"VICTOR BABEȘ" National Institute of Pathology Annual Scientific Meeting & 13th National Pathology Symposium

November 5-7, 2020

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BOOK OF ABSTRACTS

Topics:

Cellular Pathology - Molecular Pathology - Histopathology -
- Nephropathology - Omics in Pathology -
- Neuropathology and Neuroregeneration -
- Varia -

“VICTOR BABEȘ” NATIONAL INSTITUTE OF PATHOLOGY

ANNUAL SCIENTIFIC MEETING

& 13TH NATIONAL PATHOLOGY SYMPOSIUM

BOOK OF ABSTRACTS

NOVEMBER 5 – 7, 2020

BUCHAREST, ROMANIA

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"Victor Babeș" National Institute of Pathology, Bucharest

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PROGRAM

Day 1 – Thursday, November 5

09:30 – 10:00

Opening ceremony

Prof. Univ. Dr. Mihail Eugen Hinescu – Director General al Institutului Național de Patologie „Victor Babeș" București

Prof. Univ. Dr. Ing. Adrian Curaj - Director General al Unității Executive pentru Finanțarea Învățământului Superior, a Cercetării, Dezvoltării și Inovării

Prof. Univ. Dr. Viorel Jinga - Rector al Facultății de Medicină și Farmacie „Carol Davila" București

10:00 – 11:30

"Victor Babeș" Annual Conference

New therapeutic targets in malignant disorders of the hematopoietic system. Prof. Ștefan N. Constantinescu - Cell Signaling Pole at the Université catholique de Louvain's de Duve Institute; Ludwig Institute for Cancer Research, Brussels, Belgium

11:30 – 12:00 break

12:00 – 13:00

Plenary Lecture 1

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13:00 – 14:00 break

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ARTEMIS Workshop - Genomic mapping in areas polluted by pro-carcinogens

Moderators – Prof. Ioana Berindan-Neagoe & Dr. Gina Manda

The ARTEMIS project - Genomic population mapping of radioactive and heavy metals in order to increase national security. Ioana Berindan-Neagoe

Histological evaluation in BALB/c mice chronically exposed to toxic compounds. Claudiu Gal, Mihaela Diaconu, Gina Manda, Ioana Berindan-Neagoe, Adrian Onu, Crina Stăvaru

Physiopathological aspects induced in BALB/c mice acutely exposed to manganese(II) chloride. Mihaela Diaconu, Claudiu Gal, Mariana Văduva, Petronica Gheorghiu, Ana-Maria Teodoru, Simona Popoiu, Gina Manda, Ioana Berindan-Neagoe, Adrian Onu, Crina Stăvaru

Dosimetry of wastewater from thyroid carcinoma patients treated with radioactive iodine I-131. Doina Piciu, **Cristina Moisescu-Goia**, Elena Olariu (Barbus), Eduard-Alexandru Bonci, Katalin Gabora, Marius Badan, Adrian Stoian

Mass spectrometric analysis of differential protein expression in the blood plasma of healthy versus cancerous individuals from radioactively and/or heavy metal contaminated regions. Aura-Elena Ionescu, Simona Călăraş, Doina Piciu, Ştefan Eugen Szedlacsek

Expression of stress responsive genes in human monocytes exposed to ionizing radiation. Gina Manda, Maria Dobre, Ionela Victoria Neagoe, Elena Milanesi, Cristian Postolache, Ulrich Weber, Nicole Averbeck

Arsenate target specifically cell death mechanisms: implication in breast cancer management. Cornelia Braicu, Lavinia-Lorena Pruteanu, Dezsö Módos, Maria-Ancuţa Jurj, Oana Zanoaga, Lajos-Zsolt Raduly, Crina Stavaru, Eugen Gurzău, Andreas Bender, Ioana Berindan-Neagoe

16:00 – 16:15 break

16:15 – 18:00

Session 1: UpToDate in Gynecological Pathology

Moderator – Prof. Gabriel Becheanu

UpToDate in the diagnosis of cervical squamous lesions: WHO 2020 classification. Prof. Raji Ganesan - Birmingham Women's and Children's NHS Foundation Trust, Birmingham, UK

UpToDate in the diagnosis of endocervical glandular lesions: WHO/IECC 2020 classification. Prof. Simona Stolnicu - University of Medicine, Pharmacy, Sciences and Technology of Targu Mures, Romania

18:00 – 18:15 break

18:15 – 20:00

Session 2: Research and Communication

Prof. Dan Vasiliu

Prof. Mihail E. Hinescu

Day 2 – Friday, November 6

09:00 – 11:30

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Genetic disorders affecting oral health. **Emilia Severin**, George Gabriel Moldoveanu, Andreea Pădun-Moldoveanu

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11:30 – 11:45 break

11:45 – 13:15

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	Pregnancy-onset nephrotic syndrome in a patient with type 1 diabetes mellitus. **Ioana Ailincăi,** Gener Ismail
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Day 3 – Saturday, November 7

10:00 – 12:30

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Moderator – Dr. Ana-Maria Enciu

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Neuroprotective effects of allicin on the hippocampus in a rat model of traumatic brain injury. **Bogdan Dume,** Dragoș Mihalache, Ioana Roman, Bogdan Sevastre, Vlad. Al. Toma

The role of β-cyclodextrins in improving the anti-fibrotic effectiveness of silymarin in a mouse model of liver fibrosis. **Sami Gharbia**, Cornel Balta, Hildegard Herman, Marcel Rosu, Judit Váradi, Ferenc Fenyvesi, Anca Hermenean, Marieta Costache

Lipopolysaccharide-induced inflammation triggers cytoskeletal reorganisation and calcium signaling alteration in brain microvascular endothelium. **Călin Mircea Rusu,** Roberta Stoica , Antonia Teona Deftu , Alexandra Bîngă , Adela Banciu , Daniel Dumitru Banciu, Mihai Radu, Beatrice Mihaela Radu

The award for the best presentation of a young researcher *– a high performance laptop sponsored by RoneXprim.*

12:30 – 13:00	break
13:00 – 14:30	**Session 8: Telocytes in regeneration and pathology** **Moderator – Dr. Mihaela Gherghiceanu** **Telocytes in salivary gland regeneration. Mihnea Ioan Nicolescu** **Telocytes in skin pathology. Cătălin Gabriel Manole** **Telocytes in cardiac regeneration. Mihaela Gherghiceanu**

DAY 1 – THURSDAY, NOVEMBER 5

Opening Ceremony

„Victor Babeș" Annual Conference

Plenary Lecture 1

Workshop ARTEMIS

Session 1: UpToDate in Gynecological Pathology

Session 2: Research and Communication

"Victor Babeș" Annual Conference

NEW THERAPEUTIC TARGETS IN MALIGNANT DISORDERS OF THE HEMATOPOIETIC SYSTEM

Prof. Ștefan N. Constantinescu
Cell Signaling Pole at the Université catholique de Louvain's de Duve Institute; Ludwig Institute for Cancer Research, Brussels, Belgium

Plenary Lecture 1

BLOOD TRANSCRIPT SIGNATURES IN MILD DEMENTIA: A TARGETED TRANSCRIPTOMICS STUDY

Antonio Cuadrado [1,2], Elena Milanesi [1], Maria Dobre [1], Anca Cucos [1], Gerard Piñol-Ripoll [3], Gabriela Niculescu [4], Gina Manda [1]

[1] *"Victor Babes" National Institute of Pathology, Bucharest, Romania*
[2] *Instituto de Investigaciones Biomédicas "Alberto Sols" UAM-CSIC &Faculty of Medicine, Autonomous University of Madrid & Instituto de Investigación Sanitaria La Paz (IdiPaz) & Centro de Investigación Biomédica en Red Sobre Enfermedades Neurodegenerativas (CIBERNED), Madrid, Spain*
[3] *Unitat Trastons Cognitius, Hospital Universitari Santa Maria-IRBLLeida, Lleida, Spain*
[4] *Medinst Diagnostic & Polytechnic University of Bucharest, Bucharest, Romania*

Low-grade inflammation and alteration of the redox balance underly many chronic aging-related diseases (neurodegenerative, cardiovascular and metabolic diseases), sometimes starting many years before the occurrence of clinical symptoms.
Although extensive evidence of inflammation and redox alterations in the brain of patients with Alzheimer disease (AD) or mild cognitive impairment (MCI) is available, the fingerprint of these processes in blood leukocytes, as sensors and triggers of pathological events in neurodegeneration, has not been fully clarified yet.
In this context, the case-control study performed in the REDBRAIN project aimed to characterize the expression profile of 168 inflammation and redox genes in the blood of 38 MCI patients and 38 matched controls. Conventional disease-specific biomarkers (Aβ, phosphorylated Tau and total Tau) were comparatively assessed in cerebrospinal fluid and plasma. Gene expression was assessed in whole blood by qRT-PCR using targeted arrays. Particular genes that were found significantly over-expressed in MCI patients as compared to controls were gradually selected using various bioinformatic tools for identifying the most promising candidate biomarkers and potential therapeutic targets. MCI patients were investigated before the initiation of disease-specific treatment and again after one year of therapy with rivastigmine or donepezil.
A panel of seven inflammation genes involved in the canonical and non-canonical NFkB signalling pathway, toll-like receptor and interferon signalling pathways, complemented by eight redox genes participating in reactive oxygen species (ROS) production, mainly superoxide, genes that are responsive to increased ROS levels and antioxidant genes involved glutathione and thioredoxin metabolism, part of them being targets of the cytoprotective NRF2 transcription factor, were shown to make the distinction between MCI patients and controls in whole blood. The correlation study between inflammation and redox genes highlighted that by pharmacological targeting of redox genes a simultaneous inhibition of the chronic low-grade inflammation might be obtained. Moreover, the study showed that

the treatment with rivastigmine or donepezil induced after one year the normalization of both inflammation and redox markers in MCI patients.
Concluding, the REDBRAIN case-control study highlighted that blood leukocytes carry a pathological transcriptional fingerprint of inflammation and redox alterations in MCI patients. The identified genes exhibiting over-expression in patients could be valuable drug candidates for keeping under control the disease-associated systemic inflammation and redox disturbances.

Acknowledgement: Research was supported by the Competitiveness Operational Programme 2014 2020 through the grant P_37_732 (REDBRAIN).

Workshop ARTEMIS -
Genomic Mapping In Areas Polluted By Pro-Carcinogens

Moderators – Prof. Ioana Berindan-Neagoe & Dr. Gina Manda

THE ARTEMIS PROJECT - GENOMIC POPULATION MAPPING OF RADIOACTIVE AND HEAVY METALS IN ORDER TO INCREASE NATIONAL SECURITY

Ioana Berindan-Neagoe

HISTOLOGICAL EVALUATION IN BALB/C MICE CHRONICALLY EXPOSED TO TOXIC COMPOUNDS

Claudiu Gal [1], Mihaela Diaconu [1,2], Gina Manda [3], Adrian Onu [1], Crina Stăvaru [1]

[1] *Cantacuzino National Medical Military Institute for Research and Development, Bucharest, Romania*

[2] *University of Bucharest, Faculty of Biology, Bucharest, Romania*

[3] *Victor Babeș National Institute of Pathology, Bucharest, Romania*

Water pollution represents a constant issue for the ecosystem, with residues from the mining and chemical industries being some of the main pollutants affecting the groundwater, lakes and rivers from which many toxic compounds have hazardous effects on human health, some with implications yet to be discovered. Consequently, the aim of this study was to create an experimental mouse model for oral chronic toxicity through drinking water, using manganese (II) chloride (MnCl2) and γ-hexachlorocyclohexane (Lindane), two compounds found in high concentrations in some regions of Romania.

Three groups of Balb/C female mice, 4-6 weeks old, were each exposed to 10 g/L MnCl2, respectively 5 mg/L γ-HCH in drinking water for 6 months, along with a third group that received normal tap water and served as control. Tissue samples of liver, kidney, lung, stomach, small intestine and pancreas from 10 mice of each group were formalin fixed, paraffin embedded, 5 µm thick sectioned and hematoxylin-eosin stained.

The manganese-treated group presented moderate to severe diffuse glycogen accumulation in hepatocytes whereas both lindane and control groups showed minimal to mild cytoplasmic changes. Small to moderate foci of mainly lymphocytes, plasma cells, and rarely neutrophils were present in the manganese-exposed group, sometimes accompanying hepatocyte necrosis. Similar inflammatory infiltrate was also present in the portal spaces in a small number of mice, sometimes with bile duct hyperplasia and incipient fibrosis. Similar foci were rare in the lindane and control group. Some mice of the lindane-treated group presented multifocal kidney lesions, such as thickening of the basal membrane of the glomeruli and basophilia of the proximal tubule epithelial cells. The other examined organs of both manganese and lindane treated groups did not seem to present any morphological lesions.

The encountered lesions can be graded as minimal to moderate and appear to be specific to chronic toxicity and related to the specific ways of excretion of each compound. Thus, manganese biliary excretion might influence the hepatocellular metabolism, leading to excess storage of glycogen in the hepatocytes as well as cellular necrosis. Lindane is also metabolized in the liver but it is usually readily conjugated and excreted as various metabolites through the kidney. Indeed, high amounts of lindane have been previously associated with tubular necrosis of the kidney, but the exact mechanism is unclear.

Keywords: histology, manganese, lindane, toxicity, chronic

Acknowledgement: This work is supported by the Ministry of Research and Innovation, project number PN-III-P1-1.2-PCCDI-2017-0737 (ARTEMIS).

PHYSIOPATHOLOGICAL ASPECTS INDUCED IN BALB/C MICE ACUTELY EXPOSED TO MANGANESE (II) CHLORIDE

Mihaela Diaconu [1], Claudiu Gal [1,2], Mariana Vaduva [1], Petronica Gheorghiu [1], Ana-Maria Teodoru [1], Simona Popoiu [1], Gina Manda [3], Ioana Berindan Neagoe [4], Adrian Onu [1,5], Crina Stavaru [1]

[1] *Cantacuzino National Medical Military Institute for Research and Development, Bucharest, Romania; University of Bucharest, Faculty of Biology, Bucharest, Romania*

[2] *University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Bucharest, Romania*

[3] *Victor Babes National Institute of Pathology, Bucharest, Romania*

[4] *Iuliu Hatieganu University of Medicine and Pharmacy, Research Center for Functional Genomics and Translational Medicine, Cluj-Napoca, Romania*

Manganese is an essential trace element in the human diet, required for various processes, including bone growth, immune function, blood sugar regulation, digestion, and hemostasis. However, manganese normal levels of the body can be exceeded due to dietary or respiratory exposure, or to malfunctions of its absorption, transport or excretion. Consequently, this metal accumulates in specific tissues, producing organ system impairments, out of which the best characterized is manganism, an extra-pyramidal neurological disorder, clinically similar to Parkinson's disease.

Although manganese is toxic at high levels, very little is documented about its effects on the hematological and biochemical profiles of the affected individuals. Thus, to further explore manganese time and dose-dependent toxicity and its associated functional and histological modifications, we carried out an in vivo study, using mouse as a model. Specifically, two groups of 5-15 BALB/c females, 7-9 weeks old, were acutely treated with manganese(II) chloride thrice a week, as follows: one group received 150 mg/kg BW of this compound by eight subcutaneous (SC) injections, and the other received ten doses of 500 mg/kg BW manganese salt by oral gavage (GV). Next, clinical signs, weight gain, hematological, biochemical and histological profiles of these animals were monitored, and compared with the corresponding sham control animals, which received 0.9% saline solution.

SC acutely exposed mice had a faster systemic absorption and a lower clearance of manganese, as compared with GV-treated group. Histological examination of these animals indicated that the SC group developed fulminant hepatitis, with necrosis of the liver parenchyma, whereas only a few mice of the GV group had mild hepatitis. Indeed, the parameters of hepatic panel were substantially altered in the SC group with respect to the control values. The albumin level was drastically decreased, while globulin level was highly increased, in the SC mice versus control (P 0.0001 and P 0.0001, respectively). Moreover, the activity of liver enzyme alanine aminotransferase was notably higher in the same group, when compared with control (P = 0.0002). The hematological profile indicated a remarkable increase of the absolute counts of both neutrophils and monocytes (P = 0.0002 and P = 0.013, respectively), whereas the absolute number of lymphocytes was significantly decreased (P = 0.036) in the SC group versus control. Notably, both SC- and GV-treated groups had a

significant increase in the absolute number of reticulocytes, compared with the matched controls (P = 0.033 and P = 0.006, respectively).
The study showed that manganese toxicity is primarily associated with liver dysfunctions. In addition, neutrophilia and monocytosis indicated the innate immune cellular response to manganese acute exposure, whereas reticulocytosis and other variations of the erythrogram values are suggestive of microcytic anemia in the SC group.

Keywords: biochemistry, hematology, histology, toxicity
Acknowledgement: This work was supported by the Ministry of Research and Innovation, under Grant number PN-III-P1-1.2-PCCDI-2017-0737 (ARTEMIS).

DOSIMETRY OF WASTEWATER FROM THYROID CARCINOMA PATIENTS TREATED WITH RADIOACTIVE IODINE I-131

Piciu Doina, Moisescu-Goia Cristina, Olariu (Barbus) Elena, Bonci Eduard-Alexandru, Gabora Katalin, Badan Marius, Stoian Adrian

Institute of Oncology Prof.Dr.Ion Chiricuta Cluj-Napoca

The thyroid carcinoma is one of the most common types of cancer, ranking 20th in the world in terms of number of cases, and 9th in the European ranking. This value is also reflected in the cases in Romania, where there is an annual increase in the number of cases. Particularly, in the Institute of Oncology „Prof.dr.Ion Chiricuță" Cluj-Napoca (IOCN) the number of cases increased progressively from 5-10 cases/year in the 70s, now reaching over 800 new cases/year annually. The treatment of choice for differentiated thyroid cancer is radiotherapy, which consists of the oral administration of radioactive iodine to these patients. The dose administered is individualized according to several criteria and varies between 1.11-3.7 GBq. Radioactive iodine is eliminated through perspiration, saliva and urine, which is why it is important to monitor effluents in a nuclear medicine therapy laboratory for hospitalized patients. The national nuclear protection legislation requires careful monitoring of effluents before they are discharged to the common sewer, imposing limits on radioactive activity, such as a maximum activity/discharge of 2.5 ALImin but not more than 100 MBq, and in one month it cannot the maximum total activity of 25 ALImin is exceeded, so as not to exceed the total value/year of 100 GBq. Careful monitoring can be achieved by repeatedly taking water samples and performing gamma spectrometry analyzes. The samples taken are subjected to a double test by measuring the activity of each sample in the dose calibrator, then being subjected to the spectrometric test. The gamma spectrometer we have requires small amounts of material (15 ml) and can identify traces of radioactive isotopes with Bq/ml activity. At the end of the spectrometry, a report is generated containing the identification data of the sample, the quantity, date and time of the analysis, as well as the identified isotopes, their energy and activity. The values obtained are related to the volume of the pool from which the sample was taken to calculate the total activity of the pool. The analyzes performed showed values between 0.00 MBq/pool (after 10 halves times) and 5856 GBq/pool; the discharge to the common sewerage is made according to national regulation and environmental security.

Conclusion: Proper monitoring of effluents from nuclear therapy laboratories helps to avoid contamination of surface waters with radioactive material.

Keywords: thyroid cancer, radioactive iodine, gamma spectrometry, wastewater

MASS SPECTROMETRIC ANALYSIS OF DIFFERENTIAL PROTEIN EXPRESSION IN THE BLOOD PLASMA OF HEALTHY VERSUS CANCEROUS INDIVIDUALS FROM RADIOACTIVELY AND/OR HEAVY METAL CONTAMINATED REGIONS

Aura-Elena Ionescu [1], Simona Călăraş [1], Doina Piciu [2], Ştefan Eugen Szedlacsek [1]

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[2] *Department of Endocrine Tumors and Nuclear Medicine, The Oncology Institute "Prof. Dr. Ion Chiricuţă", 34-36 Republicii Street, 400015 Cluj-Napoca, Romania.*

Biomarkers are measurable indicators of normal or pathological processes, which can be used to interpret the response to a therapeutic approach. An important category of biomarkers is represented by proteins. The remarkable technological advances achieved in proteomics, make possible the in-depth as well as large scale analysis of complex protein samples. Blood is an example of complex biological matrix. Because blood flows through every tissue and organ, its proteome reflects any changes that occur in the organism. Plasma proteome profiling is a continuously developing method used for the discovery of new biomarkers. One of its purposes is the early assessment of a pathology. In cancer, early detection is one of the crucial factors which increases survival rate. That is why, identification of novel biomarkers for the early detection of cancer is very useful in clinical applications.

In this study, our objective has been the identification of potential protein biomarkers for two types of cancer, pulmonary and thyroid, using mass spectrometry. The analyzed plasma samples belonged to cancerous and healthy individuals who live in radioactively and/or heavy metal contaminated regions in Romania.

We describe a protocol for protein biomarker discovery using liquid chromatography-tandem mass spectrometry (LC-MS/MS), particularly by relative label-free protein quantification. The blood was collected from subjects who provided a written informed consent and the study was approved by the Ethics Committee of the „Iuliu Haţieganu" University of Medicine and Pharmacy. Plasma was initially depleted of the first twelve most abundant proteins, using human plasma protein immunodepletion resins. The remaining proteins were migrated in SDS-PAGE, next Commassie stained, then subjected to in-gel digestion protocol. To obtain a more in-depth investigation, a fractionation on the protein level was implemented. During the LC-MS/MS analysis, the obtained peptides were first separated on a C18 column, then eluted and directly injected into a mass spectrometer, to be fragmented by collision-induced dissociation. All resulted files were processed with MaxQuant software, against a human UniProt database and a common contaminants database, using the integrated Andromeda search engine. Relative quantification of protein abundances was computed using the peptide label-free quantification values. The MaxQuant output was processed and statistical analyses were performed with Perseus software.

We identified proteins with statistically significant differential expression in the plasma of patients suffering from pulmonary as well as thyroid cancer, compared to the plasma of healthy subjects. Although the differential expression of these proteins in healthy and

cancerous individuals needs to be validated through complementary methods, they constitute potential biomarkers for these cancer types.

Keywords: biomarker, pulmonary cancer, thyroid cancer, label-free quantification
Acknowledgement: CNCS-UEFISCDI grant "ARTEMIS" with project code PN-III-P1-1.2-PCCDI-2017-0737, which has contract no. 35/01.04.2018.

EXPRESSION OF STRESS RESPONSIVE GENES IN HUMAN MONOCYTES EXPOSED TO IONIZING RADIATION

Gina Manda [1], Maria Dobre [1], Ionela Victoria Neagoe [1], Elena Milanesi [1], Cristian Postolache [2], Ulrich Weber [3], Nicole Averbeck [3]

[1] *Victor Babes National Institute of Pathology*

[2] *Horia Hulubei National Institute for R&D in Physics and Nuclear Engineering*

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Professional and accidental exposures to low doses of ionizing radiation and their biological consequences in the long and short run remains an important health issue in the context of intensive nuclear activities for various economic purposes. Moreover, the new era in space exploration brings into focus the investigation of cellular responses to the web of stressors specific for the deep-space. Accordingly, re-evaluation of the biological mechanisms underlying the interaction of ionizing radiation with organisms is highly needed for improving radioprotection and for developing efficient biomedical countermeasures.

Through a transcriptomic approach the study aims to unravel the responses of human proliferating monocytes to the stresses induced by exposure to space-relevant 56-Fe beams. Human monocytes (CRL9855 cells) were exposed at GSI (Darmstadt, Germany) to 56-Fe beams (0.1 – 2 Gy). The gene expression profile was assessed by qRT-PCR at 24h, 48h and 72h post-irradiation regarding the expression of 84 genes critically involved in cellular responses to oxidative stress, hypoxia, osmotic stress, DNA damage and repair, unfolded protein response and cell death (apoptosis, necrosis and autophagy). Exposure to gamma rays was used for comparison.

Proliferating monocytes responded to 56-Fe irradiation by cell cycle arrest mediated by the over-expression of the CDKN1A and GADD45A genes that are under the transcriptional control of p53 and NRF2. An increased expression of inflammatory genes (IL1A, IL1B, CXCL8 and TNF) was registered in radiation-exposed cells, especially at low doses, paralleled by the up-regulation of the antioxidant molecular fingerprint of NRF2. Time-dependent cellular responses were evidenced, characterized by early over-expression of particular genes, followed by their delayed down-regulation.

The study highlights several processes underlying the response of human proliferating monocytes exposed to space-relevant 56-Fe beams, encompassing cell cycle arrest even at low doses, production of pro-inflammatory cytokines (confined to low-dose exposure) and alteration of cellular defense mechanisms against oxidative stress. Therapeutic activation of NRF2 with dimethyl fumarate may sustain repair mechanisms related to DNA damage, antioxidant and inflammatory responses.

Keywords: astrobiology, transcriptomic, cell cycle arrest, redox signalling

Acknowledgement: Work was supported by the European Space Agency through the IBER 2017 programme, and by the Ministry of Education and Research through the grant PCCDI 35/2018 (ARTEMIS).

ARSENATE TARGET SPECIFICALLY CELL DEATH MECHANISMS: IMPLICATION IN BREAST CANCER MANAGEMENT

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The medical applications of arsenic derivatives are over 2000 years ago, in the last years was capture the attention as therapeutic strategy cancer treatment or as an environmental toxic agent. In the human body, inorganic arsenic compounds are converted to arsenite (+3 oxidation state) and arsenate (+5 oxidation state). Therefore, in our study we assess the impact on cellular and molecular pathways of breast cancer cells of a low dose of arsenate.
It was assessed the impact of a single dose of 50 nM arsenate in normal (HMEC) and tumoral breast cancer cells (double positive: MCF7, triple negative: Hs578T and MDA-MB-231) using basic test for assessment cell proliferation, apoptosis and autophagy, followed by the microarray evaluation of the gene expression pattern.
The treatment with arsenate was related to the activation of the apoptosis and autophagy as demonstrated by fluorescence microscopy and confirmed at mRNA by microarray data, these effects being more pronounced in the case of Hs578T and MDA-MB-231. In this two cell lines arsenate was proved to inhibit key genes related to autophagy, apoptosis, epigenetic alteration and DNA damage. These effects being less pregnant for the case of MCF-7and not present at all in the HMEC cell line.
The mechanism of action of arsenate is cell type specific, being more pronounced in Hs578T and MDA-MB-231, producing activation of autophagy and apoptosis, in parallel with cytoskeletal alteration, that impact cell survival, confirmed by microarray data.

Acknowledgement: Work was supported by Ministry of Education and Research through the grant PCCDI 35/2018 (ARTEMIS).

Session 1: UpToDate in Gynecological Pathology

Moderator – Prof. Gabriel Becheanu

UpToDate IN THE DIAGNOSIS OF CERVICAL SQUAMOUS LESIONS: WHO 2020 CLASSIFICATION

Raji Ganesan

Birmingham Women's and Children's NHS Foundation Trust, Birmingham, UK

The talk provides an outline of preneoplastic and invasive squamous cell lesions of the cervix including handling of specimens, use of p16 and changes in the FIGO 2018 staging of cervical cancers.

UpToDate IN THE DIAGNOSIS OF ENDOCERVICAL GLANDULAR LESIONS: WHO/IECC 2020 CLASSIFICATION

Simona Stolnicu

University of Medicine, Pharmacy, Sciences and Technology of Targu Mures, Romania

The incidence of endocervical adenocarcinoma, the second most common cervical cancer in the world, has been on the rise. While most cervical cancers are squamous cell carcinomas and associated with high-risk oncogenic human papillomavirus (HPV), approximately 15% of endocervical adenocarcinomas, which now represent about one quarter of all cervical cancers, are HPV-independent. The lecture will focus on the shortcomings of historical histologic classification systems of female genital tract tumors as they pertain to invasive endocervical adenocarcinomas and precursor lesions, and will highlight the advantages of the new International Endocervical Adenocarcinoma Criteria and Classification (IECC) system, which forms the basis for the WHO 2020 classification. The presentation will also cover the various histologic types, subtypes, and variants of endocervical adenocarcinoma with regard to morphology, immunophenotype, molecular genetics, HPV status and differential diagnosis, and will provide International Society of Gynecological Pathologists (ISGyP) recommendations for diagnosing these tumors.
The Silva pattern-based classification for HPV-associated invasive adenocarcinoma has emerged as a reliable system to predict risk of lymph node metastasis and recurrences. Although not part of any staging system yet, it has been incorporated in synoptic reports as established by the College of American Pathologists (CAP) and the International Collaboration on Cancer Reporting (ICCR). Moreover, the current National Comprehensive Cancer Network (NCCN) guidelines include this classification as an "emergent concept". In order to facilitate the understating and application of this new classification by all pathologists, the lecture will present all the current evidence on the Silva classification also providing recommendations for its implementation in practice, including interpretation, reporting, and application to biopsy and resection specimens.

Session 2: Research and Communication

Prof. Dan Vasiliu
Prof. Mihail E. Hinescu

DAY 2 – FRIDAY, NOVEMBER 6

Session 3: Genomics and proteomics

Plenary Lecture 2

Session 4: Neurosciences

Session 5: Nephropathology

Session 6: Short communication - varia

Session 3: Genomics and proteomics in regeneration and tumorigenesis

Moderators – Prof. Cristiana Tănase & Dr. Aurora Arghir

IMMUNOLOGICAL TRAITS IN NON-MELANOMA PATIENTS

Monica Neagu [1], Carolina Constantin [1], Mihaela Surcel [1], Adriana Narcisa Munteanu [1], Ana Caruntu [2], Constantin Caruntu [3], Sabina Zurac [4]

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Skin cancers that develop from aberrant proliferation of keratinocytes comprise the vast majority of skin cancers in humans. Baso (BCC) and squamocellular carcinomas (SCC) have a rising incidence and just in USA 4 million new cases are diagnosed every year, with a similar incidence in Europe. Although the metastasis rate is reduced in these non-melanoma skin cancers, therapy resistance and recurrence raises clinical issues. Evaluating immunity in these types of skin cancers and seeking immune parameters that can be found deregulated in BCC and SCC can be bring new information on clinical evolution prediction.
Patients diagnosed with BCC in Central Military Hospital were investigated pre-surgery for circulating immune cells. Flow-cytometry investigation and IVD kit BD Multitest™ IMK Kit was used to analyze: T lymphocytes (CD3+), B lymphocytes (CD19+), T helper subpopulation (CD3+CD4+), T cytotoxic subpopulation (CD3+CD8+), natural killer lymphocytes (NK) (CD3–CD16+ and/or CD56+). CD4+/CD8+ ratio was calculated and statistic analysis was performed using a control group with identical sex and age as the enrolled patients. In all the investigated cases before tumor excision circulating T lymphocytes are in the normal ranges. We have obtained statistical low values of circulating CD4+ sub-population while high values for cytotoxic/suppressor CD8+ subpopulation. Hence, the low ratio CD4+/CD8+ in BCC patients was found. Another statistic significant immune parameter compared to controls were the low circulating B lymphocytes and the high circulating NK cells. As reported by our group in several other pathologies, this B-NK unbalance was found also in BCC patients reinforcing the general immune compensation mechanisms.
Circulatory immune cells can be taken into account in monitoring BCC patients although all the investigated patients had non-metastatic localized tumors.

Keywords: basocellular carcinoma, immune parameters

Acknowledgement (optional): Study financed by PN-III-P1-1.2-PCCDI-2017-0341/2018, PN 19.21.01.01/2019.

IMMUNITY IN SARS-COV2 INFECTION

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As we are facing the most important pandemia after the Spanish flu in 1918, immunology has to answer several seminal questions. The dynamics of the immune response in SARS-Cov2 infection is partially known while the persistence of immunity in this infection is completely unknown. The establishment of herd immunity in this pandemia, the main point in halting infection spread, is also an on-going topic to be solved. In our quest to establish some traits of humoral immunity in RT-PCR negative and positive individuals we have started a study on cured or healthy, exposed to SARS-COV2 virus individuals. Therefore in 83 health care workers that are exposed to infected biological samples (tissue, blood) and to deceased patients positive for SARS-COV2 we have tested the levels of specific IgG and IgA from May to July 2020. We have used the ELISA Kit Euroimmun (Perkin Elmer) for specific IgG and IgA, antibodies raised against viral structural protein (domain S1). The test has 20% cross-reactivity with anti SARS-Cov, antibodies, but taking into account that this infection did not circulate in the tested population, this cross-reactivity was not taken into account. We did not tests IgM levels because there have been abundant reports on the irrelevance of IgM level as indicator of primary response, so we have switched our study to IgA testing. At the first testing done in May 2020, 15.6% out the tested group were positive for specific IgG. Three out of the 83 individuals had been confirmed with positive RT-PCR test, two with mild symptoms and one with minimum symptoms. For these three cases the May analysis was done 3 weeks after RT-PCR negative testing. The other entire group members were RT-PCR negative prior and the entire period of testing. The same group was retested in June and all the IgG titers tend to drop and in July testing the antibody titers reduce once more. Interestingly, the asymptomatic individual did not have positive levels of IgG from the beginning to the end of the test period. Our study shows that in the group that has contact with infected samples there is a humoral specific response in IgG that diminishes in time and that asymptomatic recovered patients can lack detectable specific antibodies. This last assertion does not exclude the existence of memory immune cells that can be re-activated upon a subsequent re-infection.

Keywords: COVID-19, specific IgG antibodies

SOLUBLE BIOMARKERS IN SKIN CANCER - A MULTIPLEX PROTEOMIC VIEW

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The immune setting in tumorigenesis holds a key role in antitumor therapy efficacy assessment. Evaluating potential biomarkers for therapy response prediction or disease monitoring is nowadays a continuously expanding domain. Omics methodologies among other modern biomedical assays are a robust tool mandatory for assuring data consistency, accuracy and reproducibility, and for providing robust biomarkers useful in disease management.
Within proteomics platforms, protein microarray plunges in a specific and important niche for biomarkers discovery, both for research and clinic aims. Moreover, in the last decade flexible arrays layouts have been successfully developed for diagnostic purposes.
In skin cancer, especially in cutaneous melanoma, high tumor heterogeneity and complex immune interactions claims a combined analysis that would cover the outline from genes signatures to cellular phenotype decoding. As proteins are those "pointers" that delineate the cellular phenotype, proteomic approaches would accurately measure the magnitude of biological effects caused by different genomic alterations. Soluble biomarkers such as cytokines or different growth factors (e.g., MIF, IL-6, IL-10 etc.) could generate an inflammatory milieu representing a steady component of malign process.
Some unconventional markers studied in the tumor framework, such as leptin, could correlate immune and metabolic axis helping in tumor therapy assessment and/or disease monitoring. Thus, recent data reveal that T lymphocytes functionality could be directly modulated by leptin, a hormone which is increased in obesity, and further this connection would impact the PD-1 immune axis blockade response. This recent finding is a good example that draws attention to the fact that deciphering mechanism underlying pathological processes would be insufficiently accomplished only by genomic lens. Proteomic approaches detect protein alterations levels but also expand knowledge through important physiological axis to new therapeutic targets characterization.

Keywords: microarray, melanoma, squamous cell carcinoma, leptin, immunobiology
Acknowledgement: Study financed by PNIII-P1-1.2-PCCDI-2017-0341/2018 (acronym PATHDERM) and PN 19.29.01.01/2019.

GENETIC DISORDERS AFFECTING ORAL HEALTH

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Oral health is a determinant factor for quality of life across the life course; it is essential to general health and well-being and greatly influences quality of life. Most oral health conditions are largely preventable and can be treated in their early stages. (WHO fact sheet on oral health, 2020). How genetics impacts on oral and dental health has not often been studied.
This study aimed to use genetic tests to confirm or rule out a diagnosis in a symptomatic individual, and to identify individuals at risk of getting an oral health problem with delayed onset.
Of a sample of 235 pediatric patients, 6 (2.55%) children (1 male and 5 females) between 4 and 17 years of age were selected based on the occurrence of two or more different cranio-oro-dental features in the same person. Chief complaints were unaesthetic facial appearance, reduce capacity in biting, chewing, swallowing, speaking or smiling. Extraoral and intraoral examination, available clinical and laboratory testing were performed, followed by detailed investigation into the personal and family medical history. Genetic testing was performed according to phenotype.
Different dysmorphic features were found on physical examination. Mandibular enlargement and micrognathia was associated with dental anomalies and malocclusion. Tooth number anomalies were noticed in two forms, multiple missing teeth and multiple supernumerary teeth. Enamel hypoplasia, premature and delayed eruption, and dental size anomalies were noticed as well. The combination of clinical features, including the physical appearance of the patient, laboratory abnormalities, and aspects of family history suggested a certain genetic condition. Genetic tests showed that the disorder was the result from a gene mutation or chromosomal anomalies. Each cluster of findings in a patient was determined by its genetic makeup. Both familial and sporadic cases were reported.
Genetic tests are particularly useful if an individual has a family history of a specific oral condition and an intervention is available to prevent the onset of disorder or minimize its severity.

Keywords: genetic disorder, tooth anomalies, oral health

GENETIC TESTING IN PEDIATRIC MOVEMENT DISORDERS – CLINICAL EXAMPLES

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Movement disorders encompass a broad category of neurological disorders associating abnormal involuntary movements or impaired voluntary movements, abnormal postures and/or impaired muscular tonus , having various etiologies. There are two main categories – hypokinetic and hyperkinetic syndromes, with different features. In children various movement disorders will overlap, with a changing clinical picture through the psychomotor development as a dynamic process and also with an evolutive clinical picture depending on the underlying condition.

In the search of etiology the focus should be on treatable disorders. The recent development of genetics had a major impact on the definition and classification of these medical conditions. The presentation aims to present a targeted diagnostic algorithm in pediatric movement disorders, with emphasis on the genetic testing from the clinician's point of view through clinical examples. Dystonia as an episodic presentation at onset in a small child or as a prominent feature in a teenager with neurological deterioration, a child with ataxia and chorea will be some of the examples, trying to focus on clinical signs and also on the place of the genetic testing in the diagnostic achievement.

It is very important for a clinician to know whom to test and what to test for, how to choose the right investigations, knowing that the early semiological recognition of the pattern of abnormal movement, the identification of model of evolution, with targeted investigations, will shorten the time to diagnostic and help the children to get the appropriate treatment and have a better quality of life. Genetic diagnosis will further allow genetic counseling of the family.

Keywords: movement disorder, hypokinetic, hyperkinetic, dystonia, chorea, ataxia

CHARACTERIZATION OF MOLECULAR EVENTS RELATED TO DISEASE PROGRESSION IN A CASE OF POLYCYTHEMIA VERA USING WHOLE-EXOME SEQUENCING

Ana Maria Vlădăreanu [1,2], Cristina Mambet [1,3,4], Anca Botezatu [4,5], Petruța Gurban [4,6], Laura G. Necula [3,4], Horia Bumbea [1,2], Minodora Onisai [1,2], Andreea Neculce [2], Andreea Spanu [2], Alina Mititelu [2], Diana Bonea [2], Elena Andrus [2], Cristina Enache [2], Ionut Dumitru [2], Jean-Philippe Defour [8,9], Pascale Saussoy [9], Gabriela Anton [4,5], Carmen C. Diaconu [4,5], Ștefan N. Constantinescu [4,7,8]

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Polycythemia vera (PV) is a Philadelphia-negative myeloproliferative neoplasm (MPN) defined by erythrocytosis, high incidence of thrombotic events and a tendency to undergo fibrotic or leukemic transformation. Several mutations in genes coding for epigenetic regulators and messenger RNA splicing machinery have been associated with inferior fibrosis-free or leukemia-free survival in PV.

We report the case of a 64-year old patient diagnosed in 2011 with *JAK2* V617F-positive PV and treated with hydroxyurea and phlebotomy, that developed secondary myelofibrosis in February 2019, with rapid progression to blast phase of MPN. Whole-exome sequencing (WES) was performed on DNA samples obtained at diagnosis of MPN chronic phase and leukemic conversion respectively, to characterize the molecular events related to disease progression. Under treatment with hypomethylating agent azacytidine disease stabilization with blast cell reduction in peripheral blood and bone marrow was achieved.

WES analysis did not detect new mutations in blast phase compared to MPN chronic phase. However, an increase of *JAK2* V617F allele burden from 35% to 85% was noticed. Also, the allelic frequency of the missense *KMT2A* c.6232C>T (p.R2078C) mutation identified in the initial sample increased from 34% to 42%. In addition, an acquired trisomy X was detected by the conventional karyotype analysis during disease transformation.

KMT2A (MLL, 11q23) gene encodes Lysine Methyltransferase 2A that methylates histone H3 on lysine 4 (H3K4), being involved in the epigenetic regulation of transcription through chromatin remodeling. The somatic missense *KMT2A* mutation identified in our patient is indexed in COSMIC database with a pathogenic score of 0.99 (COSM6910464), and it was

previously reported only in solid tumors. As WES data cannot fully explain the molecular events leading to leukemic conversion of PV, future transcriptome and chromatin structure analyses are necessary.

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PHARMACOLOGICAL INVESTIGATION FOR THE PRODUCTS NATURASTIM-SOL AND NATURASTIM-VET

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Naturastim-SOL is an original product developed by the company NATURASTIM - SATU-MARE. The product is registered as food supplement, and in the initial investigations the lack of toxicity, as well as a series of beneficial effects, among which liver regeneration, an increase in the number of lymphocytes,

The product is a solution for oral administration, but originally also other routes of administration were investigated, like intramuscular or intraperitoneal. For such routes of administration, there is the need to associate it with a local anesthetic since the characteristics of the product (mainly the pH) makes it injection without an anesthetic difficult to support. The idea was that the product could be used with beneficial effects on animals, in the injectable form.

The two forms of the products were tested in vitro, using as model cultures of human monocytes -macrophages (SC-ATCC CRL 9855), mouse macrophages (RAW 264.7) and on mouse fibroblasts ATCC CCL 92. Cultivation of the cells was according to manufacturer's specification, using a 96 well layout for the exposure. Both products were added to the culture media to concentrations of 10 and 20%. To avoid the pH shock, the diluted samples were maintained in the incubator for 24 hours, prior to the exposure of the cells. The cells were exposed to the preparations for 24 and 48 hrs., then observation of cell aspects, cell count and MTS assays were performed.

All the three cell systems offered good results (as evaluated by cell counts and MTS assays), indicating a slight stimulation of cell multiplication (an increase of cell index of 17, 21 and 16% respectively at a 10% ratio) at 24 hrs., and of 23, 28 and 29 respectively, at 48 hrs. For the human monocytes-macrophages an assay for IL-1 alpha was performed, and this revealed, upon stimulation with LPS and treatment, an increase in secretion of 22%.

The results are suggesting that Naturastim-Vet could be used for a veterinary injectable composition, proving so far the potential to stimulate proliferation of some immunocompetent cells and the release of cytokines that may prove helpful as adjuvant in the treatment of animals.

The data were supplemented with evaluations of microbial contamination (both forms were found sterile), estimation of mutagenic potential using the micronucleus assay, and underwent physico-chemical characterization.

Keywords: immunomodulators, cytotoxicity, cell porliferation

Acknowledgement The studies were supported from the contract POC-INOVOPRODFARM – SUBCONTRACT C 9/2020

Plenary Lecture 2

THE EMERGING PATTERN OF SHARED POLYGENIC ARCHITECTURE OF PSYCHIATRIC DISORDERS - CONCEPTUAL AND CLINICAL IMPLICATIONS

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During the last decade witnessed a series of discoveries in psychiatric genetics, and today a significant proportion of the genetic architecture of a range of psychiatric disorders have been discovered. Interestingly, most psychiatric disorders and traits seem to be polygenic, i.e. with a large number of gene loci affecting disease risk, each with a small effect.

We have developed methodology customized to investigate a scenario of many small genetic effects, and to study overlapping polygenic architecture beyond genetic correlation that can identify genetic loci shared between disorders and traits.

This approach has provided novel insight into the genetic architecture across the neurodevelopmental, affective, psychotic disorders, including a large proportion of overlapping gene loci. Further, there were a range of different patterns of overlap with behavioral and mental traits, such as cognition, personality, substance use. Further, recent mathematical models can estimate the number of gene loci overlapping between mental disorders and traits.

There is a potential for clinical translation of these findings. While the polygenetic risk can be translated into prediction and stratification approaches, the potential for elucidating the disease mechanisms to guide future drug development has also a large potential impact.

Keywords: Mental illness, molecular genetics, pleiotropy, clinical translation

Acknowledgement: Partial funding from Research Council of Norway, EU H2020, NIH and the EEA Grant 2014-2021, under the project contract No 6/2019.

Session 4: Neurosciences

Moderator - Prof. Bogdan O. Popescu

TARGETING INTRAMURAL PERIARTERIAL DRAINAGE AS A THERAPEUTIC STRATEGY IN ALZHEIMER'S DISEASE

Roxana O Cărare

University of Southampton, Southampton General Hospital

Accumulation of the β-amyloid (Aβ) protein in cerebral blood vessels is a hallmark of Alzheimer's disease. Soluble Aβ from the extracellular spaces of the brain is removed along the basement membranes of capillaries and basement membranes surrounding smooth muscle cells of arteries towards the surface of the brain, as intramural periarterial drainage (IPAD). This process depends on the biochemical integrity of the extracellular matrix and the strength of arterial smooth muscle cells. With ageing, possession of Apolipoprotein E4 (APOE4) genotype, hyperlipidemia, maternal high fat, immune complexes, IPAD fails, resulting in the accumulation of proteins in the walls of cerebral arteries as cerebral amyloid angiopathy. The motive force for IPAD is derived from contractions of vascular smooth muscle cells and targeting their function appears to be a promising therapeutic avenue for Alzheimer's disease. Clusterin (Apolipoprotein J) appears to be a chaperone for Aβ, facilitating IPAD and understanding how it acts upon IPAD is a key direction in therapeutic interventions for Alzheimer's disease.

TREATMENT AND DIAGNOSTICS OF DEMENTIA – ABC TRANSPORTERS A NEW AVENUE OUT OF A DESPERATE SITUATION

Jens Pahnke

Professor at the University of Oslo (UiO) and the Head of the Department of Neuropathology at the Oslo University Hospital, Norway

All treatment studies against Alzheimer's disease (AD) or mild cognitive impairment failed so far. What is the reason for that? Ninety-nine percent of all AD patients develop the sporadic form of the disease that is not linked to any of the known genes of familiar AD. Familiar AD is a disease that is caused by an overproduction of a toxic peptide (Abeta) due to problems in degradation of a larger, complex transmembrane protein called APP. The toxic Abeta accumulation leads to further devastating effects finally resulting in the death of neurons and the clinical signs of dementia with memory deficiency, orientation problems, speech abnormalities, behavioural changes and many more. Treatment trials aimed so far at reducing Abeta overproduction or destroying Abeta aggregates in the brain. These tests aimed at either reducing the production with small-molecule drugs or increasing the removal of plaques by antibody treatment, so called AD vaccination.

We have been working on another major general problem of dementia: the brain vessels and the blood-brain barrier since AD is accompanied by major vascular problems and vascular dementia. The blood-brain barrier hosts important active transport proteins that can be used for diagnostics and treatment of dementia and neurodegenerative disease in general. These transport proteins, ABC transporters, are known from cancer research and treatment since the 1970ies.We have discovered that some of these transporters are impaired in the brain's vessels and thus lead to increased amount of toxic peptides resulting in aggregation and storage. Activation of ABC transporters can be used for treatment and diagnostics. The presentation will describe the mechanisms and explain possibilities for diagnostics and treatment of patients with dementia and movement disorders. These treatments are currently under exploration in patients.

IN VITRO MODELLING OF THE HUMAN BLOOD-BRAIN BARRIER TO STUDY AMYLOID TRANSPORT IN ALZHEIMER'S DISEASE

Fabien Gosselet

Professor at University of Artois, Head of Lens Blood-brain barrier, Arras, France

COTININE AND 6-HYDROXY-L-NICOTINE IMPROVES NEUROBEHAVIORAL CHANGES AND REDUCE OXIDATIVE STRESS IN A ZEBRAFISH (DANIO RERIO) MODEL OF ALZHEIMER'S DISEASE

Razvan Stefan Boiangiu [1], Marius Mihasan [1], Lucian Hritcu [1]
Department of Biology, Alexandru Ioan Cuza University of Iasi

Almost 47 million people worldwide suffer from the most common form of dementia, namely Alzheimer's Disease (AD), which is a progressive neurodegenerative disorder characterized, among others, by cognitive decline, mood changes and loss of forebrain cholinergic neurons. Zebrafish (Danio rerio) is emerging as an increasingly successful model for translational research on human neurological disorders and has been successfully used to simulate AD pathology. The presence of nicotinic acetylcholine receptors (nAChRs) in the cholinergic neurons suggests their involvement in higher brain functions, such as memory, learning, and cognition. There is considerable interest in modulating nAChRs to treat nervous system disorders, such as AD. Nicotine is an exogenous agonist of nAChRs and was shown to improve memory, attention, and learning. However, its therapeutic use in AD was limited by cardiovascular and addictive side-effects. Interestingly, two structural related nicotine derivatives, cotinine (COT) and 6-hydroxy-L-nicotine (6HLN), were shown to improve cognition without exhibiting nicotine's side-effects. We aimed to investigate the effects of COT and 6HLN on memory deficits, anxiety and oxidative stress in a zebrafish model of AD induced by scopolamine. Doses of 1 and 2 mg/L of COT and 6HLN were administered acutely by immersion to zebrafish immediately after scopolamine (100 µM) treatment. Memory performances and anxious behavior were assessed using in vivo tasks such as Y-maze and novel object recognition test (NOR) and novel tank diving test (NTT) respectively. Also, we evaluated the impact of the two nicotine derivatives on oxidative stress by measuring the level of reduced glutathione and carbonylated proteins. In this study, we have shown that 6HLN and COT improve memory performances in Y-maze and NOR tasks and reduced the anxious behavior in NTT. Moreover, these nicotinic derivatives increased the content of reduced glutathione and decreased the level of carbonylated proteins thus suggesting that COT and 6HLN reduce the oxidative stress in scopolamine-treated zebrafish. Our results demonstrate that COT and 6HLN promote brain antioxidant action and mitigate scopolamine-induced anxiety and memory deficits in zebrafish. These findings suggest that COT and 6HLN could be used as neuropharmacological agents in AD.

Keywords: Nicotine derivatives, Scopolamine, Memory, Anxiety, Antioxidants
Acknowledgement: This work was co-funded by the European Social Fund, through Operational Programme Human Capital 2014-2020, project number POCU/380/6/13/123623, project title "PhD Students and Postdoctoral Researchers Prepared for the Labour Market!"

Session 5: Nephropathology

Moderators – Dr. Mihaela Gherghiceanu & Dr. Gener Ismail

CHALLENGING CASES IN NEPHROPATHOLOGY

George Terinte-Balcan

Victor Babes National Institute for Pathology, Bucharest, Romania

A 29 year old Caucasian male presented with right flank pain associated with nausea and non-bloody vomit. He denied fever, hematuria or dysuria. The patient was a heavy drinker, but reports that in the past 2 weeks he didn't drink due to the abdominal pain.
Blood tests revealed acute renal failure, with a serum creatinine of 5.5 mg/dL. His serum albumin had a value of 3.0 g/dL. A kidney biopsy was performed in order to establish the cause of his acute renal failure. The patient is also noted to have angiokeratomas and low leukocyte alpha-galactosidase. A kidney biopsy was performed.
Immunofluorescence microscopy showed very intense staining for IgG, kappa light chains, lambda light chains in a liniar pattern along the capillary walls. Multiple breaks in the glomerular basement membranes were also observed. There was focal strong segmental staining in the glomeruli for fibrin, consistent with segmental fibrinoid necrosis and crescent formation. The tissue for light microscopy showed that 5 of the 6 glomeruli present in the sample had segmental fibrinoid necrosis and large cellular crescent formation. The intact glomerular segments had no endocapillary hipercellularity and no thickening of the capillary walls. Red blood cell casts were also observed in the lumen of some tubules. Podocytes demonstrated marked vacuolisation of the cytoplasms. Electron microscopy revealed dense myelin figures in the podocyte and in the parietal epithelial cells. Rare inclusions were also noted in the endothelial cells, mesangial cells and tubular epithelial cells. No dense deposits were found in the mesangium or in the capillary walls of the glomerulus.
A final diagnosis of severe anti-GBM crescentic and necrotising glomerulonephritis associated with Fabry disease was made based on the findings observed by immunofluorescence, light and electron microscopy.

Keywords: Fabry disease, anti-GBM glomerulonephritis

UPDATES IN THE HISTOLOGICAL DIAGNOSIS AND TREATMENT OF LUPUS NEPHRITIS

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Lupus nephritis (LN) affects nearly 60% of patients with systemic lupus erythematosus (SLE) and is the most important factor contributing to the overall morbidity and mortality of these patients. Despite the intensive immunosuppressive (IS) regimens used for LN treatment, remission is achieved in only 50-70% of patients and up to 30% may still develop end-stage renal disease (ESRD). Moreover, despite improvement in overall mortality of SLE patients, the incidence of ESRD has remained stable over the last decades. This reflects the limits of current IS therapy and the ongoing need for better predictors of renal outcome to individualize the intensity and duration of IS treatment. Significant progress has been made in the understanding of LN pathogenesis that translated into several novel therapeutic agents tested in phase 2 and 3 trials that show an improvement in renal outcome as compared with the current standard of care. Additionally, the prognostic value of histological data is emerging and several revisions of the ISN-RPS classification have been proposed. Nonetheless, histological lesions and patterns of injury that are not captured in the current ISN/RPS classification are relevant in terms of patient management and outcome. Moreover, a kidney-biopsy management of immunosuppressive therapy is increasingly being recognized as useful, safe and may ameliorate outcome of such patients. We provide an update in the histological diagnosis and treatment of lupus nephritis.

Keywords: Lupus nephritis; kidney biopsy; immunosuppressive treatment

DIFFERENT REJECTION PHENOTYPES IN KIDNEY TRANSPLANT RECIPIENTS WITH POSITIVE ANGIOTENSIN II TYPE 1 RECEPTOR ANTIBODIES

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Angiotensin II type 1 receptor antibodies (AT1R-Ab) are among the most investigated types of non-HLA antibodies in kidney transplantation (KT). The prevalence of AT1R-Ab in KT varies from 2.1% to 59%. Even though it has been shown to belong to the IgG1 and IgG3 complement fixing subclass, AT1R-Ab seem to mediate graft injury in a complement independent and antibody dependent cell mediated manner. The pathophysiological chain of graft injury involves an interplay between allo and autoimmunity, activation of proinflammatory and procoagulant pathways, which are responsible for renal graft injury, including vascular lesions, but also for graft disfunction. Preformed or de novo AT1R-Ab may cause different rejection phenotypes, for instance antibody-mediated rejection (AMR), T-cell-mediated rejection (TCMR) and vascular rejection. According to current Banff criteria, the diagnosis of active AMR requires histological evidence of acute tissue injury (including microvascular inflammation >0), associated with evidence of recent antibody interaction with vascular endothelium (including microvascular inflammation ≥2 or C4d positivity in peritubular capillaries) and serologic evidence of HLA- donor specific antibodies or non-HLA-Ab (including AT1R-Ab). The main histological findings associated with AMR are decreased Cd4 positivity and increased microvascular inflammation (glomerulitis and peritubular capillaritis). For this reason, microvascular inflammation could be a more reliable indicator than C4d in AT1R-Ab mediated rejection. The mechanism by which AT1R-Ab lead to TCMR is unclear, but there is a proposal for a bidirectional relationship. Antibody-mediated vascular rejection was proposed as a distinct kidney graft rejection phenotype characterized by hypertension, presence of antibodies, decreased C4d positivity, microvascular inflammation, intimal arteritis, fibrinoid necrosis, subintimal fibrosis and vascular occlusion and was associated with the lowest graft survival. Understanding the complexity of AT1R-Ab mediated graft injury could provide a complementary, integrated assessment of immunological risk, help stratify the risk of graft rejection and may guide the treatment approach.

Keywords: AT1R-Ab, rejection, microvascular inflammation, C4d, intimal arteritis

CASE PRESENTATION OF RAPIDLY PROGRESSIVE GLOMERULONEPHRITIS

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IgA nephropathy is one of the most common causes of glomerulonephritis in the world and is characterized histologically by the deposition of IgA within the mesangium and along glomerular capillary walls causing mesangial hypercellularity and matrix expansion. While these are common in IgA nephropathy, additional glomerular pathology can include endocapillary proliferation and cellular crescents. Many studies demonstrated IgA Nephropathy to be a rare cause of rapidly progressive glomerulonephritis with crescent in more than 50% of glomeruli and the best treatment option is pulse and oral glucocorticoids associated with an alkylating agent.

A 40 years old women presented to the hospital with progressive bilateral oedema for 6 months and she was diagnosed with nephrotic syndrome with a nephritic component associated and a serum creatinine of 1,8 mg/dl (3 months before was 1,3 mg/dl); the kidney biopsy showed IgA nephropathy with mesangio-capillary pattern and diffuse extracapillary proliferation, having a MEST-C score of M1E1S1T0C2. She was started on Methylprednisolone and gradually reduced doses of Prednisone and intravenous Cyclophosphamide for 6 months and then she continued with Azathioprine and later on, Hydroxychloroquine for 17 months. Proteinuria was significantly reduced from the beginning and despite the fact that she had a minor increase in her serum creatinine, after 4 months of treatment it remained stationary at around 2 mg/dl. After 1 year and a half the kidney biopsy was repeated to asses the histological response to treatment. The results showed 2 glomeruli with endocapillary proliferation and crescents absent from glomeruli, but there was severe interstial fibrosis, tubular atrophy and 7 out of 9 glomeruli with global glomerulosclerosis. In spite all these lesions seen in the second biopsy, having certain other parameters under control, like proteinuria, normal blood pressure, surely had a favourable prognostic in this patient's evolution. The patient was stable for 2 months more after the second kidney biospy, but then she presented with acute pyelonephritis and acute kidney disfunction (serum creatinine of 7 mg/dl). At the end of this episode her creatinine level remained stable at around 4,5 mg/dl. Eventually the patient got to the point where she needs to be prepared for kidney transplant or replacement therapy.

This case illustrates the severity of IgA Nephropathy with mesangio-capillary pattern and crescent in more than 50% of glomeruli and how even after intensive immunosuppression, the histological response is with severe fibrosis and global glomerulosclerosis. Pregnancy-onset nephrotic syndrome in a patient with type 1 diabetes mellitus.

A RARE CAUSE OF ACUTE KIDNEY INJURY IN SYSTEMIC LUPUS ERYTHEMATOSUS– THE IMPORTANCE OF KIDNEY BIOPSY

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A 49-year-old woman presented with altered clinical status, subfebrile temperature and dry cough. The laboratory tests showed renal dysfunction (serum creatinine level of 3.2 mg/dL), leukocytosis (23000 cells/µL, neutrophilia), with multiple electrolytic deficiencies (severe hyponatremia 115 mmol/l, hypokalemia, hypochloremia, hypophosphatemia, hypomagnesemia). A diagnosis of type B Influenza was established. The patient had thrombocytopenia and signs of microangiopathic hemolytic anemia, leukocyturia, hematuria and subnephrotic range proteinuria. Ultrasonography showed normal renal dimensions and serositis (pericardial and right pleural effusion). It should be mentioned that the patient had two positive titres for ANA and one positive titre for lupus anticoagulant (LA) in her medical history. An immunologic assay was performed, with ANA and anti dsDNA antibodies being negative, but with serum complement C3 fraction consumption and positive LA and anticardiolipin antibodies. Taking into account the two positive titres for ANA, the laboratory criteria for antiphospholipid syndrome, the presence of thrombotic microangiopathy (TMA) signs, renal dysfunction with proteinuria and serositis, a diagnosis of systemic lupus erythematosus was established. The patient was treated with diuretics, antibiotic, antiviral and antimycotic treatment and with Methylprednisolone 16 mg/day. Kidney biopsy was postponed due to low platelet count and was effectuated after 2 weeks. In immunofluorescence, Ig G stained weakly positive, with a linear pattern, C3c staining was weakly positive in vessels and glomeruli, at the vascular pole, with negative Ig A, Ig M and C1q. In light microscopy, the glomeruli showed permeable capillaries, with areas of mesangial focal segmental proliferation, without evidence of intracapillary thrombi, endo or extracapillary proliferation. Interstitial inflammatory infiltration was not abundant, most of the inflammatory cells being conglomerated in the peritubular area. In the tubular wall, there were apoptotic cells, with pycnotic nucleus. Also, in tubular lumina, cellular detritus was present, some of the tubules being obstructed. In electron microscopy, endotheliosis was evident, with lack of endothelial fenestrations, thickened glomerular wall and the GBM tending to form a neomembrane. Low grade podocyte foot process effacement was present also and lymphocytes were captured in the tubular wall (tubulitis). No dense deposits were seen. In this setting, we had no criteria for lupus nephritis and the kidney biopsy established the diagnosis of severe acute tubular interstitial nephritis, with acute tubular necrosis and TMA associated with the antiphospholipid syndrome. The tubular lesions were attributed to the type B influenza virus infection, which probably was the trigger for the TMA lesions also. In conclusion, the kidney biopsy was essential in establishing the cause of acute kidney injury in this patient with SLE.

PREGNANCY-ONSET NEPHROTIC SYNDROME IN A PATIENT WITH TYPE 1 DIABETES MELLITUS

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A 31-year-old woman with a history of type 1 diabetes mellitus diagnosed at the age of 14 yo was referred to our department at 13 weeks gestation for evaluation of a new-onset arterial hypertension, peripheral edema and nephrotic range proteinuria.
At physical examination, she had pale skin and important palpebral and lower limbs edema. Blood pressure was 140/90 mmHg, heart rate 66 beats/min, and she had a diuresis of 2000 mL/day, with foamy urine.
Laboratory tests revealed mild renal dysfunction (serum creatinine level of 1.21 mg/dL), mild normocytic, normochromic anemia and nephrotic syndrome (serum albumin 2.1 g/dL and proteinuria 10g/day). Urinalysis showed microscopic hematuria with dysmorphic red blood cells 31/µL and leukocyturia 75/µL. The immunology was negative (ANA, anti-dsDNA, anti-Sm, anti-Ro, anti-La, anti-RNP, anti-Sm, anti-C1q, anti-PLA2R, CRP and rheumatoid factor within normal range), with a slightly decrease of the C3 fraction of the complement (79 mg/dL).
A kidney biopsy was performed. In immunofluorescence (IF), there was mild linear staining along the capillary walls for IgA, IgM, k and λ, with moderate linear IgG and granular C1q and C3c deposits involving both the glomerular and tubular basement membranes (TBM). Although the patient had negative serum ANA, speckled staining of cell nuclei for IgG was revealed (tissue ANA). Light microscopy showed 2 out of 9 glomeruli with endocapillary hipercelularity and small crescent formation, with 5 glomeruli having nodular segmental mesangial matrix expansion and with periglomerular lymphoplasmacytic inflammatory cell infiltrate and arteriolar hyalinosis. The tubulointerstitial compartiment showed thickening of the TBM, peritubular fibrosis, mild tubular atrophy and moderate lymphoplasmacytic inflammatory cell infiltrate. The electron microscopy revealed severe endocapillary hipercellularity, mesangial hypercellularity, small electron-dense subendothelial deposits and effacement of foot processes.
A diagnosis of immune complex-mediated membranoproliferative glomerulonephritis was made, also with class II diabetic nephropathy lesions. We considered the diagnosis of lupus nephritis, given the high specificity of tissue ANA for lupus nephritis.
Treatment with Methylprednisolone and Azathioprine was started. The serum creatinine was stable during pregnancy and at 31 gestational weeks the patient underwent a successful cesarean section and delivered a healthy premature baby boy. Postpartum, induction immunosupressive (IS) therapy with IV pulses of Cyclophosphamide and Methylprednisolone was started, continued by maintenance therapy with Rituximab. At last follow-up 14 months after initiation of IS therapy, renal function is stable (serum creatinine 2.3 mg/dL), but with persistent proteinuria.

Session 6: Short communication – varia

Moderator – Dr. Laura C. Ceafalan

IMMUNOLOGICAL PARAMETERS OF CHILDREN WITH RECURRENT RESPIRATORY INFECTIONS

Adriana Narcisa Munteanu [1,2], Mihaela Surcel [1], Gheorghita Isvoranu [1], Ioana Ruxandra Pirvu [1], Ovidiu Bratu [3], Carolina Constantin [1,4], Monica Neagu [1,4]

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Recurrent respiratory infections (RRI) represent a high percentage of general childhood pathology, in conditions of an immune system without major apparent defects. Because they may be associated with an altered cellular immune response, the aim of the study was to identify possible immunological changes with an impact on the pathogenesis of RRI, by quantifying T and B lymphocyte subpopulations.
The casuistry includes two groups of children (1-7 years), thus: i) RRI group (30) – children with at least 6 episodes of RI/year; ii) control group (10) – clinically healthy children. Blood samples were taken for dosing serum immunoglobulins (IgG, IgA, IgM) by nephelometry and for lymphocyte immunophenotyping (LIF) by flow cytometry (BD FACSCanto II). By LIF were quantified: total T-lymphocytes (CD3+) with T-helper subpopulations (CD4+) and T-suppressor/cytotoxic (CD8+), double-negative T cells (CD4-CD8-CD1d-), NKT cells (CD3+CD16/56+CD1d+), NK cells (CD16/56+) and total B-cells (CD19+CD20+) with mature/naive B cells subpopulations (CD27-IgD+), memory B cells (CD27+) and plasmocytes (CD10-CD27+CD38bright).
Serum Ig values were normal in 70% of cases. The most important changes observed in T lymphocytes were the decrease of the average values of T-CD8+ ($p=0.009$) and the increase of the T-CD4+/T-CD8+ ratio ($p=0.002$). Although the values obtained for NK cells in the RRI group are higher ($p=0.003$) than the control, they are within normal limits. 86% of cases showed decreases in B lymphocytes, with low mean values ($p=4.5x10-5$). Analysis of B lymphocyte subclasses revealed a decrease in mature/naive B lymphocytes and an increase in the percentage of memory B lymphocytes ($p=0.027$). No statistically significant differences were observed between the groups tested for the other analyzed cell subpopulations.
Investigation of cellular immunological parameters may complete the clinical diagnosis, especially in cases where humoral parameters are within normal limits. Considering that RRI can cause disorders in children's development, the detection of the causes and prophylaxis

of these infections are major elements for improving the living conditions of the affected child population.

Keywords: flow cytometry, immunophenotyping, nephelometry
Acknowledgement: This work was carried out through the Core Program, carried out with the support of ANCSI, the projects PN19.29.01.01; PN19.29.02.03, Grant PN-III-P1-1.2-PCCDI-2017-0341/2018 and 7PFE/16.10.2018.

DYING OF COVID19 VERSUS DYING WITH COVID19 – WHICH ARE THE SCIENTIFIC DIFFERENCES?

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** Popp&Nichita are first authors in equal proportion*

COVID-19, a multisystemic viral disease, represents the most significant medical challenge of 2020, for pathologists as well as for the entire medical system. As pathologists in a COVID hospital, we present a short review of fatal cases of COVID recorded in the first 3 months of the pandemia, trying to identify diferences between patients who died of COVID19 and patient with COVID19 who died from another disease.

We reviewed the files from 122 patients that died in Colentina University Hospital in March, April and May 2020. 90 patients died of SARS-COV2 infection (main cause of death according to their death certificate - group A), while 32 patients died from another cause while being hospitalized for SARS-COV2 infection – group B. We registred age, sex, causes of death, co-morbidities, blood oxygen level at admission and other significant parameters.

Results: No significant differences were identified between the two groups regarding age (mean age 69,27 for group A and 70,81 for group B). Group A included significantly more males than females (52 males and 38 females) comparing with group B (13 males and 19 females). Also, significant data were obtained regarding the presence of pneumonia (more frequent in group A) and of malignancies (more frequent in group B). Blood oxygen level at admission was an important outcome marker, being significantly lower in group A. A broad spectrum of malignancies were identified in both groups, significantly more frequent in group B (28% vs 18% in group A), where they represented main cause of death in some patients.

Sometimes, SARS-COV2 infection is not the main death cause, being just another disease that contribute to the patients'death. Males with SARS-COV2 infection are more likely to die of the disease, while the presence of pneumonia and blood oxygen level at admission are significantly different in patients that died of COVID19 versus patients that died with COVID19.

Keywords: COVID19, pneumonia, pathology

INTERVENTIONAL CHALLENGES IN RAPIDLY WORSENING ATHEROMATOSIS

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Cholesterol molecules can diverge from their physiological pathway and end up accumulating on the inside of arterial walls, triggering an inflammatory response which further strengthens the newly formed entity- the atheroma plaque. From this point on, depending on the patient's habits, diet and lifestyle, atheromatosis can suffer a continuous development and further restrict the underlying blood flow.

We present the case of an overweight 65 year old male who came in accusing thoracic pain while resting. At that time his clinical picture was already not in his favor: a chronic smoker with ongoing stage III hypertension, dyslipidemia, and previously confirmed chronic ischemia in the lower left extremity due to type II aorto-iliac occlusive disease. Laboratory findings showed high levels of fibrinogen (691 mg/dL), VSH (52mm/1h) and cholesterol (275mg/dL). Carotid echography showed bilateral atheromatosis hemodynamic significantly and led to a surgical approach of the atheroma plaques.

Coronary and peripheral angiography revealed multiple calcifications as follows: calcifications in the left and right coronary arteries, with even a 95% stenosis in certain segments, calcifications in the abdominal aortic walls, 50-60% stenosis for the left common iliac artery with further occlusion of the left external iliac artery, severe calcifications with a deep and irregular atheroma plaque in the right external iliac artery. A CAGB (coronary artery graft by-pass surgery) was performed for the coronary arteries. After the invasive mapping of the atheromatous multiple targets and surgery, the patient developed chronic renal failure that required dialysis (creatinine clearance 15 mL/min/1.73m^2). Further clinical findings show bilateral stage III calcaneal ulcerations.

Atheromatosis and atheroembolism are unpredictable phenomena. Rapid evolution and the inability to observe it by the minute are two key elements which explain the dramatism of this type of clinical case, and also the skepticism towards invasive surgical interventions.

Keywords: atheroma plaque, atheroembolism, calcifications, angiography

NEUROREGENERATIVE EFFECTS OF THE LAMIUM ALBUM L. EXTRACT BASED ON SHANZHISIDE - METHYL ESTERS ACTION, IN RESTRAINT STRESS CONDITION

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Stress is a strong factor in the occurrence of hypertension, endocrine disorders or digestive illness. The stressors can be widely varied; for instance, restraint stress induces a disturbance of the hippocampus-hypothalamic-pituitary-adrenal (HHPA) axis and generates oxidative stress with ramifications in many ROS-cellular-associated-pathologies.
In this experiment we investigated the stress-counteracting and neuroregenerative action of the shanzhiside-methyl esters, an iridoid from Lamium album, on female Wistar rats exposed to repeated restraint stress 3 h/d during 5 days. Another group of animals were exposed to Restraint Stress + L. album extract (RS+LA group). The experiment was performed over the course of five days. The extract was administered by enteral route in a dose of 100 mg dry substance/kg b.w, daily.
During stress induction, the oxidative stress markers (TBARS, catalase) and restraint stress parameters (cholesterol, adrenaline and corticosterone) were increased in stress and decreased after extract administration via shanzhiside-methyl esters agonistic properties on glucagon-like peptide 1 receptors from hippocampus and TNFα inhibition. Histological evaluations identified gliosis in dentate gyrus marked by thinning of granular cell layer and dark neurons in subgranular area. However, Lamium album stimulates the cellular regeneration and encourages the cell survival in granular layer from dentate gyrus.
We may assume that in stress conditions, Lamium album have a brain-homeostatic modulator effects, based on shanzhiside-methyl esters properties.

Keywords: dentate gyrus, oxidative stress, restraint stress, iridoids
Acknowledgement: This work was supported by a grant of the M.R.I. by Program 1, Subprogram 1.2 - Institutional Performance - Projects for Excellence Fiancing in RDI 22PFE/2018 and the Core Program PN2019-2022- BIOSERV 19270201/25N/2019.

INTEGRATION OF METABOLOMICS AND CHEMOSENSITIVITY DATA FROM THE NCI-60 STUDY

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In cancer patients, high mortality rates are associated to the onset of drug resistance post-treatment [1]. One of the challenges in personalized oncology is determining a priori whether or not a tumor will respond to treatment. One possible solution is identifying biomarkers that could act as proxies of tumor response. In the past years, metabolomics studies have gained attention due to their cumulative effects associated with pathologic phenotypes [2]. Here we investigate whether metabolite abundances pre-treatment can predict a cancer cell's response to a drug, as recorded by the National Cancer Institute (NCI) in 57 human tumor cell lines corresponding to 9 cancer types (breast, colon, brain, lung, ovarian, prostate and renal cancer, melanoma and leukemia) [3].
We took in consideration a panel of 128 previously identified metabolites and 518 drugs with known mechanisms of action or targets. For each drug, three concentrations were described as relevant for the cell's dose response curve - we limited our analysis to the concentration that inhibits half of the cell population growth (GI50). Additionally, we analyzed the potential confounding effect of a set of 42 known oncogenic mutations on some of the metabolite – dose response associations. After preprocessing, 46 metabolites and 457 drugs were selected for further processing. Integration of metabolomics and chemosensitivity was achieved using Spearman's rank correlation, a method robust to outliers that summarizes the strength of the relationship between the two variables.
After the Benjamini-Hochberg correction for multiple testing, we identified 333 significant correlations between metabolites and drug GI50s (padj < 0.05). Lipid metabolism representatives such as cholesterol and inositol 1-phosphate led to significant associations with a variety of drugs such as platinum-based alkylating agents (Oxaliplatin, Tetraplatin), microtubule stabilizers (Paclitaxel) or selective estrogen receptor modulators (Tamoxifen). Amino acid metabolism representatives such as valine, threonine and leucine were predominantly associated with DNA binding drugs such as Bleomycin and topoisomerase inhibitors (Camptothecin). Associations between mutation status and either metabolites or drug GI50s were computed using Kendall's correlation. After the Benjamini-Hochberg correction, no significant correlation (padj < 0.05) was found.
In conclusion, several metabolites significantly correlate with dose response parameters such as GI50 across a variety of drugs and mechanisms, indicating their potential to predict tumor chemosensitivity, although experimental validation is needed in order to move forward.

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Keywords: cancer research, biomarkers, metabolomics, GI50, integrative bioinformatics
Acknowledgement: This work was supported by the STOP Cancer Association, Bucharest, Romania, represented by Dr. Doru Paul.

HIGHLY AGGRESSIVE METAPLASTIC CARCINOMA WITH DIVERGENT CHONDROSARCOMATOUS DIFFERENTIATION

Dana Tapoi [1], Oana Patrascu [1,2], Ana Ciongariu [1], Ionel Dandu Colceriu [1], Maria Sajin [1,2], Mariana Costache [1,2], Adrian Dumitru [1,2]

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Metaplastic breast carcinoma represent a rare heterogenous group of high grande carcinomas defined by the differentiation of the epithelium into squamous cells and/or sarcomatoid cells. We report the case of a metaplastic breast carcinoma diagnosed in a 71 year old woman. The tumour measured 5.5x5 cm, presenting areas of necrosis and haemorrhage. On histopathological examination, the tumour exhibited a highly pleomorphic cellular proliferation, composed of a hazardous admixture of epithelial and mesenchymal looking cells, with frequent atypical mitotic figures. The epithelial cells were markedly atypical, displaying a solid growth pattern with less than 10% glandular differentiation. The intermixed mesenchymal population consisted mostly of large, atypical chondrocytes (90%) and a few rhabdoid looking cells displayed against a blue chondroid matrix with focal necrosis. As metaplastic carcinoma is a very aggressive, yet infrequent neoplasia, careful analysis, including immunohistochemical exams and extensive sampling should be performed in order to identify all the cellular types and avoid the misdiagnosis of a soft tissue sarcoma.

Keywords: breast, cancer, epithelial mesenchymal, sarcomatoid

ENDOCERVICAL ADENOCARCINOMA AND HPV INFECTION

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Endocervical adenocarcinoma is not a frequent cancer of the cervix, as compared to squamous cell carcinoma, representing 10-25% of all cervical carcinomas. The majority are associated with high-risk HPV: types 16, 18, 45. Other risk factors are long duration usage of oral conraceptives or genetic predisposing conditions like Peutz-Jeghers syndrome.
Based on literature data, histological subtypes of endocervical adenocarcinoma more commonly associated with HPV infection are: usual type, endoemtrioid, villoglandular, mucinous intestinal subtype and small cell neuroendocrine tumors.
Other types, like: serous, clear cell, mucinous signet ring cell subtype, are sometimes associated with positivity for HPV infection, and rare types like mesonephric carcinoma and mucinous gastric subtype didn't demonstrated HPV etiology.
Patient's age is usually around 50 years old, but can occur in younger ages, for examples for villoglandular carcinoma the mean age is 35 years old.
Clinical presentation can be as exophytic, polypoid, papillary or nodular mass, often with hemorrhage.
Immunohistochemistry markers are useful in assessing diagnosis towards endocervical origin in problematic cases and for demonstration of and HPV infection, with a diffuse and strong positivity for p16, nuclear and cytoplasmatic co-expression.
The influence of determining a certain subtype or the virus subtype affinity for endocervical epithelium remains an open path for continuous research, and a red flag for the importance of implementing anti-HPV vaccination programs in countries with high incidence of HPV infection and lack of supportive preventing programs.

Keywords: endocervical carcinoma, HPV infection, histological subtype

DAY 3 – SATURDAY, NOVEMBER 7

Session 7: Short communication - young researchers

Young Investigator Award

Session 8: Telocytes in regeneration and pathology

Session 7: Short communication - young researchers

Moderator – Dr. Ana-Maria Enciu

ANTIBACTERIAL EFFECTS OF A SYNTHETIC FLAVONOID AGAINST PENICILLIN-RESISTANT STRAIN OF STAPHYLOCOCCUS AUREUS

Cristina-Veronica Moldovan
Universitatea "Alexandru Ioan Cuza" Din Iași

The World Health Organization considers antibiotic resistance as one of the most important public health issue around the world, who leads to a high level of morbidity and mortality, increased medical costs required to treat patients infected with different microorganisms and a decreased number of different antibiotics that are still effective against multidrug resistant strains. In order to solve this problem, different alternative solutions are being explored in order to counteract the antibiotic resistance phenomenon. One of these includes testing new combinations of antimicrobial compounds that show a synergistic effect.
The main goal of our study was to determine the synergistic effect produced by a synthetic tricyclic flavonoid (Br-Cl flavonoid) with penicillin G against penicillin-resistant strains of Staphylococcus aureus. Our results revealed a 15.62 µg/mL minimum inhibitory concentration (MIC) of the tested flavonoid, while a concentration equivalent to 2 × MIC caused a bacteriostatic effect for more than 12 hours. Using the checkerboard method, we established a synergistic effect of different combinations of flavonoid Br-Cl (1⁄4 and 1⁄8 × MIC) and penicillin G (1⁄4 and 1⁄16 × CMI). In all cases, the growth of S. aureus strain being inhibited for 24 hours.
Given the obtained results, we consider that the tested synthetic flavonoid showed a high antibacterial effect against S. aureus strains. Also, in combination with penicillin G, the flavonoid decreased the bacterial resistance to penicillin G. The Br-Cl flavonoid may be an alternative solution to the antibiotic resistance, but further investigations are required.

Keywords: Br-Cl flavonoid, antibiotic resistance, synergistic effect

THE SHIFTING OF EUSTRESS TO DISTRESS: BEHAVIOR, STRUCTURAL AND MOLECULAR FEATURES OBSERVED IN THE REPEATED RESTRAINT STRESS MODEL

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We investigate the neuroglobin (Ngb) and oligodendrocytes (Olig) activation in the CA3 hippocampus field after repeated stress exposure and their dynamic relation with MeCP2 expression and morphological and behavioral features in the context of the repeated restraint stress. Materials and Methods. Rats were exposed to 2 and 6 consecutive days to immobilization. Three experimental cohorts were used: Control (C), Restraint Stress for 2 days (S2), and Restraint Stress for 6 days (S6), according to a previous study. Oxidative stress and hormones were assayed by UV-VIS and ELISA whereas Ngb, Olig, and MeCP2 were detected by IHC. Brain slices were then stained with Nissl and Bielschowsky methods and standard procedures were done for EM and behavioral analysis. Results. Restraint stress exposure increased corticosterone as well as oxidative stress and decreased the testosterone concentration in CA3. Repeated stress stimulates CNP+ oligodendrocytes and Ngb expression whereas MeCP2 immunoreactivity was decreased in CA3 neurons whereas TET1 modulates the Ngb expression. Nissl staining revealed that repeated stress exposure decreased the neurosecretion deposits in CA3 neurons without morphological changes, confirmed by Bielschowsky silver impregnation. The ultrastructure of the CA3 area revealed slight modifications in neurons and the proliferation of the oligodendrocytes after 6 days of stress exposure. The behavioral echo of the stress was identified after 6 days of repeated stress exposure which induced anxiety-like behavior in rats. Conclusion. 2 days of repeated stress exposure induced eustress patterns whereas 6 days of stress exposure were associated with distress specific changes, marked by mitochondrial disruption, platelets aggregation, Ngb inhibition, and anxiety. Keywords: eustress, distress, neuroglobin, oligodendrocytes, CA3, anxiety.

Keywords: eustress, distress, neuroglobin, oligodendrocytes, CA3, anxiety
Acknowledgement: Project IZO-MOL-EA PN19 35 02 03 is gratefully acknowledged; National Authority for Scientific Research and Innovation: IZO-MOL-EA PN19 35 02 01.

EVALUATION OF CD36 INVOLVEMENT IN THE PROINFLAMMATORY RESPONSE OF ASTROCYTES

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Astrocytes are the main population of cells of the nervous tissue, yet their role in inflammation is under-evaluated. Although microglia are the main players in neuroinflammation, there are several reports of pro-inflammatory activity of astrocytes under amyloid load. CD36 is a scavenger receptor which binds amyloid beta peptide (Aβ) and acts also as a fatty acid transporter.

The aim of this study was to assess whether CD36 blockade in astrocytes impacts on the pro-inflammatory response elicited by Aβ

Cell line and cell treatments: Normal human astrocytes were treated with Aβ 10 μM in the presence and absence of SSO, a CD36 inhibitor. Oleic acid (OA) 40 μM was used as positive control for CD36 binding. Palmitic acid (PA) 20 μM was used as positive control for CD36 related inflammation. Inflammatory response in cell medium was assessed by multiplexing and validated by ELISA. *Animal groups and neurocognitive testing:* Memory impairment of aged CD36 KO mice was compared to age-matched control and NRF-2 KO mice using 8 the arm-maze.

Normal human astrocytes produced and secreted IL-6 and IL-8 following fatty acids treatment and Aβ treatment, in a time-dependent manner. The pro-inflammatory response was mitigated by CD36 blockade with SSO. Next, we investigated whether absence of CD36 could improve memory performance of aged CD36 KO mice. In addition to breeder's recommended control, we tested also age-matched control of NFR2 KO mice, as positive control of neuroinflammation. CD36 KO mice were the best performers in terms of learning and memory, as assessed by 8-arm radial maze test.

In conclusion, CD36 blockade mitigates the proinflammatory response of astrocytes challenged with fatty acids and its KO improves learning and memory. Therapeutical blockade of CD36 could prove a valuable option in neurodegenerative diseases.

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STUDIES OF THE INFLUENZA M1-PROTEIN LAYER USING SUBTOMOGRAM AVERAGING

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Influenza virus epidemics remain an important global threat, with some estimates suggesting over half a million associated deaths each year. Given the more recent SARS-CoV-2 pandemic, it has become even more important that we develop efficient and easily accesible influenza vaccines for the population at risk. Influenza A is a pleomorphic and enveloped virus, characterized by the presence of hemagglutinine and neuraminidase spikes on the outer surface. The shape and rigidity of the viral envelope is supported by the assembly of matrix protein 1 (M1) monomers, a process which has been shown to be influenced by changes in environmental conditions, such as pH and the presence of Na^+ and K^+ ions. This has implications to viral assembly, fusion and potentially vaccine development. Here, we investigated by cryo-electron tomography (cryo-ET) and subtomogram averaging whether changes in the concentration of environmental K+ can lead to alterations of the M1-matrix layer.

Cryo-ET was done on influenza A virus strain X175, plunge frozen in either 100 mM NaCl or KCl solutions (adjusted for pH). Tilt series from -50^0 to $+50^0$ were acquired at 45000x magnification and -5 to -10 µm defocus, giving a final resolution of 0.32 nm/px at sample level. Tomograms were then reconstructed and filtered in the software IMOD-eTomo and finally processed by subtomogram averaging using the software PEET.

Contours of virions with visible M1-matrix layers were manually drawn on digital sections of the filtered tomograms. Coordinates for viral spikes manually set on their circumference, then aligned to increase the signal to noise ratio of the viral envelope and allow more precise measurements. We revealed nanometer-differences between viral envelopes of the two datasets (Na^+ and K^+-treated virions) in favour of K^+ treatment, which indicates that K+ likely alters the association of M1 monomers and/or their three-dimensional conformation.

We implemented and employed subtomogram averaging to study viral structure, a new method for our lab. Initial results suggest that K+ based salts interfere with the formation of the M1-protein layer in influenza virions, which impacts our understanding of viral infection mechanisms, and may assist future vaccine development.

Keywords: influenza virus, M1 protein, cryo-electron tomography, PEET, image processing
Acknowledgment: Project funded by the Ministry of Research and Innovation, CCCDI - UEFISCDI, project number PN-III-P1-1.2-PCCDI-2017-0529 / 62PCCDI / 2018, from PNCDI III.

TOMOGRAPHY AND THREE DIMENSIONAL RECONSTRUCTION OF INTRACELLULAR HEPATITIS B SUBVIRAL PARTICLES

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During infection, hepatitis virus B (HVB) hijacks the cellular machinery and drives the assembly and release of new virions. The viral envelope comprises S (small) and L (large) proteins, which can also associate to form subviral particles (SVPs), essentially empty shells, devoid of genetic material. Despite being non-infectious, they represent an important component of vaccines, owing to they high immunogenic potential. These SVPs can adopt different shapes depending on the representation of each of the two proteins, specifically octahedral spheres when only the S protein is present, and filaments when both S and L proteins are present. The scope of this work was to assess the formation and morphology of SVPs in different subcellular compartments, following transfection of cells with a plasmid encoding a mutated S protein, with potentially higher immunogenicity. This was done using electron tomography (ET) and subsequent 3D reconstruction of tomographic volumes.

Cells from the HEK 293 line, expressing either wild-type (WT) or mutated S proteins, were fixed with glutaraldehyde, embedded in epoxy resin and then 120 nm sections were placed on formvar and carbon coated electron microscopy grids. ET was done using the STEM (scanning-transmission electron microscopy) mode of a Talos F200C system (ThermoFisher Scientific). Tomogram reconstruction and manual and/or semi-automated segmentation were done using the IMOD-eTOMO software package.

ET and 3D reconstruction allowed a precise morphological analysis of SVPs and their intracellular cycle. SVPs localized in dilated regions of the perinuclear space and cisternae of the endoplasmic reticulum (ER) for both datasets. After manually tracing 42 mutant and 18 WT SVPs it was found that both samples contained mostly filamentous shapes, of variable length and with diameters ranging from 20 to 40 nm. SVPs appeared to be mostly in intermediate stages of formation, as they disassemble from filamentous shapes to the spherical shapes that are released in the extracellular space. No obvious morphological changes were observed between WT and mutated S protein SVPs, suggesting similar assembly, cellular trafficking and release. This has potential implications for the development of future vaccines.

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IDENTIFICATION AND 3-D RECONSTRUCTION OF ALPHA-2-MACROGLOBULIN - A CONTAMINANT OF CRYO-EM SAMPLES

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This study aimed to determine the 3D structure of a contaminant protein present in high concentration in cryo-electron micrographs of various cell culture media isolates in our lab. For this purpose we used the software Relion (for REgularised LIkelihood OptimizatioN), an open-source computer program specialized in single particle reconstruction (SPR). In the past decades this technique boosted the 3D reconstruction of biomolecules and viruses imaged by cryo-electron microscopy (cryo-EM), offering new insights into their structure and interactions at the atomic level.

We employed SDS-PAGE and LC-MALDI to identify the contaminant protein as alpha 2 macroglobulin (A2M). Cryo-EM micrographs were acquired on a 200 keV FEI Talos system (ThermoFisher Scientific) at nominal -10 µm defocus and 45000x magnification (0.32 nm/px). Then, we manually selected and processed 4025 particles from 289 cryo-EM micrographs to generate 20 two-dimensional classes, each class representing a possible projection in space for A2M. Of these, we selected four to render the initial 3D model.

Despite a relatively low number of particles and technical limitations associated with both sample and the cryo-EM system, we were able to obtain a 21.7Å resolution model which was tentatively docked with the X-ray crystallographic structure of the receptor binding domain of bovine A2M, using the software Chimera.

In conclusion, we identified bovine A2M as likely an ubiquitous contaminant protein in cell culture supernatants enriched with fetal bovine serum. We also performed an initial 3D reconstruction of A2M by SPR, which although widely used in structural biology elsewhere, represents a novel approach at a national level.

Keywords: single particle analysis, 3D reconstruction, cryo-electron microscopy, alpha 2 macroglobulin, Relion

Acknowledgment: Project funded by the Ministry of Research and Innovation, CCCDI - UEFISCDI, project number PN-III-P1-1.2-PCCDI-2017-0529 / 62PCCDI / 2018, from PNCDI III.

NEUROPROTECTIVE EFFECTS OF ALLICIN ON THE HIPPOCAMPUS IN A RAT MODEL OF TRAUMATIC BRAIN INJURY

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Allicin is one of the main organosulfur compound found in species from the genus Allium. Allicin has been demonstrated to have various anti-oxidative and anti-inflammatory activities both in vitro and in vivo. In this study, we investigated the potential neuroprotective effects of allicin in a rat model of traumatic brain injury (TBI).

Female Wistar rats were divided into 3 groups (Control, Valproic acid (VPA) treatment and Allicin treatment). Allicin (5 mg/kg b.w.) and VPA (150 mg/kg b.w.) were administered daily by enteral route for 20 days. One day after TBI brain tissues were dissected out and prepared for histopathological evaluation (H&E, Golgi-Cox staining) and biochemistry assays. Astrogliosis, glial cell activation and neuronal damage were checked by immunohistochemistry staining for GFAP, MAP2, HMOX1, Neuroglobin and Neurofilament-L. The ipsilateral brain tissues were homogenized and centrifuged under refrigeration. Total protein content and malondialdehyde (MDA), protein carbonyl levels were determined from the supernatant. The enzyme activities of acetylcholinesterase (AchE), catalase (CAT), succinate dehydrogenase (SDH), glutathione peroxidase (GPx), cytochrome c oxidase (CyOX) in tissue homogenate were measured.

Both allicin and VPA treatment decreased the expression levels of MDA and protein carbonyl and preserved the endogenous antioxidant enzyme activities. Allicin increased GFAP, MAP2, HMOX1, neuroglobin levels.

Allicin attenuates neuronal injury and suppresses oxidative damage (increases GPx levels), neuronal inflammation and had protective effects on brain cells nuclei and mitochondrial membranes after TBI.

Keywords: allicin, valproic acid, traumatic brain injury, neuroglobin

Acknowledgement: This work was supported by the project IZO-MOL-EA PN19 35.

THE ROLE OF B-CYCLODEXTRINS IN IMPROVING THE ANTI-FIBROTIC EFFECTIVENESS OF SILYMARIN IN A MOUSE MODEL OF LIVER FIBROSIS

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Silymarin, a standardized flavonolignans extract (silybin, silychristin and silydianin) obtained from milk thistle seeds, has a high capacity to treat gastrointestinal diseases, including liver fibrosis and cirrhosis. Unfortunately, silymarin has a lower solubility and bioavailability because of the presences of polyaromatic rings, which increase hydrophobicity and therefore limits its biological effects after oral administration. This study was performed to demonstrate that inclusion in cyclodextrins (RAMEB/HPBCD) improved the anti-fibrotic and anti-inflammatory effects of the silymarin in CCl4-induced liver fibrosis to mice.

White male mice CD1 were orally treated for two weeks with 50 mg/kg Sy-RAMEB, Sy-HPBCD, and with free silymarin after the induction of liver fibrosis by daily i.p. injection for 7 weeks with carbon tetrachloride (CCl4) dissolved in olive oil (20% v/v ml/kg). The potential effect of the Sy-RAMEB and Sy-HPBCD complexes compared with the free silymarin and spontaneous fibrosis recovery groups were assessed after 2 weeks of treatments.

CCl4 increased TIMP-1, MMP-2, MMP3 and MMP-9 gene expression, decreased MMP-1 gene expression and induced the collagen deposition. Treatment with complexes for 2 weeks remodels ECM components and significantly decreased collagen accumulation via MMPs and TIMPs rebalancing.

The gene expression of the inflammatory markers (NF-κB, TNF-α, and IL-6) were marked increased after CCl4 i.p. injections, and were restored after two weeks of RAMEB/HPBCD-silymarin oral administration, whereas in the group of spontaneous regression of fibrosis, they remained significantly higher.

In this study we demonstrated that the RAMEB and HPBCD increased the anti-inflammatory and anti-fibrotic effects of silymarin and could be considered viable options for its oral delivery and for further applications in the treatment of liver fibrosis.

Keywords: silymarin, carbon tetrachloride, B-cyclodextrin

Acknowledgement: This study was supported by research project fund no. PN-III-P1-1.1-PD-2019-0337

LIPOPOLYSACCHARIDE-INDUCED INFLAMMATION TRIGGERS CYTOSKELETAL REORGANISATION AND CALCIUM SIGNALING ALTERATION IN BRAIN MICROVASCULAR ENDOTHELIUM

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One of the many roles of the blood-brain barrier (BBB) is to protect the central nervous system (CNS) from neurotoxic substances transported by the blood. The BBB permeability is affected by several factors such as inflammation, encephalitis, meningitis, but also endotoxins in the outer membrane of Gram-negative bacteria in its purified form of lipopolysaccharide (LPS). LPS endotoxins are often used in studies for models of bacterial infections, but also to stimulate inflammation associated with infection. The resident macrophages in the (CNS) are the microglia. They play a key role in the process of neuroinflammation, as they are activated by various pathogens invading the CNS. The goal of our study was to analyze the effects of E. Coli LPS (1 µg/mL for 24 hours) on the BBB following the preparation of three in vitro models. Model 1 consists of LPS treated mouse brain microvascular endothelial cells (mBMEC), Model 2 consists of mouse microglial conditionated medium (LPS activated) exposure of mBMEC, and for the Model 3 we co-cultured mBMEC with mouse microglia then exposed to LPS. All three neuroinflammation models revealed a significant upregulation of β-actin filaments staining with phalloidin in mBMEC, revealed by the fluorescence intensity analysis. Confocal microscopy imaging along with digital image analysis performed with the FiberScore algorithm, revealed that LPS is contributing to the remodelling of mBMEC cytoskeleton by inducing fragmentation and a reduction of the mean filament length, as well as changing the distribution of filaments, hence modifying the polarity of the actin network. Viability tests revealed that LPS stimulates cell viability at low concentrations (between 1-100 µg/mL, exposed for 24 hours) and decreases cell viability at high concentrations (over 100 µg/mL, exposed for 24 hours). Following our preliminary calcium imaging results, we performed calcium transients parameters analysis. This way, we observed that the mBMEC exposed to LPS are more sensitive and respond more quickly to the interaction with extracellular ATP molecules. Among the three in vitro models that we tested, Model 2 proved to be the most suitable in mimicking the bacterial-induced inflammation at the level of the BBB. Calcium imaging experiments indicated that LPS modulate the degree of correlation between different pairs of parameters in calcium transient records, which may indicate their effect on various molecular components involved in the ATP-activated processes at the level of mBMEC. In conclusion, multiple processes confined at the level of the BBB are induced by neuroinflammation in the CNS. These processes may imply both direct and indirect (by microglial activation) remodelling of the mBMEC cytoskeleton, as well as changes in the sensitivity and response of mBMEC to extracellular ATP molecules.

Keywords: actin, BBB, lipopolysaccharides, inflammation, cytoskeleton, calcium, endothelium

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Session 8: Telocytes in regeneration and pathology

Moderator – Dr. Mihaela Gherghiceanu

TELOCYTES IN SALIVARY GLAND REGENERATION

Mihnea Ioan Nicolescu

TELOCYTES IN SKIN PATHOLOGY

Cătălin Gabriel Manole
"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
"Victor Babeș" National Institute of Pathology, Bucharest

The presence of telocytes (TCs) was previously documented at the level of dermis, where they microscopically proved morphological similitudes as TCs described in stroma of other organs. Their (ultra)structural phenotype was already coined by previous published data. Even former studies immunohistochemistry showed that dermal TCs have positive expression for several markers, a combination of CD34 and PDGFR□ and β, associated with additional morphological studies, being the most reliable (to date) method of TCs identification. Particularities of their localization within dermis, along blood vessels, nerve endings, among immune cells (lymphocytes, basophils, eosinophils, mast cells, macrophages), they are also coming in relation with, indicated few possible roles of TCs either in skin homeostasis, or in other skin pathologies (in terms of immune surveillance, communication, integration of stromal signals, or contribution to repairing processes). These presumable functions are assumed to be supplementary supported by their involvement in a stromal network by homocelullar and heterocelular junctions. Moreover, in pathologies (e.g. systemic sclerosis, psoriasis), TCs are quantitatively and qualitatively affected, thus they could contribute to (or are affected by) the altered homeostasis of the skin. However, the existence of TCs within the dermis, and their presumable roles in skin homeostasis, corroborated with their involvement in vascular recovery during the lesions healing (as it was demonstrated in heart, for example), indicate attractive directions to be investigated in regard with the further contribution the TCs to skin repair/regeneration processes after injury, or in chronic dermatological pathologies.

TELOCYTES IN CARDIAC REGENERATION

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"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

Although the role of the mesenchymal compartment in tissue homeostasis or disease is increasingly recognized, the relative contribution of distinct cell subsets remains poorly understood. The main limitation has been the lack of specific markers available to discriminate between different interstitial cells. Telocytes (TCs), a type of interstitial cells, are essential involved in heart function through multiple interactions with cardiac progenitors, cardiomyocytes or other cardiac cells, but appreciation of their contribution has suffered from the incomplete characterization and lack of the molecular identity. A highly heterogeneous population of stem and progenitor cells has been described by light immunohistochemistry in the mammalian adult heart, but the ultrastructural identity of cardiac stem cells remains unknown. Using electron microscopy, we demonstrate the presence of cells with stem features in the adult mouse heart. These putative cardiac stem cells are small, round cells, with an irregular shaped nucleus, large nucleolus, few endoplasmic reticulum cisternae and mitochondria, but numerous ribosomes. Stem cells located in a stem cell niche undergo mitosis and apoptosis. Cells with intermediate features between stem cells and cardiomyocyte progenitors have also been seen. Moreover, electron microscopy showed that cardiomyocyte progenitors were added to the peripheral working cardiomyocytes. Telocytes form a supportive interstitial network for stem cells and progenitors in a stem cell niche. Our study enhances the hypothesis of a unique type of cardiac stem cell and progenitors in different stages of differentiation. In our opinion, stem cells, cardiomyocyte progenitors and telocytes sustain a continuous cardiac renewal even low process in the mouse adult mammalian heart, in a restricted coronary pericardial space.

Keywords: telocytes, stem cells, cardiac progenitors, regeneration
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