood flow by more than 30%. These actions by LDL-apheresis on viscosity suggest that hemorheology is a strong stimulant for vascular disease. The measurement of blood viscosity may assist in the identification of patients at risk of developing atherosclerosis and help to evaluate the success of treatment for the prevention of vascular disease.

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RIASSUNTI

LETTURE

l1

EMOREOLOGIA ED AFERESI

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Sotto il profilo patogenetico e fisiopatologico le sindromi da iperviscosità ematica primaria possono essere distinte in policitemica (caratterizzata da aumento della massa cellulare circolante), sierica (caratterizzata dalla presenza di una frazione proteica anomala) e sclerocitemica (caratterizzata da ridotta deformabilità eritrocitaria, dovuta a trasformazioni irreversibili, quasi sempre di origine genetica, di uno o più componenti del globulo rosso). La sindrome da iperviscosità ematica secondaria può essere presente, in forma completa e non, in alcune condizioni cliniche quali il diabete mellito, la cardiopatia ischemica, la vasculopatia cerebrale e periferica, la cirrosi biliare primitiva, la sindrome nefrosica, le eteroplasie solide, etc.). Per quanto riguarda le tecniche aferetiche, nella pratica clinica sono disponibili l'eritroaferesi, la leucoaferesi e la plasmaferesi con le sue varianti comprendenti la Heparin-induced extracorporeal LDL precipitation (HELP) e il Rheosorb. Numerosi sono i dati ottenuti con l'impiego dell'eritroaferesi nella policitemia vera, nella poliglobulia secondaria e soprattutto nell'anemia falciforme. Incoraggianti risultati sono stati ottenuti con tecniche di leucoaferesi nelle leucosi iperleucocitiche, mentre la plasmaferesi trova applicazione nel mieloma multiplo, nella macroglobulinemia di Waldenström, nelle crioglobulinemie e nelle malattie del connettivo (in particolare nell'artrite reumatoide). Del trattamento con plasmaferesi possono avvalersi anche la malattia e la sindrome di Raynaud, la vasculopatia cerebrale e periferica, la retinopatia diabetica e la cirrosi biliare primitiva. L'utilizzo dell'HELP ha dato ottimi risultati nell'ischemia critica, nello stroke e nella malattia cerebrale multiinfartuale, ma anche nella cardiopatia ischemica e nella sordità improvvisa. Il Rheosorb, che consente di rimuovere dal plasma in modo specifico il fibrinogeno, ha trovato finora applicazione nelle lesioni trofiche degli arti inferiori.

l2

MICROCIRCULATION INVOLVEMENT IN CVI

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Microcirculation is the terminal part of the systemic circulation, and a connecting system between arterial, venous macrovessels and tissues.It is called "exchange circulation" since it is the site of hematotissular exchanges. Countless microcirculatory units, according to their organ function, set up the microcirculatory system.

The microcirculatory unit consists of a terminal arteriola, a metarteriola, the capillary network,the initial venula, artero-venous anastomoses, lymphatic capillaries and nervous fibers, all held in a matrix of connective tissue .

The local blood perfusion is regulated through two main ways: 1) the metabolic pathway, conditioned by changes in tissue PO2 and PCO2, which uses EDRF or NO and adenosine as vasoactive mediators; 2) the myogenic pathway, conditioned by changes in pressure or flow (1,2,3).A third mechanism invoked is arteriolar vasomotion.It consists of rhythmic arteriolar dilations and constrictions at a frequency of 1 to 20-30 cycles per minute, inversely proportional to the diameter of the terminal arteriola (4,5). These cyclical changes in arteriolar diameter are accounted for by the presence of a sphincter-type endarterial thickening acting as a pacemaker (6). Vasomotion is thought to be responsible for: 1) a redistribution of the blood flow in the capillary network; 2) changes in capillary blood viscosity, in the oncotic-hydrostatic pressure ratio (7). In turn, the variations of frequency of arteriolar vasoconstriction and vasodilation, seem to be determined by changes in interstitial pressure and PO2, through adenosine.

Capillary haemodynamics result from arteriolar vasomotion and rheological changes according to stochastic laws (Casson's law ω ½= A ν ½+B).

Capillary endothelium lies on a basement membrane and lacks underlying muscle cells. It sets up a continuous barrier which allows diffusion of fluids and molecules through direct and indirect mechanisms of selective transport (8,9,10). Such a continuous barrier is altered in the presence of both stasis and hypoperfusional ischemia, resulting in an uncontrolled outflow of liquids (11) and corpuscles into the the interstitial tissue with consequent tissue edema and ultimately tissue necrosis.

The primum movens of venous ulcers is chronic venous insufficiency, due to both primary and secondary venous pathology. The common pathogenetic denominator is venous stasis .

Venous ulcers resulting from primary venous insufficiency and those resulting from the post-phlebitic syndrome differ in the clinical evolution, more rapid in the secondary forms, but not in the pathogenesis (9).

By venous or phlebostatic ulcers we mean lesions of the skin of the lower limbs, typically in the internal malleolar area, caused by prolonged venous hypertension with consequent irreversible microcirculatory failure.
The initial venous macrocirculatory hemodynamic failure deeply affects the microcirculation so that the final event,i.e. venous ulcer, is a purely microcirculatory phenomenon of interstitial overflow (12,13,14).
The valvular incompetence of the large veins with flow inversion through the perforating veins, mostly damages the lower third of the leg where the effects of the gravitational force are greater.
Specifically at Cockett's perforating veins, two flows meet: the reflux from the great saphenous vein and the short horizontal reflux from the perforating veins, which join to increase the intravenous pressure, especially during walking when deep venous pressure is sensibly higher.
When an active venous hypertension or passive venous hypertension from stasis occur, the microcirculatory flow regulation system (MFRS) intervenes so to prevent capillary overflow.
The defence mechanism causes vasomotion opening and closing periods to change, with prevalence of closing periods of the precapillary sphyncters, limiting capillary hypertension and resulting in capillary hemoconcentration (3,5).
This phenomenon promotes drainage of fluids from the interstitial spaces and prevents hypoxyc alterations of the hematotissular barrier. Therefore, in mild chronic venous insufficiency no significant functional changes are observed (10). On the other hand, in severe chronic venous insufficiency, vasomotion fades out and arteriolar vasoparalysis occurs. Consequently, the capillary bed is flooded and the intracapillary pressure significantly increases. The outflow of liquid and corpuscles from the capillaries into the interstitial tissue causes relevant anatomic and functional changes of the latter (15). The decreased microcirculatory perfusion pressure favours capillary plugging by white cells (16), the activation of which seriously damages the endothelium through the release of proteolytic enzymes, oxygen metabolites and lipid products, finally resulting in increased permeability with passage of fibrinogen and fibrin deposition (Fig.4).So a fibrin cuff is formed around the capillary which initially protects the capillary, preventing further liquid filtration, but later blocks oxygen exchanges.
The microhemodynamic and microhemorheological events lead to capillary thrombosis with exclusion of microcirculatory units, consequent tissue ischemia and trophic lesions.
The flooding of the interstitial tissue overloads lymphatic microvessels which together with venous capillaries act as a drainage system, the failure of which accounts for the phlebolym-phedema of chronic venous insufficiency.

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l3

LA MICROCIRCOLAZIONE, DAL LABORATORIO ALLA CLINICA

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Nel 1628 W. Harvey (De Motu Cordis 1628) descrisse molto dettagliatamente le grandi linee del sistema circolatorio erano ben note, salvo la connessione tra il sistema arterioso e quello venoso, ipotizzata mediante anastomosi della porosità della "carne". Nel 1661 Malpighi (De Pulmonibus: Abservationes Anatomicae) descriveva i "vasa per capillamenta resoluta" che rappresentano la prima osservazione diretta della microcircolazione, confermati dalle osservazioni di van Leeuwenhoe (Epistola alla Scientific Royal Society of London, 1686) che nei piccoli vasi descritti da Malpighi aveva individuato i "globuli rossi fluttuanti nel plasma". Centocinquanta anni dopo, alle descrizioni morfologiche si aggiunsero alcuni dati "funzionali", come l'endo ed esosmosi descritta (E. Dutrochet, 1828) e la legge di Starling (Starlin EH: J Phisiol 19:312-326, 1895) sul ruolo delle pressioni osmotica ed oncotica nel meccanismo della filtrazione e del riassorbimento vaso-tessuto. La nascita ufficiale della microcircolazione come scienza va tuttavia datata agli inizi del secolo scorso, con la descrizione della formazione dell'eritema e dell'edema nella risposta infiammatoria (Th. Lewis 1917), le fondamentali ricerche di Krogh (A. Krogh, Anatomy and Physiology of Capillaries, New Haven Academic Press 1922), e la descrizione dello schema di Chamber e Zweifach, riferimento morfofunzionale attuale, capace di rispondere alle esigenze metaboliche tessutali distrettuali come una entità organica indipendente (J Cell Comp Physiol 15:255-272, 1940). I decenni successivi sono stati caratterizzati da tre tappe fondamentali; gli studi sulla viscosità ematica e sulla emoreologia (L. Dintenfass 1971, T. DiPerri Angiology 1979, S.Forconi Cl. Hemorheology 1987; G.Caimi Clin Hemorheology 1988); gli studi sulla microemodinamica che misero in evidenza che il flusso nel microcircolo segue le leggi della stocastica e non regole matematiche (R.DelGuercio e G.Leonardo, Microv Res 1986); l'osservazione della perfusione dal versante del tessuto (GM.Andreozzi CV World Rep 1990), valutando anche l'aspetto metabolico mediante la misura dell'acidosi (GM.Andreozzi, J Cardiov Diagn Proc 1996). I metodi ricordati hanno permesso di conoscere nuovi aspetti delle malattie vascolari, di osservare a misurare in vivo il fenomeno dell'ischemia riperfusione. La biologia molecolare e cellulare sono gli attori più recenti apparsi sulla scena, svelando la interazioni endotelio-cellule circolanti, il ruolo del cell-coat di GAG, il rolling e l'adesione leucocitaria. Fenomeni governati da numerose molecole, espressione della funzione paracrina delle cellule microcircolatorie, in continuo equilibrio dinamico. Un'altalena continua tra attivatori e inibitori che caratterizza lo stato di quiete, della sollecitazione funzionale, del ristoro, e lo stato di malattia. Ma la microcircolazine non è una dsciplina medica, una specialità, è soltanto una scienza, come disse Krogh nel discorso per la consegna del Nobel. Una scienza, una metodologia di studio, volta a capire fenomeni finalizzati alla specifica funzione dell'organo in studio, fenomeni che, pur sganciati dalle leggi della macrocircolazione, sono ad essa strettamente connessi! Una scienza la cui ricaduta pratica in clinica sarà considerevole non solo per lo studio della fisiologia e della patologia circolatoria, ma anche per tutte le altre branche della Medicina! Di tutto ciò, cosa portiamo in clinica? Come bagaglio di conoscenza certamente tutto; ma le misure della microcircolazione sono indispensabili nella quotidianità clinica? Certamente no; le decisioni terapeutiche rimangono sostanzialmente cliniche, anche se spesso "aiutate" da poche misure di microcircolazione, divenute ormai "esami clinici".

SIMPOSIO

s1.1

LA MICROEMODINAMICA NELLA ARTERIOPATIA OBLITERANTE PERIFERICA AL II STADIO

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La microemodinamica nella arteriopatia obliterante periferica (AOP) può essere indagata utilizzando la metodica laser Doppler (LD), che permette di rilevare e misurare la perfusione cutanea, sia in condizioni basali, che in risposta a stimoli. La stessa metodica può essere applicata allo studio della perfusione del muscolo scheletrico. I tests più utili nello studio della microemodinamica nella AOP utilizzano come stimolo l'ischemia o sostanze farmacologiche come l'acetilcolina (Ach) ed il nitroprussiato di sodio (NPS). La risposta perfusionale all'ischemia è considerata globalmente indice del grado di compenso o di scompenso microemodinamico presente nel distretto esaminato. La risposta iperemica cutanea alla cessione iontoforetica di Ach è considerata indice di funzionalità endoteliale, essendo mediata in principale misura dall'ossido nitrico, prodotto e rilasciato dall'endotelio. La risposta iperemica cutanea alla cessione iontoforetica di NPS, vasodilatore diretto sulla muscolatura liscia vasale, è considerata indicativa del grado distensibilità arteriolare. Lo studio della microemodinamica nella AOP può essere ulteriormente approfondito attraverso l'analisi spettrale del segnale LD. Tale analisi permette di esaminare la flowmotion e di misurarne le varie componenti, in termini di intensità spettrale. Alcune di esse sono ritenute direttamente correlate ad attività, come quella endoteliale, quella simpatica e quella miogena spontanea della parete arteriolare, direttamente implicate nella regolazione del microcircolo. L'esame LD della perfusione cutanea dell'arto inferiore in pazienti con AOP al II stadio ha evidenziato valori di perfusione quantitativamente normali in condizioni basali e una ridotta e/o ritardata risposta iperemica all'ischemia. Il grado di riduzione della iperemia post-ischemica appare correlato alla gravità della arteriopatia. Anche la risposta iperemica cutanea alla Ach, come pure quella al SNP, sono risultate ridotte in pazienti con AOP al II stadio. Tale rilievo, tuttavia, è stato ottenuto anche in distretti non interessati dalla arteriopatia ed è pertanto da considerare indicativo di una condizione sistemica di disfunzione endoteliale e di ridotta distensibilità arteriolare, che caratterizza i pazienti con AOP al II stadio. L'analisi spettrale del segnale LD cutaneo rilevato in condizioni basali, in arti affetti da arterioptia al II stadio, ha mostrato un aumento delle componenti di flowmotion correlate all'attività endoteliale, simpatica e miogena. Al contrario, diversamente da quanto avviene nei soggetti normali, l'analisi spettrale del segnale LD, registrato durante iperemia post-ischemica, non ha evidenziato in pazienti con AOP al II stadio, alcun significativo incremento delle componenti di flowmotion riferibili all'attività endoteliale, simpatica e miogena. Questi dati suggeriscono che in arti con AOP al II stadio la perfusione cutanea sia mantenuta normale, in condizioni basali, grazie ad un meccanismo di compenso, in parte favorito dalla attivazione della flowmotion. L'utilizzo di tale meccanismo di riserva, già in condizioni basali, impedirebbe l'entrata in funzione dello stesso meccanismo in condizioni di iperemia post-ischemica, concorrendo ad alterare in modo caratteristico la microemodimica del distretto interessato dalla arterioptia periferica, nella stadio II della malattia.

s1.2

ASPETTI ENDOTELIALI ED EMOREOLOGICI NELL'ARTERIOPATIA OBLITERANTE PERIFERICA

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L'arteriopatia obliterante periferica (AOP) è una patologia che colpisce soprattutto il sesso maschile, la patogenesi è da ricondurre alla aterosclerosi delle arterie degli arti inferiori. I fattori di rischio sono sempre gli stessi, ma assumono una prevalenza maggiore il diabete ed il fumo di sigaretta. In alcuni studi, effettuati negli anni precedenti, avevano mostrato che nella AOP si riscontrava un peggioramento reologico che si poteva valutare con aumento della viscosità ematica e riduzione dell'EMI (indice morfologico eritricitario) maggiore che nelle altre vasculopatie ischemizzanti come quella cardiaca e cerebrale. Negli ultimi anni, con lo studio della funzione endoteliale abbiamo valutato anche i parametri endoteliali e i fattori del rischio trombotico in pazienti affetti da AOP al II stadio di Fontaine. Abbiamo valutato 20 pazienti,(Gruppo A:13 maschi e 7 femmine, età media 74±6) i pazienti sono stati confronatati con un gruppo di controllo C di 20 soggetti non affetti da AOP di pari età. Abbiamo valutato i seguenti parametri: la viscosità ematica totale (Reolist 500) a $10s^{-1}$ e $25s^{-1}$ di shear rate, l'esame emocromocitometrico (Coulter-Count), il fibrinogeno plasmatico, la morfologia eritrocitaria con l'EMI (Indice Morfologico Eritrocitario) metodo di Zipursky-Forconi, l'omocisteina, i metaboliti dell'NO (L-arginina ed L-citrullina ed il loro rapporto) e l'ADMA e l'omocisteina con la metodica HPLC, la VCAM-1 (metodo ELISA). I fattori del rischio trombotico (PT-INR, aPTT, Fibrinogeno, antitrombina III, proteina C; proteina S, LAC 1, APCr protrombina, Fattor V, VII, VIII, XI con il metodo coagulativo BCT), anticorpi anticardiolipina (ACA) e anti beta2 glicoproteina I(metodo ELISA). I nostri risultati mostrano un aumento significativo della viscosità ematica totale: (Gruppo A: 12,34±0,56cPs, Gruppo C: 8,5±0,6 cPs $10s^{-1}$) e dell'omocisteina (A:28,6±8,9; C: 14,7±3,9 µmol/l); una riduzione dell' EMI rispetto al Gruppo di controllo (A:0,58±0,04; C: 1,15±0,05). Il rapporto L-Citrullina e L-Arginina (espressione dell'attività della NOs) è diminuito (0,36±0,12); rispetto al gruppo C: (0,55±0,1) e la Dimetil arginina asimmetrica (ADMA, inibitore endogeno dell'NOs) è aumenta (A: 0,55±0,08; C:

0,38±0,09 μM/L). L'analisi di questi risultati evidenzia che nei pazienti con AOP è presente una marcata alterazione degli indici di disfunzione endoteliale (riduzione del rapporto L-arginina/L-citrullina e aumento dell'ADMA) che favorisce il peggioramento reologico con aumento della viscosità ematica e riduzione dell'EMI. Rispetto alla altre vasculopatie ischemizzanti notiamo che le alterazioni endoteliali e quindi anche il peggioramento reologico è maggiore in questi pazienti sia perché l'aterosclerosi occupa un territorio più vasto, i fattori di rischio sono numerosi e spesso l'AOP rappresenta una complicazione ulteriore di in cardiopatia ischemica e di altre localizzazioni che devono essere considerate molto importanti nelle prevenzione dell'ATS e delle sue complicanze.

s1.3

ASPETTI EMOGASANALITICI TRANSCUTANEI NELL'ISCHEMIA CRITICA

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Non è ancora del tutto chiarita la concatenazione di eventi macro-microemodinamici e biochimici, nell'ischemia critica, che conducono ad un progressivo esaurimento della riserva metabolica distrettuale. La caduta della pressione di perfusione tissutale innesca una serie di risposte complesse microcircolatorie, creando multipli circoli viziosi, che costituiscono la causa ultima del dolore a riposo e delle lesioni trofiche. L'ossimetria transcutanea, fornendo informazioni sul metabolismo tessutale a cui è finalizzato l'intero sistema macro-microvasale, è a buon diritto la metodica di studio microcircolatorio compresa nei protocolli di indagine dell'ischemia critica, indicando valori limite di TcPO2 compatibili con la definizione stessa di ischemia critica. L'utilità della metodica si rivela nella quantificazione della severità della sofferenza tessutale, valutazione del grado di reversibilità dello scompenso metabolico locale, valutazione e monitoraggio terapeutico, previsione dell'esito di procedure invasive, previsione di cicatrizzazione di amputazioni distali. I protocolli di studio prevedono sia rilievi topografici in condizioni basali sia rilievi dinamico-funzionali. Questi ultimi indagano le risposte a variazioni sia rapide, come le variazioni tensive locali indotte dalla posizione dell'arto (test posturali), sia lente come la reattività microcircolatoria all'ischemia (test di iperemia post-ischemica). Tali test funzionali considerano, oltre all'andamento dei valori di TcPO2, condizionati dalla perfusione ematica tessutale e dal consumo tessutale di O2, e di TcPCO2, interpretabili come indice di adattamento all'ischemia, anche i tempi di reazione, di cui il più significativo si è dimostrato finora il tempo di recupero, inteso come il tempo necessario al ritorno della TcPO2 e della TcPCO2 ai valori basali o anche il tempo di semirecupero, per il ritorno a valori pari al 50% dei valori basali. Altre prove funzionali sono il test di inalazione di O2 e, meno praticabile, il test di esercizio muscolare. La nostra esperienza ci ha rivelato particolarmente utili ai fini prognostici, i test posturali con l'arto sollevato a 45° e quindi abbassato fuori del letto, al punto di impiegarli ormai di routine, in tale categoria di pazienti, anche per la semplicità di esecuzione. Abbiamo impiegato l'ossimetria transcutanea con le prove posturali, per valutarne il valore predittivo ai fini della reversibilità o meno dell'ischemia, in 57 pazienti con ischemia critica non rivascolarizzabile degli arti inferiori. Il follow-up dei pazienti, sottoposti a terapia medica (prostanoidi, pentossifillina), è durato 3 mesi. Abbiamo rilevato che l'incremento della TcPCO2 ad arto abbassato poteva essere considerato un indice predittivo di evoluzione sfavorevole, essendosi verificato a breve termine, in 3 mesi, il più alto indice di peggioramento clinico, espresso dalla estensione delle lesioni trofiche, dalla ingravescenza del dolore ischemico a riposo fino alla necessità di amputazione maggiore. I dati iniziali, confermati da successiva ulteriore esperienza, autorizzano a considerare la stazionarietà o il decremento dei valori di TcPO2, ma soprattutto la stazionarietà o l'incremento dei valori della TcPCO2 ad arto abbassato, espressione del prevalere del metabolismo anaerobio per mancato apporto di ossigeno, indici attendibili di non reversibilità dello scompenso assoluto.

s1.4

FLOWMOTION IN CRITICAL LIMB ISCHEMIA AND IN VENOUS ULCERS

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Flowmotion, periodic rhythmic variation of blood perfusion in microvessel, is determined by the control systemic and local of myocells of arteriolaes. To study flowmotion in lower limbs with critical limb ischemia and with chronic venous insufficiency laser doppler has been widely utilised. Twenty patients with unilateral CLI and 13 with venous ulcer were compared with control group 10 healthy participants. Laser Doppler was used to evaluate the foot skin microcirculation simultaneously at four different areas. Flowmotion was expressed using fast Fourier transformation as low frequency waves (LFW) and high frequency waves (HFW). All patients, those with CLI and venous ulcers showed HF waves . These were absent in healthy controls. There were no regional differences in frequency in the critically ischemic feet while in venous ulcers patients difference were found. In conclusion HFW are associated with CLI and venous ulcers showing a similar way of sufferance of skin microcirculation regardless the main vascular pathology . The areas of sufferance was markedly higher in CLI.

COMUNICAZIONI ORALI

c1.1

MONITORAGGIO DELLE DETERMINANTI EMOREOLOGICHE NELL'INFARTO MIOCARDICO GIOVANILE

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I contributi riguardanti la valutazione delle variabili emoreologiche nell'infarto miocardico acuto (IMA), non solo nella fase acuta ma anche nelle prime settimane, nei mesi e negli anni successivi a tale evento clinico, hanno riguardato pazienti non suddivisi per età e comunque quasi sempre con un'età media avanzata. Recentemente la nostra attenzione si è indirizzata all'assetto emoreologico nell'IMA giovanile, da alcuni ritenuto quasi un'entità nosologica autonoma. L'IMA giovanile si caratterizza per quanto attiene la prevalenza dei fattori di rischio, la familiarità, gli aspetti genetici ma anche le caratteristiche del danno coronarico (elevata è la percentuale dei pazienti con coronarie indenni o malattia monovasale) e soprattutto per la prognosi a breve e a media distanza. La nostra ricerca ha finora riguardato 96 soggetti con IMA insorto in età < 46 anni (89 M e 7 F; età media 39.0±6.0 anni (range 19-45 anni), studiati inizialmente a distanza di 13±7 giorni dall'esordio dei sintomi (T1), successivamente a distanza di 3 mesi (T2) e di un anno (T3) dalla prima valutazione. Dei 96 soggetti inizialmente arruolati solo 41 (39 M e 2 F) hanno completato il follow-up ad un anno. La valutazione dell'assetto emoreologico (viscosità ematica, plasmatica e sierica, ematocrito, filtrabilità ematica ed elongation index) mostra in fase iniziale una chiara sindrome da iperviscosità, solo parzialmente influenzata dall'estensione del danno coronarico (angiograficamente documentata) e dal sommarsi dei principali fattori di rischio (fumo, familiarità, ipertensione, diabete mellito, dislipidemia). Tale condizione di iperviscosità, diversamente da quanto nella nostra esperienza si verifica nell'IMA che insorge in età più avanzata, permane quasi immodificata a distanza di tre mesi o di un anno dall'evento iniziale. I dati ottenuti sembrano suggerire che la sindrome da iperviscosità ematica, con il suo indiscusso potenziale protrombotico, possa preesistere nei giovani adulti che vanno incontro ad IMA e soprattutto non venga influenzata dal trattamento farmacologico che ciascun soggetto ha praticato dal momento della dimissione.

c1.2

CARDIOPATIA ISCHEMICA CRONICA: PATTERN INTEGRINICO DEI LEUCOCITI POLIMORFONUCLEATI DOPO ATTIVAZIONE IN VITRO E DURANTE TEST ERGOMETRICO

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È conosciuto il ruolo dei leucociti polimorfonucleati (PMN) nella cardiopatia ischemica, suggerito dalla significativa associazione della loro conta con il rischio cardiovascolare e la prognosi delle coronaropatie. Sia il numero elevato che, soprattutto, un'inappropriata attivazione dei PMN possono rappresentare fattori patogenetici del danno ischemico, e tra i fenomeni associati all'attivazione è compresa l'espressione fenotipica delle $beta_2$-integrine. È noto inoltre che l'esercizio fisico si accompagna, anche nei soggetti normali, a modificazioni del numero e dell'assetto funzionale dei leucociti circolanti. Lo scopo di questa ricerca è stato quello di determinare, in pazienti con cardiopatia ischemica cronica, l'assetto integrinico dei PMN sia in condizioni di base che durante attivazione in vitro e durante test ergometrico. Sono stati arruolati 15 pazienti affetti da cardiopatia ischemica cronica (14 uomini e una donna, età media 55,1±9,5 anni), nei quali l'espressione fenotipica delle catene costitutive delle $beta_2$-integrine sui PMN (CD11a, CD11b, CD11c e CD18) è stata valutata mediante citofluorimetria; l'attivazione in vitro è stata condotta incubando i PMN per 5 e 15 minuti con forbolo-miristato-acetato (PMA) o con formil-metionil-leucil-fenilalanina (fMLP); il test ergometrico è stato effettuato secondo il protocollo di Bruce e il profilo integrinico è stato valutato al picco dello sforzo e in fase di recupero. L'esame dell'assetto integrinico dei PMN mostrava di base differenze significative fra volontari sani e cardiopatici ischemici: in questi ultimi si notava infatti un aumento dell'espressione del CD11a, del CD11c e del CD18, accompagnato da una riduzione del CD11b. Durante attivazione in vitro si osservava, nei volontari sani, un aumento significativo nell'espressione di tutte le molecole integriniche; nei cardiopatici ischemici era evidente un aumento del CD11b, associato a riduzione significativa del CD11a, mentre CD11c e CD18 rimanevano immodificati. Durante test ergometrico nei volontari sani si osservava un incremento solo a carico del CD11a e del CD18; nei cardiopatici, oltre ad un aumento più accentuato del CD18, era evidente un incremento significativo del CD11b, sia all'acme dello sforzo che in fase di recupero. Quanto descritto evidenzia un diverso comportamento dell'espressione integrinica nei cardiopatici ischemici cronici rispetto ai volontari sani, e in particolare la diversità delle modificazioni che si osservano a carico dei PMN dopo attivazione in vitro e dopo test ergometrico. Sembra rivestire un significato particolare la risposta marcata del CD11b allo sforzo, presente esclusivamente nei cardiopatici ischemici, in considerazione del suo ruolo chiave nella partecipazione dei PMN alla genesi del danno ischemico.

c1.3

LA SINDROME DEL DITO BLU: QUANDO È ISCHEMIA CRITICA?

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Definizione: Com'è noto la sindrome del dito blu rappresenta un quadro relativamente frequente in Angiologia.
Può essere definita come una ischemia digitale del piede con localizzazione variabile all'estremità distale di uno o più dita, in presenza dei polsi tibiali (rilevabili alla palpazione o all'esplorazione doppler). Eziopatogenesi: Il quadro può essere correlato a varie cause, quali microembolia da ateromi prossimali (ateroembolia), condizioni di iperviscosità ematica, alterazioni emoreologiche, insufficienza renale, displasie arteriose del piede. Dal punto di vista clinico si manifesta con una cianosi fissa interessante parzialmente uno o più dita dei piedi. Per valutare la correlazione di tale quadro clinico con i suoi differenti possibili meccanismi patogenetici abbiamo effettuato uno studio retrospettivo di tutti i pazienti osservati presso il Servizio di Angiologia del Presidio Ospedaliero di Anagni o del Poliambulatorio Specialistico dell'Azienda ASL di Frosinone nel periodo gennaio 1988 - dicembre 2004.
Casistica: sono stati selezionati 78 pazienti affetti da sindrome del dito blu con netta cianosi digitale o plantare senza lesioni trofiche e con presenza dei polsi sottopoplitei alla palpazione o alla esplorazione strumentale. A tutti i pazienti sono stati effettuati i seguenti esami:
Esame emocromocitometrico – conta piastrine – glicemia – azotemia - es. urine - protidemia con elettroforesi - t.d.p. - PTT - Rx torace – Esame doppler ad onda continua degli arti inferiori ecodoppler e/o ecocolordoppler aorta addominale e distretto arterioso degli arti inferiori.
12 pazienti (15,3%) sono stati inviati a consulenza specialistica del Chirurgo Vascolare e sottoposti ad esame arteriografico)
In rapporto alla *patogenesi* è stato possibile rilevare: *cause emoreologiche* in 28 paz. (35,9%) e segnatamente trombocitosi 7 pazienti (25%), diabete 3 pazienti (10,7%), poliglobulia 7 pazienti (25%), insuff. renale cronica 5 pazienti (17,8%), neoplasie 6 pazienti (21,4%) . *Cause emboligene* in 25 paz. (32,05%) per patologia aneurismatica sopradiaframmatica 5 pazienti (20%), patologia ,aneurismatica aorto-iliaca 10 pazienti (40%), patologia aneurismatica arterie femorali e poplitee 10 pazienti (40%). *Cause miste* in 25 pazienti (32,05%) e precisamente angiodisplasie delle arcate plantari 8 pazienti (32%), angiodisplasia associata a diabete 3 pazienti (12%), angiodisplasia associata a poliglobulia 6 pazienti (24%), angiodisplasia associata a patolologia aterosclerrotica aorto-iliaca 8 pazienti (32%).
L'evoluzione dei vari quadri clinici è stata generalmente favorevole e con prognosi benigna. Il trattamento terapeutico è stato necessariamente correlato alla patogenesi vista la molteplicità delle condizioni all'origine delle diverse forme. I quadri più importanti dal punto di vista della evoluzione e della prognosi e che hanno determinato un quadro di ischemia critica distrettuale seppur limitata sono state le forme correlate ad una patogenesi embolica.
I quadri correlati invece ad una patogenesi emoreologica hanno avuto una migliore evoluzione in rapporto alla correzione e/o alla eliminazione delle suddette alterazioni.

c1.4

ISCHEMIA CRITICA D'ARTO NON RIVASCOLARIZZABILE – RISULTATI DELLA STIMOLAZIONE CORDALE SPINALE (S. C. S.)

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In ambito vascolare la S.C.S. ha la sua indicazione più mirata nella ischemia critica "cronica" degli arti inferiori con dolore a riposo (III stadio) e/o con lesioni trofiche di limitata estensione (IV stadio) in cui non è possibile il trattamento chirurgico (ischemia critica non rivascolarizzabile) e/o farmacologico con prostanoidi. Per alcuni pazienti si tratta del tentativo estremo di evitare o procrastinare l'intervento demolitivo dell'arto ischemico: in una significativa percentuale di questi pazienti si registra un esito positivo poiché si realizza una riduzione del dolore, una regressione fino a scomparsa delle lesioni parcellari ed un conseguente miglioramento della qualità della vita. Dal genn. 99 al dic. 2004 sono stati sottoposti ad impianto di S.C.S. 50 pazienti (15 donne - 35 uomini - età 41 - 96 anni), affetti da insufficienza arteriosa degli arti inferiori da arteriopatia obliterante al III ed al IV stadio (iniziale) sec. Lériche-Fontaine.Le lesioni erano parcellari e localizzate a livello acrale o periungueale. Nessun paziente risultava passibile di intervento di rivascolarizzazione diretta, sia per la compromissione delle condizioni generali, sia per la presenza di localizzazioni multiple o distali sia per espresso rifiuto di soluzioni chirurgiche proposte. I criteri utilizzati per la valutazione dell'efficacia del trattamento sono stati: *1) Valutazione della riduzione dell'uso di analgesici* utilizzando una scala arbitraria con punteggio semiquantitativo a 5 gradini. *2) Riduzione o scomparsa del dolore a riposo o notturno* con regressione al II stadio di Lériche-Fontaine. *3) Riduzione della estensione o scomparsa della lesioni trofiche* con regressione della stadiazione clinica. *4) Valutazione dell' indice di Winsor* mediante rilevazione con metodica doppler c.w. *5) Presenza di effetti emodinamici sistemici 6) Effetti avversi o collaterali*
I controlli sono stati effettuati al tempo 0, dopo 1 mese e dopo 6 mesi di trattamento. In 24 pazienti (48%) si è avuta scomparsa del dolore. In 16 pazienti (32%) pur non variando la stadiazione si è avuta netta riduzione del dolore da consentire il riposo notturno e la riduzione dell'utilizzo di analgesici. In 10 pazienti (20%) non si è avuta alcuna modificazione del quadro clinico

In 1/3 dei 16 pazienti con lesioni trofiche si è osservata la completa risoluzione delle lesioni medesime. I risultati raggiunti appaiono confortanti, pur nel limitato numero dei pazienti reclutati, soprattutto se si considera che gli stessi non erano suscettibili di rivascolarizzazione chirurgica, non avevano tratto apprezzabile beneficio dai protocolli farmacologici con eparina, prostanoidi e/o metabolici o presentavano condizioni generali compromesse. Da una preliminare osservazione è scaturita l'evidenza di un miglioramento della qualità della vita legata ad una azione antalgica protratta. Escludendo un'azione sul macrocircolo i risultati conseguiti possono essere correlati ad una attività emodinamica per quanto attiene all'incremento dell' indice di Winsor e ad una azione emoreologica la riduzione o la risoluzione delle lesioni trofiche. L'azione antalgica inoltre incidendo favorevolmente sulla posizione declive antalgica determina indirettamente un miglioramento della perfusione distale per riduzione dell'edema: da qui il convincimento di una doppia azione antalgica ed emodinamica (anche se prevalente la prima) suscettibile di ulteriore e progressiva verifica ed osservazione clinico-strumentale su una casistica più ampia da valutare nei semestri successivi.

c1.5

L'ISCHEMIA E RIPERFUSIONE NON INDUCE DISFUNZIONE ENDOTELIALE A LIVELLO DEL MICROCIRCOLO CUTANEO: CONFRONTO IN VIVO IN UOMINI CON IL CIRCOLO ARTERIOSO DI CONDUTANZA

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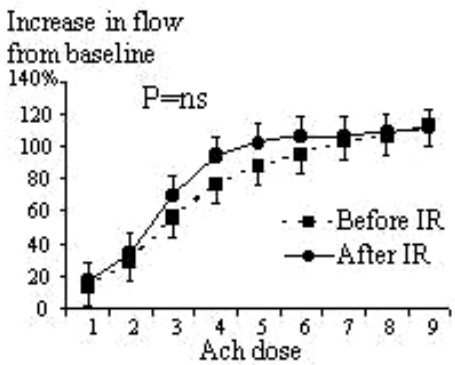
Introduzione: Studi in modelli animali ed umani hanno dimostrato che l'esposizione ad un ciclo di ischemia e riperfusione (IR) causa disfunzione endoteliale a livello delle arterie di conduttanza. Nonostante l'importanza fondamentale che il microcircolo riveste nella fisiopatologia dell'infarto miocardico (ad esempio, nel fenomeno denominato no-reflow), nessuno studio ha valutato se esistano differenze nella sensibilità al danno da IR tra microcircolazione ed arterie di conduttanza.
Metodi e risultati: 10 soggetti sani non fumatori (età 23-45 anni) sono stati arruolati per questo studio. La vasodilatazione endotelio dipendente è stata studiata a livello delle arterie di conduttanza mediante vasodilatazione flusso mediata (FMD) della arteria radiale ed a livello del microcircolo cutaneo mediante microvascular cutaneous reactive hyperemia ed acetylcholine-induced microvascular vasodilation (laser doppler iontophoresis). Tali dati sono stati acquisiti prima e dopo un ciclo di IR (15' di ischemia a livello della arteria brachiale seguiti da 15' di riperfusione). Tutti i dati sono stati analizzati in cieco. La FMD (funzione endoteliale delle arterie di conduttanza) era significativamente ridotta dopo IR (prima di IR: 7.5±4.2%; dopo: 4.2±0.8%, $p<0.05$). Al contrario, la IR non aveva alcun effetto sulla iperemia reattiva (prima di IR: 386±96%; dopo: 443±96%, p=ns) e sulla vasodilatazione acetilcolina-dipendente (p=ns, vd figura) a livello del microcircolo cutaneo.
Conclusioni: In un modello in vivo su uomini, dimostriamo che il microcircolo è più resistente nei confronti del danno da IR paragonato al circolo di conduttanza.



c2.1

LO STUDIO DELLA FLOWMOTION CUTANEA MEDIANTE ANALISI SPETTRALE DEL SEGNALE LASER DOPPLER COME METODO DI VALUTAZIONE DEI MECCANISMI DI REGOLAZIONE LOCALE DEL MICROCIRCOLO CUTANEO

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L'analisi spettrale del segnale laser Doppler rilevabile a livello cutaneo, rende possibile lo studio della flowmotion, fenomeno consistente in una variazione ritmica del flusso microcircolatorio, provocata dalla dilatazione e contrazione della arteriole (vasomotion). Come dimostrato dagli studi di diversi Autori, la vasomotion arteriolare influenza la distribuzione del flusso nel microcircolo cutaneo, riducendo le resistenze nel letto microvasale e garantendo un flusso intermittente ma adeguato in presenza di un ridotto apporto di flusso al tessuto. Cinque differenti componenti di flowmotion sono state descritte grazie all'analisi spettrale del segnale laser Doppler cutaneo. Tre di esse sono state attribuite a specifici meccanismi di origine microcircolatoria cutanea: una componente con intervallo di frequenza di 0.007-0.02 Hz, è stata attribuita all'attività endoteliale; una, con intervallo di frequenza di 0.02-0.06 Hz, all'attività simpatica ed una, con intervallo di frequenza di 0.06-0.02 Hz, all'attività del muscolo liscio arteriolare (attività miogena spontanea). Due altre componenti della flowmotion cutanea, una con intervallo di frequenza di 0.6-1.8 Hz ed una con intervallo di frequenza di 0.2-0.6 Hz, sono state attribuite alla trasmissione al livello del microcircolo cutaneo di variazioni emodinamiche sincrone, rispettivamente, con l'attività respiratoria e con quella cardiaca. Lo studio della flowmotion cutanea mediante l'analisi spettrale del segnale laser Doppler permette di migliorare le conoscenze sui meccanismi di regolazione del microcircolo cutaneo in diverse condizioni fisiologiche e patologiche. Saranno discussi i presupposti teorici e sperimentali su cui è basata l'indagine.

c2.2

STUDIO DELLA FLOWMOTION CUTANEA IN RISPOSTA ALL'ESERCIZIO ACUTO IN SOGGETTI ALLENATI E SEDENTARI

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Precedenti studi hanno dimostrato che la cute va incontro, durante l'esercizio acuto, ad una intensa vasodilatazione, finalizzata alla dispersione di calore da parte dell'organismo. Il meccanismo di tale vasodilatazione appare legato alla riduzione della tono simpatico vasocostrittore ed all'aumento del tono simpatico vasodilatatore. L'allenamento allo sport di resistenza favorisce una più intensa vasodilatazione cutanea in risposta all'esercizio acuto, ma i meccanismi implicati in tale adattamento sono ancora poco conosciuti. Abbiamo ipotizzato che il training fisico si accompagni ad una maggiore attivazione della flowmotion cutanea in risposta all'esercizio acuto, contribuendo ad una migliore distribuzione della perfusione nel microcircolo cutaneo, con conseguente aumentata capacità di dispersione termica da parte della cute stessa. Allo scopo di verificare tale ipotesi abbiamo studiato la flowmotion cutanea, mediante l'analisi spettrale del segnale laser Doppler registrato a livello della cute dell'avambraccio sinistro, prima e dopo esercizio acuto massimale al cicloergometro, in 15 oggetti soggetti sani con età media di 30±6 anni, praticanti sport di resistenza e in 15 soggetti sani di controllo, sedentari, omogenei per sesso ed età. In condizioni basali non si sono osservate differenze significative tra soggetti allenati e sedentari nel valore medio di intensità spettrale della flowmotion totale (2.52±1.99 PU/Hz versus 2.05±3.07 PU/Hz, rispettivamente), né nel valore medio di perfusione cutanea (11.3±6.31 PU versus 9.18±3.21 P.U., rispettivamente). L'esercizio fisico acuto ha indotto nei soggetti allenati un più elevato incremento nel valore medio di intensità spettrale della flowmotion totale rispetto ai soggetti sedentari (6.65±4.13 Hz/PU, $p<0.001$ e 4.17±1.86 Hz/PU, $p<0.05$), cui ha corrisposto un significativo maggiore incremento nella perfusione cutanea. Sia in condizioni basali, che dopo esercizio acuto entrambe le componenti di flowmotion cutanea riferibili all'attività endoteliale e a quella miogena spontanea hanno mostrato una intensità significativamente più un elevata nei soggetti allenati rispetto ai soggetti sedentari. Questi dati indicano che il training fisico si accompagna ad un aumento della flowmotion cutanea e delle sue componenti endoteliale e miogena spontanea, in risposta all'esercizio fisico acuto, che può concorrere ad una più adeguata distribuzione del flusso nel microcircolo cutaneo durante esercizio.

c2.3

STRESS OSSIDATIVO E REATTIVITA' PIASTRINICA IN MENOPAUSA

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Studi clinici ed epidemiologici hanno convalidato l'ipotesi secondo cui il maggior rischio per la malattia cardiovascolare che si osserva nelle donne in menopausa è conseguente alla perdita del ruolo protettivo multifattoriale degli estrogeni. La maggiore vulnerabilità a sviluppare la malattia aterosclerotica e/o eventi ischemici acuti in menopausa sembra essere legata ad una significativa modificazione che la caduta degli estrogeni determina sul profilo lipidico e lipoproteico. E' noto che le lipoproteine a basso peso molecolare (LDL), soprattutto nella forma ossidata (ox-LDL), possono influire sul tono vascolare ed

aumentare la reattività piastrinica, predisponendo ad una maggiore suscettibilità per eventi trombotici. In questo studio abbiamo voluto verificare se la maggiore aggregazione piastrinica che si osserva di frequente in menopausa sia riconducibile ad una globale modificazione sistemica dello stato redox dovuta alla perdita di estrogeni. A tale fine, sono state ammesse allo studio 10 donne in menopausa, di età compresa tra 54 e 62 anni, non trattate con terapia ormonale sostitutiva. Dieci donne sane in età fertile (20-40 anni) hanno costituito il gruppo di controllo. Campioni ematici sono stati utilizzati per la determinazione dell'aggregazione piastrinica *ex vivo* su sangue intero mediante metodo impedenziometrico. Come indici di funzionalità piastrinica sono stati, inoltre, valutati la concentrazione citoplasmatica dei metaboliti finali stabili nitrati/nitriti (NO_x), quale espressione del pathway intrapiastrinico L-arginina/NO, la concentrazione citosolica di calcio e magnesio e la fluidità di membrana. Lo stato redox sistemico è stato analizzato mediante determinazione plasmatica della concentrazione delle sostanze reattive all'acido tiobarbiturico (TBARS) e della capacità antiossidante totale (TEAC) e mediante valutazione della suscettibilità delle LDL all'ossidazione *in vitro*. E' stata, infine, misurata la biodisponibilità plasmatica di NO_x quale indice della funzione endoteliale. I risultati ottenuti hanno evidenziato alterazioni statisticamente significative della aggregazione, della concentrazione citosolica di calcio e di magnesio insieme a ridotti livelli di NO_x e ad una diminuita fluidità di membrana. Inoltre, è stato rilevato un aumento della concentrazione di TBARS ed un diminuito contenuto di NO_x nel plasma così come una maggiore suscettibilità delle LDL all'ossidazione. Complessivamente, i risultati ottenuti supportano la speculazione che la perdita del ruolo antiossidante degli estrogeni in menopausa possa favorire una maggiore formazione intravasale di ox-LDL e che la maggiore aggregazione piastrinica sia un epifenomeno conseguente all'effetto inibitorio che le ox-LDL esercitano sulla effettiva bioattività di NO, plasmatico ed intrapiastrinico, determinando una minore efficacia del meccanismo a feedback NO-mediato preposto a prevenire l'attivazione piastrinica intravasale.

c2.4

IL GENE eNOS INFLUENZA LA VISCOSITÀ EMATICA E LA DEFORMABILITÀ DEI GLOBULI ROSSI: RUOLO DEI POLIMORFISMI T-786C, G894T E 4a/4b DEL GENE eNOS

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La viscosità plasmatica e l'aggregazione eritrocitaria svolgono un ruolo importante nella regolazione del microcircolo. Alcuni studi *in vivo* ed *in vitro* hanno suggerito un ruolo del nitrossido (NO) nella modulazione della vasodilatazione indotta dal flusso e nella deformabilità eritrocitaria. Un'alterazione della disponibilità di NO dovuta a mutazioni del gene eNOS potrebbero contribuire ad alterare i parametri emoreologici. Lo scopo di questo studio è stato quello di analizzare il ruolo dei polimorfismi dell'eNOS T-786C, G894T e 4a/4b in relazione all'alterazione dei parametri emoreologici. Abbiamo per questo studiato 80 pazienti affetti da sordità improvvisa neurosensoriale idiopatica (ISSHL) e 80 soggetti sani di controllo. Dall'analisi multivariata è emersa un'associazione significativa tra la variante rara del gene eNOS 894T e l'ISSHL (OR 894TT+GT=2,08; p=0,03). Il test di filtrazione eritrocitaria (EFT) è risultato alterato in una più alta percentuale sia di pazienti che di controlli, portatori delle varianti rare del gene eNOS rispetto ai portatori del tipo wild. Inoltre, sia nei pazienti affetti da ISSHL che nei soggetti sani di controllo, i polimorfismi dell'eNOS T-786C e G894T sono risultati in grado di influenzare indipendentemente l'EFT (OR -786CC+TC=2,81; p=0,01 e OR 894TT+GT=2,5; p=0,02 rispettivamente) in particolare nei soggetti in cui è stata osservata la contemporanea presenza dei due alleli rari (OR del genotipo combinato -786CC+TC e 894TT+GT =6,9; p<0,0001). Il nostro studio ha dimostrato che il gene dell'eNOS è in grado di influenzare la deformabilità eritrocitaria, contribuendo così eventualmente a determinare l'insorgenza di ISSHL, che rappresenta un buon modello di patologia del microcircolo.

c2.5

EFFETTI DEL NEBIVOLOLO SUL MICROCIRCOLO SUPERFICIALE IN PAZIENTI VACULOPATICI IPERTESI NON DIABETICI. STUDIO VIDEOCAPILLAROSCOPICO (VCS)

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I ß-bloccanti sono farmaci di grande utilizzo in molti campi di interesse internistico. Il loro precipuo meccanismo di azione ne limita l'impiego in alcune patologie internistiche quali le arteriopatie obliteranti periferiche ed il diabete. Il Nebivololo ß-bloccanti di ultima generazione presenta un interessante meccanismo d'azione che sembra interessare l'asse Adenosina-Nitrossido in sinergia con l'effetto di blocco sui recettori ß. Tale condizione ne potrebbe far ipotizzare l'utilizzo in pazienti ipertesi con vasculopatia periferica. L'ipotesi di questo studio è quella di verificarne l'effetto in cronico (5 mg./die in unica somministrazione) sul microcircolo in pazienti ipertesi di nuova diagnosi, vasculopatici con l'utilizzo della VCS. Questa tecnica elettiva di valutazione del microcircolo in vivo appare ripetibile, sensibile specifica. Per questo studio sono stati arruolati 18 pazienti (12 ♂, 6 ♀) di età media 68±3.6, vasculopatici (AOP) II A (4 ♀, 2 ♂) e stadio II B (2 ♀, 8 ♂) con pressione sistolica 165.6±24.3 e diastolica 87.5±9.9. È stata selezionata una popolazione analoga come gruppo di controllo (8

‖ , 5 ⁞) di età media 66±2 con pressione sistolica 128.6±8.3 e diastolica 80.5±3.9. Tutti gli arruolati sono stati sottoposti a VCS di: congiuntiva, letto periungueale (mano, piede), antecubito di base, dopo 7 gg., 15 gg., 30 gg. e dopo 6 mesi sono inoltre stati sottoposti ad esame ecocolordoppler ed è stato valutato con VCS l'arto più colpito (AOP). Il gruppo AOP presentava diminuita densità capillare, aumento della tortuosità venulare, ectasie circoscritti, microaneurismi, microemorragie (Stadio A, B di Fragrell e Lundberg). Normale la VCS nei sani. Già dal 15° giorno, al dosaggio di 5 mg al giorno, si segnalava un miglioramento del quadro periferico, a livello del letto ungueale del piede. Al 15° giorno si assisteva ad una significativa diminuzione delle ectasie e della tortuosità venulare ed un incremento della densità capillare (+18%), invariato sostanzialmente al 30° giorno (+24%), ulteriormente migliorato al controllo del 180° giorno (+32%). Sono inoltre migliorati la visibilità e diminuita la profondità dei solchi interpapillari, indice di sofferenza tissutale. Migliorata la abitazione delle papille dermiche e diminuito il loro piattismo. A livello della congiuntiva migliorato il flusso e diminuito lo sludging. Invariata la morfologia a livello del letto periungueale della mano, migliorato il flusso e lo sludging. A livello delle reti poligonali antecubitali nessuno significativa modificazione.

c2.6

ALTERATA DEFORMABILITA' ERITROCITARIA ASSOCIATA AD UNA RIDOTTA RISPOSTA PIASTRINICA A FARMACI ANTIAGGREGANTI IN PAZIENTI CON SINDROMI CORONARICHE ACUTE.

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In questi ultimi anni una ridotta risposta antiaggregante a farmaci antipiastrinici è stata oggetto di numerosi studi, ma nessun dato è disponibile in letteratura sul possibile ruolo delle variabili emoreologiche nella funzionalità piastrinica e nel fenomeno della resistenza agli antiaggreganti. Scopo del nostro studio è stato quello di valutare la funzionalità piastrinica e i parametri emoreologici in pazienti con sindromi coronariche acute (SCA) sottoposti a duplice terapia antiaggregante con Ac. Acetilsalicilico (ASA) e Clopidogrel. Su 301 pazienti (231 M/ 70 F; età: 66±13) con SCA sono stati valutati: tempo di chiusura (CT) PFA-100 (Dade Behring), con collageno-epinefrina (CT/EPI) e collageno-ADP (CT/ADP); viscosita' del sangue totale (WBV) alle shear rates (s.r) 0.512 e 94.5 sec-1, viscosita' plasmatica (PLV) alla s.r.20.4 sec-1 (microviscosimetro rotazionale Contraves LS 30), ed il test di filtrazione eritrocitaria (Filtrometro Myrenne MF4) per la valutazione della deformabilita' eritrocitaria (DI). Resistenza all'aspirina (definita per valori di PFA/EPI <203 sec.) è stata documentata in 104 pazienti (Gruppo 1) (34,5%). Tra i due gruppi nessuna differenza è stata evidenziata nei valori di PLV [gruppo 1= 1,43±0.12; gruppo 2= 1,55±0,84, p=ns] né di WBV alle s.r. 94.5 sec-1 [gruppo 1= 4,43±0,25; gruppo 2= 4,45±0,61, p=ns]. I valori di WBV alle s.r. 0.512 sec-1 erano più elevati nel gruppo 1, ma non raggiungevano la significatività statistica [gruppo 1= 23,37±4,68; gruppo 2= 22,54±3,90]. I valori di DI erano significativamente più bassi nei pazienti resistenti all'aspirina [gruppo 1= 4,05±2,93; gruppo 2= 5,71±3,30, p<0.0001]. La conta leucocitaria è risultata significativamente più elevata nei pazienti resistenti all'aspirina [gruppo 1= 11464±35044; gruppo 2= 7867±2162, p<0.0001]. Anche la funzionalità piastrinica valutata tramite PFA/ADP è risultata associata in modo significativo ai valori di DI: in particolare, i valori di PFA/ADP erano progressivamente più lunghi dal primo al quarto quartile di distribuzione dei valori di DI [Q1= 115,71±69,62; Q2=139,86±86,90; Q3=162,33±95,34; Q4=168,39±99,29,p<0.0001]. Questi risultati indicano:1) l'esistenza di una associazione tra DI e la risposta al trattamento antiaggregante con acido acetilsalicilico; 2) nei pazienti con SCA l'effetto antiaggregante di farmaci antipiastrinici è influenzato non solo dall'azione farmacologia diretta sulle piastrine, ma anche dalla deformabilita' eritrocitaria e dalla risposta leucocitaria.

c3.1

STUDIO DEI MECCANISMI DELL'ATTIVITA' VASODILATATRICE DELL'INSULINA MEDIANTE ESAME DELLA FLOWMOTION CUTANEA IN RISPOSTA ALLA IONTOFORESI DI INSULINA

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Recenti dati sperimentali e nell'uomo dimostrano che l'insulina possiede un'attività vasodilatatrice, che contribuisce, favorendo una maggiore disponibilità di glucosio nei confronti delle cellule, all'espletarsi dell'attività metabolica di tale ormone. Alcuni dati sperimentali suggeriscono che il meccanismo dell'attività vasodilatatrice dell'insulina, risieda nello stimolo alla sintesi e al rilascio endoteliale di ossido nitrico da parte di tale ormone. Tuttavia, l'osservazione di una conservata risposta vasodilatatrice dall'insulina in pazienti coronaropatici con disfunzione endoteliale, suggerisce anche altri meccanismi alla base della attività vasodilatatrice di tale ormone. Allo scopo di chiarire i meccanismi dell'attività vasodilatatrice dell'insulina abbiamo esaminato la flowmotion cutanea mediante l'analisi spettrale del segnale laser Doppler (LD) durante cessione iontoforetica cutanea di insulina (0.1 ml Humulin R 100 IU/ml diluita 1/10 in soluzione fisiologica) in 20 soggetti sani, con età di 28±9 anni, senza familiarità per diabete mellito. La stessa analisi è stata effettuata sul segnale LD cutaneo registrato durante cessione iontoforetica di sola soluzione fisiologica (SF). Sia la perfusione cutanea, sia l'intensità spettrale della flowmotion cutanea, sono aumentate in misura significativamente più elevata in risposta alla iontoforesi di insulina, che di sola SF (rispettivamente: $p<0.001$, ANOVA per misure ripetute; $p<0.05$). Considerando le varie componenti di flowmotion, la iontoforesi di insulina, ma non quella di SF, ha indotto un incremento significativo della intensità spettrale della componente riferibile all'attività miogena spontanea (da 0.21±0.15 P.U./Hz a 0.37±0.22 P.U./Hz, $p<0.0001$), mentre le componenti riferibili all'attività endoteliale e simpatica hanno mostrato un aumento significativo della loro intensità spettrale, sia in risposta alla insulina, che alla S.F. Questi dati indicano che l'insulina agisce direttamente sulla microcircolazione cutanea, aumentando la perfusione ematica e la flowmotion totale. L'aumento in risposta alla iontoforesi di insulina, e non di SF, della componente di flowmotion correlata con l'attività miogena, suggerisce una azione dell'insulina su tale meccanismo di regolazione locale del microcircolo, da riferire ad una azione diretta di tale ormone sulla muscolatura liscia vascolare.

c3.2

VIDEOCAPILLAROSCOPIA COMPUTERIZZATA. STUDIO MORFOLOGICO DELLA MICROCIRCOLAZIONE: ESPERIENZE DEL C.E.M.O.T. - BARI

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Introduzione: Molto importante risulta essere la valutazione oltre che della plasticità e della aggregabilità delle emazie e della situazione emoreologica dei leucociti, anche della situazione morfologica dei capillari nei quali il sangue fluisce, nelle varie patologie di interesse sia internistico che chirurgico. E' infatti a tutti noto il grande valore che ha la emoreologia nel diabetico, nell'iperteso, nel dislipidemico, nei soggetti con vasculopatie periferiche (POAD) e sistemiche (LES,SSP,ecc.) nonché nei trapiantati (fegato,ecc.).
Scopo: Scopo di questo lavoro è stato quello di valutare le alterazioni morfologiche presenti in diverse patologie correlandole agli indici emoreologici ottenuti usando il LORCA (Laser assisted Optical Rotational Red Cell Analyzer).
Materiale e Metodi: Abbiamo considerato diversi gruppi di pazienti:
- gruppo 1 Controlli pz 21 (12 M e 9 F età 35±4 anni)
- gruppo 2 Diabetici pz 28 (IDDM n° 15: 8 M e 7 F età 44±3 anni; NIDDM n° 13: 6 M e 7 F età 47±4 anni)
- gruppo 3 Ipertesi pz 49 (Fumatori n° 28: 12 M e 16 F età 40±4 anni; non fumatori n° 21: 12 M e 9 F età 38±3 anni)
- gruppo 4 Glaucoma pz 30 (16 M e 14 F età 42±5 anni)
- gruppo 5 Epatopatici pz 6 (3 M e 3 F età 44±5 anni).

A tutti è stata effettuata la valutazione emoreologica usando il LORCA e la morfologica usando il videocapillaroscopio computerizzato Videocap (Mitzubishi) con ingrandimento 200 x.
Risultati e Conclusione: Le anse capillari nei soggetti con microangiopatia diabetica (MAD) presentano nel 50% dei casi (11 casi su 22) una immagine a corna di cervo, una immagine a naso di elefante nel 72% dei casi (16 su 22) e a cavaturaccioli nel 48% dei casi (10 casi su 22). Nei soggetti vasculopatici si è notato una marcata rarefazione capillare nel 26% dei casi (12 casi su 43). Nei soggetti ipertesi non fumatori si è avuta la presenza di alterazioni morfologiche nel 25% dei casi (5 casi su 21) e nei fumatori l'incidenza è stata del 47% (13 casi su 28). Un miglioramento della perfusione delle anse, comunque non funzionanti, si è avuta nei soggetti epatopatici dopo 1 settimana del trapianto di fegato da cadavere nel 90% dei casi (5 casi su 6), nel glaucoma si ha nell'84% dei casi (25 su 30) vasi tortuosi e a pettine; la rarefazione capillare nei casi complicati è del 70% (18 casi su 30). La deformabilità eritrocitaria è stata valutata calcolando con il LORCA l'elongation Index (E.I.). la aggregabilità valutando con il LORCA il t½ in sec. del picco di aggregabilità eritrocitario. Gruppo controllo EI: 0,59±0,02 ; t½: 3±1 sec. Gruppo 2 IDDM E.I.: 0,55±0,01 t½: 2±0,5 sec. $p<0,05$ rispetto ai controlli ; NIDDM E.I.: 0,56±0,01 ; t½: 2±0,2 sec. $p<0,04$. Gruppo 3 ipertesi fum. E.I.: 0.56±0,01 ; t½: 2±0,6 sec. $p<0,04$; non fum.: E.I.: 0,57±0,02 ; t½: 2±0.7 sec. $p<0,05$ rispetto ai

controlli; Gruppo 4 glaucoma: E.I.: 0,56±0,01 ; t½ 2±0,4 sec. p<0,05; Gruppo 5 epatopatici: E.I.: 0,56±0,02 ;t½: 2±0,4 sec. Pertanto la videocapillaroscopia computerizzata insieme al LORCA sembrerebbe essere una metodica molto utile, affidabile e semplice per la valutazione emoreologica di soggetti con vari tipi di vasculopatie comunque indotte e con alterazioni della ossigenazione tissutale.

c3.3

LE MODIFICAZIONI DELLA UNITÀ MICROCIRCOLATORIA NELLA PATOGENESI DELL'ULCERA FLEBOSTATICA

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Tra le ulcere degli arti inferiori a genesi primitivamente vascolare , quelle venose o "flebostatiche" per usare una terminologia più aderente alla fisiopatologia, rappresentano la percentuale nettamente preponderante (70-80%). Queste lesioni correlano sempre con una significativa ipertensione ortostatica ed ortodinamica del sistema venoso distrettuale che produce severo danno sulla unità microcircolatoria fino al suo sfacelo. Tale unità costituita da differenti elementi anatomici quali l'arteriola, i capillari, la venula, il linfatico ed il connettivo perivascolare di sostegno, rappresenta un "unicum"dal punto di vista funzionale. Il letto capillare, per le peculiari funzioni di scambio metabolico chiamato a svolgere, ha una grande estensione ancor più significativa nei distretti cutanei e sottocutanei. In condizioni di ipertensione venosa distrettuale i capillari vanno incontro ad alterazioni strutturali quali allungamento, dilatazione e tortuosità delle anse ed a modificazioni della architettura macromolecolare della membrana basale, del collagene, delle glicoproteine e degli eparani . Tali alterazioni determinano un incremento della adesività piastrinica al sub-endotelio con formazione di trombosi capillare cui consegue un incremento della permeabilità con fuoriuscita di fibrinogeno che determina la formazione di manicotti di fibrina pericapillari Tale apposizione di fibrina è all'origine dei processi di indurimento e di depositi siderinici che va sotto il nome di dermatosclerosi: è proprio in queste aree cutanee distrofiche, sede di processi di dermo-ipodermite siderinica talora con zone di atrofia bianca che si manifesta l'ulcera flebostatica. Nel determinismo della lesione assume un ruolo preponderante proprio l'ostacolata diffusione dell'O2 a causa dei manicotti di fibrina che incapsulano le anse capillari: il laboratorio conferma questo dato poiché l'O2 presente nel sangue refluo di un arto sede di lesione ulcere flebostatiche è in concentrazione superiore a quello presente nel sangue venoso di un arto sano. A questo meccanismo principale si associano altre alterazioni che riducono la concentrazione di O2 a livello dei tessuti perilesionali quali l'edema, l'ipoperfusione secondaria alla riduzione del numero delle arteriole perfuse nell'area, all'intrappolamento ed al sequestro di aggregati leucocitari concorrendo in varia misura al determinismo dell'area ischemica causa di ulcerazione. Il sequestro leucocitario e la successiva attivazione cellulare è all'origine del rilascio di varie sostanze compresi enzimi litici istolesivi e derivati altamente reattivi dell'ossigeno quali il perossido di idrogeno ed i radicali liberi superossido (O2-) ed idrossile (°OH). In conclusione è possibile ritenere che le modificazioni della emodinamica venosa indotte dalla condizione stasi-ipertensione costituiscono il primum movens fisiopatologico a cui conseguono le alterazioni strutturali dell'unità microcircolatorio Queste sono basate principalmente sulla iperpermeabilità microdistrettuale, sulla conseguente formazione di manicotti di fibrina e sui processi di microtrombosi intracapillare con secondaria ipovascolarizzazione, alterazioni cellulari sia di ordine strutturale che metabolico con ulteriore peggioramento della diffusione dell'O2: il complesso di questi processi si estrinseca con una azione di sommazione che dà luogo alla lesione ulcerativa, fase evolutiva più avanzata della insufficienza venosa cronica ed espressione dello scompenso istangico secondario ad alterazioni di ordine emoreologico, microcircolatorio e metabolico.

c3.4

EMOREOLOGIA E FIBRILLAZIONE ATRIALE: UN MARKER NUOVO PER LE COMPLICANZE EMBOLICHE?

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La fibrillazione atriale (FA) è una tachiaritmia frequente, caratterizzata da un aumentato rischio di stroke. La terapia con anticoagulanti orali (TAO) riduce l'incidenza di stroke di circa due terzi. Alcuni studi recenti hanno dimostrato che l'iperviscosità è frequente nei pazienti affetti da FA. Scopo di questo studio è stato quello di valutare se alcune alterazioni emoreologiche possono avere un ruolo nell'insorgenza di eventi cerebrovascolari ischemici in pazienti affetti da FA. Abbiamo pertanto studiato 23 pazienti affetti da FA [13 M, 10 F; età mediana 75 (54-85) anni] con anamnesi positiva per almeno un evento embolico e 63 pazienti affetti da FA ed anamnesi negativa per eventi embolici [35 M, 28 F; età mediana 74 (59-84) anni]; i due gruppi erano paragonabili per età e sesso. Sono state analizzate le seguenti variabili emoreologiche: la viscosità del sangue totale (WBV) a 0,512 s^{-1} e 94,5 s^{-1}, la viscosità plasmatica a 94,5 s^{-1} ed il test di filtrazione eritrocitaria (EFT). La WBV e la PLV sono state analizzate con un viscosimetro rotazionale Contraves LS 30 e l'EFT è stata analizzata con un filtrometro di Myrenne MF4. I prelievi ematici sono stati effettuati almeno un mese dopo l'evento embolico e durante un periodo di anticoagulazione ottimale. Nessuna differenza significativa è stata trovata tra i due gruppi per quanto riguarda la WBV a 0,512

s^{-1}, la PLV, l'EFT ed i valori di ematocrito e fibrinogeno. Per quanto riguarda la WBV a 94,5 s^{-1}, i valori erano considerati normali o alterati secondo un nomogramma che prendeva in considerazione diversi range di valori di ematocrito. Un'alterazione della WBV a 94,5 s^{-1} è stata osservata più frequentemente in pazienti con pregresso evento embolico sia all'analisi univariata (OR: 4,60; CI 95: 1,41-15,06; p=0,012) che all'analisi multivariata (OR: 5,72; CI 95: 1,39-23,50; p=0,016) corretta per età, sesso, ipertensione arteriosa, diabete mellito, cardiopatia ischemica, disfunzione ventricolare sinistra, esposizione tabagica e dislipidemia. Questi risultati stimolano a studiare ulteriormente i meccanismi che potrebbero determinare uno stato di iperviscosità nei pazienti affetti da FA, in particolare per quanto riguarda un aumento dei valori di WBV 94,5 s^{-1}. Devono essere inoltre analizzati i possibili meccanismi attraverso cui l'iperviscosità possa determinare un aumento del rischio di complicanze emboliche in pazienti affetti da FA.

c3.5

LA VISCOSITA' EMATICA CAPILLARE NELLA MICROCIRCOLAZIONE

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A livello arteriolare si ha , come risaputo, la maggiore resistenza al flusso dovuta alla sezione e alla velocità con caduta media della pressione di 50 mmHg (da 85 a 35 mmHg). Tale resistenza è espressa dal rapporto W/2r (shear-rate per unità di lunghezza, espressa in sec^{-1}). Tale rapporto è molto alto, in media 332 sec^{-1} e la viscosità a queste shear-rates elevate è trascurabile. A livello capillare la caduta di pressione è di 15 mmHg (da 38 a 20 mmHg) ma la resistenza vascolare W/2r è molto minore, in media 33 sec^{-1}. Si può dire che se ad una resistenza di 333sec^{-1} corrisponde una caduta di pressione di 50 mmHg, ad una resistenza di 32 sec^{-1}dovrebbe corrispondere una caduta di pressione di 4,8 mmHg. La maggiore caduta di pressione è dovuta ad un'altra resistenza che si può definire "Viscosità Capillare" in quanto dovuta alle caratteristiche emoreologiche e strutturali del sangue come tessuto. Il nostro apparecchio, che riproduce in un modello sperimentale la struttura del letto capillare, è in grado di misurare la Viscosità Ematica generale e la Viscosità ematica Capillare alle stesse shear-rates ed in particolare alle basse shear-rates quando nei liquidi non-newtoniani si manifesta il maggior incremento di viscosità. Risulta che, a livello capillare, la viscosità diventa dominante rispetto alle altre resistenze geometriche e fisiche.Il rapporto tra Viscosità generale e viscosità capillare espresso in percentuale dà poi una misura fisica della deformabilità. La conoscenza della viscosità alle shear-rates del sistema circolatorio, ci permette di ricavare la grandezza degli aggregati nei vari distretti e le loro caratteristiche espressa come "forza di aggregazione". Nel campo clinico le modificazioni della viscosità capillare rappresentano una reale possibilità di migliorare il flusso circolatorio del distretto capillare perchè non è accertato che modulazioni del calibro arteriolare possano migliorare il flusso nei capillari o piuttosto favorire le anastomosi artero-venose. Ancora in campo clinico, a livello del circolo generale, la misurazione degli aggregati permette di evidenziare quel fenomeno di adesione cellulare che, per la presenza di numerosi recettori glicoproteici interessa anche le altre cellule ematiche. Infine è la stessa grandezza e tenacia degli aggregati che può modificare la tromboresistenza endoteliale.

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