O2: Clinical Hemorheology

O2-1 Pilot clinical study of quantitative ultrasound spectroscopy measurements of erythrocyte aggregation within superficial veins of 50 volunteers

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An enhanced inflammatory response is a trigger to the production of blood macromolecules involved in abnormally high levels of red blood cell (RBC) aggregation. This study aimed to demonstrate the clinical feasibility of a non-invasive ultrasound-based erythrocyte aggregation measurement method for potential application in critical care medicine.
RBC aggregation was evaluated using modeling of the ultrasound backscatter coefficient with the structure factor size and attenuation estimator (SFSAE). SFSAE spectral parameters W (packing factor describing spatial organization of RBCs) and D (fractal dimension of RBC aggregates) were measured within the antebrachial vein of the forearm and tibial vein of the leg in 50 healthy participants at two flow shear rates. Recordings were performed under natural flow or reduced flow controlled by a pressurized bracelet applied on the skin. Blood samples were also collected to measure RBC aggregation ex-vivo with a laser erythroaggregometer (parameter S_{10}).
W and D measured in-vivo were positively correlated with ex-vivo S_{10} index for both measurement sites and shear rates (correlations between 0.35 - 0.81, $p < 0.05$). SFSAE W and D measurements on the forearm were correlated with values over the leg for similar shear rates ($p < 0.05$). For both venous sites and shear rates (natural flow at 37 s-1 on average, and reduced flow at 0.8 s-1 on average), intra-observer variability for 5 repeated measures of D varied between 26.3 - 28.2%, whereas it was higher (34.1 – 48.9%) for W. Repeatability might be improved by readjusting the bracelet applied pressure at each measure.
In conclusion, designed bracelet, ultrasound system, and SFSAE software may found application for continuous patient monitoring in critical care unit to predict sepsis events.

O2-2 Rapid clinical assessment of the sublingual microcirculation - visual scoring using microVAS in comparison to standard semi-automated analysis

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Rationale: Alterations in human microcirculation occur in many disease states leading to morbidity and mortality, however assessing the microcirculation is not standard clinical practice. Standard microcirculation analysis using semi-automated analysis is expensive, time consuming, and expertise dependent making it unfeasible. We proposed a novel visual scoring system (microVAS) for the analysis of microcirculation videos that can be performed at the patient bedside in real time.
Objective: Validate our microVAS score by training health professionals unfamiliar with the microcirculation field to use our microVAS score and compare their scores to the standard method of semi-automated analysis using AVA3 software.
Methods: Using a prospective double-blind study design, we recruited and trained 20 participants to use our microVAS score. Participants scored 40 videos (from 22 healthy and

18 septic patients) for MFI and PPV. The same 40 videos were analyzed by an expert using the gold standard semi-automated method of analysis.
Results: Overall correlation of MFI was r = 0.3283 (95% CI 0.27 – 0.39), $p < 0.05$; overall correlation of PPV was r = -0.1123 (95% CI -0.18 to -0.04), $p < 0.05$. The Krippendorff's alpha for MFI was 0.56 (healthy videos: $\alpha = 0.34$, sepsis videos: $\alpha = 0.31$). For PPV Krippendorff's alpha was 0.43 (healthy videos: $\alpha = 0.56$, sepsis videos: $\alpha = 0.17$).
Conclusions: Overall for both MFI and PPV, there was a small correlation between our microVAS score and AVA 3 scores. Regarding inter-rater reliability both MFI and PPV showed fair agreement between raters. Going forward multiple improvements to the microVAS scoring system as well as the training program are suggested to improve reliability and consistency.

O2-3 L-cysteine improves blood fluidity that has been impaired by acetaldehyde

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Introduction: Regular heavy consumption of alcohol increases the risk of stroke and ischemic cerebrovascular diseases. Acute heavy alcohol consumption leads to increased whole-blood viscosity, decreased erythrocyte deformability, and impaired fibrinolytic potential. Acetaldehyde (ACD) causes abnormal erythrocyte morphology due to cross-linking of erythrocyte ghost proteins, and decreased ability of erythrocyte deformability. It was reported that blood ACD levels are reduced in mice pretreated with L-cysteine (L-cys). However, there is no study on the effect of ACD and/or L-cys on blood fluidity. In this study, we evaluated whether ACD impaired whole-blood fluidity. In addition, the effect of L-cys on blood fluidity that had been impaired by ACD was examined.
Methods: Blood samples were obtained from 10 healthy, non-smoking, male volunteers (age: 23.3±1.3 years, body mass index: 21.6±2.6 kg/m^2). ACD or ACD and L-cys were added the blood samples before each experiment. We measured blood passage time (100 μL and consecutive 20-μL volumes) using Kikuchi's microchannel method (MC-FAN: Hitachi Haramachi Electronics Co., Ltd., Japan).
Results: The blood passage time increased after adding ACD in a dose-dependent manner. The blood passage time that increased after adding ACD decreased after adding L-cys in a concentration-dependent manner. The sequential blood passage time at 20-μL intervals after adding ACD gradually increased, but this was not observed after adding ACD and L-cys.
Conclusion: Blood fluidity is impaired by adding ACD in a dose-dependent manner. Adding L-cys improves blood fluidity that has been impaired by adding ACD.

O2-4 Hemorheological studies in a group of patients with Waldenström's macroglobulinemia

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Waldenström macroglobulinemia (WM) is defined by the World Health Organization as lymphoplasmacytic lymphoma (LPL). Increased concentration of IgM is one of the factors that lead to increase of blood viscosity. Blood hyperviscosity in patients with Waldenström's macroglobulinemia is serious clinical problem. The aim of this work was to observe the rheological parameters in a group of Waldenström's macroglobulinemia patients in a two year period. During this time the blood samples from each patient were collected five times. The evaluation included such factors as whole blood viscosity, plasma viscosity, hematocrit value and the tendency to aggregation and deformation of erythrocytes. The latter features were quantified using the mathematical rheological model of Quemada. Compared to the hemorheological parameters obtained for healthy objects, elevated value of plasma viscosity and an increased tendency to aggregation were observed in the studied group. Other rheological parameters values did not differ significantly form the values in healthy objects. All patients were under constant medical control.

O2-5 Adora2b receptor activation mediates flap protection from ischemia/reperfusion injury

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Summary Background Data: Ischemic preconditioning (IPC) is defined as increasing tolerance to subsequent ischemic stress by exposing tissues to sub-lethal ischemia. Although many candidates have been suggested, recent studies have clearly demonstrated that adenosine-mediated ADORA2B receptor (ADORA2BR) activation is the main mechanism involved in IPC. While the tissue-protective role of this mechanism has been demonstrated in different ischemia/reperfusion (I/R) models, its role in flap surgery-derived I/R damage has not to date been investigated. The aim of this study is to investigate the role of adenosine and ADORA2BR activation in IPC-mediated tissue protection in an epigastric flap model.

Methods: 80 female Wistar rats were divided into five groups, which were all exposed to epigastric flap surgery comprising 6 hours of ischemia and 6 days of reperfusion in the presence or absence of IPC. No drugs were administered to Group 1. In Group2, animals were pretreated with specific CD73-inhibitor in order to inhibit adenosine generation. InGroup3,animals were pretreated with adenosine. In Group4,animals were pretreated with a specific ADORA2BR antagonist, and in Group5,animals were pretreated with ADORA2BR agonist before ischemia induction. After 6 days of reperfusion, tissue survival was evaluated via histological and macroscopic analysis.

Results: IPC application significantly enhanced tissue CD73 expressions and adenosine

concentrations ($p<0.01$). Flap survivals were increased by IPC application in Group1 ($p<0.05$).However, CD73 inhibition blocked this increase (Group2). In Group3, adenosine therapy improved flap survival even in the absence of IPC ($p<0.01$). Similarly, while an ADORA2BR antagonist attenuated the tissue-protective effect of IPC ($p<0.01$), an ADORA2BR agonist improved flap survival by mimicking IPC in groups 4 and 5.

Conclusion: These results provide pharmacological evidence for a contribution of CD73 enzyme-dependent adenosine generation and its signaling through ADORA2BR to IPC-mediated tissue protection. They also suggest for the first time ADORA2BR agonists may be used as a potential preventive therapy against I/R injury in flap surgeries.

O2-6 Purinergic regulation of erythrocyte enzyme activity

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BACKGROUND: eNOS activity in several cell types including endothelial cells, has been shown to be induced by purinergic receptors. Although erythrocytes have an active eNOS enzyme, its regulation with purinergic reseptors remains unknown. The aim of the present study is to evaluate purinergic receptor P2X mediated eNOS activation and NO production in erythrocytes.

METHODS: Erythrocytes were isolated from healthy volunteers and re-suspended in Hepes solution at a hematocrit of 0.01 l/l. Intracellular NO and Ca^{+2} levels and eNOS activation measured by flow cytometry in response to P2X receptor agonist, in the absence and presence of eNOS, P2X receptors and PI3K inhibitors.

RESULTS: Activation of purinergic P2X receptors found to induce intracellular NO generation, Ca^{+2} influx and phosphorylation of eNOS enzyme in erythrocytes. These responses were blunted in response to incubation of erythrocytes with P2X receptor agonist in the presense of NOS enzyme, P2 receptors and PI3K inhibitors.

CONCLUSIONS: The results of the study clearly demonstrated purinergic activation of eNOS enzyme in erythrocytes through Ca^{+2} dependent and independent mechanisms. Considering erythrocytes are continuously exposed to purinergic receptor ligands, such as ATP, in the plasma, our results may help to understand basal in-vivo activation mechanisms of erythrocyte eNOS enzyme activity.