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ABSTRACT BOOK

(and Meeting Program)

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SCHEDULE OVERVIEW

THURSDAY, NOVEMBER 23

08:30 - 10:00 REGISTRATION

10:00 - 10:30 **Opening Ceremony** (Victor Babeș Auditorium)

10:30 - 11:30 **Session 1: *Cellular Pathology*** (Victor Babeș Auditorium)

Chair: Prof. Dr. Monica Neagu

Extended Lymphocyte Immunophenotyping for Immuno-diagnosis of Recurrent Infections in Children without Primary Immunodeficiency

Cornel Ursaciuc[1], Mihaela Surcel[1], Radu Huică[1], Adriana Munteanu[1], Dan Ciotaru[1], Ioana Pîrvu[1], Coriolan Ulmeanu[2]

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Is there Endoplasmic Reticulum Stress in Limb Girdle Muscular Dystrophy?

Emilia Manole[1,2], Alexandra Bastian[3,4], Bogdan Ovidiu Popescu[1,4,5]

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11:30 - 11:45 COFFEE BREAK

11:45 - 12:30 **Plenary Lecture 1** (Victor Babeș Auditorium)

Porosome: The Secretory Nanomachine in Cells

Bhanu Jena

George E. Palade University Professor, Wayne State University, Detroit, MI, USA

12:30 - 14:00 LUNCH BREAK

14:00 - 16:00 **Session 2A: *Molecular Pathology*** (Victor Babeș Auditorium)

Chair: Dr. Gina Manda

The NRF2-Neuroinflammation Network in Alzheimer's Disease
Antonio Cuadrado
Biomedical Research Networking Center on Neurodegenerative Diseases (CIBERNED), Department of Biochemistry, Faculty of Medicine, Autonomous University of Madrid, Madrid, Spain & Victor Babeș National Institute of Pathology, Bucharest, Romania

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[1]"Prof. Dr. Alex. Obregia" Clinical Hospital of Psychiatry, Department of Pediatric Neurology, Bucharest; [2]Victor Babeș National Institute of Pathology, Medical Genetics Laboratory, Bucharest, Romania

14:00 - 16:00 **Session 2B: *Workshop „Know-how transfer in biomedicine - INTELBIOMED"*** (Ioan Moraru Auditorium)

16:00 - 16:15 COFFEE BREAK

16:15 - 18:00 **Session 3A: *Nephropathology*** (Ioan Moraru Auditorium)

Chairs: Dr. Mihaela Gherghiceanu & Dr. Gener Ismail

Histological Predictors of Renal Outcome in Lupus Nephritis
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[1]Fundeni Clinical Institute, Pediatric Nephrology; [2]Semmelweis University, 3rd Department of Internal Medicine, Research Laboratory, Budapest, Hungary

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Chairs: Prof. Dr. **Takako Kiyokawa**, *Jikei University School of Medicine, Tokyo, Japan* & Prof. Dr. **Simona Stolnicu**, *University of Medicine and Pharmacy, Tîrgu Mureș, Romania*

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Chairs: Dr. Mariana Bobeica & Dr. Gina Manda

11:30 - 11:45 COFFEE BREAK

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Takako Kiyokawa

Jikei University School of Medicine, Tokyo, Japan

12:30 - 14:00 LUNCH BREAK

14:00 - 16:00 **Session 5: *Omics Concepts in Pathology*** (Victor Babeș Auditorium)

Chairs: Prof. Dr. Cristiana Tănase & Dr. Cornel Ursaciuc

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Emilia Severin

Carol Davila University of Medicine and Pharmacy, Bucharest

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Cristiana Tanase[1,2], Elena Codrici[1], Ionela Daniela Popescu[1], Simona Mihai[1], Ana-Maria Enciu[1,4], Laura Georgiana Necula[1], Radu Albulescu[1,2,3]

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Epigenomic Approaches in Cancer Pathology

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Ștefan S. Nicolau Institute of Virology

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SATURDAY, NOVEMBER 25

10:00 - 12:00 **Session 6: Open Session (Varia)**

Chair: Dr. Ana-Maria Enciu

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Cătălin Gabriel Manole[1,2], Gheorghița Isvoranu[1], Laura Cristina Ceafalan[1,2], Emanuel Tudor Fertig[1], Ruxandra Drăgoi Galrinho[1,3], Bogdan Gabriel Marinescu[1]

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Maria Dudău[1], Ana-Maria Enciu[1,2]

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Aquaporin Activity Modulation and Cell Migration

Mirela Onica[1], Cristina-Mariana Niculite[1,2], Andreea-Oana Urs[2], Mircea Leabu[1,2]

[1]Carol Davila University of Medicine and Pharmacy, Bucharest; [2]Victor Babeș National Institute of Pathology, Bucharest, Romania

Extracellular Matrix Proteins Influence the Connective Tissue Cells Activity in a Mimetic Model for Post-Myocardial Infarction Regeneration

Alexandru Florea[1,2], Cristina-Mariana Niculite[1,3], Andreea-Oana Urs[3], Elisa Anamaria Liehn[2], Mircea Leabu[1,3]

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12:00 - 12:30 COFFEE BREAK

Dismantling of posters

12:30 - 13:30 **Closing Ceremony** (Victor Babeș Auditorium)

THURSDAY, NOVEMBER 23

SESSION 1: CELLULAR PATHOLOGY

PLENARY LECTURE 1

SESSION 2A: MOLECULAR PATHOLOGY

SESSION 2B: WORKSHOP „KNOW-HOW TRANSFER IN BIOMEDICINE – INTELBIOMED”

SESSION 3A: NEPHROPATHOLOGY

SESSION 3B: ANATOMIC PATHOLOGY

SESSION 1

CELLULAR PATHOLOGY

EXTENDED LYMPHOCYTE IMMUNOPHENOTYPING FOR IMMUNODIAGNOSIS OF RECURRENT INFECTIONS IN CHILDREN WITHOUT PRIMARY IMMUNODEFICIENCY

Cornel Ursaciuc[1], Mihaela Surcel[1], Radu Huică[1], Adriana Munteanu[1], Dan Ciotaru[1], Ioana Pîrvu[1], Coriolan Ulmeanu[2]

[1]Victor Babeș National Institute of Pathology, Bucharest, Romania; [2]"Grigore Alexandrescu" Children's Hospital, Bucharest, Romania

Keywords: immunology, lymphocyte populations, flow cytometry

Introduction: Standard lymphocyte immunophenotyping (SLI) (T-CD3+, T-CD4+, T-CD8+, B, NK cells) contributes to the diagnosis or exclusion of primary immunodeficiencies (PID). In recurrent infections (RI) not related to PID, SLI appearance may be inconclusive. Therefore, we investigated some additional lymphocyte subgroups that may have an impact on the pathogenesis of RI: immature B cells (CD19+CD10+), naive B cells (CD19+sIg+), memory B cells (CD19+CD27+), plasma cells (CD19+CD38+), double negative T cells (T-DN) (CD3+CD4-CD8-), NKT cells (CD3+CD16/56+CD4±CD8±CD1d+). The objective was to guide diagnosis by this extended lymphocyte immunophenotyping (ELI), revealing some cell subgroups, usually untested, that might show significant changes.

Method: SLI and ELI was applied in 25 children aged 1-9 years, presenting RI not related to PID. The control group consisted of 18 healthy subjects. The determinations were made from EDTA-collected fresh whole blood, using 8-color flow-cytometry.

The data acquisition and analysis of results was performed with Becton-Dickinson equipment: FACSCanto II flow cytometer, compensation microspheres (Anti-Mouse Ig,□ /Negative Control Particles Compensation Set), FACSDiva 6.1 software.

Results: CD19+ lymphocytes (B cell population) were low in 67% of cases, especially by diminishing naive B cell subpopulation (50% cases). Immature B cells and memory B cells decreased in either 11% cases. CD3+ lymphocytes (T-cell population) were low in 11% cases, mainly by lowering T-CD4+ subpopulation (helper T cells) in 28% of cases. T-DN were high in 22% of cases, 75% of these being associated with T-CD4+ cell decreases. NK cells were high in 39% cases, while NKT cells showed no modification. The overall improvement of immunodiagnosis by ELI was obtained in 22% cases with T-cell modifications and 72% cases with B-cell deficiencies. ELI alone was useful only in 28% patients with B-cell modifications.

Conclusions: ELI determines more accurately the origin of decreases in peripheral blood B, T or NK cell populations in IR, proving usefulness particularly in situations of B-lymphocyte and T-CD4+ cell depletion. Diagnosis features can consequently be varied and adapted to each case.

Acknowledgement: the study was funded by grant of Romanian Ministry of Education and Research, Core project 22N 03 04/2016.

IS THERE ENDOPLASMIC RETICULUM STRESS IN LIMB GIRDLE MUSCULAR DYSTROPHY?

Emilia Manole[1,2], Alexandra Bastian[3,4], Bogdan Ovidiu Popescu[1,4,5]

[1]Victor Babeș National Institute of Pathology; [2]Colentina Clinical Hospital, Research Department;[3]Colentina Clinical Hospital, Pathology Department; [4]Carol Davila University of Medicine and Pharmacy; [5]Colentina Clinical Hospital, Neurology Department

Keywords: skeletal muscle, UPR biomarkers

Introduction. Endoplasmic reticulum stress (ERs) is caused by the accumulation of unfolded/misfolded proteins and leads to a cell-specific response, the so-called ""unfolded protein response"" (UPR). It is a complex process involving three signaling pathways: IRE-1, PERK and ATF-6. The literature data confirms that there is an association between ERs, cellular metabolism impairment and autophagy. The main function of the UPR is the restoration of ER function. But if the phenomenon is excessive or prolonged, it can cause pathological changes in the muscle cell, leading to cell death/apoptosis. In the pathological skeletal muscle, an increased expression of some ERs markers was observed, especially in myositis. There are very few studies regarding ERs in muscular dystrophies (myotonic dystrophy type 1, muscular dystrophies with deficit in caveolin-3, fukutin-related protein and dystrophin), especially on animal models, the UPR mechanisms remaining poorly understood. Here we present some preliminary data on the phenotypic expression of several ERs biomarkers in the skeletal muscle of some patients with different limb girdle muscular dystrophies (LGMD): eIF2 alpha, CHOP, ATF3 (PERK signaling pathway), XBP1 (IRE-1 signaling pathway), NF-kB (linked to all three signaling pathways)

Materials and methods. The research was done on muscle biopsies from patients with different forms of LGMD. Control samples were normal muscle tissue from patients with mild peripheral neuropathy. The expression of the investigated proteins was revealed by Western blotting and immunofluorescence. Cryopreserved tissue and cryosections were used (after the pathological evaluation).

Results and discussions. Variations in the expression of UPR biomarkers in the muscle tissue of LGMD patients, compared with control samples, were observed. We hypothesized that ERs is heightened and contributes to the muscle pathology. Although the investigated proteins over-expression was observed, we did not find direct correlations between the degree of UPR activation and the severity of pathological muscle affection, LGMD being the result of complex molecular mechanisms. One of the limitations of this investigation is that LGMD are rare diseases, with a relatively small number of study subjects. Another limitation is the small amount of muscle tissue available for such extensive proteomic studies on a large number of UPR biomarkers.

Conclusions. LGMD are genetic myopathies, characterized by myofibrillar degeneration and muscle replacement with connective/fatty tissue, resulting in a nonfunctional muscle. A complex understanding of the molecular mechanisms that regulate a functional muscle mass is prerequisite. Our study may lead to the development of pharmacological therapies targeted on the various UPR signaling pathways to preserve or restore the muscle mass of LGMD patients and could have a major impact on the recovery/prolongation of the life of these patients until a genetic therapy."

Acknowledgement: The study was funded by MCI, Project PN 16.22.02.04.2016.

PLENARY LECTURE 1

POROSOME: THE SECRETORY NANOMACHINE IN CELLS

Bhanu P. Jena

Department of Physiology, School of Medicine, Wayne State University, Detroit, USA; Department of Chemical Engineering and Material Sciences, College of Engineering, Wayne State University, Detroit, USA

Secretion is a fundamental cellular process in living organisms, from yeast to cells in humans. Since the 1950's, it was believed that secretory vesicles completely merged with the cell plasma membrane during secretion. While this may occur, the observation of partially empty vesicles in cells following secretion suggests the presence of transient or so called 'kiss-and-run' mechanism that allows fractional discharge of intra-vesicular contents during secretion. This proposed mechanism is mediated by a supramolecular cup-shaped structure at the plasma membrane called 'porosome', which enable secretory vesicles to transiently fuse with the plasma membrane, expel a portion of its contents, and disengage. Porosomes range in size from 15 nm in neurons and astrocytes, to 180 nm in endocrine and exocrine cells. Neuronal porosomes are composed of nearly 40 proteins, compared to the 120 nm nuclear pore complex of >500 protein molecules. Porosome structure, its chemical composition, and functional reconstitution into artificial lipid membrane, and the molecular assembly of membrane-associated t-SNARE and v-SNARE proteins in a ring or rosette complex to establish the fusion pore at the porosome base, and the molecular mechanism of secretory vesicle volume increase required for intravesicular content release during cell secretion, collectively provide a molecular understanding of cell secretion, resulting in a paradigm-shift in our understanding of the process.

SESSION 2A

MOLECULAR PATHOLOGY

THE NRF2-NEUROINFLAMMATION NETWORK IN ALZHEIMER'S DISEASE

Antonio Cuadrado

Biomedical Research Networking Center on Neurodegenerative Diseases (CIBERNED), Department of Biochemistry, Faculty of Medicine, Autonomous University of Madrid, Madrid, Spain & Victor Babeș National Institute of Pathology, Bucharest, Romania

Inflammatory pathways chronically activated by damaged neurons underlay neurodegeneration. The mechanisms that change beneficial inflammation into detrimental and chronic neuroinflammation in Alzheimer's disease (AD) are still elusive, but preliminary evidence points towards NRF2 transcription factor, among other triggers. We have addressed this question with a mouse model of AD that combines amyloidopathy and tauopathy on a wild type (AT-NRF2-WT) or NRF2-deficienct (AT-NRF2-KO) background. The proteinopathy induced cognitive deficits and premature death of mice, with a marked spinal deformity accompanied with motor problems that were accelerated by the absence of NRF2. A transcriptomic analysis demonstrated that AT-NRF2-KO evidenced dysregulation of inflammatory process mediated by chemokines and cytokines. Accordingly, deficiency in NRF2 worsened inflammation and this resulted in more astrogliosis and microgliosis as determined by an increase in GFAP, IBA1 and CD11b. To determine whether pharmacological targeting of the transcription factor NRF2 might provide a disease-modifying therapy, we studied dimethyl fumarate (DMF), a drug already in use as antioxidant modulator of inflammation for the treatment of multiple sclerosis. Daily oral gavage of DMF during six weeks improved cognition and motor problems in the AT-NRF2-WT mice compared with the vehicle treated animals. This study demonstrates the relevance of inflammatory responses in AD, tightly regulated by NRF2 activity, and provides a new strategy to fight Alzheimer's disease.

BIOMARKER SEARCH IN CHRONIC NON-COMMUNICABLE DISEASES USING MASS SPECTROMETRY BASED PROTEOMICS

Felicia Antohe, Raluca Boteanu, Viorel Suica, Elena Uyy, Luminita Ivan, Maya Simionescu

Institute of Cellular Biology and Pathology N. Simionescu

Keywords: biomarker, non-communicable diseases, proteomics, mass spectrometry

Introduction: A wide range of chronic pathological conditions and vascular diseases are associated with inflammation that offers protections against harmful stimuli (induced cell injury, tissues damage) by means of specialized mediators and cells. Alarmins, or damage-associated molecular patterns molecules (DAMPs), are critical molecular biomarkers of the immune response to tissue suffering. These danger signals are released by insulted cells into the extracellular milieu and bind to specific receptors to stimulate and promote activation of innate immune cells, cell differentiation, cell death, or secretion of inflammatory mediators. The recently called precision medicine, aims to rapidly translate the basic research results to clinical applications for early diagnosis and personalized therapy of patients. This strategy is closely supported by the unprecedented technological development of mass spectrometry based proteomics in biomedical research. **Objective:** To evaluate the expression of alarmins released under various stress factors (as hyperlipidaemic diet, hyperglycaemia, or other insults that generate cancer) in experimental biological systems, targeting the chronic vascular diseases. **Results:** Based on biochemical and mass spectrometry proteomic data, we showed that alarmins are potential biomarkers of chronic non-communicable diseases that are involved in maintaining and amplifying inflammation. *In vivo* and in culture experiments we demonstrated that the high lipid stress induces increased expression of certain heat shock proteins (HSPs) in endothelial cells. In experimental atherosclerosis and diabetes on small animals the high-mobility group box 1 is highly associated with atherosclerotic plaques progression and proteomic alteration of membrane microdomains respectively. Mass spectrometry evidenced a specific expression pattern of S100 proteins both in human patients and pancreatic cancer cells in culture. **Conclusions:** The data complement and also support published results that underlines the multiple functions of these proteins acting as inducers, sensors and mediators of inflammation. The pattern of alarmins and signalling pathways activated in particular situations are closely related to certain types of diseases and evolution characteristics of each patient. Mass spectrometry together with the powerful bioinformatics tools will allow in the near future the smart exploitation of generated data for efficient use in clinic for the benefit of the medical doctors but most of all of the patients.

Acknowledgement: The present work was supported by the Romanian Academy and Ministry of Education and Research grant PN-II-PCCE/CNDI-UEFISCDI no. 90,135 and 153/2012-2016.

CHALLENGES IN CLINICAL INTERPRETATION OF NEW MUTATIONS IN RARE CONDITIONS

Magdalena Budisteanu[1,2], Sorina Mihaela Papuc[2], Andreea Țuțulan-Cuniță[2], Ioana Borcan[2], Raluca Colesniuc[2], Carmen Burloiu[1], Aurora Arghir[2]

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Keywords: mutations, phenotype-genotype correlation

Objective: We report on 3 cases with complex clinical phenotypes and unreported mutations detected at sequencing, which raise difficulties in interpretation.
Cases presentation: First case is a 3 years-old boy with epileptic seizures, macrocephaly and psychomotor regression, and MRI suggestive for Alexander disease (diffuse demyelization in fronto-insular white matter and striate nucleus bilaterally, with progressive aspect). Sequencing of GFAP gene was indicated. The second case is a 7 years-old with epilepsy and developmental delay. In this case a panel of 150 genes for epilepsy has been sequenced by next generation sequencing. The third case is a 2 years-old girl with global developmental delay and ataxia. Whole exome sequencing was performed in this case.
Results: The genetic testing revealed: in the first case, a heterozygous variant in exon 1 of the GFAP gene, c.292G>C, previously unreported, which was interpreted as being of uncertain significance; in the second case, 2 mutations, in heterozygous state, on SLC2A1 gene, c.1176C>T, reported as pathogenic, and c.998G>A, considered as unclassified variant of undetermined pathogenicity; in the third case, a previously unreported heterozygous variant in the CACNA1A gene, c.2122G>A.
Conclusions: In these cases, gene sequencing could not bring a clear clinical interpretation, although variants in genes relevant for their respective phenotypes were identified. New data (genetic testing of the parents, other cases with similar variant, functional studies) could bring further information about the clinical impact of these variants of uncertain significance.

SESSION 2B

WORKSHOP

"KNOW-HOW TRANSFER IN BIOMEDICINE - INTELBIOMED"

SESSION 3A

NEPHROPATHOLOGY

HISTOLOGICAL PREDICTORS OF RENAL OUTCOME IN LUPUS NEPHRITIS

Bogdan Obrișcă[1], Roxana Jurubiță[1], Vlad Berbecar[1], Bogdan Sorohan[1], Camelia Achim[1], Raluca Bobeica[1], Andreea Andronesi[1], Mihaela Gherghiceanu[2], Gener Ismail[1]

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Keywords: lupus nephritis, kidney biopsy, histopathological lesion

Lupus nephritis is encountered in 40% to 70% of patients with systemic lupus erythematosus during their natural course. The prognostic value of ISN/RPS classification is controversial and therefore we aimed to analyze clinical and pathological predictors of outcome in lupus nephritis patients.
For this study, 37 patients with lupus nephritis that underwent percutaneous kidney biopsy between 1997 and 2016 were included. 20 clinical and 25 histological variables were tested for their association with renal flare and a combined end-point of doubling of serum creatinine, ESRD and death.
During a median follow-up period of 17,5 months (IQR: 48-120 months), 5 patients experienced a renal flare, 5 progressed to ESRD and 2 died. The percentage of glomeruli with extracapillary proliferation correlated with eGFR (r= -0,45, p=0,005), serum albumin (r=-0,55, p<0,001), haemoglobin levels (r= -0,41, p=0,01) and proteinuria (r= 0,52, p<0,001). In univariate analysis those patients with a higher percentage of global (p=0,02), with the presence of crescent (p=0,004), tubulitis (p=0,048), adhesions (p=0,003), class 4 (p=0,03) with higher baseline proteinuria (p=0,031) and lower eGFR (p=0,003) were more likely to reach the combined end-point. However, none of these variables were associated with the outcome in multivariate analysis. The patients that experienced a renal flare had a lower percentage of glomeruli with global and segmental glomerulosclerosis (0% vs 14%, p=0,003 and 0% vs 13%, p=0,03, respectively) and a higher percentage of glomeruli with mesangial hypercellularity (100% vs 66%, p<0.001).
Evaluation of all histopathological lesions in lupus nephritis could add to the prognostic value of ISN/RPS classification.

A RARE CASE OF NEPHROTIC SYNDROME AND RECURRENT SUBFEBRILITY

Bogdan Sorohan[1], Marina Paraschiv[1], Mihaela Gherghiceanu [2,3], Ismail Gener[1,2]

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Keywords: secondary AA amyloidosis, familial Mediterranean fever, proteinuria

Familial Mediterranean fever (FMF) is an inherited autoinflammatory disorder, caused by mutations of the Mediterranean fever (MEFV) gene that is characterized by recurrent episodes of fever and serosal or cutaneous inflammation. Chronic inflammation can lead to secondary AA amyloidosis due to the accumulation of extracellular amyloid protein in various tissues, even in the kidneys.
A 29-year-old male patient presented with lower limbs edema and inguinal adenopathy. He had a positive family history of nephrotic syndrome, CKD, hemodialysis, amyloidosis and personal history of recurrent purpura, subfebrility and arthralgia. At admission serum creatinine was 1.02 mg/dl, serum uric acid 9.3 mg/dl, serum albumin was 2.6 g/dl with a 24h proteinuria of 3.8 g/day and urinary sediment was normal. Serology was negative for lupus, vasculitis, Sjogren syndrome and rheumatoid arthritis. The patient also had hepatosplenomegaly. We performed a kidney biopsy that showed amorphous masses in the mesangium, arterioles, positive for Congo red stain, tubulointerstitial focal fibrosis and inflammation, tubular atrophy (light microscopy), mesangial, subendothelial, tubular basal membrane deposits (electron microscopy) and no IF examination. After this result we referred him to the Haematology Department, where a lymph node biopsy was performed that showed perivascular and sinusal amyloid deposits. After bone marrow biopsy, immunohistochemistry tests, immunofixation electrophoresis, kappa/lambda ratio and genetic tests, primary amyloidosis and Glu54Gln gene mutations for transthyretin were excluded. A second renal biopsy was undertaken and the tissue was fixed in paraffin and sent to an amyloidosis specialized center for further examination. Light and electron microscopy findings were in concordance with the first one but immunohistochemistry was positive for anti-AA (mcC). Their primary proposed diagnosis was secondary AA amyloidosis caused by a chronic inflammation process, like in FMF.
FMF affects mainly ethnic groups originating from the Mediterranean basin. According to diagnosis criteria, our patient fulfilled 2 minor criteria, but we didn't perform a genetic test for FMF. In the period between the two renal biopsies the renal function remained stable but with variable values of serum albumin (min 1.1 g/dl) and 24h proteinuria (max 9.6 g/day). We started the treatment with Colchicine 1mg/day for 14 days, increased at 2 mg/day but was reduced to 1 mg/day due to digestive intolerance.
We described a case of 29-year-old Romanian man with nephrotic syndrome, secondary AA amyloidosis and FMF. Even rare this diagnostic should be considered for differential diagnosis.

COMPLEMENT IN KIDNEY DISEASE – FROM PHYSIOPATHOLOGY TO THERAPEUTICAL TARGETING

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Keywords: complement cascade, kidney disease, Eculizumab

The complement system serves as the first line defense against invading pathogens and is a component of the innate immune system. The system is composed of three distinct activation pathways: classic pathway (CP), alternative pathway (AP), and mannose-binding lectin pathway (LP). Any pathway activates the complement cascade generating C3-convertase which cleaves C3 into C3a and C3b. In normal conditions, a small amount of C3-convertase is activated by the AP and is necessary to have regulators to prevent complement attack on healthy self-cells. This regulation is provided by a combination of plasma and cell surface inhibitory proteins.

Over activation of complement is involved in Lupus nephritis, anti-glomerular basement membrane glomerulonephritis, antineutrophil cytoplasmic antibody-associated vasculitis, membranous nephropathy, C1Q nephropathy, IgA nephropathy, immune complexes-associated membranoproliferative glomerulonephritis. Dysregulation of complement is involved in atypical hemolytic uremic syndrome, C3 glomerulopathies. Complement in renal transplantation is involved in: ischemia-reperfusion injury, cell-mediated rejection, and antibody-mediated rejection Complement is apparently involved in many kidney diseases as well as in kidney transplantation. Hence targeting complement cascade at different levels may represent a new therapeutic strategy directed against the pathogenetic mechanisms. Emerging evidence has recently documented that the complement cascade as a common pathogenetic mechanism in many kidney diseases, in the constant progression of the kidney diseases and the kidney transplantation. Even if only the anti-C5 monoclonal antibody is on the market, many targets as C1, C3, C5a, and C5aR are the object of national or international trials.

Many complement system molecules proved to be effective in vitro or preclinical trials and are waiting to move to human trials in the future. For these reasons, complement system should be carefully investigated in kidney diseases assessment.

ELECTRON MICROSCOPY IN THE DIAGNOSIS OF GLOMERULAR DISEASES

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Keywords: endothelial cells, basement membrane, electron tomography

Electron microscopy is performed as a routine diagnostic technique in glomerular diseases. The EM examination of kidney biopsies with focus on glomerular ultrastructure is crucial for the diagnosis of minimal change disease, genetic defects of glomerular basement membrane, fibrillary glomerulopathies or viral infections of grafts. Besides its utility in kidney diseases diagnostic, electron microscopy assessment of glomerulus may advance research in this field.

Electron tomography was used to investigate the glomerulus and we found out that endothelial cells of glomerular capillaries present a cytoplasmic specialization which extend into the capillary lumen as folds. The cytoplasm of glomerular endothelial cells extends into the capillary lumen and forms a sieve next to the cellular body. There are no fenestrae at the level of these cytoplasmic folds, but an organization of gaps and cytoskeletal elements could be seen.

Various names were previously used to describe such structures: ridge-like, pseudopod-like, pored projections, or cytofolds. There is no theory about the involvement of endothelial cytofolds in glomerular function. Our preliminary data suggests that endothelial cells loose the cytofolds in proliferative glomerular lesions. If the function of cytofolds is to respond to the variation of blood flow velocity or to act as a membrane reserve remains to be investigated. Identification and description of new structural elements could have an impact in our understanding of renal physiology and pathology.

Acknowledgment: ANCSI PN 16.22.03.02/ 2016.

SESSION 3B

ANATOMIC PATHOLOGY

CHARACTERIZATION OF THE TUMOR-MICROENVIRONMENT IN CERVICAL EPITHELIAL NEOPLASIA

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Keywords: inflammatory response, stromal edema, oxidative stress

Cervical cancer is one of the most common causes of cancer-associated mortality in women worldwide. Although neoplastic epithelial transformation and the association with HPV infection has been widely studied, there is less research on microenvironmental response.
To study the alterations of the microenvironment associated with cervical cancer development, we analyzed 32 cervical samples. The histological spectrum of alterations varied from low grade intraepithelial lesions to invasive carcinoma. Formalin-fixed, paraffin-embedded 4-cm tissue sections were stained with various immunohistochemical antigens targeting both neoplastic epithelial cells and stromal component (ER, ki 67, MDA, 8-OHdG, 3-NT). The stromal response was heterogeneous and showed different patterns which were correlated with the epithelial alterations. Cancer is not an "all or nothing" process, but integrates various landmarks into a pathological network of events and cellular responses under the influence of many types of stress - oxidative and inflammatory, which support tumorigenesis and tumor progression, but are also effective antitumor therapeutic tools. Recent data showed that the chemo and radiotherapy efficacy requires a tumor-activated oxidative status, suggesting that the pharmacological inhibition of the endogenous antioxidant system, for example by inhibiting Nrf2 activity, may represent an adjunctive therapeutic approach in solid tumors to control resistance to conventional anti-tumoral therapy.

Acknowledgement: This paper has been supported by the Ministry of Research and Innovation, Project no. 16.22.04.04.

MANAGEMENT OF IMMUNOHISTOCHEMICAL HETEROGENOUS LESIONS OF MULTIPLE BREAST CARCINOMAS

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Keywords: breast carcinoma, invasion, multiple centers, immunohistochemistry marker

Introduction. Multiple synchronous breast carcinomas have histopathological characteristics that are still not established. In search of a complete and correct definition of multicentric and multifocal breast carcinomas, there is still much left to debate. Therefore, estimating prognosis and response to treatment remains a challenge.

Materials and methods. The medical records from the Emergency University Hospital of Bucharest between January 2015 and December 2016 reveal 36 cases of invasive multiple breast carcinomas. Tissue samples from all the tumours were evaluated both histologically and immunohistochemically. Tumour features such as diameter, distribution, grading and lymphovascular invasion were also assessed. The samples were tested for the immunohistochemical expression of ER, PR, HER2, Ki-67, E-cadherin and p53.

Results and discussions. Of all the 36 cases invasive multiple breast carcinomas, 4 cases had a multicentric distribution and the rest had a multifocal distribution. All of the multicentric distribution cases were associated with Luminal A phenotype and lymphatic invasion ($p<0.05$). The difference in tumour grading between the multicentric and the multifocal carcinomas was not statistically relevant. Im-munohistochemical tests revealed a high proportion of heterogeneity between the index tumour (the largest tumour) and the lymphatic metastasis. These contradicting findings make these cancers difficult to manage, in both terms of treatment and of reporting of the cases.

Conclusions. Lymph node invasion was the most frequent in the multicentric carcinomas. In such cas-es, it is very important to evaluate the heterogeneity between the index tumour and the lymphatic invasion. Oncologic treatment should be individualised for these patients, particularly because there is no approved consensus for managing these kind of breast cancers.

FRIDAY, NOVEMBER 24

SESSION 4A: WORKSHOP
"UPDATE IN GYNAECOLOGICAL PATHOLOGY" (I)

SESSION 4B: WORKSHOP
"BIOMEDICINE IN THE CONTEXT OF ELI-NP"

PLENARY LECTURE 2

SESSION 5: OMICS CONCEPTS IN PATHOLOGY

SESSION 4A WORKSHOP
"UPDATE IN GYNAECOLOGICAL PATHOLOGY" (II)

SESSION 4A

WORKSHOP

"UPDATE IN GYNAECOLOGICAL PATHOLOGY" (I)

UPDATE IN GYNAECOLOGICAL PATHOLOGY

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[2]University of Medicine and Pharmacy of Tirgu Mures, Romania

This workshop includes 2 lectures and 2 slide seminars. The first lecture will be presented by Takako Kiyokawa under the title "Update in ovarian tumors". After briefly reviewing the WHO histological classification of ovarian tumors revised in 2014, typical cases of ovarian carcinomas as well as borderline tumors will be presented. Some less common ovarian tumors in other categories will also be touched upon. The related interactive slide seminar, titled "Endometrial lesions" will use a multihead microscope to examine and discuss selected cases of benign and malignant endometrial lesions. In addition to histological features by H&E slides, participants will learn the utility of immunohistochemistry in the diagnosis. The second lecture, "Update in cervical pathology", presented by Simona Stolnicu, will focus on endocervical adenocarcinoma, providing information about a novel three-tiered histopathological risk stratification system (the Silva system) which better identifies which patient may be at risk of lymph node (LN) metastases and can spare unnecessary lymphadenectomy. Also, we will comment on a new classification proposal based on morphological features linked to etiology (International Endocervical Adenocarcinoma Criteria and Classification, IECC). IECC criteria distinguishes between HPV-associated adenocarcinoma (HPVA) and no or limited HPVA features (NHPVA), using H&E slides alone; both categories can be further stratified using existing morphologic criteria. The second slide seminar will focus on the morphological and immunohistochemical diagnostic criteria in different microscopic types of endocercical adenocarcinomas, precursor glandular and squamous cervical lesions, non-neoplastic cervical lesions, adenosarcoma of the uterus, mature and immature teratomas, as well as cervical rabdomiosarcoma.

SESSION 4B

WORKSHOP

"BIOMEDICINE IN THE CONTEXT OF ELI-NP"

PLENARY LECTURE 2

KRUKENBERG TUMORS OF THE OVARY

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Keywords: Ovary, neoplasm, metastatic carcinoma, Krukenberg tumor

The Krukenberg tumor is a metastatic ovarian carcinoma that contains a significant component (>10%) of mucin-filled signet-ring cells typically lying within a cellular stroma. The term has been used inappropriately by some authors, however, to include all adenocarcinomas that have metastasized to the ovary or all metastatic ovarian carcinomas that originated in the gastrointestinal tract. The eponymous designation of the tumor derives from the description of Krukenberg in 1896, even though he erroneously classified the neoplasm as a primary fibrosarcoma containing mucinous cells.

The patients' age ranges widely but it occurs in young patients more often than other common types of metastatic ovarian tumor. The average age is about 45 years, and 40% of the patients are younger than 40 years. The patients most commonly present with abdominal swelling or pain but may be asymptomatic.

The tumors are bilateral in 3/4 of the patients and are typically solid with bosselated external surfaces. The microscopic spectrum of the Krukenberg tumor is broader than that often present in the literature with various architectural patterns, prominence of signet-ring cells, epithelial cell types, and stromal changes. Glands and cysts are common, and stroma may be luteinized. Vascular invasion by tumor cells are common, most frequently in the hilus. Envelopment and sparing of intact follicles and their derivatives, which are typically destroyed by other malignant ovarian tumors are also often seen in the Krukenberg tumor. Because of wide variety of morphology of the Krukenberg tumor, pathologic differential diagnosis is in wide range.

The primary carcinoma has the propensity to be occult and it may not be identified until autopsy. The primary carcinoma is known before detection of the Krukenberg tumor in only one-third of the patients. Two-thirds of the tumors are of gastric origin, but they can originate from other organs including colon, rectum, appendix, breast, and biliary tract.

SESSION 5

OMICS CONCEPTS IN PATHOLOGY

INCORPORATION OF RARE DISEASES GENOMIC MEDICINE INTO MAINSTREAM HEALTHCARE: NEW HOPES AND CHALLENGES

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Around the world, the rare disease community represents one of the largest patient communities. Thus, rare diseases are a global health challenge posing a significant medical and economic burden for patients, communities and healthcare systems. For many rare diseases, the lack of scientific knowledge and quality information on the cause of the disease, pathophysiology, natural course of the disease and epidemiological data results in a delay of diagnosis, misdiagnosis, conflicting medical opinions and stress for patients, their families, caregivers, physicians and society as a whole.

In this review, we discuss the current status of genetic technologies properly applied, patients with poorly differentiated sets of clinical symptoms or rare combinations that could not easily be recognised by the non-specialist and how can receive a more rapid diagnosis and, thereafter, treatment and management by experts in that rare disease. We highlight successful strategies for gene identification and the implementation of genomic technologies into clinical practice for patients with rare diseases, who" in addition to patients with cancer represent the first health care beneficiaries of these revolutionary technologies". Research advances have led to the development of new diagnostic and therapeutic procedures.

PRECISION MEDICINE FOR THE FUTURE

Cristiana Tanase[1,2], Elena Codrici[1], Ionela Daniela Popescu[1], Simona Mihai[1], Ana-Maria Enciu[1,4], Laura Georgiana Necula[1], Radu Albulescu[1,2,3]

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Precision medicine is based on advanced omics technologies, such as next-generation sequencing, protein and gene microarray, laser capture microdissection, implying the integration of genomic, epigenetic, proteomic, metabolomic, and clinical phenotypes of the individual patient. The development of multiplex genotyping technologies and high-throughput genomic profiling allow the analysis of individual patient genome from peripheral blood or small biopsy material.

Next-generation sequencing (NGS) technologies and data have changed cancer investigation and provided support for clinicians in treatment decision-making. NGS technologies have permitted an "omics" approach to cancer, allowing a genomic, transcriptomic, and epigenomic characterization of the disease of individual patients.

The main target of personalized medicine is to understand the molecular mechanism of the disease and to integrate it with individual pharmacogenomics profile that defines the response to drugs. The personalized treatment implies the characterization of predictive (diagnostic), prognostic, treatment and prevention biomarkers.

Omics profiling reflects more accurately real-time physiological status. Personalized omics analyses both disease as a whole and disease processes within for a better understanding of the individualized health. Through this approach health monitoring, preventative medicine, and personalized treatment can be targeted simultaneously.

Acknowledgment: Partially supported by the grants PN 16.22.04.01, PN 16.22.05.03 and TE 101/2015.

PRECISION HEMATOLOGY: FROM KARYOTYPE TO NEXT GENERATION SEQUENCING

Aurora Arghir[1,2], Sorina Mihaela Papuc[1], Andreea Ţuţulan-Cuniţă[1], Raluca Colesniuc[1], Dan Soare[2,3], Ioana Borcan[1], Diana Cîşleanu[2,3], Silvana Angelescu[2,4], Mihaela Andreescu[5], Ana-Maria Vlădăreanu[2,3], Horia Bumbea[2,3]

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Keywords: DNA microarray, chromosomal anomalies, sequence variants

Precision medicine (PM) aims to tailor disease management to unique individual variability, most importantly to genetic variability. Hematology has long been at the forefront of PM. Genetic investigations of hematologic neoplasms allowed a better understanding of leukemogenesis and development of targeted therapies. The first targeted approaches, such as all trans retinoic acid for PML/RARA positive leukemias and tyrosine kinase inhibitors for BCR/ABL1 translocation patients provided a paradigm for treatment in oncology.

The management of patients with hematologic neoplasms strongly requires genetic testing for diagnosis, risk stratification and minimal residual disease monitoring. There is a wide range of genetic/genomic assays at use in hemato-oncology, each of them having unique advantages in specific clinical settings.

We report on the use of genetic investigations in patients with myeloid and lymphoid neoplasms and its contribution towards a better clinical care.

Bone marrow or leukemic peripheral blood samples were analyzed at diagnosis, before any therapy. Classical genetic analysis included GTG banding and fluorescence in situ hybridization (FISH) tests. Genomic profiling was performed on CGH/CGH+SNP microarray platforms with resolution varying between 60 kb to 25 kb (Agilent Technologies). Targeted next generation sequencing (NGS) testing with Ion AmpliSeq™ AML Research Panel was done on Ion PGM System (ThermoFischer Scientific).

Recurrent chromosomal anomalies, with known prognostic impact, as well as novel/rare or complex abnormalities were detected in our patient group. Rare reciprocal translocations and complex karyotypes with low level amplification of KMT2A through unbalanced translocations and other structural rearrangements (ring chromosomes) were identified. Copy-neutral loss of heterozygosity on chromosomes 6p and 13q were detected in patients with normal karyotypes. Targeted sequencing revealed mutations recurrently reported in the panel of analyzed genes; besides these, several mutations with potential deleterious effects, previously unreported in cancer databases, were identified. The cytogenetic data contributed to diagnostic refinement (e.g. acute leukemia with recurrent genetic abnormalities), risk stratification and therapeutic decision (tyrosine-kinase inhibitors in t(9;22) leukemias) in our patient group. The use of combined genetic testing (FISH testing, array-CGH and targeted NGS) allowed a better characterization of chromosomal anomalies, especially in cases with complex karyotype changes, or definition of the molecular profiles for cases without gross chromosomal anomalies. Our study highlights the value of genetic testing that informs diagnosis and prognosis in precision hematology.

Acknowledgement: This work was supported by to Ministry of Research and Innovation Projects no 16.22.01.01 and 16.22.05.01. The authors wish to thank other colleagues from Emergency University Clinical Hospital, Coltea Clinical Hospital and Colentina Clinical Hospital that contributed to this work.

DATA ANALYSIS OF SOMATIC GENE VARIANTS IN ONCOLOGY: ACUTE MYELOID LEUKEMIA STUDY

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Keywords: next generation sequencing, cancer, acute myeloid leukemia

Application of next-generation sequencing (NGS) technologies in tumor samples allowed the discovery of somatic variants (single nucleotide variants, small deletions/ insertions). Genomic data analysis revealed the presence of a high number of somatic mutations per tumor sample showing that the genetic landscape of cancer is complex, affecting a much larger number/types of genes than previously expected. Gene panels with different number of targeted genes or hot-spot regions are used in clinical practice to generate information regarding the tumor gene variant with clinical impact in diagnostic classification, prognostic insights and/or therapeutic decisions. The identification of the variants with potential impact in cancer depends on several experimental and technical aspects, the most critical being the overall assay performance and raw data quality. Accurate interpretation of clinical significance is also an essential step, based on frequency of the variant in general population and/or reports in specific cancer databases. The final aim is to distinguish between driver mutations and passenger mutations.

We present acute myeloid leukemia (AML) targeted NGS data as an example of somatic variants analysis. Genomic profiling with Ion AmpliSeq™ AML Research Panel (Ion PGM, ThermoFisher Scientific) was performed on genomic DNA extracted from diagnostic bone marrow samples from 12 patients with AML and normal cytogenetics. Raw data quality was evaluated using Torrent Suite software (ThermoFisher Scientific). The alignment and data analysis were performed using two different software: Ion Reporter v.5.2 (ThermoFisher Scientific) and NextGENe v.2.4.2.1 (SoftGenetics).

A total of 40 mutations, with a median of 4 mutations per patient (between 1 to 5 mutations/patient) were identified in our patient group. Among these, 31 are recurrent mutations reported in hematologic malignancies, while 9 mutations with deleterious effects /in silico predictions, were not previously reported in cancer databases (ClinVar, COSMIC). The most frequent mutated genes in our study group were NPM1 (10 samples), followed by DNMT3A gene (8 patients).

Targeted NGS proved a successful approach for molecular profiling of AML. Comparative analysis using two different software solutions enhanced the accuracy of somatic variant identification.

Acknowledgement: This work was supported by Ministry of Research and Innovation, Projects no 16.22.01.01 and 16.22.05.01.

EPIGENOMIC APPROACHES IN CANCER PATHOLOGY

Anca Botezatu, Iulia V. Iancu, Irina Huică, Adriana Pleșa, **Gabriela Anton**

Ștefan S. Nicolau Institute of Virology

Introduction. Epigenetic alterations are considered major features in cancer development. The most studied epigenetic change is DNA methylation. Cervical cancer being one of the malignity with viral etiology, the methylation of both viral (human papillomavirus, HPV) and host genes is link with this pathology. Taking into account that DNA methylation status has been shown to be an early event during carcinogenesis and that the magnitude of this epigenetic modification is related to disease severity, the aim of this study was to determine in cervical scraps an association between the methylation status of E6HPV and of the host cell genes.

Methods. From a cohort of 649 women who were investigated for cytological and viral screening, 54 subjects were confirmed positive for HPV 16 (Roche – LINEAR ARRAY HPV Genotyping Test). The group included women with NILM (n= 14), low grade intraepithelial lesion, LGSIL (n= 24) and high grade intraepithelial lesion HGSIL (n=19) cytology. The methylation status of E6HPV, DAPK, E- cadherin, p16 and hMLH-1 was quantified by sequencing and qMSP (FastStart Universal SYBR Green Master, Roche Molecular Biochemicals) of bisulfite treated DNAs (EpiTect Bisulfite kit, Qiagen, Valencia, California, USA) along with positive and negative controls (Millipore, Billerica, MA, USA).

Results. Methylation of the E6 gene promoter was found in 71.4% NILM, 54.16% LSIL and 42.1% HGSIL cases. Methylation in all host genes promoters significantly increased with lesion severity (p=0.002). Epigenetic changes in DAPK promoter strongly discriminated HGSIL from NILM (range of odds ratios [OR]: 4.048-13.296) while p16 gene promoter methylation showed the highest predictive value for cytology abnormalities (86.9%) (range of odds ratios [OR]: 1.65-14.051). An inverse correlation was observed between the methylation status of E6 HPV16 and hMLH-1 in LSIL/HSIL (samples (p=0.058). To determine biomarker performance in patient screening, sensitivity and specificity were calculated. The best correlation was calculated for the E-cadherin gene (OR = 21,579) and DAPK (OR = 9,711). A good correlation was observed for the hypermethylation of the promoters of p16 (OR = 6.488), and hMLH1 (OR = 3.756).

Conclusions. Epigenetic changes in viral and host genes can distinguish among precursor lesions and association with viral genotyping can be used to stratify women at risk to develop severe cervical lesion and cervical cancer.

SESSION 4A

WORKSHOP

"UPDATE IN GYNAECOLOGICAL PATHOLOGY" (II)

SLIDE SEMINARS

SATURDAY, NOVEMBER 25

SESSION 6: OPEN SESSION (VARIA)

SESSION 6

OPEN SESSION (VARIA)

EXPERIMENTAL MODEL OF HEART FAILURE - SURGICAL BANDING OF TRANSVERSE AORTA IN WISTAR RATS. PRACTICABILITY AND IMPLICATIONS

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Keywords: heart failure, aorta banding, echocardiography cardiac (ultra)structure

A variety of etiologic factors are known to cause heart failure. Among them, the myocardial infarction is the common cause of heart failure due to ischemic heart disease. Another possible cause for heart failure is hypertrophic (obstructive) cardiomyopathy, due to high-pressure gradient in the left ventricle outflow tract. Cardiac (ultra)structure is affected in both etiologies of heart failure, involving both heart muscle compartment and the interstitial compartment, respectively. In Victor Babeș National Institute of Pathology, we implemented the experimental model of heart failure by surgical banding of transverse aorta to Wistar rats. The surgical discovery of transverse aorta was made using a suprasternal thoracotomy. The diameter of the aorta was surgically reduced to a targeted value by using a Polypropylene 5-0 suture and a metal guide (with previously acknowledged diameter). The initial diameter of the transverse aorta was documented by echography. The cardiac structural and functional consequences were assessed by echocardiography, performed at specific time intervals. This experimental model allows tracing the progression of cardiac (ultra)structural and functional changes. Adapting and implementing an experimental animal model for heart failure due to hypertrophic cardiomyopathy simplify a complex disease to experimental variables. Moreover, it creates the reliable model for studying the involvement of transplanted cardiac interstitial cells (including telocytes), either alone or in tandem with cardiac stem cells to heart failure specific processes of regeneration/remodeling/repairing. Furthermore, the development of a reproducible experimental model of heart failure opens new perspectives for studying cardiac remodeling/reparative processes of failing heart and also new therapeutic methods, with possible major implications in heart translational medicine.

Acknowledgement: This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS – UEFISCDI, project number PN-II-RU-TE-2014-4-1811.

EXPRESSION OF AMYLOID PRECURSOR PROTEIN IN THE BRAIN OF CAVEOLIN-1 KNOCK-OUT MICE

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Keywords: amyloid precursor protein, cav-1 KO mouse, cerebellum

Introduction. Amyloid precursor protein (APP), is expressed in the healthy human brain and has been linked to Alzheimer's disease(AD), its pathogenic role being intensely studied. A link between APP and caveolin-1 (cav-1) -the scaffold protein for membrane microdomains called caveolae - has been proposed, but the precise role of cav-1 has not been demonstrated in the pathogenesis of AD. In this study, we investigate whether the expression and phosphorylation of APP changes in caveolin-1 knock-out mice, a model which exhibits multiple characteristics of Alzheimer's disease from an early age.

Materials and Methods. In this study we compared C57Bl6 mice (control) with STOCK Cav1tm1Mls/J mice (knock-out for caveolin-1) of the same age and gender. On membrane cell fraction from mouse cerebellum and brain lysate, we performed Western Blot and Native Electrophoresis for APP, phospho-APP, caveolin-1 and GAPDH.

Results. Amyloid precursor protein levels were found to be expressed differently between brain regions in caveolin-1 knock-out mice and the control group, particularly in the cerebellum. Phosphorylated forms of APP were expressed at varying molecular weights in the cerebellum of both control and knock-out mice, suggesting that APP may aggregate into oligomers. In the membrane fraction of the cerebellum, cav-1 and APP were highlighted at the same molecular weight.

Conclusions. The caveolin-1 knock-out mouse is an important tool for studying neurodegenerative processes, therefore it could be used as a possible model of Alzheimer's disease. Furthermore, our results indicate an interaction at the membrane level between caveolin-1 and amyloid precursor protein, APP expression and phosphorylation being influenced by caveolin-1.

Acknowledgement: NCS-UEFISCDI PN-II-RU-TE-2014-4-1534 project.

AQUAPORIN ACTIVITY MODULATION AND CELL MIGRATION

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Keywords: time-lapse videomicroscopy, immunofluorescence, focal adhesion kinase

Introduction. Aquaporins are channels that transport water across membranes, in and out of the cell, driven by the coloid-osmotic pressure. Several physiological roles for human aquaporins have been described, such as: nerve excitability, urine concentration, cell migration, but also in a diversity of pathologies: diabetes insipidus, neuromyelitis optica, cancer invasion and metastasis. This work focuses on the implication of aquaporins in cell migration, under treatment with AgNO3 and/or DHA (cis-docosa-4,7,10,13,16,19-hexaenoic acid).

Materials and methods. Two immortalized cell lines have been used in a wound healing experimental model: normal keratinocytes (HaCaT) and dysplastic keratinocytes (DOK). Cell motility has been studied by time-lapse video microscopy, under treatment with 10µM AgNO3 and/or 100µM DHA. The immunofluorescence technique has assesed the effects of the treatment on expression and distribution of certain proteins, that play a critical role in cell migration: AQP1, 2, 3, FAK (focal adhesion kinase) and pFAK (phosphorylated, active form).

Results. By time-lapse microscopy, it has been observed that AgNO3 lowered cell motility. DHA, when administered alone, favored cell movement, whereas in the combined treatment with AgNO3, markedly decreased cell migration. Regarding cell trajectories, AgNO3 and/or DHA treatment leads to slower and chaotic cell movement as compared to control cells. The immunofluorescence images taken at 2 hours after wounding, showed that AgNO3 and combined treatment promoted a perinuclear distribution of AQP1 and AQP2, suggesting a de novo synthesis. In contrast, AgNO3 treatment did not trigger any change in FAK and pFAK expression and distribution. Differences were observed between cell lines as follows: DOK cells had a higher expression level of FAK and pFAK, with a main localization at the migration pole, indicating a more advanced motility compared to HaCaT cells.

Conclusions. Normal and dysplastic keratinocytes treated with AgNO3 and/or DHA presented different behavior in terms of both cell migration and distribution of AQP1 and AQP2. However, AQP3, FAK and pFAK have not been influenced by the treatments.

EXTRACELLULAR MATRIX PROTEINS INFLUENCE THE CONNECTIVE TISSUE CELLS ACTIVITY IN A MIMETIC MODEL FOR POST-MYOCARDIAL INFARCTION REGENERATION

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Keywords: fibroblast, myofibroblast, staurosporine, TGF-β, cell proliferation

Introduction. The events following the myocardial infarction have two critical steps: acute inflammation, which tries to clear the debris created by the cytotoxic milieu, and tissue regeneration via fibrosis, where fibroblasts and myofibroblasts come into play. A challenge is represented by the development of an extracellular matrix that both protects the cells against the aforementioned cytotoxic milieu, but that is not fibrotic. Here, fibroblast and myofibroblast real-time dynamics and proliferation were assessed on different extracellular matrix proteins (i.e. collagen, fibronectin and laminin) in the cytotoxic medium created by staurosporine (STS).

Materials and Methods. To determine a useful STS concentration, fibroblast real-time dynamics was monitored using a time lapse videomicroscopy system. Cells were cultured on plain or collagen –coated surfaces and incubated for 24h in medium with serial diluted STS, between 10µM, and 0.625µM. To check the reversibility of the cytotoxic effect, cells were incubated for an additional 24h with fresh medium (without STS). Fibroblast proliferation was estimated using a proliferation assay with spectrophotometric determination. Cells were pre-incubated with 1.25µM STS, for 24h, on collagen, fibronectin or laminin-coated surfaces. Fibroblast and myofibroblast (cells transdifferentiated with TGF-β) proliferation dynamics was assessed using an electrical impedance monitoring system. Cells were cultured on collagen, fibronectin or laminin-coated surfaces and incubated with 1.25µM STS, for 24h.

Results and discussions. The time lapse videomicroscopy revealed that collagen protected fibroblasts at 1.25µM STS (32.7% dead cells on collagen vs. 53.6% on plain surface); therefore, 1.25µM was selected as the STS concentration for subsequent experiments. The proliferation assay suggested that collagen best protected fibroblast against the cytotoxic milieu created by 1.25µM STS (62.83% STS vs. control, on collagen, compared to 44.86% on fibronectin, and 31.07% on laminin).The electric impedance monitoring system confirmed the ranking suggested by the proliferation assay, in the case of fibroblasts (relative cell index: 86% on fibronectin, 76% on laminin, as normalized to collagen, considered 100%). For myofibroblasts, both collagen and fibronectin performed equally well, while laminin still underperformed (relative cell index: 106% on fibronectin, 76% on laminin, as normalized to collagen, considered 100%).

Conclusions. Our results suggest that collagen offers the best protection (in terms of survival and proliferation) for fibroblasts, while both collagen and fibronectin similarly protect myofibroblasts.

POSTERS

Hanging of posters: Thursday 18:00 - 18:30

Viewing and presentation: Friday 16:15 - 18:00

Poster dismantling: Saturday 12:00 - 12:30

IN VIVO TOXICITY ASSESSMENT OF SILICON QUANTUM DOTS

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Keywords: nanoparticle, oxidative stress, redox balance

Introduction. Quantum dots (QDs) are nanocrystalline semiconductor materials that have been recently tested for biological applications such as cancer therapy, cellular imaging and drug delivery. The purpose of this study was to evaluate in vivo the degree of oxidative stress generated at the liver level following administration of Si / SiO2 QDs.

Materials and methods. Silicon QDs toxicity was investigated by injection into the codified vein of these Si / SiO2 QDs in Swiss mice, being tested in 3 different concentrations (1, 10 and 100 mg QDs / kg body weight). After 24 hours of nanoparticle administration, the mice were sacrificed and liver tissue was sampling. From the total protein extracts, were measured the specific activities of the antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione reductase (Gred), glutathione S-transferase (GST), glucose 6-phosphate dehydrogenase (G6PDH), as well as reduced glutathione (GSH) and malonaldehyde (MDA) concentration. The results have been reported to those obtained in control mice, injected with physiological serum.

Results. The analyzes showed that the highest dose (100 mg QDs / kg body weight), decrease by 30% CAT activity, by 22% G6PDH activity, by 15% GST activity, and by 20% GPX and GSH concentration, respectively. The performed determinations demonstrate the lack of toxicity of Si / SiO2 QDs to concentrations of 10 mg/kg body, not affecting the redox balance at the liver.

AN AUTOPSY CASE REPORT OF WEIL'S SYNDROME

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Keywords: leptospirosis, silver impregnation, liver, multi-organ

Objective: Weil's syndrome, a rare infectious disorder, is a severe form of leptospirosis, transferred to humans through urine or tissue of an infected domestic or wild animal, usually work-related. It has high global incidence mainly in the tropical developing countries, making it a rare disorder in Romania, especially in urban settings.

Method: We present the case of a 42-year-old male, with fatal leptospirosis, who died due to multisystem organ failure. A complete autopsy was performed and samples from all organs were harvested. Formalin-fixed, paraffin-embedded tissue sections were studied using hematoxylin-eosin stain and Warthin-Starry silver stain.

Results: Gross examination revealed plum-colored, dark red lungs, mottled, enlarged liver and pale kidneys. Histologically, we identified intra-alveolar hemorrhage and extensive hyaline membranes indicative of diffuse alveolar damage, bilateral pneumonia, focal liver necrosis, cholestasis and fatty change and acute tubular necrosis in the kidneys. The Warthin-Starry silver impregnation stain done on liver sections showed numerous black, roundish spirochetes against a yellow to pale brown background, suggestible for the granular form of Leptospira.

Conclusion: Weil's Syndrome is a devastating disorder with multi-organ involvement and a high mortality rate. The spirochetes are usually identified by using silver impregnation techniques. This disease should always be considered in patients with sepsis who develop multiple organ dysfunctions, as appropriate treatment is life-saving.

GENETIC CHARACTERIZATION BY FISH OF A COHORT OF MATURE B-CELL NEOPLASMS PATIENTS PRELIMINARY RESULTS

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Keywords: mature B-cell neoplasms, prognosis

Mature B-cell neoplasms are a heterogeneous group of hematologic disorders from clinical and genetic perspective. Classical chromosomal analysis, fluorescence in situ hybridization (FISH), array-based comparative genomic hybridization and DNA sequencing are currently used for genetic characterization of these disorders. FISH is a widely available, robust, standardized technique that offers important diagnostic and prognostic information for mature B-cell neoplasms patients.

We analyzed a patient group consisting of 36 chronic lymphocytic leukemia (CLL), 2 mantle cell lymphoma (MCL) and 6 multiple myeloma (MM) cases. Interphase FISH studies were performed on peripheral blood samples using commercially available probes (Vysis, Abbott Molecular) for the following regions: 17p13.1 (TP53), 11q22.3 (ATM), 13q14, chromosome 12 centromere and 6q23 (MYB). Translocation probe for IGH/CCND1 fusion gene and break-apart probe for IGH locus were also used (Vysis, Abbott Molecular).

Twenty three out of 44 investigated patients showed at least one anomaly of the targeted regions. Eleven CLL patients, one MCL patient and 2 MM patients showed unique aberrations by FISH. Multiple aberrations (defined by the presence of at least two anomalies) were identified in 6 CLL patients, one MCL patient and 2 MM patients. Most of the multiple aberrations included deletion of TP53 locus (e.g. del TP53 plus trisomy 12/ del ATM/ del 13q14) which is in agreement with literature data, del TP 53 being more frequently associated with genomic instability. In 3 patients the fluorescent signals pattern was suggestive of hyperdiploidy, further testing by karyotype analysis being required.

FISH testing has been shown to be useful for genetic characterization and risk stratification of patients with mature B-cell neoplasms and most importantly for treatment initiation decision.

MICRORNA EXPRESSION IN KRAS- AND BRAF- MUTATED COLORECTAL CANCERS

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Keywords: miRNA signature, gene mutation, predictive marker

Objective: RAS and RAF gene mutations play an important role in the activation of RAS-RAF-MEK-ERK signaling pathway in colorectal cancer (CRC) progression. Recent studies have shown that microRNAs (miRNAs) signature is associated with specific tumour subtypes. This study aimed to determine the miRNA signature in KRAS- and BRAF-mutated CRC.
Method: We studied fresh-frozen fixed in RNAlater tumour fragments from 18 patients with CRC. To identify miRNA signature we used Human Cancer Pathway Finder miRNA PCR Array, Qiagen, USA, comparing 10 mutant with 8 wild-type KRAS and BRAF tumours (KRAS: codons 12, 13, 59, 61, 117 and 146, BRAF: codons 600 and 601). Analysis of the results was performed using Free miRNA PCR Array Data Analysis, Qiagen, USA.
Results: The presence of the KRAS and BRAF mutation was associated with 14 downregulated miRNAs: miR-132, let-7d, miR-138, let-7i, miR-10a, miR-15b, miR-193b, miR-181d, miR-100, miR-98, let-7f, miR-181c, miR-128 and miR-155 (p=0,004÷0,043).
Conclusions: We identified a specific miRNA signature associated with KRAS and BRAF mutation in CRC. Larger series of patients are necessary for application of these miRNAs as predictive/prognostic markers and as promising candidate in the better stratification of CRC patients.

Acknowledgement: This work was supported by PN 16.22.01.02 and POSCCE 173/2010.

OVEREXPRESSION OF HSA-MIR-143-3P AND HSA-MIR-145-5P INHIBITS CELL PROLIFERATION AND MIGRATION IN BREAST ADENOCARCINOMA CELL LINE

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Keywords: microRNA mimics, breast cancer, tumor suppressor

MicroRNAs (miRNAs) are small non-protein coding RNAs that regulate expression of a wide variety of genes including those involved in tumor initiation and progression. Increasing evidence has suggested that the components of miR-143/145 cluster have low expression level and function as tumor suppressors in several types of cancer, including breast cancer. Up-regulation of these tumor-suppressor acting microRNAs could modulate the tumorigenic process and therefore these microRNAs might be potential therapeutic tools for cancer treatment.

In the present study we analyzed the biological effect of overexpressed hsa-miR143-3p and hsa-miR-145-5p on MCF-7, breast adenocarcinoma cell line. One or both mimic miRNAs were transiently transfected and 48-72 hours post-transfection the cellular proliferation, viability and migration were assessed and compared with cells transfected with negative control miRNAs. Furthermore, target gene expression regulated by these microRNAs were studied.

The overexpression of either one or both miRNAs had significantly inhibited proliferation and reduced cellular viability in comparison with control cells, demonstrated by MTS assays and real time cellular monitoring (xCELLigence platform) experiments. The normalized cellular index (IC) values for miRNA mimics transfected cells being 1.5 to 2 times smaller than the IC values for cells transfected with negative control miRNA. The cells migration in wound healing experiments revealed that the recovery time for miRNAs transfected cells were significantly larger than for cells from control experiments. Also, couple of miRNAs target gene involved in epigenetic mechanisms were analyzed by qRT-PCR and immunofluorescence experiments.

Conclusion: In this study we demonstrated that overexpression of hsa-miR-143 and hsa-miR-145 inhibits cellular proliferation, migration and reduces viability of MCF-7 cells.

Acknowledgement: This work was supported by the PN 16.22.04.03 grant.

PRIMARY NEUROENDOCRINE NEOPLASMS OF THE RECTUM: A MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF 10 CASES

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Keywords: NET, NEC, digestive tract, immunohistochemistry

Introduction: Neuroendocrine neoplasms can arise throughout the digestive tract, with a slow increase in incidence for the colorectal region in the last decade.
Objective: The aim of this study is to present a series of 10 cases of rectal neuroendocrine neoplasms diagnosed at Fundeni Clinical Institute between 2005 and 2016, with their distinctive pattern on microscopic examination and immunohistochemistry.
Materials and methods: We studied 10 cases of primary rectal neuroendocrine neoplasms (five neuroendocrine tumours and five neuroendocrine carcinomas), surgically removed and diagnosed in Fundeni Clinical Institute between September 2005 and December 2016 (11 years).
Results: Out of 10 cases diagnosed as neuroendocrine neoplasms in our pathology department at Fundeni Clinical Institute, 5 were male patients with a median age of 66.6 years (the youngest patient being 51 and the oldest 83) and 5 were female patients with a median age of 54.2 (the youngest being 43 and the oldest 67). 2 patients (20%) were diagnosed with neuroendocrine tumours G1 (carcinoids of the rectum), 3 patients (30%) were diagnosed with neuroendocrine tumours G2 and 5 with neuroendocrine carcinomas (50%). On macroscopic examination, the neuroendocrine tumours (NET G1 and NET G2) were mostly protrusive polypoid sessile lesions (3 out of 5 – 60%) with one being a nodular submucosal lesion (20%) and one being an ulcerated infiltrative lesion (20%). The diameter of the neuroendocrine tumours was roughly 4.8 cm (between 0.8 and 15 cm). On microscopic examination NET G1 and G2 had a classic solid and trabecular pattern with occasional rosettes, positive for neuroendocrine markers (synaptophysin, chromogranin, neuron specific enolase, CD 56) and a proliferation factor (Ki67) between 2 and 10%. One neuroendocrine tumour (NET G2) developed multiple hepatic and pulmonary metastases. Neuroendocrine rectal carcinomas were mostly ulcerated, infiltrative, stenosing tumours, with a medium diameter of 5.6 cm (between 4 and 9 cm) with one multifocal carcinoma presenting as multiple nodules throughout the rectal wall between 0.5 and 8 cm. The microscopic examination revealed mixed small and large cell neoplasms with positive neuroendocrine markers, a high cellular pleomorphism and an average high mitotic rate (Ki67) of 31% (between 20 and 40%). One neuroendocrine carcinoma extended locally to the bladder and prostatic walls, one developed peritoneal metastases and two multiple hepatic metastases.
Conclusion: Neuroendocrine neoplasms in the rectum are rare, slow growing aggressive neoplasms with a tendency to extend locally to bladder wall, regionally to pelvic lymph nodes and to evolve to hepatic and pulmonary metastasis if untreated surgically or with targeted therapy.

MLL-AF9 MURINE MODEL OF HUMAN ACUTE MYELOID LEUKEMIA

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Keywords: leukemic stem cell, cellular, molecular, mice

Animal models of human acute myeloid leukemia (AML) are important for understanding the disease mechanisms as well as for testing innovative therapeutic strategies. The Mll-AF9 knock-in allele encodes a MLL-AF9 fusion protein whose expression results in development of acute myeloid leukemia in human patients and mice. Murine hematopoietic stem cells (CD45.2) were injected intravenously through the tail vein into lethally irradiated mice (CD45.1). The mice transplanted with MLL-AF9 cells displayed signs of leukemia within 6-10 weeks. PCR, flow cytometry and clinical observation were employed to evaluate the murine acute leukemia model system.
In this study, we summarize the molecular mechanism of MLL-associated leukemia and the potential applications of this model to develop better targeted therapies.

STATUS THYMICOLYMPHATICUS: REAL OR FAKE!? CASE REPORT

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Keywords: sudden death infant syndrome, thymus hypertrophy

Sudden Death Infant Syndrome still remains a problem for the coroners and anatomopathologists. For over a century, status thymico-lymphaticus was considered responsible for the sudden death in children. This case presentation proposes to highlight again that "status thymicolymfaticus" is an outdate concept: the hypertrophic thymus hiding another preexistent pathological process, the thymus being the effect and not the cause of the tanatoregenerator process.

Clinical report: There is presented the new born P. M., male, spontaneously born with cranial presentation, being resuscitated in the delivery room, undergoing all the neonatal resuscitation stages, without any success. Despite the positive pressure ventilation on the endotracheal tube, the thorax trips were of low amplitude, inefficient ventilation, and inefficient resuscitation procedures. He died after 45 minutes since birth, when the resuscitation team stops the resuscitation procedures.

Pathologic report: At opening the thorax, there was observed a hypertrophied thymus, compressing both lungs. The liver was also oversized for a term delivery newborn. There were sampled fragments of organs, which were subject to the standard histological processing, by fixation in 10% neuter formalin and hematoxylin-eosine staining. The microscopic aspect thymus highlighted a cortico-medullar ratio quite high in favor of the cortical, rich in lymphocyte population, with the dilated subcapsular sinuses. The general aspect was of a highly quantitative developed thymus, with a qualitative differentiation left from the gestational age. The lungs were practically non-respiratory, with the bronchi mucosa epithelium plicaturated and with areas of athelectasia. The liver microscopically presented a long-lasting liver suffering.

In **conclusion**, we may state that cardiorespiratory failure, which was the immediate cause of death, could have been caused by the thymus hypertrophy. Still, this hypertrophy cannot be considered as the initial cause, but more a complication of an intrapartum preexistent condition, most probably of hepatic nature.

EVOLUTION OF DIAGNOSTIC AND PROGNOSTIC BIOMARKERS IN RHEUMATOID ARTHRITIS TREATED WITH ANTI-TNF ALPHA AGENTS

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Keywords: rheumatoid arthritis, anti-TNFα therapy, biomarkers

Introduction: Rheumatoid arthritis (RA) is a complex autoimmune disease characterized by a chronic inflammatory reaction of the joint synovium that causes progressive and irreversible joint destruction. Although the biological therapies revolutionized RA, 20-40% of patients are reported to be non-responders. Side effects and high cost of therapy require the identification of key biomarkers at different stages of RA evolution. The present study follows the evolution of diagnostic / prognostic biomarkers from three to six months of anti-TNFα therapy: rheumatoid factor (RF) IgG, IgA, IgM, cyclical citrullinated peptide (anti-CCP) antibody, cartilage oligomeric matrix protein (COMP), 14-3-3 η protein, anti-mutated citrullinated vimentin (anti-MCV), C Reactive protein (CRP).
Materials and methods: The casuistry includes a batch of patients (non-responders to Methotrexate) with anti-TNFα therapy; the trials were performed before therapy (visit 0), after 3 (visit 1) and 6 months (visit 2) with anti-TNFα; the disease activity was monitored according to the evolution of the DAS28 score. Quantitative dosages of RF-IgG, IgA, IgM, anti-CCP, COMP, 14-3-3 η protein and qualitative determination of anti-MCV were performed from serum / plasma by ELISA technique. Serum CRP dosages were performed by nephelometry.
Results: The overall trend of RF evolution is a decrease seen at first at visit 1, then at visit 2. There is no tendency to normalize RF, most seropositive patients at the beginning of anti-TNFα therapy have RF above the normal range even after 6 months of treatment. At visit 2, anti-CCP values are higher than visit 0, although there is a downward trend from visit 1. The COMP values during the three visits are in normal limits and are correlated in most cases with the favourable evolution of the DAS28 score. 14-3-3η protein has a downward trend after visit 2, although 79% of cases still show pathological values. CRP has a downward / normalization trend after visit 2, but 58% of cases still have elevated levels above the normal range.
Conclusions: After six months of therapy, there was a slight decrease trend in RF, anti-MCV and 14-3-3 η protein. The marker with the most significant normalization / decrease was CRP, suggesting the reduction of inflammation. For the other markers, monitoring indicates a slower course under treatment.

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ANALYSIS OF MIXED GERM CELL TUMORS OF THE TESTIS

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Mixed germ cell tumors (GCT) represent a malignant entity which is frequently constituted by: embryonal carcinoma, teratoma, seminoma or Yolk sac tumor. To diagnose such a tumor, at least 2 components are needed, but frequently there are more than 2. The GCT components are important for the clinical implications, and it is recommended to mention in the diagnosis the percentage for each component.

We analyzed a series of 24 mixed GCT from the archives of Victor Babeș National Institute of Pathology. The age range was 18-68, with a median of 33.3 years. Of the 24 cases, 20 had a component of embryonal carcinoma (83.3%), 19 Yolk sac (79.2%), 16 teratoma (66.7%), 9 seminoma (37.5%), 1 coriocarcinoma (4.1%), 1 poliembrioma. Three cases had associated lesions of germ cell neoplasia in situ (12.5%). The most frequent combination (66.6%) was embryonal carcinoma with Yolk sac tumor. Half of the cases (50%) had embryonal carcinoma, Yolk sac tumor, associated with teratoma. Four of these cases had an associated seminomatous component. One case was constituted by: embryonal carcinoma, Yolk sac tumor, teratoma, poliembrioma. Most cases were comprised of 2 components (only 8 had 2 components; 33.3%).

Analysis of these cases revealed the increased incidence of association between embryonal carcinoma and Yolk sac tumor, the third most common component being teratoma. Most of the cases associated more than 2 components.

GENE AND PROTEIN CHANGES IN DYSTROPHINOPATHIES

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Keywords: muscular dystrophy, dystrophin, DMD gene

Background: Duchenne and Becker muscular dystrophy (DMD/BMD) also known as dystrophinopathies are a group of X-linked recessive inherited disorder characterized by progressive weakness caused by mutations in the DMD gene which encodes a large cytoskeletal protein dystrophin. The correlations between type of mutations and the corresponding protein level is an important issue in this pathology with no cure nowadays.
Aim: The aim of our study was to determine mutations that occur in DMD gene and how these mutations affect dystrophin expression in a cohort of 40 Romanian patients with dystrophinopathies.
Methods: We evaluated the expression of protein dystrophin by two main complementary techniques: immunofluorescence (IF) and immunoblotting (WB). Spectrum of mutations in DMD gene was established by multiplex ligation-dependent probe amplification (MLPA).
Results: Dystrophin protein analysis by both qualitative and quantitative methods IF and WB revealed two patterns: a total absence and a reduced level of dystrophin. 16 patients out of 40 showed a total absence of dystrophin suggesting a sever DMD phenotype. Molecular analysis for these patients permitted to identify: 12 deletions and 2 duplications. Most of deletions were located in the distal hotspot region of DMD gene that encompasses exons 40-55 from rod domain and one case presented a mutation in the proximal hot spot region (exon 2-19). The two duplications were also located in rod domain. 24 patients out of 40 with a mosaic pattern of dystrophin expression revealed by IF and WB were identified with in-frame deletions in distal hotspot region of DMD gene and BMD phenotype.
Conclusion: We observed in our study group a higher tendency for mutations in the central areas of DMD gene with a higher frequency of deletions than duplications. Based on the absence of C-terminal domain in all patients with a sever DMD phenotype as well as reduced level of dystrophin for C-terminal domain in all BMD patients, our results indicate a critical role of the C-terminal domain in producing a proper function of dystrophin.

GENETIC CAUSES OF INTELLECTUAL DISABILITY AND AUTISM: X FRAGILE SYNDROME IN A COHORT OF PEDIATRIC PATIENTS

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Keywords: X fragile syndrome, intellectual disability, autism

X fragile syndrome (XFS, Martin-Bell syndrome, OMIM #300624) is the most frequent monogenic defect associated with intellectual disability and autistic features, with a prevalence of 1/4,000–5,000 in males and 1/5,000-8,000 in females, respectively. In addition, XFS patients exhibit a particular facial dysmorphism, more obvious starting with puberty, macroorchidism, macrocephaly, seizures, variable intellectual disability, speech development delay, anxiety, stereotypic movements, conjunctive tissue abnormalities. In most cases, its genetic defect is represented by a dynamic mutation consisting of a trinucleotid expansion in FMR1 (FRAXA) gene, at Xq27.3 or, rarely, in AFF2 (FMR2, FRAXE), at Xq28; rarely, substitutions or deletions in these two genes may lead to the same outcome in the patient. Genetic testing is mandatory for the confirmation of clinical diagnosis, according to the most recent American and European guidelines, and it is essential in the case of very young patients, whose phenotype is less suggestive. However, there are very few studies dedicated to XFS in Romanian population.

The present study aimed at investigating for X fragile status a small cohort of 62 paediatric patients, aged from 2 to 14 years, referred for genetic testing by "Prof. Dr. Alex. Obregia" Clinical Hospital of Psychiatry, between 2011 and 2016. Peripheral blood was collected from paediatric patients under informed consent. DNA was isolated using PureLink (Invitrogen, Thermo Fisher Scientific). X fragile status was assessed either by triple-primed qF-PCR (FastFraX, Elucigene, performed on an Applied Biosystems 7500 Fast Real-Time PCR System, Life Science Technologies) or by methylation-specific MLPA (SALSA MS-MLPA probemix ME029-B3 FMR1/AFF2, MRC Holland, followed by capillary electrophoresis on an 3500 Applied Biosystems 3500 equipment and analysis using the dedicated Cofalyzer software, MRC Holland). Our investigation found two patients positive for the dynamic mutation in FMR1, out of 62 (1.6%), in our cohort of patients. The two patients were first-degree maternal cousins. Both triple-primed qPCR and MS-MLPA are reliable options for the evaluation of X fragile status; while qPCR-based test offers a faster turn-around time, MS-MLPA covers both FMR1 and AFF2 genes, thus being more informative.

Acknowledgement: Project PN 16.22.05.02.

IN VITRO EVALUATION OF CYTOTOXIC EFFECTS AND ANTI-INFLAMMATORY ACTIVITIES OF EXTRACTS OF MUD IN BUZĂU COUNTY

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Introduction. The use of mud extract contributes to a long term stability of therapeutic effects, thus avoiding common inconveniences of conventional drugs, like installation of therapeutic resistance and adverse effects. Active fractions obtained from mud were investigated using *in vitro* methods regarding cytotoxicity and therapeutic efficacy. The real effects of mud bath applications on the inflammatory processes are still not clarified. The purpose of the investigations is to analyze the use of such extract in more addressed applications, like injection, besides the classical use of mud and its extract in topical applications.

Material and methods. Cytotoxicity testing was performed in vitro using ATCC-CRL-9855 cell cultures and MTS (*CellTiter 96® Aqueous One Solution Cell Proliferation Assay, Promega*). The anti-inflammatory action was evaluated by cytokine measurements using *Milliplex MAP Human Cytokine/Chemokine Magnetic Bead Panel* kit and analyzed using Luminex 200 system (Luminex Corp., TX, USA). We focused to establish if these mud extracts have cytotoxic effects (MTS assay) and to what extent and if they have anti-inflammatory effects. The extracts were provided by *Pell-Amar Cosmetics* as spray-dried powders. **Results. Cytotoxic activity**. For this purpose, we used different concentrations – ranging 3 to 75 mM, considering an "average" MW of 90 for extract components, at different cell densities (5000/10000 cells/well) and incubation times (48/72h). For 10000 cells incubated for 48 hours – IC50 were 1.6M for S1, 1.5M for S2 and S3, 1.45M for S4, 1.78M for S11, 1.4M for S13 and 1.7M for S12. For 5000 cells at 72 hours – IC50 were 1.6M for S1, S2 and S3, 1.5M for S4, 1.82M for S11, 1.65M for S13, 1.58M for S12. Our results indicated the relatively low-cytotoxic effects of the mud extract analyzed. **Anti-inflammatory activity.** In the first step of the experiment we used cells and mud extracts with no inflammatory response observed. Subsequently, we used lipopolysaccharides (LPS) – 50 ng/mL (for stimulation) and dexamethasone – 40 ng/mL (anti-inflammatory control) in cell cultures. The mud extracts were demonstrated to modulate cytokine release, generating profiles that are characteristic to anti-inflammatory activities, decrease of pro-inflammatory cytokine release at high concentrations of mud. We have noticed decreased levels for some of the pro-inflammatory cytokines - IL-6, IL-1a, TNFα, and MIP-1a; $p<0.05$, with statistical significance. **Conclusion.** Using a combination of *in vitro* assays, mud extracts could be classified and ranked for their cytotoxicity and specific activity, providing an effective screening system for the discovery of potential therapeutic compounds.

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REAL-TIME MONITORING OF THE CELL PROLIFERATION UNDER THE INHIBITORY ACTION OF SEVERAL PHARMACEUTICAL PRODUCTS

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Aim: to estimate cellular responses to various exposure conditions to several pharmaceutical products, by applying real-time monitoring technology (xCELLigence)

Material and Methods: U87 cell lines were used in experiments relative to human astrocyte cultures. In a first step, cell cultures of glioblastoma and astrocytes were maintained under standard conditions, namely EMEM medium with 10% fetal bovine serum, cultured in vials of 25 cm2 until confluence. After obtaining the amounts of cells necessary for cultivation in the xCELLigence system, the cells were plated under specific conditions. Experiments on exposure patterns at different doses of biologically active compounds were performed for all types of cell cultures, using a range of active substance concentrations between 0.1 and 100µM, of the following compounds: ZSTK, SB203580, EGF, insulin, in the presence of 5-FU or cisplatin.

Results: Substances were administered either individually or in multiple combinations of both ZSTK signaling inhibitors. One can first see a net inhibition of cellular proliferation by exposure to ZSTK, with a progressive decrease in viability and cell proliferation. There is also an increase in effects by simultaneous treatment with 5 FUs. The combination of ZSTK-cisplatin shows a dose-dependent inhibition, but of lower intensity compared to ZSTK-5FU. Under these conditions, an IC50 value for combined treatment can be estimated at a value of 92 ± 7nM.

Conclusion: A continuation of research to modulate cellular behavior is need, using real-time monitoring experiments.

Acknowledgement: This work has been partially supported by the grants PN 16.22.04.01, PN 16.22.05.03.

PROTEOMIC PROFILING IN PANCREATIC CANCER

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Background: Pancreatic cancer presents a major concern, due to a complex of unfavorable conditions that include the lack of early detection markers, reduced efficacy of most therapeutical non-surgical approaches, rapid progression and invasiveness that usually lead to lethality. Serum cytokines may represent a major component of potential biomarker panels contributing to improvement of diagnostic and monitoring of pancreatic cancer patients.
Methods: Our data were obtained using two advanced proteomic profiling technologies: Luminex xMAP multiplexed biomarkers (pancreatic cancer associated cytokines, chemokines, angiogenic and growth factors – Milliplex MAP Human Cytokine Panel) and SELDI TOF-MS. Serum protein profiles from cancer and normal patients were analyzed with the ProteinChip Data Manager Software 3.0.7. Both technologies provided robust discrimination between pancreatic cancer patients and normal-matched controls.
Results: We have investigated the individual and combined utility of the two approaches for protein expression analysis in 40 cases (20 pancreatic cancer and 20 controls). Cytokines expression (IL-6, IL-10, VEGF, IL-1β, IL-8, bFGF, IL-12, TNFα) was strongly correlated with tumor stage in pancreatic carcinoma. The possible biomarkers discovered by SELDI-TOF-MS may be applied in early pancreatic cancer detection.
Conclusion: Combining serum protein profiling using multiplexed assays and mass spectrometry can prove to be an effective strategy for the discovery of new proteins in pancreatic cancer. In addition to using less-invasive techniques, these proteins may become molecular biomarkers useful for diagnosis, prognosis and could be involved in this pathology as therapy targets.

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PROTEOMICS FOR PREDICTION OF CHRONIC KIDNEY DISEASE PROGRESSION

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Background: Chronic kidney disease (CKD), despite being a silent epidemic disease, represents one of the main causes of mortality in general population. Recent advances in proteomic technology have provided an excellent opportunity to achieve high-throughput screening as well as testing that could help early diagnosis, evaluation and prognosis in CKD. The present study aims to assess the relationship between bone/vascular alterations and the circulating level of 7 biomarkers in CKD patients with different stages.

Methods: Two proteomic technologies – xMAP array and SELDI-ToF MS (surface-enhanced laser desorption/ionization time-of-flight mass spectrometry) were assessed to quantify a panel of 7 biomarkers (IL-6, TNF-α, OPG-osteoprotegerin, OPN-osteopontin, OCN-osteocalcin, FGF-23 and Fetuin-A). A total of 106 serum samples (86 with CKD - stages 4, 3, 2 and 20 normal controls) were analyzed using CM10 ProteinChip Arrays. Serum protein profiles from CKD and normal patients were analyzed with the ProteinChip Data Manager Software 3.0.7.

Results: The proteomic spectra obtained were compiled, normalized, and mass peaks with mass-to-charge ratios between 2 and 100kDa were identified. Peaks information was analyzed using univariate statistics and 10 significantly different protein peaks were selected, with AUC values ranging 0.750-0.930 and $p \leq 0.05$. The results obtained by SELDI-ToF-MS analysis confirm those obtained by xMAP array.

Conclusions: The biomarkers panel shows great potential for early detection, clinical evaluation and prognosis in CKD patients. The present study reflects the clinical utility of a multiplexed biomarker panel in CKD and was found to be more relevant than one single biomarker to detect patients in early CKD stages. Proteomic techniques shed light on clinical evaluation for CKD staging and progression.

Acknowledgment: Partially supported by the grant PNII 93/2012 and PN 16.22.05.03.

A RARE CASE OF OVARIAN MALIGNANCY IN A 47-YEAR-OLD FEMALE: CLINICO-SURGICAL AND CYTO-HISTOPATHOLOGICAL CORRELATIONS

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Keywords: ovarian malignancy, yolk sac tumor

Introduction: Malignant primitive germ cells tumors represent ≈4% of ovarian malignancy. We present the case of a 47-year-old woman with abdominal tenderness and unusual ultrasonographic (US) findings admitted for further investigations in our clinic.
Methods: Abdomino-pelvin US was repeated and revealed a 17/10 cm pelvi-abdominal mass with indistinct borders on the anterior uterine wall, no signs of metastases; findings were suspicious of uterine sarcoma. Laparotomy was performed, and revealed a polymorhous, friable, voluminous right ovarian mass adherent to caecum, small intestine, omentum and uterine adnexa with peritoneal sero-sagvinolent effusion. Subtotal histerectomy with bilateral salpingo-oophorectomy was done. The peritoneal effusion and surgical specimen were adequately processed and cyto-histopathologically and immunohistochemically examined.
Results: On microscopy, peritoneal effusion was positive for malignancy with serous fenotype and histo-processed tumor was comprised of atypical cells, frequent mitosis, with reticulo-microcystic, papillary and hepatoid growth patterns, focally with cytoplasmic clearing, myxoid areas, Schiller-Duval bodies. Immunohistochemical profile: AFP diffusely positive, p53 positive, glypican-3 positive, CK7 and EMA focally positive, WT-1 negative, Ki 67 positive in 60% of tumoral cells.
Conclusions: Histopathological and immunohistochemical profiles were characteristic of Yolk sac tumor, a very rare finding in women over 40 years. Despite the clinical appearance and phase diagnosis, a thorough differential diagnosis should always be made and rarer entities not overlooked.

COMPARISON OF UVA- AND GAMMA RADIATION-INDUCED EFFECTS ON SERUM ALBUMIN ADDRESSED BY THz SPECTROSCOPY AND Trp FLUORESCENCE MEASUREMENTS

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Keywords: biomarker of radiation exposure

Serum albumin (SA) is the most abundant soluble protein found in blood plasma. SA is involved in the transport and distribution of numerous endogenous and exogenous substances, and its biological function is known to be closely correlated to the appropriate native conformation. The goal of the study was to identify a sensitive parameter associated with radiation-induced changes of SA, as a biomarker of radiation exposure (for both ionizing and non-ionizing radiation). We investigated the effects of UVA (365 nm) and gamma radiation on SA structure, using Terahertz (THz) spectroscopy (radiation with frequencies comprised between 0.3 and 3 THz, where 1 THz = 1012 Hz), and Fluorescence spectroscopy (Tryptophan fluorescence measurements - the excitation wavelength set to 295 nm and spectra collected over 300–500 nm range). THz spectroscopy addresses collective, low frequency and high amplitude motions of biomolecules, and provides information regarding conformational changes. Intrinsic Trp fluorescence measurements can also provide information about protein dynamics and/or protein denaturation. Samples with a physiological concentration of bovine SA (40 g/L) were prepared in phosphate buffered saline. The samples were exposed to both UVA light (accumulated doses of 4.7, 9.4, 18.8, 28.2 and 37.6 J/cm2), and gamma rays (1.25, 2.5 and 5 Gy). The study found that SA exposed to gamma rays of higher quantum energy than UVA, undergo much smaller extent of changes in Trp fluorescence and THz spectra, than when exposed to UVA. After UVA irradiation, a major impact on bovine SA conformation was registered for the dose of 18.8 J/cm2 as shown by both THz spectra and Trp fluorescence measurements. We conclude that THz spectra and intrinsic Trp fluorescence are very sensitive parameters for the evaluation of the radiation exposure effects.

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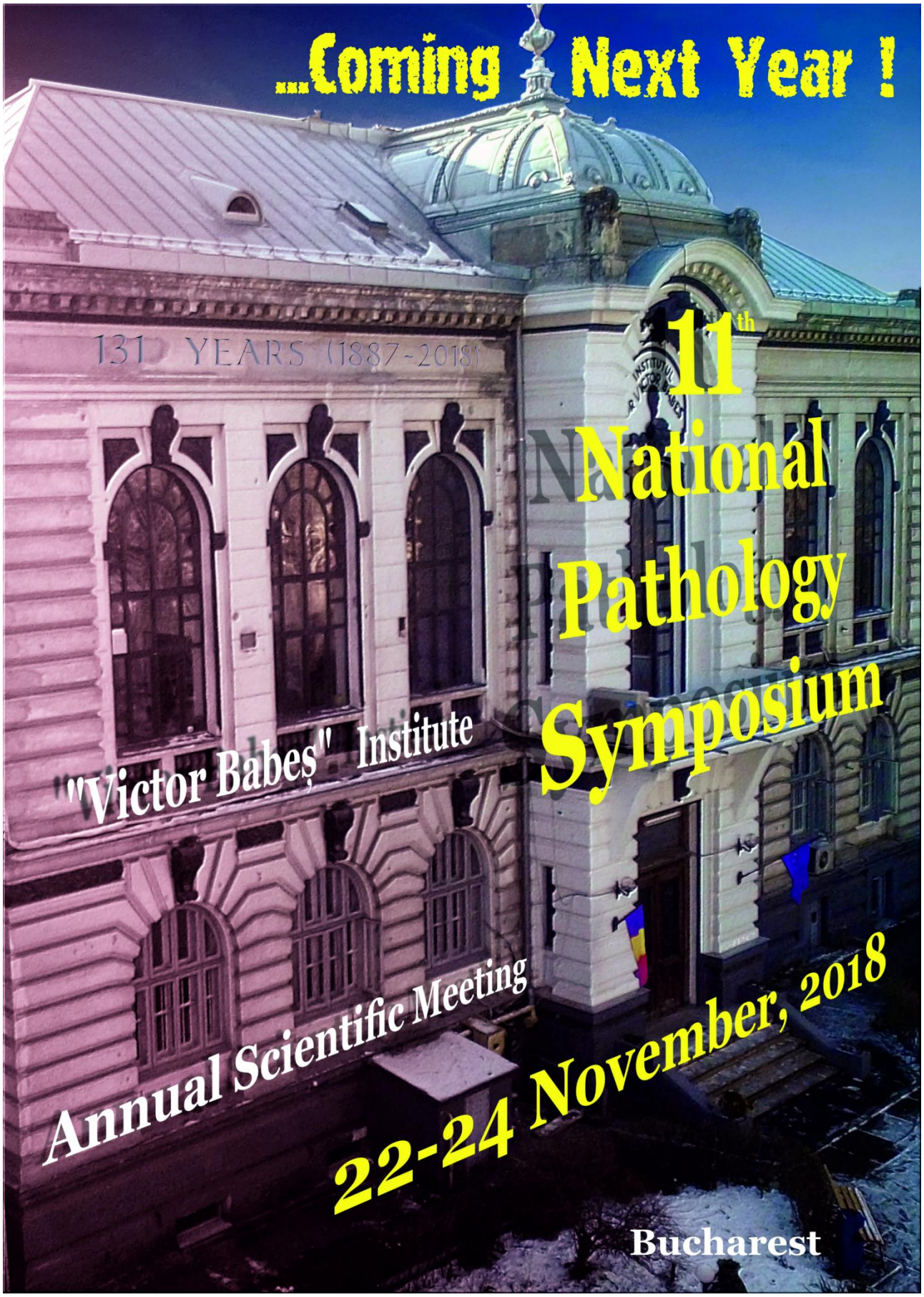
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