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PHYSIOLOGY, FROM CELL TO PATIENT ... AND BACK TO THE CELL

ABSTRACTS

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DEVELOPMENT AND MORPHO-FUNCTIONAL CHARACTERIZATION OF AN IN VITRO BRONCHIAL EPITHELIAL CELL MODE

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Background: In vitro bronchial epithelial cell models can be effectively used for the assessment of the effects of allergens that lead to allergic reactions. However, obtaining a pseudo-differentiated layer of normal human bronchial epithelial (NHBE) cells can be a real challenge. A thorough characterization of such a model using a morpho-functional analysis is necessary prior to testing experimental conditions.

Purpose: In this study, we have developed and performed a morpho-functional characterization of an in vitro bronchial epithelial cell model, with the aim of using it in further studies for testing the effects of the ragweed pollen allergens upon transepithelial barrier permeability.

Methods: NHBE cells were grown in 12-well and 24-well Transwell inserts at an air-liquid interface (ALI) using bronchial epithelial cell growth medium and a specific ALI medium up to ALI day 21, when transepithelial electrical resistance (TEER) was measured. The epithelial cell layer was analyzed using a histology protocol that allowed staining with hematoxylin and eosin (H&E) and performing microscopical observations.

Results: A functional characterization and a comparison between NHBE cell cultures grown in 12-well and 24-well transwell inserts was performed, as well as a histology protocol adapted for processing the cell cultures in order to carry out H&E staining. We have also analyzed the potential causes for the relatively low TEER values of some inserts.

Conclusions: The in vitro-bronchial epithelial model presented in this study was morphologically and functionally characterized. This was achieved by building upon existing histology protocols and adapting them to our NHBE cell cultures. However, additional studies are required in order to optimize our current model.

PREDICTION OF THE DEGREE OF PULMONARY LEUKOCYTE INFILTRATION AT 24 HOURS AFTER THE TRAUMA

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Background: Immune cells can manifest their destructive effects on their own body tissues. For example, the "remote" lesions in polytraumas can occur due to intact tissues damage mainly by neutrophils, which, activated, infiltrate unaffected by the traumatic agent organs and release proteases which could have destructive effects decreasing the physiological reserves or causing the subsequent failure of the respective organs.

Purpose: This paper is an attempt to identify some biomarkers for "remote" lesions and to develop a predictive model quantifying their degree.

Methods: The proposed goal was achieved by using the appropriate statistical methods, namely the correlational analysis of the components of the protease/antiprotease system with degree of pulmonary leukocyte infiltration (DPLI), the effects of which on the physiology of the patient's respiratory system are obvious, 24 hours after the traumatic impact. By the Backward method, all potential variables are included in the model, after which, all insignificant parameters are excluded until only the significant variables remain to predict the studied result. In predicting DPLI at 24 hours after trauma, the enzymatic activities of α1-antitrypsin, elastase, and cathepsin D and G were initially included.

Results: Considering observed associations, a predictive model capable of estimating the DPLI value at 24 hours after trauma was quantified.

Conclusions: The destructive effects of proteases are those that determine the positive associations between DPLI and the concentrations of these substances at different time intervals after trauma. By analogy, negative correlations with antiproteases are attributed to their protective effects. The obtained results, require further research on large groups of patients.

MITOCHONDRIAL EFFECTS OF ATORVASTATIN IN HUMAN PLATELETS IN HEALTH AND DISEASE

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Statins are the first-line therapy for the treatment and prevention of cardiometabolic diseases. Adherence is hindered by a couple of side effects among which, statin-induced muscle symptoms are most currently reported. Mitochondrial dysfunction plays a key role in the pathophysiology of statin-induced side effects. Platelets have recently emerged as a convenient tool to assess mitochondrial respiration as a mirror of organ-specific mitochondrial dys/function in various pathologies, including diabetes mellitus.

The aim of the current study performed in isolated human platelets was to assess whether atorvastatin induces dose-dependent mitochondrial toxicity and if these changes occur in diabetic patients chronically treated with atorvastatin. Peripheral blood platelets were isolated (on K2-EDTA) from healthy volunteers for the mitochondrial toxicity study and from diabetic patients chronically treated or not with atorvastatin for the study on chronic mitochondrial effects, respectively. Mitochondrial respiration of intact platelets (200 mil. cells/mL) was assessed by high-resolution respirometry using the Oxygraph-2k (Oroboros Ltd.) Mitochondrial respiration of intact platelets is acutely inhibited by atorvastatin applied in increasing concentrations (20-320 µM) in a dose-dependent manner with an inhibition of 46.8% ±8 of control using atorvastatin at 320 µM ($p<0.01$). In patients chronically treated with atorvastatin, we evaluated the endogenous respiration, the electron transport (ET) capacity and free-ET capacity. No significant difference was found for these respiratory parameters between the treated vs non-treated diabetic patients. In human platelets, atorvastatin presents dose-dependent acute toxicity, yet mitochondrial safety under therapeutic conditions.

THE IMPACT OF CHRONIC IVABRADINE ADMINISTRATION ON ATRIAL ARRHYTHMOGENESIS AND AUTONOMIC NERVOUS SYSTEM IN RATS

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Background: Previous studies have reported contradictory results concerning the pro-or anti-arrhythmic effect of chronic ivabradine administration.

Purpose: We aimed to evaluate the effect of chronic ivabradine administration on the occurrence of atrial arrhythmias and to identify the autonomic changes related to a possible pro- or anti-arrhythmic effect of ivabradine.

Method: Male Wistar rats were randomized into two groups (n=16 each): atrial fibrillation (AF), and treated with ivabradine (IVA). An atrial pacing protocol was applied to both groups. IVA rats were treated with ivabradine (10 mg/kg/day), starting 3 weeks prior to stimulation. All rats were implanted with ECG radiotelemetry devices and the ECG was recorded for 72 hours continuously before, during, and for three consecutive weeks after the atrial pacing protocol. The spontaneous atrial arrhythmogenicity and heart rate variability (HRV) were analyzed and compared between the two groups.

Results: Compared to the AF rats, IVA rats had a significantly higher number of AF episodes before, during, and one week after the pacing protocol (all $p<0.05$). Prior to atrial pacing, the normalized low-frequency components of HRV tended to be higher ($p=0.05$) in the IVA compared to the FA group. Higher normalized high-frequency components were also recorded in the IVA compared to the AF group before the pacing protocol ($p<0.001$) and in the last two weeks of monitoring (both $p=0.05$).

Conclusions: Chronic ivabradine administration induced a significant increase in spontaneous AF occurrence, coupled with an increase in sympathetic and parasympathetic activity. As sympatho-vagal coactivation is known to be a major trigger of AF, this change could explain, at least in part, the increased risk of ivabradine-induced AF.

PHARMACOLOGICAL INHIBITION OF THE RENINA - ANGIOTENSIN ALDOSTERONE SYSTEM

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The renin-angiotensin aldosterone system (RAAS) is a hormonal cascade that functions in the homeostatic control of blood pressure, tissue perfusion and extracellular volume. Disorder of this system is considered a major factor in the development of cardiovascular and renal pathologies. As data on the efficacy of angiotensin converting enzyme inhibitors and angiotensin II receptor blockers accumulate as drugs of fundamental importance in the treatment of cardiovascular and renal disorders, it becomes clear that the clinical benefits are suboptimal and not surprisingly different. can perform with other therapeutic interventions.

Purpose: To review the role of the renin-angiotensin aldosterone system in the development of hypertensive cardiovascular disease and related conditions and to identify an overview of the classes of pharmacological agents that inhibit this system.

Discussion: The development of agents that block the RAAS, such as beta-blockers, angiotensin converting enzyme inhibitors and angiotensin receptor blockers, began as a therapeutic strategy for the treatment of hypertension. Preclinical and clinical studies have shown important additional cardiovascular and renal therapeutic benefits of conversion enzyme inhibitors and angiotensin receptor blockers. However, blocking this system with these agents is incomplete.

Conclusion: Visiting therapeutic approaches that completely inhibit the RAAS may provide additional clinical benefits for patients with cardiovascular and renal disorders. These approaches may include double blocking, using conversion enzyme inhibitors and angiotensin receptor blockers in combination with new therapeutic modalities, such as the direct inhibition of renin with aliskiren, recently approved for the treatment of hypertension.

THE INFLUENCE OF ANGIOTENSIN ON SMOOTH MUSCLE FUNCTION

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Background: The renin-angiotensin-aldosterone system is involved in numerous physiological functions that regulate vasoconstriction, fluid volume regulation, cardiac output, cell growth and vascular wall integrity. The smooth muscle cells of the blood vessels show considerable plasticity in their phenotype. In young and healthy blood vessels, the phenotype is largely contractile and blood pressure is well self-regulated. However, during an individual's lifetime, vascular cells may switch to a synthetic phenotype, largely uncontractable, in response to proinflammatory stimuli, diet, or other factors that lead to the development of atherosclerosis or vessel remodeling. The accumulated evidence reveals the complexity of angiotensin II signal transduction in the pathophysiology of vasculature, heart, kidney, and brain, as well as several pathophysiological features, including inflammation, metabolic dysfunction and aging.

Purpose: We discuss the mechanisms that regulate vascular reactivity and contraction in physiological and pathophysiological conditions and highlight some new advances in the field, focusing especially on hypertension. Thus, hypertension is a major risk factor for many chronic diseases, such as heart failure, myocardial infarction, stroke, vascular dementia and chronic kidney disease. Pathophysiological mechanisms that contribute to the development of hypertension include increased vascular resistance, largely due to reduced vascular diameter due to increased vascular contraction and arterial remodeling.

Conclusion: Vascular smooth muscle cells are very plastic and under pathological conditions undergo phenotypic changes from a contractile state to a proliferative one.
Keywords: smooth muscles, angiotensin, implications.

ASSESSMENT OF MITOCHONDRIAL RESPIRATORY DYSFUNCTION IN PREECLAMPTIC PLACENTAS: A PILOT STUDY

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Preeclampsia (PE) is a systemic disorder with abnormal placentation with mitochondrial dysfunction being recently reported as a central pathomechanism. PE has two different subtypes: early onset, occurring < 34 weeks of pregnancy that is often associated with intrauterine growth restriction (IUGR), and late onset >34 weeks of pregnancy, without IUGR.

The aim of the present study was to characterize the mitochondrial respiration dysfunction in preeclamptic placentas. The participants (n=22) in this study were included in 3 groups: healthy (n=12) and preeclamptic pregnancies (n=10), with (n=4) or without (n=6) IUGR. Placentas samples were collected and processed immediately after delivery following caesarean section. Respiration of isolated placental mitochondria was assessed at 37°C, by means of high-resolution respirometry using the Oxygraph-2k (Oroboros Ltd.), according to the Substrate-Uncoupler-Inhibitor-Titration protocol adapted to measure complex I (glutamate + malate) and complex II-dependent (succinate) respiration.The main respiratory parameters were as follows: basal respiration, active respiration (after ADP addition) and the maximal respiration (in the presence of an uncoupler). Active and maximal uncoupled respiration for both complex I and complex II-supported respiration were decreased in pre-term preeclamptic placentas associated with IUGR vs. healthy
controls. At variance, in preeclamptic placentas without IUGR, an increase in respiratory parameters was found. PE associated with mitochondrial respiratory dysfunction that differs according to the presence vs absence of IUGR. Whether the increase in respiration found in placentas harvested from pregnancies with normal fetuses is an adaptive mechanism that might prevent IUGR requires further investigation.

BIOPRINTING OF 3D TUMOR TISSUE MODELS

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Background: 3D bioprinting, a numerically controlled, layer-by-layer deposition of cells and biocompatible hydrogels was employed to replicate the essential features of tumor microenvironment, demonstrating that 3D tumor millieu affects the efficacy of anticancer drugs.

Purpose: Evaluation of microenvironment's role in tumor development, using 3D bioprinted tumor models.

Materials and Methods: Tumor models were generated by bioprinting hydrogel droplets, with cancer cells, TAF and PBMCs, in different combinations of 2 or 3 cellular types. Employing a dedicated 3D printing software, these tumor models were embedded in hydrogel and exposed to 365 nm ultraviolet light for 2 minutes. These tissue models were cultured in vitro for 2 days, then implanted subcutaneously in the CD1 Nu/Nu immunosuppressed mice, 2 constructs per animal. The constructs were evaluated histologically ex vivo after 8 weeks of development, and compared to the in vitro-developed tissue models.

Results: In vitro studies of 3D printed tumor models showed that development is slow, the constructs reaching a soft capsular structure in about 2 weeks of culture. When implanted in vivo, the tumor models had a growing rate of about 1 mm per week. At 8 weeks, the constructs were assessed for Ki67 and Her2 markers and showed increased expression of both proteins, mainly in tumors containing all 3 cellular types, thus providing evidence for microenvironment support in tumor development. The in vivo tumor models developed intense vascularisation, adipose and conjunctive tissue, and were adherent to the mouse subcutaneous tissues.

Conclusions: Bioprinted tissue models revealed that tumor and peritumoral cells should be used together to better mimic the tumor architecture and development of solid tumors.

APPLICATIONS OF IN VITRO CELLULAR ASSAYS IN ALLERGY DIAGNOSIS AND RESEARCH

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Background: Currently, allergy diagnosis is based on skin prick tests, but these tests are not recommended to patients that had a previous severe allergic reaction to an allergen source. In vitro cellular assays such as the basophil activation test (BAT) and rat basophil leukemia (RBL) cells test, are a good diagnosis alternative. They measure the IgE-mediated hypersensitivity to allergens without exposing the patients to a risk of anaphylaxis. In research, this type of tests can be used in allergy mechanisms exploration, allergen immunotherapy development and clinical relevance evaluation of individual allergens.

Purpose: This study aims to compare two different in vitro cellular tests commonly used in allergy diagnosis and research.

Methods: Ragweed allergic patients were tested to different concentrations of ragweed pollen extract and Amb a 1, a major ragweed allergen, using BAT and RBL assays. For BAT, whole blood was harvested on heparin and incubated with the allergens. The basophil degranulation was determined by flow cytometry after anti-human CD63 monoclonal antibody staining. In RBL case, rat basophil leukemia cells transfected with human FcεRI were loaded with patients' sera. The release of the β-hexosaminidase mediator was measured after allergen incubation.

Results: The basophil activation was expressed as the percentage of CD63+ basophils at a given concentration of allergen while for the RBL assay results were shown as the percentage of total β-hexosaminidase release. The highest basophil degranulation was not induced by the same allergen concentration in all tested patients.

Conclusions: Cellular assays reproduce in vitro the IgE-mediated allergic reactions and are a useful tool for both clinical and research applications.

CLINICAL AND FUNCTIONAL EVALUATION OF BLADDER LITHIASIS

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Background: Bladder lithiasis represents 5% of the specific pathology of the reno-urinary tract. It usually occurs due to obstruction of the bladder, urinary tract infections or neurogenic bladder.

Purpose: The purpose of this study is to evaluate the prevalence of risk factors, causes and symptoms in bladder lithiasis.

Methods: The study included a group of 47 patients with bladder lithiasis, investigated between January 2017 - December 2018. These patients underwent mechanical Punch cystolithotripsy and the investigations used to diagnose the patients consisted of: blood laboratory tests, urine examination and functional examinations (renal and prostate ultrasound).

Results: The age average of the patients was 65 years, and 94% of the patients were male. The most common cause of bladder lithiasis is prostate adenoma with a predominance of 45%, urinary tract infections in 19% of cases and urethral stricture in 15% of cases. The most common clinical manifestations at patients with bladder lithiasis are: pollakiuria (23%), macroscopic hematuria (20%), dysuria (19%), hypogastric pain (12%), "two-stage urination" (9%), nocturia (7%), alguria (6%) and acute urinary retention (4%).

Conclusions: Urinary tract infection has an important role in the formation of bladder stones, and singular stones are much more common at patients in the study group. In a significant percentage (95%) pacients with endoscopically treated bladder lithiasis, the postoperative evolution is favorable and at the rest of the patients a prolonged febrile syndrome or acute urinary retention appeared. Half of these patients were discharged urologically cured, which indicates that the prognosis and course of the disease depends on: risk factors, the cause of lithiasis, symptoms and funtional status.

THE ROLE OF AMMONIA IN PREDICTING THE OUTCOME OF PATIENTS WITH ACUTE-ON-CHRONIC LIVER FAILURE

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Background: High venous ammonia values have been proven to be a part of the mechanism of hepatic encephalopathy in patient with liver cirrhosis as well as acute hepatitis. However, the role of ammonia in acute-on-chronic liver failure (ACLF) has not yet been clearly established.

Purpose: We aimed to assess the role of venous ammonia (VA) in predicting the outcome of cirrhotic patients with ACLF in a tertiary care center.

Methods: We performed a retrospective study including patients with liver cirrhosis hospitalized for acute decompensation and fulfilling the APASL criteria for ACLF. The AARC score was calculated and ACLF grade was established accordingly.

Results: 446 patients were included, aged 59(50-65) years, 57.4% men. 66.4% had ACLF grade I, 31.2% ACLF grade II, and 2.5% ACLF grade III. Overall mortality was 7.8%. Venous ammonia was 103(78-148) µmol/L. ROC analysis showed good accuracy for the prediction of in-hospital mortality for the AARC (AUC=0.886), MELD (AUC=0.816) scores, and for VA (AUC=0.812) but only a fair accuracy for the Child-Pugh score (AUC=0.799). A cut-off value for the prediction of mortality was identified for VA (152.5 µmol/L, sensitivity=0.706, 1-specificity=0.190). Univariate analysis found acute kidney injury, severe hepatic encephalopathy (grade III or IV), VA≥152.5 µmol/L, MELD score≥22.5, Child-Pugh score≥12.5, and AARC score≥8.5 to be associated with in-hospital mortality. Multivariate analysis identified AARC score≥8.5 and VA≥152 µmol/L to be independent predictors of in-hospital mortality.

Conclusions: VA could be used as a predictor of in-hospital mortality in patients with ACLF. Patients with ACLF and venous ammonia>152.5 µmol/L have a high risk for a poor outcome and could be candidates for urgent liver transplantation.

QUERCETIN REDUCES OXIDATIVE STRESS IN EXPERIMENTAL ALLERGIC RHINOSINUSITIS INDUCED BY SILICA EXPOSURE IN RATS

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Background: Silica nanoparticles are widely used in various applications, ranging from additives for plastic materials, cosmetic products or toners for printers to directed drug transport systems. Worldwide, 1.5 million tons amorphous silica nanoparticles are produced. Chronic exposure through inhaling coarse crystalline silica particles (2.5 up to 10 μm) leads to the development or exacerbation of allergic airway diseases, production of reactive oxygen and nitrogen species and finally, rhinosinusitis.

Purpose: This study was designed to investigate the effect of quercetin administration on changes in oxidative stress parameters in the nasal sinus mucosa of rats with CRS induced by chronic ozone exposure.

Methods: Allergic rhinosinusitis (ARS) was induced in Wistar rats by intranasal instillation (IN) of silicon dioxide or silica SiO2 (52.5%) in a dose of 400 μg/animal, dissolved in 100 μl physiological serum (2 administrations per week, 7 nasal instillations). At 8 hours after silica administration, the ARS rats were treated with oral quercetin (30 mg/kg body weight/day) for 4 weeks. At the end of the study, nasal sinus mucosa was isolated for the assessment of oxidative stress markers (malondialdehyde, MDA) and the activity of some antioxidant enzymes (superoxide dismutase, SOD and catalase, CAT).

Results: The rats with ARS showed significantly increased MDA levels and a reduction of SOD and CAT activity in nasal sinus tissue. A decrease in the levels of oxidative stress markers associated with elevated activity of antioxidant enzymes in the nasal sinus tissue of quercetin-treated rats with CRS was observed.

Conclusions: The results of the present study suggest the potential protective effect of quercetin in experimental inclusion in the above mentioned topics is mandatory

THE EXPRESSION OF RECOMBINANT GLYCOPROTEINS IN SPODOPTERA FRUGIPERDA (SF9) INSECT CELLS - AN INTEGRATIVE APPROACH TO COMPONENT-RESOLVED DIAGNOSIS AND ALLERGEN IMMUNOTHERAPY

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Background: Ambrosia artemisiifolia and Artemisia vulgaris are representative allergenic weeds belonging to the Asteraceae family. Both plants can occur in the same area and are a major health concern throughout Europe, inducing severe IgE-mediated allergic symptoms in late summer and autumn.

Purpose: In areas where the two species co-exist, recombinant allergens can prove to be an important tool to determine primary sensitization and to differentiate between cross-reactivity and co-sensitization. This differentiation leads to increased diagnostic specificity and ensures safer and more effective immunotherapy. Replacement of allergenic extracts with recombinant allergens is an innovative new tool in allergy diagnostic and personalized medicine.

Methods: An eukaryotic expression system (Spodoptera frugiperda, Sf9) was used to obtain recombinant glycosylated allergens. Physicochemical and immunological characteristics of the produced allergens were then analyzed to see whether they are comparable to the natural form of the protein.

Results: Analysis of physicochemical characteristics of recombinant proteins has been performed by the following techniques: protein separation by electrophoresis based on their size and load (SDS-PAGE), determination of the secondary structure of the protein (circular dichroism), and mass analysis (MALDI-ToF). Allergenicity of recombinant allergens was verified by the following methods: semi-quantitative assessment for antibodies binding (ELISA), detection of allergen-induced inflammatory reaction (RBL), and measurement of specific IgE level (ImmunoCAP).

Conclusions: Having long been used as a model in physiology, developmental and molecular biology, Sf9 cells show promising potential as candidates in protein therapeutics.

THE REMARKABLE EFFECTS OF A SPECIFIC IONIZED MEDICAL WATER (SIMW) IN 3 CASES OF DUCHENNE MUSCULAR DYSTROPHY IN CHILDREN AND THE THERAPEUTIC POTENTIAL OF THIS SIMW IN TREATING CELLULAR OXIDATIVE STRESS

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Duchenne muscular dystrophy (DMD) is severe X-linked recessive congenital muscular dystrophy and the most common type of muscular dystrophy. Long-term corticosteroid therapy (LTCT) (started from the age of 4 years) is the most accessible and used pharmacological therapy of DMD in Romania. Several studies showed that a specific ionized medical water (SIMW) (which was approved as a dietary supplement in UE) is a very potent selective NRF2 activator, thus a very potent (indirect) antioxidant, with no toxicity up to high doses (in contrast with LTCT).

This research aims at discovering dietary supplements which may show comparable or even stronger beneficial effects (with less or none adverse effects) than LTCT in children with DMD whose parents refuse LTCT. Three boy-patients with DMD (of ~4, ~5 and ~3 years of age) (whose parents refused LTCT) were treated with relatively high doses of SIMW 3-7 ml/kg/day (usually combined with medium doses of L-carnitine and omega-3 fatty acids for various intellectual disabilities of two patients): periodic consults and rhabdomyolysis, medular and liver toxicity markers assessment (blood count, GGT, ASAT, ALAT, LDH, CK, CK-MB and serum myoglobin) were accomplished.

Results: From the first months of SIMW-based treatment, all the rhabdomyolysis markers (with very high initial serum levels) dropped significantly, with no found medular/hepatic toxicity in all these three tested DMD children. This SIMW appears to have remarkable antioxidant and immunomodulatory effects (strong NRF2 selective activation and NF-kB inhibition) and should be studied on larger groups of children with DMD under the age of 4 years old (but also on other age groups of children and even young adults), as an alternative to as an alternative to early corticosteroids.

EVALUATION OF MACULAR THICKNESS IN DIABETIC PATIENTS WITHOUT DIABETIC RETINOPATHY

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Background: Recent studies suggest that neurodegeneration plays an important role in the pathogenesis of diabetic retinopathy (DR). Histological studies have revealed that alternation of metabolic pathways in diabetes can potentially cause neural cell degeneration in the retina. Some of the studies reported that neural loss may occur before any visible signs of vascular changes. The interaction between regulation of blood flow and neural activity is considered to be involved in the pathophysiologic mechanism of DR, and is described as neurovascular coupling.

Purpose: The purpose of this study is to examine early changes in macular thickness using optical coherence tomography (OCT). We evaluated to what extend macular thickness differed between patients with type 2 diabetes mellitus without diabetic retinopathy compared with healthy individuals.

Methods: 26 diabetic patients without diabetic retinopathy and 26 normal participants without any retinal and optic nerve diseases underwent ophthalmic examination, fundus photography and OCT imaging. Using Cirrus OCT we measured macular thickness in nine ETDRS subfields.

Results: Mean age in diabetic group was 61.5 years and in control group was 55.5 years. In patients with type 2 diabetes mellitus, central macular thickness was significantly thinner than that of control eyes (243.5 μm versus 269.9 μm, p value <0.001). The macular thickness in all quadrants, except the center, in the diabetic patients was not statistically significantly different from the control group.

Conclusions: Our results support the hypothesis that structural changes of the neuroretina is an early indicator of retinal impairment in patients with diabetes without diabetic retinopathy.

FROM THE SARCOMERE FORWARD TO FUNCTION AND BACK (BACKWARD) TO CARDIOMYOCYTES

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Background: Ageing process reaching in time the "elders" period arrives with different disabilities. Sarcomere and muscle function diminish influencing the cardiac output and cardiac adaptation to exertion.

Purpose: We tried to determine the presence of sarcopenia in a group of elders, and its influence on cardiac function.

Methods: From July to September 2020, we performed a clinical check-up examination on a total number of 67 elderly people, mostly women (89.55%), from different rural areas. We recorded anthropometric data, medical history, current and chronic symptoms, present and prior treatment. The muscle strength was measured bilateral on upper limbs by a dynamometer. We planned to perform one-minute step test in a 15 step-up rhythm per minute, monitoring oxygen saturation and heart rate at rest, immediately, and within the first and the second minute after the effort.

Results: The extreme age limits went from 65 years and 4 months to 95 years and 3 months, with a median age of 75 years and 3 months. The oldest old people grouped 14 women and 6 men. Women presented a higher functional longevity, but yet, a greater physical disability was seen (37%), reducing or impeding the performance on the step test (osteoarthritis, hearing, or visual impairment, elevated resting heart rate in atrial fibrillation, or bedridden for more than 6 months). There was a steep drop in upper-arm strength for the 75-79 years old group of age, more in left arm. Both right and left hand-grip test were statistically correlated to the ability to perform the step-test.

Conclusions: Our study included a clinical evaluation of the oldest old group category of age. In selected cases of advanced motor disability of different etiology or influence, the grip strength test shows the reserve capacity for rehabilitation. Comparative to men, women had a higher longevity and cardiac recovery capacity.

Key words: sarcomere, disabilities, hand-grip test, step-test, cardiac adaptation

FROM CELL TO FIBROSIS IN LEFT ATRIAL REMODELLING AND ATRIAL CARDIOMYOPATHY

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Background: Fibrosis and associated dysfunction may appear early during remodelling, preceding chamber enlargement. They are linked to an increased risk of stroke even in non-atrial fibrillation patients.

Purpose: The aim was to describe the mechanisms from cell to fibrosis in atrial remodeling and atrial cardiomyopathy.

Methods: This is a narrative review about the molecular and cellular mechanisms evolved in these atrial changes.

Results: Left atrial remodelling can be defined as the time-dependent structural, functional and/or electrical alterations in response to mechanical (pressure and/or volume overload), metabolic or electrical stressors, being the substrate for veritable atrial cardiomyopathy. Initially reversible (<1 week of exposure) and adaptive, in time, the cellular, electrical and autonomic nervous alterations will become permanent and maladaptive. The different types of left atrial remodelling (structural, functional, electrical) are interconnected, influencing both therapeutic options and prognosis. Fibrosis associated with structural remodeling leads to conduction heterogenicity, promoting re-entry and abnormal foci. Furthermore, low-voltage areas correlate with fibrotic regions in atrial fibrillation patients and low gadolinium enhancement – cardiac magnetic resonance fibrotic burden is linked to left atrial dysfunction. At a molecular level, atrial cardiomyopathy is supported by the pro-fibrotic effects of angiotensin II, aldosterone, TGF-β1 and pro-inflammatory cytokines and the reported reverse-remodelling following therapy with either aldosterone receptor blockers or angiotensin-converting enzyme inhibitors.

Conclusion: There are therapeutic and prognostic implications of atrial cardiomyopathy, properly identifying it becomes mandatory.

MICROGLIA MORPHOLOGY ACROSS THE LIFESPAN

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Background: Microglia, as the only physiological specialized immune cells of the central nervous system (CNS), have been involved in almost all CNS pathologies.

Methods:. Hetero-zygotes and wiled type TgH(CX3CR1-EGFP) mice were used asses life span and quantify microglia morphology at 5, 10 and 21 days, 20, 58, 84 and 110 weeks of age.

Results: Mouse survival was not influenced by environment, gender or social housing, while normal microglia morphology is subjected to specific age related changes.

GENETIC METHODS TO RESTORE POST STROKE CELLULAR IMBALANCE

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Ischemic stroke is the second leading cause of death and the primary reason for sustained disability worldwide. It is common knowledge that neurons are lost in the infarct core after stroke. On the other hand, astrocytes, become reactive and proliferative leading to a severe disruption of neuronal vs non-neuronal cell balance. This phenomena especially occurs in the aged brain. Therefore, restoring the balance between neurons and non-neuronal cells within the perilesional area is crucial. What is more, so called gliotic scars are formed by the proliferating glia cells. Although initially protective, these gliotic scars act like a barrier to neural regeneration in the long-term. Strategies such as transforming inhibitory gliotic tissue into an environment conducive to neuronal regeneration and axonal growth have been experimented. This idea has further improved its chances of success following the discovery that in vivo direct lineage reprogramming in the adult mammalian brain is a feasible strategy for reprogramming non-neuronal cells into neurons. However, the potential of this new methodology was not yet tested as a method to improve the restoration of structure and function of cells in the hostile environment caused by the fulminant inflammatory reaction found in the brains of aged animals following stroke. In our experiment, we used retroviral/lentiviral delivery systems encoding certain transcription factors, to target astrocytes in the neocortex of aged mice. The effects of in vivo reprogramming of reactive glia into neuroblasts and mature neurons has been assessed by cellular phenotyping over different time intervals. Considering that no restorative treatment is yet available for stroke, this method could offer a completely new approach for stroke therapy.

PREDICTION OF THE DEGREE OF PULMONARY CONGESTION AT 24 HOURS TRAUMATIC IMPACT

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Background: Immunocompetent cells activated in trauma, mainly neutrophils, can cause indirect damage of intact tissues once they infiltrate the primarily unaffected organs by the traumatic agent and secrete proteases. These are substances with a destructive potential causing the further development of the insufficiency of those organs.

Purpose: The current experimental study is an attempt to identify biomarkers of indirect lung lesions and to develop a predictive model for early estimation of their degree.

Methods: By statistical data processing tools, the correlations between different components of the protease/antiprotease system and the degree of pulmonary congestion (DPC) at 24 hours after trauma were investigated.

Results: The standardization of the coefficients showed that, from a quantitative point of view, the effects on DPC of α1-antitrypsin antiprotease measured at 2 hours after trauma are the most significant, followed by Elastase at 24 hours and α1-antitrypsin measured in the moment of trauma. The other initially analyzed factors were excluded by the Backward method. The associations and the tendencies towards them were the basis for the elaboration of a predictive model for estimating the DPC at 24 hours after the trauma.

Conclusions: The effects of proteases and antiproteases determine the associations identified between DPC and the concentrations of these substances at different time intervals after trauma. The predictive model for DPC at 24 hours after trauma included α1-antitrypsin and Elastase measured in the mentioned intervals. The model requires completion, validation and testing in clinical trials. The results obtained, despite the data of linear regression and resampling, require further research in subsequent studies on large groups of patients.

MOLECULAR DIAGNOSIS IN ALLERGIES – A NEW APPROACH OF PRECISION MEDICINE

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Background: Since the discovery of immunoglobulin E in the late 1960s, serological tests have become an integral part of allergy diagnosis, along with detailed anamnesis and in vivo diagnostic procedures.

Purpose: Allergenic extracts used to be the basis of in vitro diagnostic methods, but their accuracy was not optimal, due to standardization issues. This deficit is now progressively covered by new diagnostic options based on molecular biology methods.

Methods: Molecular allergology has emerged at an opportune time in this era of precision medicine, when precise diagnostic tools, based on detailed knowledge of the disease phenotype, are desired, in order to provide the patient with individualized treatment options. Singleplex and multiplex molecular analyses not only provide detailed information about the patient's sensitization profile, but also about possible cross-reactivities.

Results: Molecular diagnosis evaluates the patient's IgE-mediated immune response to specific allergenic molecules, providing a finer resolution compared to testing based on extracts from allergenic sources. Molecular diagnosis also allows: 1) assessment of the risk induced by sensitization, useful especially in food allergies; 2) evaluation of cross-reactivity between allergenic sources of different species, and therefore the elucidation of seemingly unrelated clinical manifestations; 3) selection of appropriate allergen immunotherapy (AIT) individually; 4) observation of the longitudinal evolution of complex molecular profiles of IgE and IgG in cohort studies at birth, essential for the explanation of complex immunological phenomena.

Conclusions: Molecular diagnostic methods can accurately identify the causes of allergies, thereby facilitating risk assessment and therapeutic decisions.

THE EFFECTS OF ISOFLURANE ANESTHESIA ON THE HEART RATE RESPONSE TO PARASYMPATHETIC STIMULATION AND INHIBITION IN RATS

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Background: The study of the parasympathetic nervous system, an important target in many cardiovascular experiments, is often affected by the use of anesthesia.

Purpose: We aimed to evaluate the effect of isoflurane anesthesia on the heart rate (HR) response to parasympathetic stimulation and inhibition in rats.

Methods: Six adult male Wistar rats were anesthetized with isoflurane (2.5%, 4L O2/min). The HR was evaluated prior to and 20-min after parasympathetic stimulation (carbamylcholine, 0.4 mg/kg) and inhibition (atropine sulfate and atropine nitrate, 2 mg/kg each). Six additional rats were used to assess HR changes in response to similar doses of carbamylcholine and atropine nitrate, in the absence of anesthesia.

Results: As expected, parasympathetic stimulation induced a significant decrease in HR compared in both the anesthetized (341.2 ± 7.5 vs. 364.4 ± 9.2, $p< 0.01$) and the non-anesthetized (139.8 ± 7.6 vs. 294.2 ± 14.4, $p< 0.001$) rats, but the HR response was significantly higher in the non-anesthetized rats (-154.3±18.2 vs. -23.2±6.6; $p< 0.001$). Parasympathetic inhibition with either atropine nitrate or sulfate had no effect on the HR in the isoflurane-anesthetized rats (both $p> 0.05$), whereas atropine nitrate significantly increased the HR in the non-anesthetized rats (421±9.6 vs. 305.2 ± 17.3, $p< 0.001$).

Conclusions: In the present study, isoflurane anesthesia canceled the HR response to parasympathetic stimulation and blunted the HR response to parasympathetic inhibition. Isoflurane anesthesia thus seems to be unsuitable for experiments aiming to evaluate the parasympathetic nervous system in rats.

PREDICTIVE FACTORS FOR BLEEDING EVENTS IN PATIENTS WITH HEPATITIS C VIRUS LIVER CIRRHOSIS TREATED WITH DIRECT ACTING ANTIVIRALS

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INTRODUCTION: The advent of direct-acting antivirals (DAAs) is a major breakthrough in hepatology representing the therapeutical standard of care in patients with chronic hepatitis C virus infection over the past few years. Despite high rates of sustained virological response (SVR), DAAs therapy doesn't eliminate the risk of bleeding events. In our study we aimed to identify the factors associated with the occurrence of bleeding events in patients treated with DAAs therapy.

MATERIAL AND METHODS: We retrospectively analyzed a cohort of patients with HCV-related liver cirrhosis treated with paritaprevir/ritonavir, ombitasvir and dasabuvir (PrOD) ± ribavirin and ledipasvir/sofosbuvir (LED/SOF) ± ribavirin for 12/24 weeks, in a tertiary gastroenterology referral center from North-Eastern Romania, between January 1st 2016 and January 1st 2020. All patients with presumption of digestive bleeding were evaluated and confirmed by upper digestive endoscopy performed in emergency.

RESULTS: The study included 874 HCV-infected cirrhotic patients treated with PrOD or LED/SOF ± RBV, with documented SVR, mean age 58,7 ± 6,2 years, predominantly female (58%). Of the total number, 443 (50.68%) received PrOD and 431 (49.31%) patients were treated with LED/SOF ± RBV. Of the patients included in study, 572 (65.34%) Child-Pugh class A, 226 (25.96%) class B and 76 (8.7%) class C cirrhotic patients. Mean period from SVR and the occurrence of bleeding events was 230±121 days. Bleeding complications after SVR were reported in 16 (1.83%) patients: 9 (56.25%) with variceal hemorrhage and 7 (43.75%) with non-variceal hemorrhage.

CONCLUSIONS: Bleeding events in patients with HCV-related liver cirrhosis treated with DAAs are influenced by the hemodynamic changes induced by the status of advanced liver disease. The most bleeding events were variceal bleeding due to persistent portal hypertension despite viral eradication. Despite SVR liver fibrosis persists, and so does portal hypertension and consequently the risk of bleeding.

SPONTANEOUS SEROREVERSION OF ANTI-HEPATITIS C VIRUS DURING THE NATURAL COURSE OF HEPATITIS C VIRUS INFECTION IN THE PATIENTS EVALUATED FOR TREATMENT - A SINGLE CENTER EXPERIENCE

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I**NTRODUCTION:** Hepatitis C virus (HCV) infection is common worldwide but there are different prevalence rates in different countries. Spontaneous viral clearance occurs in 10–25% of infected individuals after acute infection yet controversy exists regarding the frequency of spontaneous clearance during the natural course of HCV infection in the general population.

AIM: In our study we aimed to evaluate undetectable ARN HCV prevalence in antibodies HCV population evaluated for IFN free treatment.

MATERIAL AND METHODS: We performed a prospective study in which we included all patients with positive anti -HCV antibodies who were admitted in a tertiary referral center from North-Eastern Romania and were evaluated for IFN free treatment between June 1st 2018 and July 1st 2020. All patients included in the study performed the quantitative determination of HCV viraemia. The patients with sustained virological response to interferon (IFN) therapy were excluded from the study.

RESULTS: The study included 473 persons with positive anti -HCV antibodies; mean age was 54.6±5.2 years, predominantly female (293- 61.94%). Among all patients with positive anti-HCV antibodies, in 45 (9.51%) cases we found undetectable HCV viraemia. Seroreversion occurred in individuals with a median age of 58 years (range 45–66). Of the patients with undetectable HCV viraemia, 28.8% had elevated transaminases.

CONCLUSIONS: The frequency of spontaneous viral clearance in a homogeneous population of immunocompetent subjects not related to treatment with IFN was low (9.51%). These data are consistent with the literature where viral spontaneous clearance was 2-10% in a cohort of HCV infection.

KEYWORDS: hepatitis C virus infection, spontaneous seroreversion, direct antivirals

ASPECTS REGARDING HAEMATHOLOGIC PROFILE IN PATIENTS WITH CORONAVIRUS INFECTIOUS DISEASE

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Background: COVID-19 stands for coronavirus disease outbreak which emerged in year 2019. It is caused by the SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2). Nowadays, coronavirus infection (COVID-19) represents an important global disease with high impact for medical practice. Clinical and paraclinical investigations of this patients category are variable and still not enough known.

Purpose: The purpose of the present study was to analyse the variations of haematological parameters in patients diagnosed with SARS-CoV-2 infection.

Methods: Were used electronic medical records of 78 patients, aged between 35-89, both gender, hospitalised in "Sf. Spiridon" County Clinical Emergency Hospital, Iasi, Romania, during april-june 2020. Informed consent was obtained from the entire patients included in the study. The obtained data shows that were haematological parameters modifications in the hospitalised patients investigated.

Results: We compared haemathological variations of parameters such as neutrophils and lymphocyte count, platelet count, and coagulation parameters between the study group (32) and control one (46) which include patients with different diseases but without SARS-CoV-2 infection. Subjects with SARS-CoV-2 infections and comorbidities and the controle one. The most frequent comorbidities were represented by obesity, diabetes, cardiovascular and hepatic diseases.

Conclusions: The monitoring haemathological parameters might represent an important step for predicting the severity manifestations in patients with COVID-19 disease and can contribute to the improving of patients quality of life.

Keywords: coronavirus disease, haematologic parameters, SARS-CoV-2 infection, paraclinical investigations.

WORSENING HEART FAILURE DUE TO VERY LOW SODIUM INTAKE

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Background: Hyponatremia is a frequent manifestation of advanced heart failure and holds an important prognostic value with increase morbidity and mortality. Restrictive sodium diet enhances sodium reabsorbtion and water retention in heart failure due to elevated levels of arginin vasopressin and increased activity of renin-angiotensin-aldosterone system (RAAS) and sympathetic nervous system (SNS) secondary to low cardiac output and impaired renal perfusion.

Purpose: We analysed the correlation between very low sodium intake complicated with severe hyponatremia and worsening of heart failure due to impaired water free excretion.

Methods: A 71 yo female with a history of heart failure with low ejection fraction, permanent atrial fibrillation and severe degenerative aortic stenosis was admitted with congestive heart failure (peripheral oedema, pericardial and pleural effusion) and severe hypotonic hyponatremia (Na=116 mg/dl, serum osmolality=259 mOsm/kg). We mention serum creatinine=0.9 mg/dl, serum K=4,3 mmol/l, HR=80 bpm, BP=90/60mmHg. She understood that following a "no sodium diet" was optimal in heart failure.

Results: Sodium replacement therapy, angiotensin-converting enzyme inhibitor, aldosterone receptor antagonist and diuretics contributed to favorable outcome with improved diuresis, reduction of oedema and symptom amendment.

Conclusions: A very restrictive sodium intake contributed in this case to heart failure decompensation because of high levels of vasopressin and low renal perfusion that overrides the normal vasopressin inhibition in low plasmatic osmolality. Aortic stenosis and the loss of atrial contraction lowers even more the cardiac output.

THE PHYSIOLOGICAL RELEVANCE OF SOME DIGESTIVE FUNCTIONAL DISORDERS AND MICROBIOTA IN PARKINSON'S DISEASE

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The mutifactoriality of Parkinson's disease (PD) pathological mechanisms is a highly debated topic. In this mini-review we are covering previous work of our group in highlighting the main features of the microbial communities which have been termed "the second brain" that seem and could be an important factor for the etiopathophysiology of PD.

METFORMIN MITIGATES MONOAMINE OXIDASE-DEPENDENT OXIDATIVE STRESS IN TWO RAT MODELS OF ACUTE AND CHRONIC ENDOTHELIAL DYSFUNCTION

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Metformin, the first-line drug in the therapy of diabetes mellitus, has been reported to elicit cardio- and vasculo-protective effects via partially elucidated mechanisms. Monoamine oxidase (MAO) has recently emerged as major source of mitochondrial reactive oxygen species (ROS) in cardio-metabolic pathologies.

The present study was aimed to assess the effects of metformin on MAO expression, ROS production, and vasomotor function of rat aortas by using two experimental models of acute and chronic endothelial dysfunction. In acute experiments, aortic rings (n=8-10/group) prepared from thoracic aortas of healthy rats were treated (incubation for 12 hours, organ culture) with metformin (10 μM) in the presence vs. absence of angiotensin II (AII, 100 nM), lipopolysaccharide (LPS, 1 mg/dL) or high glucose (HG, 400 mg/dL). In chronic experiments, aortas were harvested from high-fat cafeteria diet-induced obese rats (n = 8) and incubated (12 h) or not with metformin. Measurements of MAO expression (immune histochemistry and quantitative PCR), ROS production (spectrophotometry and immune fluorescence), and vascular reactivity (myograph studies) were performed in both experimental models.MAO expression was up-regulated in aortic rings after acute ex vivo exposure to AII, LPS and HG, and also in the rat model of obesity-related chronic endothelial dysfunction. Metformin decreased MAO expression and ROS generation, reduced vascular contractility, improved the endothelium-dependent relaxation and in all diseased vascular preparations, either incubated to mimic acute endothelial dysfunction or harvested from the obese animals. Metformin mitigated MAO-related oxidative stress and improved endothelial function in both acute and chronic vascular pathologies in the rat.

PERINATAL BRAIN ASPHYXIA - AN EPIGENETIC PERSPECTIVE

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Perinatal asphyxia (PA) is a severe generalised condition, especially causing impactful neurological disorders, ranging from cognitive impairment to cerebral palsy. The neurological conditions secondary to PA mainly depends on the severity of the asphyxia episode and the impact it has on the selective vulnerable areas of the immature brain. Recent epigenetic studies using novel molecular tools are opening new perspectives needed to understand the brain response to PA and to develop early diagnostic and prognostic markers, and more effective therapeutic solutions. The current view of epigenetics includes active DNA methylation, demethylation, acetylation, and deacetylation, processes which imply changes in DNA tertiary structure, or histone covalent changes, and synthesis of small, non-coding RNA molecules, such as microRNAs, that modulates DNA translational potential at the ribosomal level. Alteration of the chromosomal structure due to epigenetic DNA changes interferes with gene silencing mechanisms. The impairment of histone-DNA bindings modifies the DNA translational potential, while miRNA synthesis affects protein synthesis. This presentation will focus on epigenetic mechanisms involved in the brain response to PA, impacting on the neuroinflammation and injury processes, homeostatic synaptic plasticity, neuronal metabolism, and neurorepair. Also, new diagnostic and therapeutic opportunities for PA will be discussed from an epigenetic perspective.

ASSESSMENT OF PLATELET MITOCHONDRIAL DYSFUNCTION IN CHILDREN WITH BLOOD MALIGNANCIES

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Assessment of mitochondrial respiration in peripheral cells has recently emerged as a potential biomarker for the assessment of blood malignancies.

The present study was double aimed: a) to characterize the platelet mitochondrial respiration in pediatric patients newly diagnosed with blood malignancies, and b) to investigate whether platelet respiration is age-dependent. Blood samples were taken in K2EDTA tubes during routine testing from pediatric patients and healthy donors. Platelet mitochondrial respiration was measured at 37°C by means of high resolution respirometry (Oxygraph-2k, Oroboros Instruments, AT) according to a classical Substrate-Uncoupler-Inhibitor-Titration protocol protocol. After stabilization at the routine state, the following respiratory parameters were measured: active respiration (State 3, ADP-induced), non-phosphorylating respiration (State 4, oligomycin-induced), and maximal uncoupled respiration (ETS state, FCCP-induced). The non-mitochondrial, residual oxygen consumption (ROX) was subtracted from all respiratory rates for data analysis. The major finding is that platelet routine mitochondrial respiration (but not the active respiration) is decreased in pediatric patients already at the onset of disease. However, respiratory rates appear to decrease with age; this observation underlies the crucial importance of selecting appropriate controls when assessing platelet mitochondrial dysfunction in children. In conclusion, mitochondrial respiratory dysfunction in peripheral platelets appears to occur early in children newly diagnosed with blood malignancies. Further experiments are required to assess the effects of chemotherapy on platelet respiration and its changes during the remission phases.

THE CHALLENGE OF TEACHING PHYSIOLOGY DURING CONFINEMENT: A LITERATURE REVIEW VS. PERSONAL EXPERIENCE

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The present medical crisis raised numerous problems in various fields, including medical education system. In Romania, medical universities were among the first to close their physical doors and switch to online teaching, in order to continue interacting and working with their students. The task was not and is not an easy one, as 'face to face' interaction between students and teachers, between students and patients, and between students themselves is an integral and essential part of any teaching strategy. Also, especially in medial universities, the applicative aspect is of utmost importance, and replacement strategies had to be found. Online teaching is not a completely new approach. Preoccupations, though not applied on a 'general' scale, existed for a long time. Studies have also been published. The contemporary medical situation lead to a general application of the online model of teaching, thus creating a 'natural' experimental setting for studies concerning online teaching. The aim of the present review consists in identifying the studies concerning teaching preclinical medical disciplines, in general, and human physiology, in particular, related to the present medical crisis, in performing a critical analysis of the described methods used to online teach physiology to medical students, and to compare these studies with the personal experience, in order to find new methods and/or to improve the methods already used.

HEART RATE VARIABILITY IN SUBJECTS WITH BORDERLINE TYPE OF PERSONALITY

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Background: Heart rate variability (HRV) is considered as a measure of autonomic nervous system balance, and therefore it may provide a quantification of the physiological changes associated with mental illness

Purpose: Borderline personality disorder (BPD) is associated with a high rate of cardiovascular mortality, which requires to study the heart rate variability.

Methods: The study included 103 subjects, who completed Personality Inventory for DSM-5 (PID-5) prior to recording of ECG. Based on the results obtained from the PID-5 test, people were divided into 2 groups, the first - control group - 69 people (N = 69), the second group BPD - 34 people (N = 34). The electrocardiogram was recorded using Biopac MP36 system during the rest (R) - 5 minutes; pain test (P) - 3 min; post-pain (pP) -5 minutes. The spectral analysis of ECG was performed.

Results: The low frequency (LF) component of spectral density, which is an index of sympathetic modulation of heart rate, and the high frequency (HF) component, used to evaluate the vagal activity, did not show significant differences between two groups included in the study. Within the second group, LF has increased by 16.3% in pP (56.57 ± 3.39) compared to R (48.62 ± 3.99) and with 12% compared to P (50.30 ± 3.60). Reciprocally, HF is lower with 15.5% in pP (43.28 ± 3.37) than R (51.22 ± 3.97) and with 13% lower in pP than P (49.44 ± 3.58).

Conclusions: Subjects with BPD presented an increase in sympathetic influences on heart rate and a reduction of vagal modulatory effects during pain test , and these changes accentuated further after removing the painful stimulus, which can be proof of the inertia of autonomic influences in these subjects.

THE ROLE OF MALADAPTIVE PERSONALITY TRAITS IN THE SLEEP QUALITY OF PATIENTS WITH INSOMNIA

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Background: There are a few factors that negatively affect sleep quality in patients with insomnia; these factors are organized into three main groups: predisposing, precipitating, and perpetuating. Maladaptive personality may act as a predisposing, and potentially perpetuating factor. Increased neuroticism, internalization, anxious concerns and perfectionism were associated with disturbed sleep.

Purpose: To determine maladaptive personality traits which are the most frequently present among insomnia patients with poor sleep quality.

Methods: In this study participated 56 patients (age range 25-71 years). The patients completed Pittsburgh Sleep Quality Index (PSQI) and Personality Inventory for DSM-5 (PID-5).

Results: From 56 patients, 35 (63%) showed poor sleep quality and 21 (37%) - good sleep quality. From 25 personality trait facets assessed of PID-5, higher average scores were observed in the following facets: Anxiousness (in 7% of cases), Attention Seeking (7%), Emotional Lability (7%), Hostility (7%), Intimacy Avoidance (9%), Separation insecurity (9%), Suspiciousness (9%) and Rigid Perfectionism (11%). The only trait domain detected was Negative Affect.

Conclusions: The most prominent personality characteristic determined in our study is Rigid perfectionism which includes concern over mistakes and excessively high personal standards and is associated with worry and rumination that at bedtime are assumed to lead to increased sleep latency and sleep maintenance difficulties. The predominant domain of Negative Affect detected in our study shows that people with insomnia have frequent and intense experiences of a wide range of negative emotions. Correction of these maladaptive personality characteristics could improve sleep quality in insomnia patients.

PARTICULARITATI DE DIAGNOSTIC SI TRATAMENT IN SINDROMUL MENIER CU TRANSMITERE GENETICA

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Sindromul Menier -doua sau mai multe episoade de vertij cu durata crizei inte 20min si 12ore, insotita de Hipoacuzie neurosenzoriala pe frecventele joase si acufene Se poate efectua investigarea genelor care contribuie la transmiterea bolii - transmitere monogenica determina hipoplazia de sac endolimfatic. In diagnostic si ulterior pentru personalizarea tratamentului se pot face cateva diferentieri intre Boala Menier cu transmitere autosomal dominate si Boala menier cu character sporadic sau idiopatic Boala Menier cu transmitere autosomal dominanta prezinta debut timpuriu, penetrarea incomplete a caracterelor de simptomatologie si prin teste genetice se poate anticipa. Raspunsul la tratmentul cortizonic este adeseori slab. In acest caz mecanismul fiziopatologic este hipoplazia de sac endolimfatic. Boala Menier idiopatica apare pe fond inflamator, prezinta debut tardive si este adeseori insotita de migrene. Aceasta poate fi socotita ca boala autoimuna, cu raspuns bun la tratamentul cortizonic. In acest caz mecanismul fiziopatologic este inflamatia sacului endolimfatic.

CONCLUZII Boala Menier reprezinta un spectru pathologic cu afectare cohleo-vestibulara, care poate fi transmisa genetic sau idiopatica. Diferentierea celor doua variante fiziopatologice si eventual testarea genetica poate fi utila in diagnosticul de certitudine iar ulterior in conduita terapeutica si schema de tratament.

EFFECTS OF DIETARY MANIPULATIONS OF THE GUT MICROBIOTA ON GENES INVOLVED IN THE ASTROCYTE-NEURON LACTATE SHUTTLE IN THE HIPPOCAMPUS

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Background: The gut microbiota modulates brain physiology, development, and behavior and has been implicated as a key regulator in several central nervous system disorders. Its effect on the metabolic coupling between neurons and astrocytes has not been extensively studied to date, even though this is an important component of brain energy metabolism and physiology and it is perturbed in neurodegenerative and cognitive disorders.

Purpose: In this study, we have investigated the mRNA expression of 8 genes encoding proteins implicated in the Astrocyte-Neuron Lactate Shuttle (Atp1a2, Ldha, Ldhb, Mct1, Gys1, Pfkfb3, Slc1a2, Slc2a1), in relation to dietary manipulations known to modulate gut microbiota composition, in the mouse brain hippocampus, a region with critical functions in cognition and behavior.

Methods: Tissue samples from two independent studies focused on an extended feeding trial in a diet-induced obese mouse model and a prebiotics feeding trial in chronically stressed mice were analyzed. RNA was extracted from hippocampi that were separated from whole brains, frozen, and stored at -80 °C previously. Gene expression was evaluated by RT-qPCR. Data was normalized using β-actin and transformed using the $2^{-\Delta\Delta CT}$ method.

Results: We have discovered that Atp1a2 and Gys1, were significantly increased in the hippocampus of mice undergoing a high-fat diet compared to a low-fat diet. Atp1a2 and Pfkfb3 were upregulated upon diet supplementation with prebiotics in the hippocampus of mice that underwent a protocol inducing chronic psychosocial stress.

Conclusions: These findings indicate an influence of dietary manipulations known to affect the gut microbiota on mRNA expression of genes implicated in the metabolic coupling between neurons and astrocytes.

IN SILICO DESIGN OF ALLERGEN-SPECIFIC VACCINES BASED ON SYNTHETIC PEPTIDES INDUCING T CELL- AND B CELL-MEDIATED IMMUNE RESPONSES

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Background: Allergen immunotherapy (AIT) is currently considered the only disease-modifying treatment that is able to stop the atopic march, exerting long-lasting effects. AIT with whole allergens or allergen extracts has been in use for a long time, however it is associated with adverse events. Peptide-based allergen immunotherapy is an alternative to whole-allergen AIT. It uses small synthetic peptides representing T cell and B cell epitopes from the target allergen, with the ability to induce T cell tolerance and immunoregulation, and IgG blocking antibodies.

Purpose: In this study, we have used in silico methods to identify continuous linear B-cell and T-cell epitopes from two ragweed pollen allergens, Amb a 4, and Amb a 6.

Methods: For T-cell epitope prediction, the amino acid sequences of Amb a 4 and Amb a 6 were input into the IEDB MHC II binding prediction tool using the full human HLA reference set and prediction was run using the Consensus 2.2 method. Core peptide sequences with an adjusted rank below 10% were indicated as strong binders. For B-cell epitope prediction, the following algorithms were used: Bepipred 2.0, ABCpred, Kolaskar & Tongaonkar Antigenicity, and Emini Surface Accessibility Prediction. Peptides identified by Bepipred and validated by at least one other method were considered.

Results: The following T-cell epitopes with strong binding confidence were predicted for Amb a 4 (aa residues): 74-82, 76-84, 65-73, 46-54, and 77-85; for Amb a 6, predicted T-cell epitopes were 59-67, 52-60, 53-61. The following B-cell epitopes were identified: 5-24, 26-43, 59-84, 88-107 for Amb a 4, and 19-27, 37-44, 57-84 for Amb a 6.

Conclusions: In this study, we have identified in silico, candidate peptides for ragweed Amb a 4 and Amb a 6 allergen-specific vaccines.

COMPARATIVE STUDY FOR DETERMINING CELLULAR VIABILITY IN ISCHEMIA

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Background: Cell viability can be assessed using cell toxicity assays that provide a readout on markers of cell death, such as a loss of membrane integrity, fluorescent dye, colorimetric assays or DNA replication. Assays to measure cellular proliferation, cell viability, and cytotoxicity are commonly used to monitor the esponse and health of cells in culture.

Purpose: We intend to investigate comparatively two laboratory methods and to establish which of them expresses the most efficient the neural viability under ischemic conditions.

Methods: The LDH test quantitatively measures lactate dehydrogenase which results in the conversion of a tetrazolium salt into a formazan product. (Control test). Incubate the cytotoxicity assay plate for 3 hours in a humidified chamber at 37°C, 5% CO2, glucose free medium (Ischemia test). The WST-1 test is a quantitative colorimetric method based on the ability of metabolically active cells to cleave WST-1 to formazan (Control test).Incubate the cytotoxicity assay plate for 3 hours in a humidified chamber at 37°C, 5% CO2, glucose free medium (Ischemia test).

Results: There is a correlation of results obtained by LDH and WST-1 as methods for determining the neuronal redox status, but the difference between the value of viability in ischemia 3 hours by WST-1 (45%) and of viability in ischemia 3 hours by LDH (29,71%) is about the existence of neurons that can transform WST-1 (are metabolically active) but appear dead in LDH determination.

Conclusions: WST-1 determination allows the detection of the lesion before the loss of integrity of the cell membrane.We recommend the LDH test, but the amount of information is limited to the viable cell-dead cell response. This experiment shows the importance of separate counting of affected cells.

THE DIPPER STATUS AND THE ACC/AHA HIGH BLOOD PRESSURE GUIDELINES (2017). ARE THEY COMPATIBLE?

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Background: Elevated blood pressure is a condition affecting over 150 million patients in central and Eastern Europe, with a prevalence of 30-45% in the adult population. The american guideline defines normal blood pressure as lower than 120/80 mm Hg.

Purpose: The aim of our study is to determine if treated patients towards this threshold can still achieve a dipper status (10-20% dipping of BP overnight).

Material and Method: We have monitored (ABPM technique) 200 patients from the TopMed Health Center in Targu Mures and compared the dipper status with the final mean daily BP values.

Results: Of the studied group, only 45% patients had correct blood pressure control (below 140/90mm Hg daily mean values) and 15% had values defined as "normal" by AHA/ACC guidelines. In this group, only 10% of patients achieved the dipper status (mean values below 108mm Hg SBP overnight)

Conclusions: Only a very small percentage of the treated patients to "normal" values as defined by ACC/AHA guidelines can achieve a dipper status.

Discussions: 1. It is possible that aggressive BP control leads to lower blood pressure reduction overnight, making the dipper status difficult to attaint. 2. Further studies are needed to establish if this loss of the dipper status is associated with an increase of the cardiovascular risk or not.

THE ROLE OF NEUTROPHILIC EXTRACELLULAR TRAPS (NET) IN INITIATING INFLAMMATION IN PSORIASIS

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Recent research has drawn attention to activated neutrophils and neutrophilic extracellular traps (NETs) in psoriasis vulgaris, both in the skin lesions and in the serum of these patients. The immune theory in psoriasis vulgaris indicates a disorder of T lymphocytes, but the new theory (Katayama, 2018) speaks of a positive feedback loop, which is initiated by neutrophils (innate immunity) and produces IL-17 A before the classical activation of IL-23: 1. Normal dermal neutrophils are attracted to the epidermis by LTB4 2. The migration of neutrophils through the basement membrane transforms the cycle of keratinocytes from the G0 phase into G1 (which stimulates keratinocyte proliferation) 3. Neutrophils secrete IL-17 that produces CCL20 a ligand for T lymphocytes (which have CCR6) 4. Neutrophil-activated keratinocytes produce HSP70, which activates an endogenous ligand for TLR4 followed by production of IL-23. 5. Natural Th17 lymphocytes that possess CCR6 are recruited into the epidermis, where they become mature and pathogenic. Under the influence of IL-23, IL-1beta and IL-6, Th17 lymphocytes are activated to produce IL-17A. This creates a framework that promotes the IL-23 / IL-17A axis that maintains and aggravates psoriasis. In patients with psoriasis vulgaris, neutrophils are pre-activated and form NETs, which continue the inflammation even after neutrophils have undergone apoptosis.

ZERO ASTHMA – NEW INITIATIVE AGAINST ASTHMA ATTACKS AT 1 YEAR SINCE IMPLEMENTATION IN ROMANIA

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Background: Asthma is associated with a high disease burden. Emergency care for the treatment of asthma attacks is frequently used by asthma patients.

Purpose: The aim of this research was to understand the impact of severe asthma (SA) exacerbations (EX) and symptoms, as well as patients' perspectives regarding disease and treatment.

Methods: A quantitative research combining physicians and patients' questionnaires with medical charts review was performed in 2019 in Romania. 70 physicians and 433 asthma patients were enrolled. Patients were stratified according to disease severity; prescribed treatment, attitude to asthma treatment, disease outcome in terms of exacerbations and healthcare interventions: GPs, specialists, emergency room (ER) or hospitalizations (H), were analyzed. Physicians and patients were asked to rank treatment goals connected with asthma attacks, chronic symptoms, daily activities, access to the best therapy, adverse effects, pulmonary function, nighttime symptoms/sleep.

Results: 19.4% of patients had mild, 60.5% moderate and 20.1% severe asthma. For mild/ moderate/SA the mean number of EX/last year were 1,0/1,5/3,2. In the SA group 66% of patients went to ER visits, 71% had overnight H, 84% visited GPs, 94% visited a specialist. 82% from SA patients declared EXs were managed without a medical consultation. In contrast to milder asthma groups, the SA group placed preventing chronic symptoms higher (2.9 vs 2.0) and showed a decreased interest in performing daily activities (2.3 vs 3.2). Preventing asthma attacks was included in the top 3 priorities by both patients and physicians, with a mean score of 3.1, and 3.3 respectively.

Conclusions: For patients in severe stage, asthma was more likely to lead to hospitalizations, ER and GP visits. As asthma severity increases, patients tend to value symptoms improvement more than the ability to perform daily activities. It seems that the burden of severe stages makes patients to be satisfied with less.

CORRELATIONS BETWEEN FETAL HEART RATE AND MATERNAL BLOOD PRESSURE

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Background: The present study analyzed the variations in fetal heart rate depending on both maternal blood pressure values and other variables. The fetal heart rate was chosen as a parameter due to the multitude of physiological and pathological factors that can lead to its modification.

Purpose: The data available in the current literature have not yet provided conclusive results on these correlations, but due to the close link between the maternal and fetal circulatory system and possible clinical applications, fetal heartbeat modulation requires further study.

Methods: Our study is based on a collaboration between the Physiology Discipline I and the Obstetrics-Gynecology Clinic of the "Bucur" maternity hospital. We evaluated a group of 39 patients aged between 18 and 41 years. In addition to maternal systolic blood pressure and fetal heartbeat, we evaluated several parameters: gestational age, maternal heart rate, maternal body mass index, fetal birth weight and APGAR score obtained. The investigation of fetal heartbeat was performed by ultrasound method. All mother signed informed consent and their data were processed according to GDP regulations.

Results: We identified a tendency to increase fetal heart rate with increasing maternal systolic blood pressure and with increased maternal body mass index. We also identified the decrease fetal heart rate in babygirls with birth weights exceeding 3500 These results, as well as their reduced statistical significance, could be due to the fact that fetal heart rate is influenced by a multitude of factors, some with inter-individual variability, the design of the present study not including the possibility of analyzing several variables at the same time.

Conclusion: Due to this complexity of both physiological and pathological factors in

ELECTROCARDIOGRAPHIC CHANGES ASSOCIATED WITH ANXIETY

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Background: The prevalence of anxiety in the general population is 25%, with a higher prevalence in female. 50% of cases of anxiety disorders are misdiagnosed.

Purpose: Anxiety is one of the most debated health problems in recent years due to its association with an increased risk cardiovascular disease development. The special interest towards this subject is justified by the general impairment of the cardiovascular function with variable intensity.

Methods: We compared 14 subjects diagnosed with anxiety, based on Hamilton Anxiety Rating Scale, admitted in the "Al. Obregia" Psychiatry Hospital, Bucharest. All patients signed an informed consent at the admission in hospital, and their data were processed according to GDRP. We analyze their ECG recording and the blood pressure value compared to control (14 healthy students).

Results: Patients diagnosed with anxiety presented higher TAS and TAD values versus control group. We identified a significant increase in heart rate in patients with anxiety compared to the control group ($p < 0.05$). We identified significant increases in both the duration of the PR segment and the PR interval, correlated with the duration of atrioventricular conduction ($p < 0.05$). The duration of the P wave (atrial depolarization) was identified as significantly increased in patients with anxiety compared to the control group ($p < 0.05$). The duration and amplitude of the T wave were significantly higher in patients with anxiety compared to the control group ($p < 0.05$). T-wave variability is associated in the literature with increased arrhythmogenic potential leading to sudden death.

Conclusion: Identifying electrocardiographic changes associated with anxiety may be a measure to prevent cardiovascular disease.

CARDIOVASCULAR CHANGES ASSOCIATED WITH ENERGY DRINKS CONSUMPTION

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Background: Consumption of energy drinks is one of the health issues intensely debated in recent years due to its association with an increased risk of developing cardiovascular disease and the increased mortality rate caused by this behavior worldwide.

Purpose: In a 2010 study, the effect of energy drinks on platelets and endothelial function showed an increased platelet aggregation and decreased endothelial function, both changes being associated with myocardial infarction and sudden cardiac death.

Methods: In order to clarify these aspects, we analyzed the evolution of the following parameters: systolic and diastolic blood pressure, electrocardiogram changes (P,Q, R, S, T waves), PQ segments, PQ and QT intervals within a group of students. We investigated 32 students, who ingested ED (energy drink), in fact one dose of Red Bull wich contains 114 mg of caffeine and we measured all the parameters before, 5 minutes and 30 minutes after ED ingestion.

Results: The ED ingestion produced a significant increase ($p<0,05$) of the systolic and diastolic blood pressure at both sexes, 5 min after ingestion as well as 30 min after ingestion of ED. We observed a significant increase ($p<0,05$) of heart rate, at both sexes, only 30 min after ED ingestion. There were significantly reduced ($p<0,05$) the durations of the QRS complex, PQ and QT intervals, 30 min after ED ingestion, at both sexes. There was a significant increase ($p<0,05$) of the T wave amplitude at both sexes, 30 min after ED ingestion.

Conclusions: Consumption of energy drinks influences the fundamental properties of the myocardium (excitability, conductibility, contractility, automatism) and so predisposing to arrhythmia and contractility disorders, including young, healthy individuals.

HYPERVENTILATION INDUCED CEREBRAL VASOCONSTRICTION: A NEAR INFRARED SPECTROSCOPY INSIGHT

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Background: Hyperventilation test, widely used to identify cerebral hyperexcitation states and vestibular diseases, is accompanied by changes in the cerebral perfusion.

Purpose: In our study, we tested if an increase in the respiratory rate (RR) from 10/minute to 20/minute can trigger cerebral vasoconstriction and how the end-tidal CO2 and cerebral oxygenation change with the RR.

Material and Method: In eighteen healthy volunteers aged from 18 to 38 years, to whom a signed informed consent was previously obtained, we continuously monitored the tidal volume, RR, and end-tidal CO2 (mmHg), using an anesthesia monitor, and the cerebral oxygenation, using near infrared spectroscopy (NIRS), while performing an increase in ventilation from a baseline RR value of 10/minute to 20/minute, and then for 4 minutes at a RR of 20/minute. The end-tidal CO2 variation, decrease in oxyhemoglobin, and minute volume were analyzed.

Results: Our findings showed that consecutive to a RR of 20/minute for 4 minutes, only 12 subjects out of 18 presented cerebral vasoconstriction, and for these subjects, the end-tidal CO2 level decreased with 29% - 40% from the baseline value ($p<0.05$).

Conclusion: Considering that a respiratory rate of 20/min is not sufficient to induce cerebral vasoconstriction, more complex monitoring of the end-tidal CO2 and cerebral oximetry are recommended to avoid a negative test to hyperventilation.

NOVEL VACCINATION PLATFORM FOR COVID-19

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Introduction: Most vaccine strategies against SARS-CoV-2 infection currently in development are based on prerequisite platforms, such as non-replicating viral vectors, DNA, RNA, inactivated virus or protein subunits. The candidate vaccine proposed by OncoGen makes use of synthetic long peptides (SLPs), a cancer treatment derived strategy, aiming to induce T cell response mainly from CTLs with cytotoxic effect on infected self-cells.

Materials and methods: SLPs were constructed from S protein immunogenic epitopes, specific for MHC class I and class II molecules particular to the Romanian population immunophenotype. At the SLPs' N-terminus a 15 AA epitope MHC class II-restricted is linked using 6 AA linker with an MHC class I epitope of 8-9 AA. HLA class I and II-restricted epitopes were predicted using NetMHCpan software and IEDB tools. Toxicity prediction was assessed using the ToxinPred, IFN-gamma secretion was predicted with IFNepitope, while the allergenicity used AllerCatProde software. 3D structure prediction was generated with trRosetta, validated by Ramachandran graph. SLPs pool was tested in vitro on PBMCs cell culture from both healthy and COVID-19 healed patients, and activation of T cells was expressed as percentage of CD69+/CD134+ cells at days 7 and 14.

Results: Our SLPs pool encloses 9 SLPs, which are predicted to be immunogenic for 85-90% of the population. Activation of T cells was present in 0.5-1.5% after stimulation with SLPs' pool in vitro. The advantage of this construct is that APC uptake results in SLP's cleavage at the linker site, splitting in 2 distinct peptides available for both MHC classes to be presented to specific T cell populations.

Conclusion: As evidence is building that SARS-CoV-2 infection triggers mainly robust cellular immunity and only secondarily a humoral immune response, maybe it is time for a paradigm shift in anti-viral vaccine strategy ultimately leading to a more efficient prevention of COVID-19 occurrence.

CAR-BASED IMMUNOTHERAPY

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Introduction: Results of the CAR-T cells therapies showed complete remission of 70%-94% of the relapsed or refractory B-cell malignancies, targeting CD19, CD20 or CD30. In solid tumors, the limitations of these therapies are related to instability of the tumor cells, tumor heterogeneity, tumor microenvironment, or lack of target antigen. Here we show the possibility of obtaining a population of peripheral blood-derived killer cells transduced with CAR that can overcome the limitations of CAR-based therapies.

Materials and methods: The effector cell population was generated by cytokine-induced activation of primary cells. PBMCs isolated from WB from healthy donors were cultured in XVIVO medium, HuPlasma, IL-2, IL-21 and IL-15, adding CD3/CD28 T cell activation beads. Mixt populations were transduced with third generation of CAR @CD19 scFv-CD28-4-1BB-CD3z, expressed with the PGK promoter, or with cetuximab-based second generation of anti-EGFR CAR. Control cells were transduced and sorted at 5 days following transduction using FACSAria, based on GFP expression. Cytotoxic effects of CAR-T and CAR-NK cells were demonstrated by killing assays against specific cell lines: Nalm-6 (CD19+), U266 and K562 (CD19-), MDA-MB-468, SK-BR3, HT-29 (EGFR+).

Results: No significant difference in transduction efficiency per entire cell culture between PBMC and CD3+ or CD56+ enriched fraction. Highest viability was observed when transducing all PBMC. Transduction of T cells could be increased to 80% if stimulated with CD3/CD28 activation beads. Anti-CD19 CAR-T cells and CAR-NK cells kill target cells specifically with 70% cytotoxic effect on K562 and Nalm-6 cells, when the E:T is 10:1. Lentiviral transduction of the EGFR-CAR vector into activated cells resulted mostly in effector memory CD56+ CAR-expressing cells, with potent MHC-unrestricted cytotoxicity.

Conclusion: CAR-T cells and CAR-NK cells anti-CD19 and anti-EGFR demonstrated their efficiency in killing target cells, while NK cells also retaining NK cell receptor mediated recognition and killing of EGFR negative cells, making them a powerful tool for solid tumor therapy.

SMOKING-INDUCED CHANGES AND CORRELATIONS REGARDING PLASMA AND SALIVARY IMMUNOGLOBULINS IN HEALTHY INDIVIDUALS

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Background and purpose: We proposed to determine plasma and salivary immunoglobulins A and G and to establish the differences that occur in smokers compared to non-smokers, as well as the possible correlations.

Material and method: The study was performed on a number of 20 subjects, men and women, with a mean age of 37.85 years. Subjects were divided into 2 groups (10 healthy, smoking subjects, and 10 healthy, non-smoking subjects). Blood and saliva samples were collected and IgA and IgG concentrations were determined.

Results and discussions: In non-smokers we determined the following average values: plasmatic IgG = 14.06 ± 2.5 g/L, plasmatic IgA = 2.07 ± 0.64 g/L, plasmatic IgA/IgG ratio = 0.15 ± 0.3, salivary IgG = 2.26 ± 0.28 mg/dL, salivary IgA = 10.11 ± 2.94 mg/dL, salivary IgA/IgG ratio = 4.57 ± 1.55. In smokers we determined the following average values: plasmatic IgG = 12.32 ± 2.32 g/L, plasmatic IgA = 2.67 ± 0.67 g/L, plasmatic IgA/IgG ratio = 0.22 ± 0.29, salivary IgG = 2.56 ± 0.33 mg/dL, salivary IgA = 13.48 ± 5.02 mg/dL, salivary IgA/IgG ratio = 5.27 ± 15.19. The correlation of plasma and salivary values, in smokers, showed for IgG p = -0.23, for IgA p = 0.65, for the IgA/IgG ratio p = 0.11, and in non-smokers, for IgG p = 0.38, for IgA p = 0.31, and for the IgA/IgG ratio p = -0.44.

Conclusions: In smokers, plasmatic IgA was increased and plasmatic IgG was significantly decreased. Also, in smokers' saliva, IgG and IgA immunoglobulins were significantly increased. In non-smokers, we did not establish significant correlations between plasmatic and salivary values of any parameter we determined. Even in smokers, plasmatic IgG and IgA immunoglobulin levels do not correlate significantly with salivary ones.

ELLAGIC ACID EFFICACY IN EXPERIMENTAL INFLAMMATION

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Ellagic acid, a natural phenol found in fruits and vegetables, is a potent antioxidant and anti-inflammatory compound. The aim of this study was to evaluate the oxidative stress in experimental-induced carrageenan inflammation in rats that previously received ellagic acid in two forms: or in carboxymethyl cellulose (CMC) solution or as gold nanoparticles functionalized with ellagic acid (AuNPs-EA) in CMC. The animals received for 15 days 0.5 mL/day of CMC with or without medication through oral gavage. On day 15, the posterior right paw of the rats was injected with 0.2 ml Carrageenan 1%, to produce inflammation.

Plethysmometry was used to evaluate the paw's inflammation, at 2 hours and at 24 hours after injection. On day 15, inflamed paws' skin and blood samples were taken to investigate the oxidative stress through malondialdehyde, glutathione and glutathione disulfide. Plethysmometry showed the anti-oedematous effects of gold nanoparticles functionalised with ellagic acid. In inflamed skin, the lipid peroxidation was inhibited significantly by the ellagic acid ($p<0.05$) and AuNPs-EA form presented a bigger antioxidant effect ($p<0.01$). In serum, ellagic acid administration presented antioxidant effects, especially in the nanoparticles form ($p<0001$). Indomethacin administration (1 mg/kg/day) had anti-oedematous ($p<0.001$) and antioxidant effects in inflamed skin ($p<0.001$) and provided antioxidant protection ($p<0.001$) in serum of rats with carrageenan inflammation.

ANTIBIOTIC RESEARCH AND DEVELOPMENT IN THE THIRD MILLENNIUM

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Background: : A decline in both financial investment and scientific factors in antibiotic research and development is undermining global efforts in combating drug resistant infections and poses a serious threat to public health worldwide.

Purpose: The ongoing pandemic is a stark reminder of our vulnerability to infectious disease. Drug resistance is a problem that was observed since the early use of antibiotics which increased in severity over the past few decades. With the advent of technology in various areas of science, alongside the rapid emergence of drug resistant pathogens, it is crucial that we focus on innovation and creativity in order to yield new drugs that can rapidly offer a solution to the pressing issues of modern medicine. Our study sheds light on two new antimicrobial drugs, discovered using novel methodology in antimicrobial drug development, both of which display distinct mechanisms of action compared to any other known antibiotic.

Methods: Alternatives to the classic pipeline in antibiotic discovery have yielded two new molecules with antibiotic properties: Irresistin and Halicin.

Results: Two re-discovered drugs, relevant to antibiotic therapy, display activity against World Health Organization (WHO) priority pathogens. SCH-79797 (renamed Irresistin) has a dual mechanism of action: inhibits folate and affects cell membrane integrity. SU-3327 (renamed Halicin) temporarily disrupts protein flow across cell membrane.

Conclusions: New and innovative methods employing advanced molecular biology and artificial intelligence can potentially offer a new paradigm in antibiotic research and development, bypassing traditional hurdles such as time constraints, high costs, and antibiotic resistance.

CORRELATIONS BETWEEN AORTIC ROOT MOVEMENT AND E/A RATION IN LEFT VENTRICLE DIASTOLIC DYSFUNCTION PATIENTS

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Introduction: Diastolic dysfunction occurs when the left ventricular myocardium is non-compliant and not able to accept blood return in a normal fashion from the left atrium. This can be a normal physiologic change with aging of the heart or result in elevated left atrial pressures leading to the clinical manifestations of diastolic congestive heart failure. Usually the diastolic dysfunction is evaluated by sonography, using various methods (E/A ratio, tissue Doppler, etc). One of the easily measurable parameters by B, Doppler or M mode echography is the aortic ring movement, which should be affected by a decrease of left ventricular compliance.

Purpose: The aim of our study is to establish a correlation between E/A ratio and the amplitude of the aortic root movement.

Material and method: The study included 100 patients examined by 2D echocardiography at the TopMed medical center in Tg. Mures. E/A ratio and aortic movement were assessed in Doppler/M mode for each patient. In this study, patients with deceleration of the E wave below 200ms were not included.

Results: The mean aortic movement for patients with E/A ratio > 1 was 18mm (+/- 3mm). In the diastolic dysfunction group (E/A < 1) we measured a mean movement of 13mm (+/- 2mm), with a reduction of 27.8% ($p<0.05$).

Conclusion: According to our study, diastolic dysfunction induces a restriction in aortic ring movement which can be used as a diastolic dysfunction parameter.

Discussions: 1. Our study is small, further investigations are needed to establish the validity of aortic ring movement as a criteria for impaired relaxation. 2. In our study patients with grade II, III and IV diastolic dysfunction were not included, therefore our results are limited to impaired relaxation stage.

EXPRESSION OF PHYSIOLOGICAL VARIATIONS IN THE IMAGING OF THE ENDOMETRIUM

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Background: A great challenge in imaging the female pelvis is accounting for hormone-induced physiological variations. The endometrial lining is a particularly interesting topic, as diffuse anomalies may also be encountered in malignancies, infectious diseases, various proliferative disorders, and iatrogenic causes. Therefore, a good knowledge of the specific imaging features of the endometrium can lead to a solid diagnosis.

Purpose: This study aims to correlate the imaging aspects of the endometrium with physiological changes, in diagnosed patients. The pearls and pitfalls of endometrial imaging by Magnetic Resonance Imaging (MRI) are also presented.

Methods: Forty-one premenopausal female patients with endometrial anomalies on MRI were included in this study, investigated between January-July 2020. A standard scanning protocol for female pelvis was employed, using contrast media. The zonal anatomy, thickness, and homogeneity of the endometrium as well as focal lesions or intraluminal content, when found, were noted.

Results: Endometrial hyperplasia was found in 35 cases. Nine patients showed post-partum thickening of the endometrium, 5 cases were diagnosed as intrauterine device-related endometritis while 4 patients showed hyperplasia related to estrogen-stimulating drugs. Six cases had intrauterine adhesions. Four patients showed focal anomalies induced by a Cesarean section. Seven patients were diagnosed with endometrial carcinoma, while another 3 patients
showed small endometrial polyps. Hyperplasia was idiopathic in 3 cases.

Conclusions: Physiological changes account for an important percentage of cases with endometrial anomalies. Identification of specific imaging features and correct integration of clinical data is paramount for establishing a correct diagnosis.

CROSS-FREQUENCY COUPLING IN THE EEG OF PILOCARPINE-INDUCED EPILEPTIC RATS

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In continuous electrophysiological signals interaction of rhythms in different bands can be observed, which is denominated cross-frequency coupling. Neuronal oscillations determine rhythmic changes in cortical excitability which affect local computation and long-range communication. Low frequencies influence activity over large regions in long temporal windows, while high frequencies are generated transiently within smaller regions.

Cross-frequency coupling can be phase-phase, amplitude-amplitude or phase-amplitude coupling. The latter is the most studied and means a statistical dependence between the phase of a low-frequency oscillation and the power (amplitude) of a high-frequency oscillation. At the moment there are several quantitative methods in use to measure cross-frequency coupling, but none of these can be regarded as a gold standard, rather each has advantages and disadvantages and is more suitable for a specific goal. Most of these methods require long segments of experimental data, therefore are not optimal for studying fast changes in coupling related to behavior. Time-resolved phase-amplitude coupling measure (tPAC) is a novel method with high temporal resolution and sensitivity to obtain a comodulogram and to detect the peak value of coupling strength between slow and fast rhythms over sliding time windows.

We tested tPAC by determining the modification of coupling strength between delta (1-4 Hz) oscillations and gamma (30-100 Hz), high-frequency oscillations (HFO, 100-150 Hz), ripples (150-250 Hz) and fast ripples (250-600) in the early chronic phase of the pilocarpine model of temporal lobe epilepsy. A robust increase of coupling strength was observed in the pilocarpine treated animals compared to healthy ones. The difference was most outstanding in the delta-fast ripple coupling.

These results indicate that phase-amplitude coupling analysis can be a useful tool for diagnosis of the disease, and highlights the importance of very high-frequency bands (e.g. fast ripples) in the diagnosis of epilepsy.

THE DIFFERENTIATION POTENTIAL OF MESENCHYMAL STEM CELLS CO-CULTURED IN VITRO WITH PERIPHERAL BLOOD MONONUCLEAR CELLS

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Background: Stem cells provide a source of target cells with multipotent differentiation capacity, promoting tissue homeostasis, growth and repair. Currently, the co-culture strategies are being discussed for several tissues restoration, including the bone.

Purpose: The present study is an in vitro experiment evaluating how the relationship of mesenchymal stem cells (MSCs) with peripheral blood mononuclear cells (PBMCs) may affect the differentiation of stem cells.

Methods: Hoffa MSCs, isolated by explant method, were initially frozen after flow-cytometric confirmation of phenotype. PBMCs were isolated by density gradient centrifugation of healthy volunteers' peripheral blood samples in parallel with MSCs thawing procedures. The cells were placed in direct co-culture, in MSCs proliferation media as well as adipogenic or osteogenic differentiation media. The evaluation was performed by cytochemistry (alkaline phosphatase, Oil Red O).

Results: After freezing-thawing procedures, MSCs in specific media maintained their differentiation potential to adipocytes and osteoblastic lineage. In adipogenic differentiation experiments, the best response was obtained in case of MSCs monoculture in adipogenic medium, while in vitro co-culture with PBMCs reduces the turn to adipocytes whether differentiation medium or proliferation medium was used. The osteoblastic differentiation was favoured by MSCs in vitro co-culture with PBMCs in osteogenic as well as in simple proliferation medium.

Conclusions: The presence of PBMCs changes the in vitro balance of MSCs differentiation from a slight prevalence for adipogenesis to a shift to the osteoblastic line, opening the prospect of using mononuclear cells as adjuvant therapy when procedures based on stem cells are used for bone recovery.

ONLINE MEDICAL EDUCATION - BETWEEN CHALLENGES AND OPPORTUNITIES

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The Covid19 pandemic lockdown brought about the need to conduct the teaching process exclusively online. UMF Timișoara, initially unprepared, adapted to this situation in order to avoid the postponement of the academic year. Our discipline took the initiative early on, and was the first to teach online - lectures and practical laboratories - using Zoom, GotoMeeting and Microsoft Teams. The results appear to have been superior to the classical method – however we do not yet know if that is an accurate reflection of reality. Specialty literature argues a number of advantages and disadvantages of online education. Its strengths are flexibility of time and place, ease of access, real-time contact, low cost, independence of location. In opposition, its weaknesses are need for self-motivation and discipline, impersonal (limited social interaction), liable to technical errors, some technological requirements, difficulties in practical work, and especially difficulties in student assessment. A study by Yazzie-Mintz (2010) showed that even under typical teaching conditions, ~30% of students lose interest during class due to the absence of direct interaction. It seems very likely that this will worsen in the virtual environment - which is why we need various methods to increase the interest of students. One solution comes from a Harvard University research team, who concluded that interspersing multimedia content and online learning materials with short tests at regular intervals can improve student involvement. The inclusion in the above mentioned topics is mandatory.

THE INFLUENCE OF SURGICAL TECHNIQUE ON OXIDATIVE STRESS IN PATIENTS WITH VENOUS INSUFFICIENCY

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Background: Chronic venous insufficiency is a common medical problem and can manifests in various clinical aspects, from cosmetic problems to severe symptoms, such as venous ulcer. Oxidative stress, as result of the imbalance between reactive oxygen species formation and enzymatic and nonenzymatic antioxidant systems, plays an important role in the pathophysiology of venous insufficiency. Open surgical therapy of varicose veins with high ligation and stripping of the great saphene vein (GSV) combined with the excision of large varicose veins has been the standard of care, with complications including bleeding, hematoma, infection, and nerve injury. Nowadays endovenous ablation therapy has largely replaced this classic ligation and stripping.

Purpose: Current study examined the oxidative stress parameters in patient submitted to clasical versus laser therapy of varicose veins.

Methods: We studied two groups of pacients undergoing classic surgery versus laser surgery, and we measured the oxidative stress parameters.

Results: MDA levels were found significantly ($P < 0.005$) higher in patients with open surgical therapy compared with laser group. There were no significant differences between the groups in terms of the other parameters ($P > 0.05$).

Conclusion: This study showed that patients with open surgical therapy have increased oxidative stress compared with pacient with laser therapy.

Keywords: oxidative stress, venous insufficiency

CEREBELLUM AS A MODULATOR OF THE CEREBRAL CORTICAL ACTIVITY – AN EXPERIMENTAL STUDY IN MICE

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The cerebellum is involved in motor learning and coordination, and in cognitive processing. It sends projections to a broad array of cortical brain areas, such as sensory and motor areas, prefrontal cortex, and the limbic system. Cerebellar lesions can cause movement disorders such as dystonia. We assessed the effects of repeated cerebellar cortex stimulation on behavioral and oscillatory brain activity in albino mice. The cerebellar cortex was stimulated by microinjections of kainate into the vermis and the left cerebellar cortex, for five consecutive days. The electromyography (EMG) of neck muscles, and the electrocorticogram (ECoG) of somatosensory, motor, and parietal cortices were recorded. Our results showed that repeated microinjections of kainate into the cerebellar cortex generate dystonic behavior. The vermal application induced generalized dystonia, decreased coherence between the motor cortex and somatosensory or parietal cortices, and between the left and right motor cortices, compared to baseline, except for the gamma bands that gradually increased until the last day of experiment. The left cerebellar cortex stimulation generated left side lateralized dystonia, reduced the interhemispheric coherence between left and right primary motor cortices in the delta, theta, and beta bands. The ECoG-EMGcoherence of the affected body part decreased in the delta and theta bands in the first two days, increased in theta and beta bands in days 4 and 5, and increased gradually until day 5 in gamma band, compared to baseline. In conclusion, we showed that altered cerebellar output disrupts the communication between the cortical sensorimotor and parietal network, and between primary motor cortices, suggesting that cerebellum is a modulator of the cortical activity.

ASSESSMENT OF THE EFFECTS OF A BENZYLAMIDE DERIVATIVE OF MASLINIC ACID ON CELLULAR BIOENERGETICS IN A375 HUMAN MELANOMA CELLS

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Maslinic acid belonging to the class of pentacyclic triterpene has been reported to possess various therapeutic effects, including antitumor properties. Furthermore, derivatives of maslinic acid have been indicated to elicit improved anticancer activity compared to the parent compound.

The present study was purposed to evaluate the effects of a benzylamide derivative of maslinic acid (benzyl (2α, 3β) 2,3-diacetoxy-olean-12-en-28-amide) on mitochondrial bioenergetics in A375 human melanoma cells. Mitochondrial bioenergetics was measured by using Seahorse Bioscience XF24 extracellular flux analyzer. A number of 10K cells/well were seeded in culture plates and further stimulated with two concentrations of the derivative (1 and 5 μM). The oxygen consumption rate (OCR) and the extracellular acidification rate (ECAR) were evaluated at 72h post-treatment. Cells were metabolically perturbed by 3 successive compound additions that shifted the bioenergetic profile of the cells: oligomycin, FCCP, and antimycin A plus rotenone. The OCR parameters recorded were: (i) the basal respiration; (ii) the leak state (after oligomycin addition); (iii) the maximal respiration (after FCCP addition); (iv) the ATP turnover (the difference between the basal respiration and the leak state); (v) the reserve capacity (the difference between the maximal and the basal respiration). At both tested concentrations, the compound elicited a decrease in the OCR parameters. Furthermore, treatment with EM2 decreased ECAR, indicating that the compound induced an impairment in mitochondrial respiration on melanoma cells. The data obtained indicate that administration of EM2 provoked an alteration of the bioenergetic profile in A375 melanoma cells, an effect that may underlie its beneficial antitumor effects.

ASSESSMENT OF THE CYTOTOXIC AND ANTIMIGRATORY EFFECTS OF A BENZYLAMIDE DERIVATIVE OF MASLINIC ACID ON B16F0 MURINE MELANOMA CELL LINE

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Maslinic acid is a naturally occurring triterpene found in the species Olea europaea L., (Oleaceae family), reported to possess various beneficial effects including antitumor properties [1]. In order to improve the anticancer activity several maslinic acid derivatives were obtained [2].

The present study was aimed to determine the cytotoxic and antimigratory properties of a benzylamide derivative of maslinic acid, "EM2" (Benzyl (2α, 3β) 2,3-diacetoxy-olean-12-en-28-amide) on B16F0 murine melanoma cell line. Various concentrations of EM2, namely 1, 5, 10, 25 and 50 μM were tested on B16F0 murine melanoma cell line. Cells viability was assessed by means of MTT assay at various periods of time (24, 48 and 72h); the antimigratory properties were evaluated using Scratch assay. At 24hpost-stimulation, EM2 provoked a decreased in tumor cell viability only at the highest dose tested (50 μM), whereas at 48h after stimulation a significant reduction in tumor cells viability was obtained at 25 and 50 μM. The data obtained at 72h after stimulation showed that EM2 elicited a cytotoxic effect starting from the concentration 10 μM. Regarding the antimigratory activity, the compound EM2 produced a reduction in the migration capacity following stimulation with the concentration 5 μM. Our results showed that EM2 had a significant dose and time dependent cytotoxic effect against the tumor cells especially at 72h post-stimulation. The compound also reduced melanoma cells migration at concentrations raging from 5 to 50 μM.

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