Session 9: Clinical biochemistry

Lectures

L.09.1

Multimodal Imaging Agent for theranostic approach into endothelial pathologies

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The development of personalized medicine has led to the increasing demand for molecular probes that allow imaging of the biochemical phenomena within specific tissues and organs in real time at the molecular and cellular level.
One of the receptors that requires further study - an image-based strategy to evaluate the expression in biological systems - is Receptor for Advanced Glycation End-products (RAGE) localized on the surface of many cells, including endothelium.
We developed nanoparticle construct based on PAMAM dendrimer, functionalized with the specific RAGE ligand Nε-(carboxymethyl)lysine-modified human serum albumin, labeled with both radionuclide and fluorophore for dual-modality PET-optical imaging. The 'bench to bedside' approach (synthesis, chemical and biological characterization and *in vivo* imaging) was possible due to international collaboration.
Using numerous techniques, we determined the cellular binding characteristics and clinical usefulness of our targeted probe in murine model of hindlimb ischemia and prostate cancer.
Despite limitations, new probe allows for easy modifications with functional groups including targeting ligands and drugs, making this probe easily modifiable into a theranostic agent. Combining radionuclides and fluorophores in one structure allows for multimodal – optical and nuclear imaging, for *in vitro* single cell or microscopic evaluation, *in vivo* quantitative nuclear imaging, histological *ex vivo* analysis, and targeted drug delivery.

Oral presentations

O.09.1

Hematological and coordination skills evaluations in response to a High-Intensity Interval Training in MS patients

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Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS) which the etiology is currently not completely understood. Is already known that physical activity plays an essential role to improve quality of life, especially in neuropathological conditions. The aim of the study was to investigate the possible benefits of high-intensity interval training (HIIT) on haematological parameters and neuromotor abilities in MS patients.
We enrolled in our study, from a cohort of 130 subjects diagnosed of MS to the neurology unit of the 'Paolo Giaccone' University Hospital, 16 patients fulfilled the inclusion criteria. The patients were randomly assigned in two groups: a control group that didn't perform any physical activity and the other one performed HIIT protocol. The study was conducted online by a clinical kinesiology from the sport research unit to the University of Palermo. The training program was administered biweekly for 12 weeks at the beginning of the study (T0) and at the end of the study (T1).
The results showed a change in reduction of Total and LDL-cholesterol levels ($p=0.02$) and in increase of osteocalcin and vitamin D levels ($p<0.05$); as regarding coordination tests, we highlighted a improve in wall squat test ($p<0.002$).
Considering the relevance of this preliminary results, further investigations will be necessary by increasing the sample size and extending the intervention period.

O.09.2

Lipoxygenase-derived hydroxyeicosatetraenoic acids – novel perioperative markers of early post-transplant allograft function?

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Background. Active metabolites of arachidonic acid (AA), eicosanoids, strongly influence renal homeostasis. The aims of this study were to measure perioperative variations in lipoxygenase (LOX)-derived 5-, 12- and 15- hydroxyeicosatetraenoic (HETE) acids levels.
Methods. Sixty-nine kidney recipients were divided into early, slow and delayed graft function (EGF, SGF and DGF, respectively) groups. Blood was taken directly before, and in the consecutive minutes of graft reperfusion. HETE concentrations were measured using liquid chromatography.
Results. Our results demonstrated significant differences in the concentrations and dynamics of HETE changes between the examined groups. Moreover, observed changes in HETE concentrations were strongly associated with post-transplant graft function and perioperative 20- HETE synthesis. Application of cut-off limits for newly introduced markers, that is 71.72 ng/mL for 5-HETE(5), 12.3 ng/mL for 12-HETEΔ(5-0) and −6.1 ng/mL for 15-HETEΔ(5-0), resulted in 72.5–81.5% sensitivity and 50–54% specificity for SGF/DGF prediction.
Conclusion. We hereby report that human kidney transplantations are accompanied by significant changes in LOX AA metabolism, which strongly influences and predicts early (1 year) post-transplant graft function.

Posters

P.09.1

Renalase, dopamine and norepinephrine – potential markers of the development of hypertension in patients with chronic kidney disease

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Introduction: Chronic kidney disease (CKD) irreversibly changes the function and structure of the kidneys. Arterial hypertension (HT) develops in > 90% of patients with CKD. It is suggested that renalase dopamine and norepinephrine are involved in the pathogenesis of HT and are factors in the progression of kidney disease.
Aim of the study: Determine of renalase, dopamine, and norepinephrine levels in CKD patients as potential markers of HT development.
Materials and methods: The study group consisted of 117 patients with CKD, divided into 4 groups: hemodialysis patients (before and after the end of hemodialysis) – 32, peritoneal dialysis – 31, patients with kidney transplantation – 24 (before transplantation and 2-5 days after kidney transplantation) and conservatively treated – 30 patients. The control group consisted of 31 healthy volunteers.
Results: The influence of the cause of CKD on renalase, dopamine, and norepinephrine levels were demonstrated ($p=0.046$; $p=0.035$; $p=0.023$). The lowest renalase concentration was found in patients with ADPKD and HT as the cause of CKD. The highest concentration of dopamine was found in patients with CKD due to glomerulonephritis, and high values were also obtained in patients with HT. The lowest concentration of norepinephrine was obtained in patients with HT and DM.
Conclusions: The concentration of renalase, dopamine, and norepinephrine may inform about the progression of CKD, the probability of cardiovascular events, the patient's prognosis.

P.09.2

The importance of selected arachidonic acid derivatives as potential biomarkers of survival and course in COVID-19 patients

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Introduction: COVID-19, caused by the SARS-CoV-2 virus, induced a global pandemic. Unsaturated fatty acids, such as arachidonic acid (AA), can inhibit the reproduction of enveloped viruses and attenuate the body's inflammatory response during COVID-19 infection.
Aim of the work: Determination of the concentration of arachidonic acid derivatives in COVID-19 patients.
Materials and method: The study group consisted of 53 patients (28 women and 25 men) with a mean age of 61±13 years. Material from patients was collected from peripheral blood for K2EDTA at the time of disease detection and 7, 14, and 28 days consecutively after disease detection. The determination was performed by ELISA.
Results: A significant relationship was found between the concentration of the tested arachidonic acid derivatives (5-HETE, 15-HETE, TXB2) in the test and control groups ($p<0.001$; $p=0.003$; $p<0.001$). The relationship between the concentration of the tested arachidonic acid derivatives and survival (5-HETE $p<0.001$, thromboxane $p=0.005$) and the course of the disease (15-HETE $p=0.001$ in SARS-CoV-2 (COVID-19) virus infection was demonstrated.
Conclusions: 5-HETE, 15-HETE, and TXB2 concentrations can be used as biomarkers in monitoring the course of the disease and prognosis of patients. The inhibitors for synthesizing the tested arachidonic acid derivatives may be used as a therapy that increases the survival of patients with COVID-19.

P.09.3

N-glycosylation of CD4+ T cells correlates with TNFα and IL-4 levels in Hashimoto's thyroiditis

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Hashimoto's thyroiditis (HT) is an autoimmune disease accompanied by T cell-dependent destruction of thyrocytes, which results from a loss of immune tolerance to thyroid antigens. CD4+ T cells produce pro-inflammatory cytokines. Most of T cell receptors is highly *N*-glycosylated. The structure and content of *N*-glycans, and cytokine secretion have been found to be altered in inflammatory and autoimmune conditions. The aim of our study was to analyze a correlation between cytokine serum levels and *N*-glycosylation of CD4+ T cells in HT and healthy donors.
The study was performed on blood and serum samples from donors with elevated levels of autoantibodies and no symptoms of hypothyroidism (HT1), HT patients treated with L-thyroxine (HT2), and healthy subjects (CTR). CD4+ T cells were purified from PBMCs by magnetic sorting. *N*-glycans, released from CD4+ proteins by *N*-glycosidase F, were analyzed by MALDI-TOF MS. Serum levels of cytokine were analyzed by flow cytometry.
N-glycosylation of CD4+ T cells was significantly altered in both HT groups compared to CTR. Results showed that TNFα may affect the content of oligomannose structures and fucosylated *N*-glycans. The serum level of IL-4 correlates with the content of fucosylated *N*-glycans in HT2.
The determined correlations between T cell *N*-glycosylation and the cytokines may be important to understand the regulation of this modification in autoimmunity.

Acknowledgements

The study was supported by the NCN (grant no. UMO-2015/18/E/NZ6/00602).